Analysis of RAPD Polymorphisms in *Rastrelliger kanagurta* off India

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Abstract

Analysis of RAPD loci in *R. kanagurta*, as generated by the arbitrary primer OPA 07 (GAAACGGGTG), revealed maximum within-region genetic variability for samples from the east coast of India. Dendograms did not show clear centre-specific clusters. Restricted intermixing among the individuals between the east and west coasts is suggested.

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 in Indian waters. 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). Observations based on tagging studies indicate two possible movements of mackerel in Indian waters, 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 and Nicobar Islands.

Some attempts have been made to delineate the stock structure of Indian mackerel from the east and west coasts based on morphometry and meristic characteristics, and more recently by studying protein (allozyme) polymorphisms (Seshappa 1985; Menezes et al. 1990, 1993; Verma et al. 1994, 1996). These studies reveal low genetic differentiation in the species.

DNA-level markers have several advantages over morphometric, meristic or protein (allozyme) markers for studying stock structure in fishes (Fergusson et al. 1995; O'Reilly and Wright 1995; Jayasankar 1997). Random Amplified Polymorphic DNA (RAPD) is a DNA approach for detecting genetic polymorphisms (Welsh and McClelland 1990; Williams et al. 1990). Dispensability of prior knowledge of the target sequence and technical simplicity make RAPDs attractive for population genetic studies.

Our earlier study (Jayasankar and Dharmalingam 1997) illustrated the generation of RAPD fingerprints in 30 mackerel specimens collected from commercial landings at two locations on the west coast (11 from Mangalore and 8 from Fort Kochi) and one on the east coast (Mandapam, 11 specimens) of India (9°17'-12°52'N, 74°52'-79°15'E). This paper analyzes the fingerprints to evaluate genetic variation.

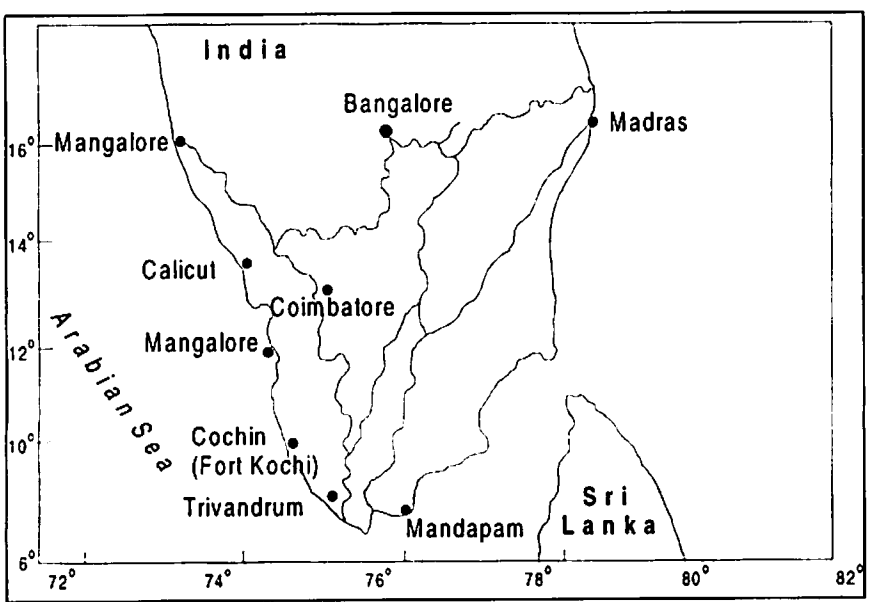

Fig. 1. Map of India showing sampling locations (Mandapam, Mangalore and Fort Kochi).

