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Genetic characterization of *Metapenaeus affinis* (H. M. Edwards, 1837) using RAPD markers

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Abstract Genetic structure of four populations of *Metapenaeus affinis* from Maharashtra, Orissa, Kerala and Tamil Nadu in India was studied using RAPD markers. Five selective primers provided distinct and consistent RAPD profiles in all the four populations. The bands in the range 225–1,900 bp were scored for consistent results. The RAPD profiles generated by all the five primers revealed varying degrees of polymorphism, ranging from 25.00% (primer E-03) to 65.00% (primer E-06). Nei's (Nei M, Natl Acad Sci Proc USA 70:3321–3323, 1973) genetic diversity (h) among the four populations varied from 0.2565 ± 0.2146 (Orissa population) to 0.3576 ± 0.1897 (Maharashtra population).

Keywords *Metapenaeus affinis* · RAPD · Genetic characterization

Introduction

India is blessed with several species of shrimps supporting the commercial fisheries. The insatiable demand for crustaceans in the international markets has resulted in tremendous increase in their exploitation along Indian coast during the past four decades. The widespread decline in shrimp production in Indian waters emphasizes the need to characterize the natural populations for aquaculture and biodiversity conservation and management.

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Metapenaeus affinis [1] commonly called as king prawn is an important commercial shrimp species in India. The general distribution of the species covers Indian seas through Malaysia and part of Indonesia and Hong Kong to Japan. It is distributed through the West and certain parts of East coast of India. The mitochondrial 16S rRNA gene and the protein coding cytochrome *c* oxidase subunit I (COI) genes have been sequenced in various invertebrate and vertebrate taxa [2–4]. Studies using randomly amplified polymorphic DNA (RAPD) have been made to determine genetic diversity, taxonomic identity, and systemic details of various organisms in the past [5–14].

The present article describes genetic structure of four populations of *M. affinis* from Indian waters. This is the baseline data for future research on population structure of *M. affinis*, as no previous report exists on this species.

Materials and methods

Collection of samples

A total of 160 *M. affinis* individuals, 40 from each population were collected from four geographic regions of India viz., Mumbai (Maharashtra; Lat 18°99′ N, Long 72°87′ E); Paradeep (Orissa; Lat 20°3′ N, Long 86°55′ E); Kochi (Kerala; Lat 9°58′ N, Long 76°17′ E) and Tuticorin (Tamil Nadu; Lat 8°48′ N, Long 78°1′ E). Tissue samples from pleopods, telson and 2nd pleura were preserved in 95% ethanol and stored at −20°C until DNA was extracted.

Genomic DNA extraction

Total genomic DNA was isolated from the muscle tissue of pleopods according to the method of SDS–Phenol/

Chloroform as described by Williams et al. [6] with some modifications. Shrimp muscle tissue (~50 mg) was cut into small pieces and crushed using a sterile micropestle in the presence of 1 ml chilled TEN buffer (50 mM Tris–HCl, pH 8.0,10 mM EDTA, 100 mM NaCl), and transferred to a 2 ml Eppendorf tube. Proteinase K (200 μg/ml), Sucrose (1%) and SDS (1%) were added in the tube. Samples were incubated at 55°C for 12–14 h, after incubation samples were extracted once with Tris saturated phenol (pH 8.0) and twice with chloroform:isoamyl alcohol (24:1). The DNA was precipitated with 3 M sodium acetate (pH 5.5) and isopropanol, washed once with 70% ethanol and finally dissolved in TE (10 mM Tris–HCl, 2 mM EDTA pH 8.0). DNA quality and quantity were determined by agarose gel electrophoresis and by spectrophotometer (Eppendorf, Germany).

RAPD–PCR amplification and product analysis

Fifty decanucleotide primers were screened and five primers (E1, E2, E3, E4 and E6; Operon, USA) which gave consistent RAPD profiles were selected for the genetic characterization of *M. affinis*. The RAPD polymorphism was examined for 25 individuals from each of the four populations (Maharashtra, Kerala, Tamil Nadu, and Orissa) following Williams et al. [6] and Welsh and McClelland [5] with necessary modifications.

