Available Online through
www.ijpbs.com

IJPBS |Volume 1| Issue 4 |OCT-DEC |2011|619-624

GEO-GENETIC POLYMORPHISMS OF INDIAN PENINSULAR LOBSTER *PANULIRUS HOMARUS* USING RAPD MARKERS

T. S. Rejinie Mon* and V. Aldous J Huxely
Biotech Research Laboratory, Department of Zoology, Thiru.Vi.Ka Govt Arts College,
Thiruvarur, Tamilnadu-610003, India
***Corresponding Author Email:** rejinie@gmail.com

PHARMACEUTICAL SCIENCES Research Article
RECEIVED ON 12-09-2011 ACCEPTED ON 05-10-2011

ABSTRACT
This study examines the population genetic structure and of the spiny lobster Panulirus homarus in the South Western and Western Indian Ocean. A total of 250 individuals were collected from five different spots along the coast of Indian Peninsula region such as Cochin, Vizhinjam (Vizhijam), Muttom, Chinnamuttom and Tuticorin Out of eight primers tested the primer 5'- AGCATTAGGG -3' produced reproducible bands in all populations. The banding patterns produced show a large amount of variations in each population being clearly distinguishable from one another.

KEYWORDS: *Lobster, Panulirus homarus, Polymorphisms, RAPD*

INTRODUCTION

Panulirus homarus is found throughout the Indo-Pacific region especially South West and West coast of India, east Africa and Indonesia [1]. The scalloped spiny lobster *P. homarus* catch is highly valuable and improved live hood of fishers [2]. Major fishing gears used in South west and west coast are gillnet, trammel-net and traps. *P. homarus* inhabits shallow waters between 1 and 90 m depth, mostly between 1 and 5 m; among rocks, often in the surf zone, sometimes in somewhat turbid water [3]. However, they respond to different environmental conditions that prevail in their respective areas [4]. Over exploitation, environmental pollution, fishing pressure and habitat loss have threatened *P. homarus* stocks in many parts of Peninsular India, but little is currently known about the levels and patterns of genetic diversity of this species. Generally marine species analyses revealed less spatial variation in genetic structure than populations inhabiting freshwater environments. This is due to the large population size, high potential for dispersal and the perceived lack of barriers to dispersal [5]. In this regard *P. homarus* in Peninsular India has special interest, because peninsula has different geographical regions, it restricted movements and depleted population. These reasons might restrict the gene flow in *P. homarus*.

Genetic sub-structuring of a species is important knowledge for managing harvested species and can be used to predict whether a locally depleted population will be successfully repopulated by immigrants. Information on genetic diversity is a basic requirement for any stock identification, stock enhancement and breeding program for the improvement of any aquatic species [6]. To avoid reduction in diversity and elimination of distinct stocks, understanding their population dynamics, including structuring of populations and genetic diversity, is critical [7]. The loss of genetic variability may possibly result in the loss of disease resistance or reduction in a population's capability to adapt a new environment. The application of genetic markers has been used to understand the population structure of taxa at inter and intraspecific levels. Information on genetic diversity is a basic requirement for stock identification, stock enhancement and breeding program for the improvement of any aquatic species [8, 9]. The genetic structure of a population is important information with ecological and commercial value. It is important for managing harvested species and can be used to predict whether a locally depleted population will be successfully repopulated by immigrants [10]. Apart from that, the geo influences among populations are also studied using these genetic markers. The gene or DNA polymorphisms have been extensively employed as a means of

assessing genetic diversity in aquatic organisms. The PCR provide a simple, fast and inexpensive means for genome analysis. Under PCR, a single, short oligonucleotide primer can be used to amplify specific sequence of genomic DNA. Random oligonucleotide primers produce random amplified polymorphic DNA (RAPD) that has been extensively used as molecular markers [11, 12].

Random Amplified Polymorphic DNA (RAPD) is a DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence [13, 14]. RAPDs produce DNA profiles of varying complexity, depending on the primer and template used. RAPD is an inexpensive yet powerful typing method for many animal species.

2. MATERIALS AND METHODS

2.1. Sampling

A total of 250 individuals were collected from five different spots along the coast of Indian Peninsula region such as Cochin, Vizhinjam (Vizhijam), Muttom, Chinnamuttom and Tuticorin. The tissue samples were dissected from the lobsters and preserved in 70% (v/v) ethanol.

2.2. DNA extraction

Total genomic DNA was extracted using a modified CTAB protocol [15]. Prior to extraction, the ethanol was evaporated from the muscle sample. Approximately 5 mm^3 of muscle tissue was incubated for 45 min at 60°C in 800µl extraction buffer (100mM Tris-HCl, 1.4 NaCl, 20mM EDTA, 2% CTAB), 2 µl β-mercaptoethanol, and 6 U proteinase K (Promega, Madison, USA), followed by two chloroform-isoamyl alcohol (24:1) separations. DNA was precipitated with 500 µl of ice-cold isopropanol, followed by storage at minus 20°C for 45 min, and centrifugation (14000 rpm) for 20 min at 4°C. The DNA pellet was rinsed with 80% ethanol, washed with 70% ethanol, air dried, and resuspended in 150 µl of sterile filtered dH_2O.