Materials and Methods

Details of sampling sites, sampling methods, extraction of genomic DNA and optimization of DNA amplification by RAPD-PCR are given elsewhere (Jayasankar and Dharmalingam 1997). Briefly, total genomic DNA was extracted from frozen/alcohol-preserved muscle by a modified phenol-chloroform procedure and was amplified in 25µl reaction mixtures containing 10 mM Tris HCl pH 8.3, 50 mM KCl, 0.001% gelatin, 2.4 mM $MgCl_2$, 0.03 mM each of dATP, dTTP, dGTP and dCTP, 0.64 µM random primer and 1 U *Taq* DNA polymerase. PCR was programmed for 1 initial cycle of 30 sec denaturation (94°C), 30 sec annealing (36°C) and 1 min extension (72°C) followed by 45 cycles of 30 sec denaturation, 30 sec annealing and 2 min extension. A final extension for 7 min was performed at the end. Amplified products were resolved in 1.4% agarose (Sigma) gel electrophoresis in 1 X TAE. They were stained in ethidium bromide solution and polaroid photographs were taken under UV illumination. Of the RAPD markers generated by 35 arbitrary primers from kits A, F and G of Operon Technologies, Inc., those produced by OPA 07 were considered to interpret genetic relationships in view of their high percentage of amplification (amplification refers to the presence of at least one polymorphic band per gel) and better reproducibility. Data sets generated by two commercial brands of *Taq* DNA polymerase enzymes, *Taq* 1 (Amresco) and *Taq* 2 (Rama Biotechnologies) were analyzed.

Molecular weights of RAPD bands (=loci) were estimated using a simple BASIC program based on the relative mobility of the fragments with respect to a standard set of DNA size markers (1 *Hind* III or 1 *Hind* III + *Eco*RI). This was followed by cluster analysis. A distance measure, Simple Matching Coefficient (SMC) was calculated by pairwise comparison of loci. SMC is given as the ratio of total number of matches for two individuals compared (i.e., both loci absent or present) to the total number of possible loci. SMC value of 1 indicates that two individuals have completely similar patterns.

The genetic distance between the two individuals is 1-SMC. The distance matrix was analyzed using the Fitch-Margoliash program version 3.56c of the PHYLIP package. Trees (dendograms) were produced using Saitou and Nei's (1987) Neighbor Joining Analysis. RAPD polymorphisms were scored as a "1" for the presence of a fragment and "0" for its absence.

Results and Discussion

Taq 1 generated more loci with wider range (Tables 1 and 2). Within-region variability was maximum for Mandapam (east coast) samples while Mangalore and Fort Kochi (west coast) samples

Table 1. RAPD markers generated using Amresco Taq *DNA polymerase in* R. kanagurta *collected from three locations in India. "1" denotes presence of a locus and "0" denotes the absence of the same. M, Mandapam; ML, Mangalore; F, Fort Kochi.*

	Individuals/loci (kb)													
Site	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.3	1.4	1.5	2.0
M1	1	1	0	0	1	0	0	1	0	1	1	0	1	0
M2	1	0	0	0	1	1	0	1	0	1	1	0	1	0
M3	1	1	0	0	0	1	0	0	0	0	0	0	0	0
M4	0	0	0	0	0	0	0	0	0	0	0	1	0	0
M5	1	1	0	0	0	1	0	0	1	0	0	0	1	0
M6	0	0	1	0	0	0	0	0	0	0	0	1	0	0
M7	0	1	0	0	0	0	0	1	0	0	1	1	0	0
M8	0	1	1	0	1	1	0	1	1	0	1	1	0	0
ML1	1	1	1	1	0	0	0	0	1	0	0	0	1	0
ML2	0	1	1	0	0	0	0	1	0	0	0	1	0	0
ML3	0	1	1	0	0	0	0	0	0	0	0	1	0	0
F1	1	0	0	0	0	0	0	1	0	1	0	0	1	0
F2	1	0	0	0	1	1	1	1	0	1	1	0	1	1
F3	1	0	0	0	1	0	0	1	0	0	0	0	1	0
F4	1	1	1	0	0	0	0	0	0	0	0	0	0	0
F5	1	0	0	0	0	0	0	1	0	0	0	0	1	0
F6	0	1	0	0	0	1	0	1	0	0	0	0	1	0
F7	1	1	0	0	0	0	0	0	0	0	0	0	1	0
F8	1	1	0	0	0	0	0	1	0	1	1	0	1	0
F9	0	1	0	0	1	0	0	1	0	0	1	0	1	0
F10	0	1	0	0	1	1	0	0	1	0	0	1	0	0
F11	1	1	1	1	1	1	0	1	1	1	0	1	0	0
F12	0	0	0	1	1	1	0	1	0	0	0	1	0	0