The polymerase chain reaction (PCR) was carried out in a 25 μl reaction volume containing $MgCl_2$ 2.0 mM, 1× PCR buffer, 200 μM each dNTPs, 0.20 μM random primer, 0.75 U Taq DNA polymerase and 40 ng genomic DNA. PCR conditions included initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 36°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 8 min. Amplified DNA was separated by electrophoresis through 2% agarose gel containing ethidium bromide (0.5 μg/ml) in 0.5× TAE buffer at a constant voltage of 80 V [15]. The gels were imaged using Syngene gel documentation system, UK. Gene runner DNA ladder was used as a molecular weight standard.

Data analysis

To maintain consistency, only the repeatable major bands ranging from 200 to 2,200 bp were scored. Bands observed in each lane were compared with all the other lanes of the same gel and reproducible bands were scored as present (1) or absent (0). Fragment sizes were estimated based on the 100 bp Plus DNA Ladder (Bangalore Genie, India) according to the algorithm provided in the Gene Tools Software. Genetic variation was analyzed using the POPGENE version 1.31 software [16]. Dendrogram was constructed based on Nei's [17] genetic distances and the unweighed pair-group method with arithmetic averages (UPGMA) of Sneath and Sokal [18] using Tools for Population Genetic Analysis (TFPGA, ver. 1.3) [19].

Results

The RAPD profiles of populations from Mumbai, (Maharashtra); Paradeep, (Orissa); Kochi, (Kerala) and Tuticorin, (Tamil Nadu) were generated for four geographically different populations of *M. affinis*. The RAPD fingerprints of total 160 individuals of *M. affinis* were carried out using optimized RAPD–PCR conditions for five selected primers. The polymorphism pattern obtained for four populations is shown in Table 1.

All the selected five primers gave distinct and consistent RAPD profiles for *M. affinis* from all the four populations (Figs. 1, 2, 3). The primers generated bands in the range of 200 to 2,200 bp. However, only the repeatable major bands ranging from 225 to 1,900 bp were scored for consistency. The RAPD profiles generated by all the five primers revealed varying degrees of polymorphism, ranging from 25.00% (primer E-03) to 65.00% (primer E-06). The range of number of bands and band size were 2–9 and 225–1,900 bp, respectively.

The present study revealed a wide variation of polymorphic loci (31.10–57.20%) among the four populations. The highest level of polymorphism (57.20%) was exhibited by the Maharashtra population where as the lowest level of

Table 1 The polymorphism pattern of four populations of *M. affinis*

Primer number	Range of number of bands	Band size (bp)	Total DNA bands	Polymorphic bands (percentage of polymorphism: %)
E-01 (CCCAAGGTCC)	2–6	310–927	52	28 (53.86%)
E-02 (GGTGCGGGAA)	3–7	225–890	46	16 (34.77%)
E-03 (CCAGATGCAC)	2–4	370–850	40	10 (25.00%)
E-04 (GTGACATGCC)	2–5	260–1150	64	35 (54.69%)
E-06 (AAGACCCCTC)	3–9	560–1900	84	55 (65.48%)

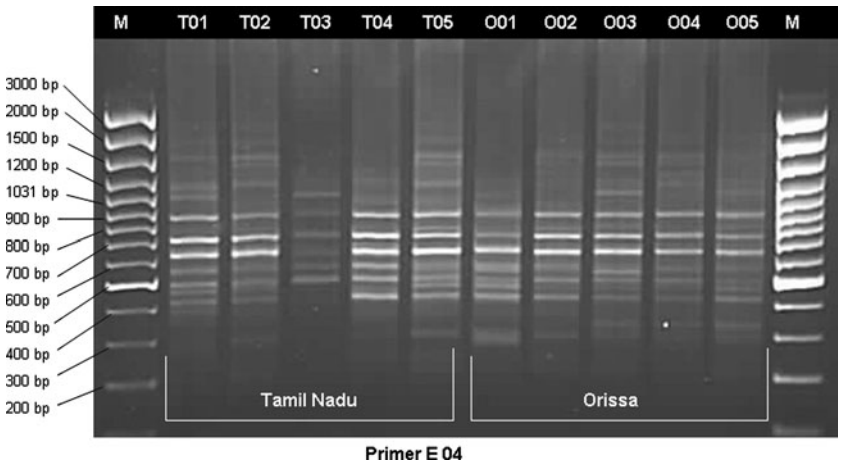

Fig. 1 RAPD profile of individuals from Tamil Nadu and Orissa population using primer E-04, MW marker: gene runner; Lanes T01–T05; individuals of Tamil Nadu population and lanes O01–O05; individuals of Orissa population

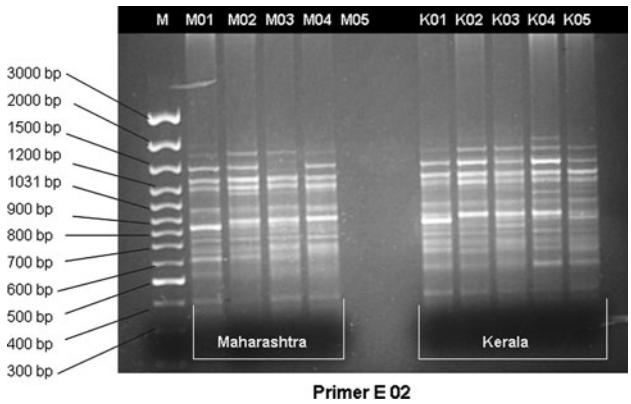

Fig. 2 RAPD profile of individuals from Maharashtra and Kerala population using primer E 02, MW marker: gene runner; Lanes M01–M05; individuals of Maharashtra population and lanes K01–K05; individuals of Kerala population

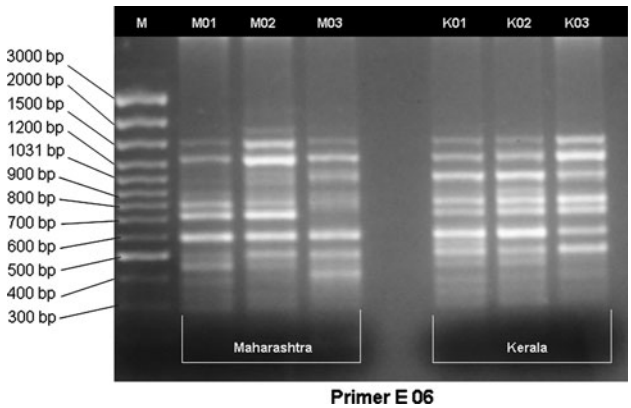

Fig. 3 RAPD profile of individuals from Maharashtra and Kerala population using primer E-06, MW marker: gene runner; Lanes M01–M03; individuals of Maharashtra population and lanes K01–K03; individuals of Kerala population

polymorphism (31.10%) was exhibited by the Orissa population. Nei's [20] genetic diversity (h) among the four populations varied from 0.2565 ± 0.2146 (Orissa population) to 0.3576 ± 0.1897(Maharashtra population). Average Similarity Index within population samples was also estimated (Table 2).

Table 2 Genetic diversity of four populations of *M. affinis*

Population	Polymorphic loci (%)	Average genetic diversity	Average similarity index within population samples (S)
Maharashtra	57.20	0.3576 ± 0.1897	0.904
Kerala	41.15	0.2976 ± 0.1376	0.912
Tamil Nadu	34.65	0.2794 ± 0.0138	0.923
Orissa	31.10	0.2565 ± 0.2146	0.894

Table 3 Nei's unbiased measures of genetic distance

Population	Maharashtra	Kerala	Tamil Nadu	Orissa
Maharashtra	xxxxxx			
Kerala	0.1345	xxxxxx		
Tamil Nadu	0.1675	0.1956	xxxxxx	
Orissa	0.2245	0.2065	0.1986	xxxxxx

Interestingly, two population specific bands were found in the population of Maharashtra (400 bp in E-06 primer) and Tamil Nadu (1200 bp in E-04 primer).