Table 2. RAPD markers generated using Rama Biotechnologies Taq DNA polymerase in Indian mackerel collected from three locations in India. "1" denotes presence of a locus and "0" denotes the absence of the same. M, Mandapam; ML, Mangalore; F, Fort Kochi.

Site	Individuals/loci (kb) 0.6	0.7	0.9	1.0	1.1	1.3	1.4	1.5	1.6	1.7	1.9	2.0
M1	0	0	0	0	0	1	0	0	0	1	0	0
M2	0	0	0	0	0	0	0	0	0	1	0	0
M3	0	0	0	0	0	0	0	1	0	1	0	0
M4	0	0	0	0	0	0	0	0	0	1	0	0
M5	0	1	0	0	0	0	1	0	1	0	0	0
M6	0	0	0	0	0	0	1	0	1	0	0	0
M7	1	0	0	0	0	0	0	1	0	0	0	0
M8	1	0	0	0	1	0	1	0	1	0	0	0
M9	0	0	1	0	1	0	1	1	0	0	0	0
M10	0	0	1	0	0	0	0	1	0	0	0	0
M11	0	0	1	0	0	0	0	1	0	0	0	0
ML1	0	0	0	0	0	0	0	1	0	1	0	0
ML2	0	0	0	0	1	0	0	1	0	1	0	0
ML3	0	0	0	0	0	0	0	0	0	1	0	0
ML4	0	0	0	0	1	0	0	0	0	1	0	0
ML5	0	0	0	0	0	0	0	1	0	1	0	0
ML6	1	1	0	0	1	1	1	0	1	0	0	0
ML7	0	0	0	1	1	1	1	0	1	0	0	0
ML8	1	0	1	0	0	0	1	0	1	0	0	1
ML9	0	0	0	0	1	0	0	1	1	0	0	0
ML10	0	0	0	0	1	0	1	1	0	0	0	0
ML11	0	0	0	0	1	0	0	1	0	0	0	0
F1	0	0	0	0	0	0	1	0	0	1	0	0
F2	0	0	0	0	0	0	0	1	0	1	0	0
F3	0	0	0	0	0	1	0	0	0	1	0	0
F4	0	0	0	0	0	0	0	0	0	1	0	0
F5	0	0	1	0	0	0	1	0	1	0	0	0
F6	1	0	0	0	0	0	1	1	0	0	1	0
F7	0	0	0	0	0	0	0	0	1	0	0	0
F8	0	0	0	0	1	0	0	1	0	0	0	0

showed relatively more homogeneity (Table 3). A perusal of the dendograms (Figs. 2 and 3) based on 1-SMC matrices shows an absence of clear center-specific clusters in both data sets. Observation of greater branch lengths between Fort Kochi and Mandapam individuals (F11 and M8 in Fig. 2) and those between Mangalore and Mandapam individuals (ML6 and M8 in Fig. 3) as well as uniformity of mean SMC values in the individuals from the two centers of the west coast suggest restricted intermixing of mackerels between the east and west coasts of India. It is noted in this context that samples of orange roughy from two distinct New Zealand spawning sites did not reveal obvious differences, although the authors conclude that the degree of intersample identity would be premature (Baker et al. 1992).