Estimates of Nei's [21] genetic distance demonstrated sufficient genetic divergence to discriminate the samples of different species (Table 3). The highest genetic distance was observed between the populations of Maharashtra and Orissa where as the lowest genetic distance was observed between the populations of Maharashtra and Kerala. A dendrogram based on Nei's genetic distance is shown in Fig. 4. Three clades were identified on the dendrogram with the Maharashtra and Kerala populations appearing on same cluster, while the Tamil Nadu and Orissa populations appearing on separate clade.

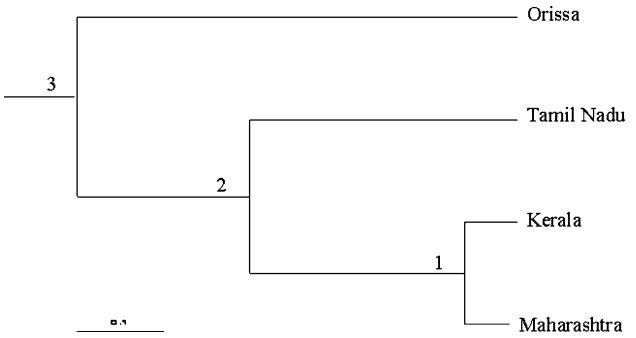

Fig. 4 Dendrogram of four populations of *M. affinis* based on Nei's [28] Genetic distance: Method = UPGMA-Modified from NEIGHBOR procedure of PHYLIP Version 3.5

Discussion

The assessment of genetic diversity is critical to conservation and management of aquatic genetic resources. Characterization of the genetic structure of the populations being harvested assists in defining the biological units and genetic stock concept [22]. Genetic variation has the potential to play a key role in selective breeding programs for shrimps [23]. Taxonomic and population studies on *Metapenaeus* species in the past have been mainly on analysis of external morphological traits which was found to be strongly influenced by the environment in several species, and may not be indicative of underlying genetic divergence [24]. To date, the RAPD technique has proven very powerful and informative in many decapods where biochemical markers have proven conservative. The RAPD data shows levels of polymorphism that are higher than allozymes [25].

Gracia et al. [26] reported a higher genetic variability in *Penaeus vannamei* populations as polymorphic bands ranged from 39 to 77%. The percentage of polymorphic fragments within a geographic sample of *Penaeus monodon* ranged from 46.7 to 61.4% [27]. Mishra et al. [12] reported 22.3–40.9% polymorphic loci in three populations of *M. dobsonii* based on RAPD markers. The high level of genetic diversity observed in *M. affinis* will be useful for selective breeding, maintaining stock diversity and distinguishing hatchery stocks from the wild populations. Gracia and Benzie [8] identified three polymorphic RAPD markers from parents and offspring of six families of *P. vannamei* and these family-specific markers for selective breeding programmes at the family level.

The highest genetic distance observed between the populations of Maharashtra and Orissa and the lowest genetic distance between the populations of Maharashtra and Kerala may be justified by the closer geographic distribution between Maharashtra and Kerala and distant geographic distribution between Maharashtra and Orissa.

Tassanakajon et al. [28] and Mishra et al. [12] observed population specific bands in *P. monodon* and *M. dobsonii*, respectively. The population specific band observed in *M. affinis* could be useful for development of Sequence Characterized Amplification Region (SCAR) marker.

Liu et al. [29] suggested that six or seven primers were sufficient to assess genetic variability within and among populations of highly polymorphic species. In the present study, the identified five primers provided consistent and distinct band patterns in the four populations of *M. affinis*. The highly polymorphic loci generated by the RAPD primers in the present study will be helpful for genetic upgradation and conservation of *M. affinis* through selective breeding.

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