In our earlier work (Jayasankar and Dharmalingam 1997), RAPD loci produced by the same random primer showed markedly different patterns between Indian mackerel (*R. kanagurta*) and king seer (*Scomberomus commerson*). Similarly, arbitrary primers of different sequences yielded different banding patterns in the same template of both scombroid fishes, indicating utility of RAPD's manipulation of primer sequence to generate amplification products of desired complexity to suit different purposes like genetic mapping or genotyping.

Concentrations of DNA, $MgCl_2$ and *Taq* DNA polymerase, primer choice and gel staining methods can influence the number and intensity of amplification products (Hadrys et al. 1992). RAPDs of Indian mackerel are sensitive to the concentrations of primer, $MgCl_2$ and the brand of *Taq* DNA polymerase enzyme (Jayasankar and Dharmalingam 1997). RAPD-based analyses of *Oreochromis* have shown that while primers differed in the level of information they afforded, general consensus between each of the primers was supported statistically (Bardakci and Skibinski 1994). Southern-

Table 3. Simple Matching Coefficients (SMC) of RAPD loci in R. kanagurta *collected from three locations in India.*

Center	Amresco *Taq* data set		Rama Biotechnologies *Taq* data set	
	Range	Mean ± sd	Range	Mean ± sd
Mandapam	0.18 - 0.82	0.47 ± 0.18	0.33 - 1.00	0.62 ± 0.18
Mangalore	0.50 - 0.75	0.67 ± 0.14	0.27 - 1.00	0.63 ± 0.21
Fort Kochi	0.29 - 0.93	0.62 ± 0.16	0.33 - 0.89	0.63 ± 0.16
Pooled	0.09 - 0.93	0.60 ± 0.17	0.36 - 1.00	0.69 ± 0.16

blotting of RAPDs with oligonucleotide repeat probes can be more informative (Cifarelli et al. 1995).

We suggest a combination of greater sample size, higher number of informative primers and a *Taq* DNA polymerase enzyme which can yield more loci for generating adequate data for interpreting genetic stock structure in fish populations.

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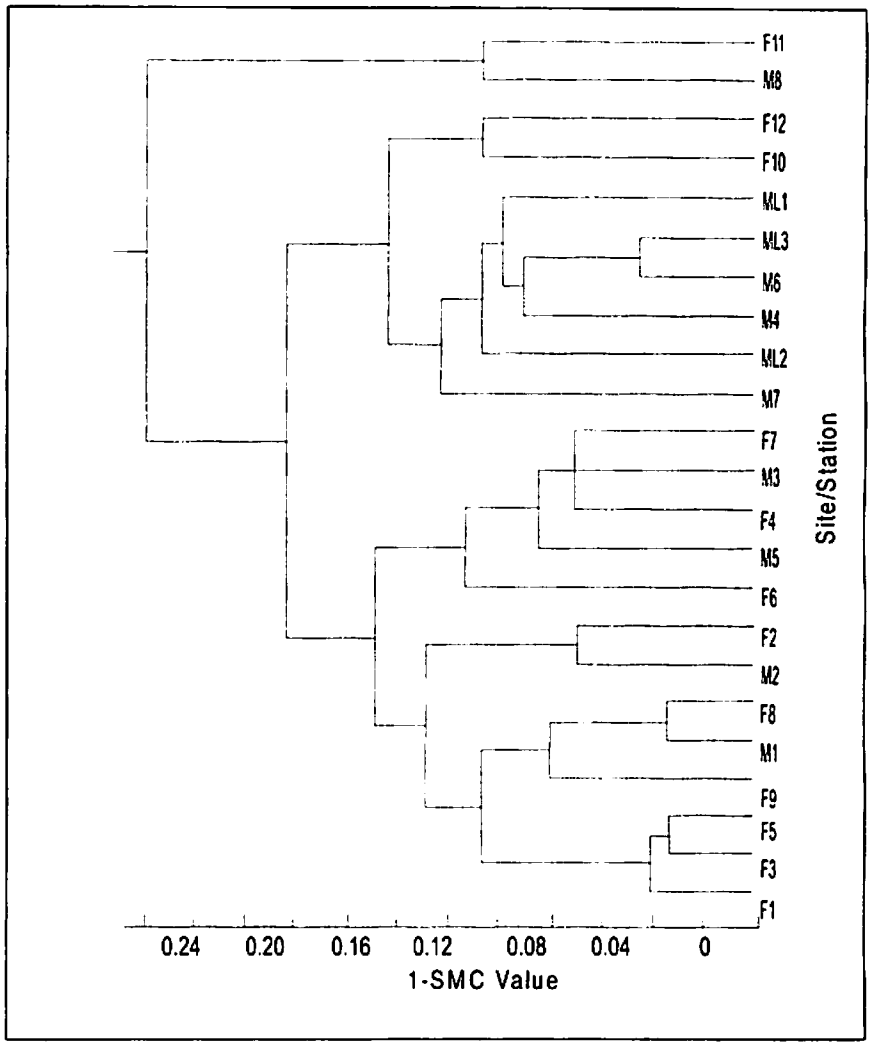

Fig. 2. Dendrograms illustrating the genetic relationship among different mackerel specimens collected from three locations in India based on 1-SMC matrix using Neighbor Joining Analysis. RAPDs were generated by Amresto Taq polymerase. M, Mandapam; ML, Mangalore; F, Fort Kochi.

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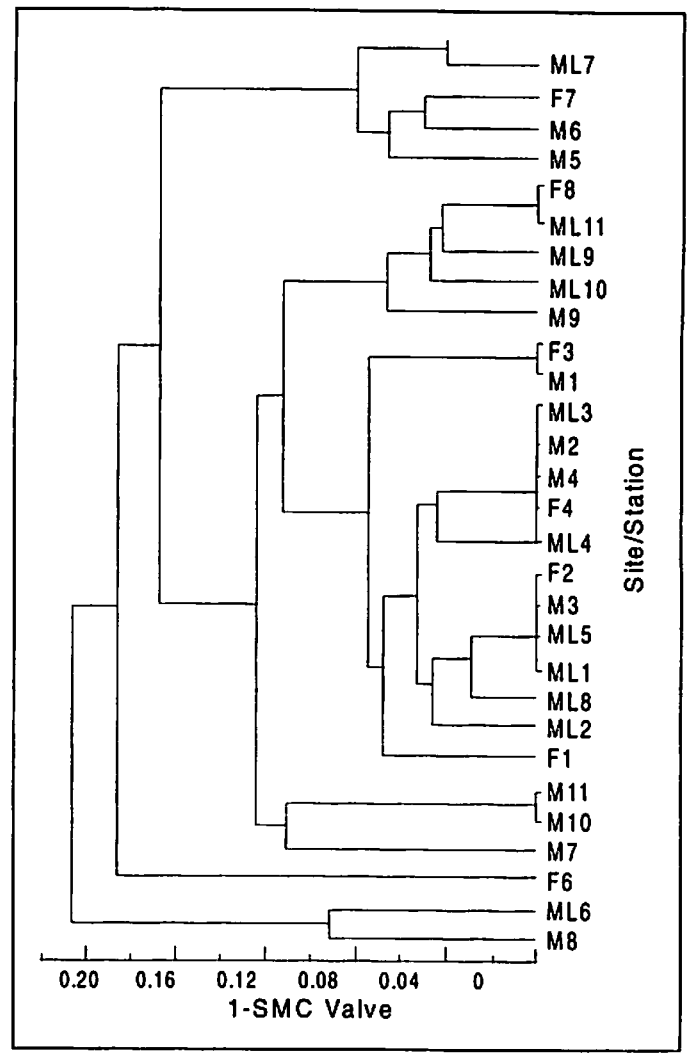

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