2.3. RAPD Primer

Eight random lobster primers (5'- CCACACTGTC -3', 5'- CCCTTCATTG -3', 5'- AGCATTAGGG -3', 5'- GGTGAACTGG -3', 5'- GGAACTGGCG -3' , 5'- CTTCTTTCGC -3', 5'- TTTCCCGACG -3' and 5'- GGAGATCGCA -3 were used in this study. 1 µM concentration of primer was used according to the manufacturer's instructions.

2.4. RAPD analysis

The RAPD reaction was performed in a 25 µl volume containing 2.5 µl 10× PCR buffer (750 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 3.5 mM $MgCl_2$, 200 µM deoxynucleoside triphosphates, 1.25 U of Taq DNA polymerase (Hi Media, Mumbai), 1 µM of OPG-11 primer, 11 µl of dsH_2O (sterile distilled water) and 2.5 µl of template DNA. Reactions were amplified in a Thermocycler (Vector, Japan). The amplification cycles were as follows; 50 cycles of 94 °C for 30 s, 37 °C for 1 min and 72 °C for 1 min 30 s. A final step of extension was applied at 72 °C for 10 min. The amplified DNA products were resolved by electrophoresis on a 2% agarose gel with Tris-borate EDTA buffer and stained with ethidium bromide, for 30 min and photographed under UV transillumination using Polaroid Gel Cam. A 100 bp DNA ladder (Promega, Maddison, USA) was used as a molecular weight marker on each gel. RAPD assays were performed at least three times each to check reproducibility.

2.5. Statistical analysis

RAPD patterns were visually analyzed and scored from the gel image. For the analysis and comparison of the patterns, only reproducible and distinct amplified fragments were scored. The data was calculated in 1 and 0 matrix and is used for establishing genetic variability between populations. Nei [16] unbiased genetic identity and genetic distance (Dn) values between populations were calculated from the data generated RAPD profile using POPGENE 1.31 [17]. Genetic distance values were utilized to construct a dendrogram through clustering analysis (UPGMA) to determine the relationship among the five populations.

The genotypes were determined by regarding the presence (1) and absence (0) of the bands. Nei's [16] unbiased genetic identity between populations was calculated using the data generated from the RAPD profiles using POPGENE [17]. Genetic identity values were utilized to construct a dendrogram through clustering analysis (UPGMA) to determine the relationship between the five populations.

3. RESULTS

The presence of variability within species is essential to survive and successfully respond to environmental changes. In the present study eight different decamer primers were tested in five

populations of *P. homarus* fish landing areas of Indian south peninsula. Out of eight primers tested the primer 5'- AGCATTAGGG -3' produced reproducible bands in all populations. The banding patterns produced show a large amount of variations in each population being clearly distinguishable from one another). The primer produced 5 to 7 RAPD fragments. The primer produced least bands in Vizhinjam populations (5 bands) and six RAPD fragments in Chinnamuttom populations. Seven RAPD fragments were produced in Cochin, Muttom and Tuticorin populations. In Vizhinjam population two DNA fragments were not produced (Fig. 1). This might be due to the gene mutation. 3 to 5 polymorphic loci were produced among the five populations.

Among the five populations the genetic distance was found between the populations Cochin and Muttom are 0.93103. Lowest genetic distance was found between the populations Muttom and Tuticorin (0.83636). Based on Phylogenic tree analysis three clusters were formed. First cluster contains Vizhinjam populations, second cluster contains Cochin populations and third cluster contains Chinnamuttom, Muttom and Tutucorin populations (Fig. 2).

4. DISCUSSION

RAPD is a popular molecular marker to differentiate geographically and genetically isolated populations; and has been used to verify the existence of locally adapted populations within a species that may have arisen either through genetic selection under different environmental conditions or as a result of genetic drift [18]. The useful methodology to assess genetic variations in fish populations is the RAPD technique, which is used in fishery management and conservation genetics of wild populations. Based on the amplification of genomic DNA by PCR (polymerase chain Reaction) with arbitrary nucleotide sequence primers, RAPD can detect high levels of DNA polymorphisms and can produce various genetic markers [19]. The potentials of RAPD in genetic mapping and population genetics have been widely documented for a large variety of organisms, including fish [20-22]. RAPD analysis has a number of advantages over RFLP and other techniques [23].

In the present study, the Geogenetic variations of spiny lobster were studied using RAPD marker. Based on the earlier sequences, eight random primers were designed and synthesized were used. The results clearly indicated that each geographical site has their influences on the gene pool of *P. homarus* and support the results of 18S rRNA gene polymorphisms of

P. homarus [24]. Different reproductive histories of hatchery and wild populations may generate differences in genetically based traits involving molecular, morphological and behavioral characteristics [25]. Many marine population genetic studies have linked areas of strong genetic structure to major boundaries in oceanographic and environmental features. In the present study, statistical analysis showed considerable genetic variation among individuals of *P. homarus* from five collection sites, i.e. Cochin, Vizhinjam, Muttom, Chinnamuttam and Tuticorin coast. It was found that there is a distinct genetic variation among the DNA from different populations. The populations from Cochin, and Vizhinjam, gene pool grouped together as a single cluster, and Muttom and Tuticorin grouped together as a cluster whereas the population from Chinnamuttam had distinct genetic cluster and is unique in fingerprints. This variability can be accounted due to the ecological differences of the Arabian Sea and Bay of Bengal. Populational genetic differentiation can be driven by ecological, evolutionary, over exploitation, geographical barriers and historical factors.

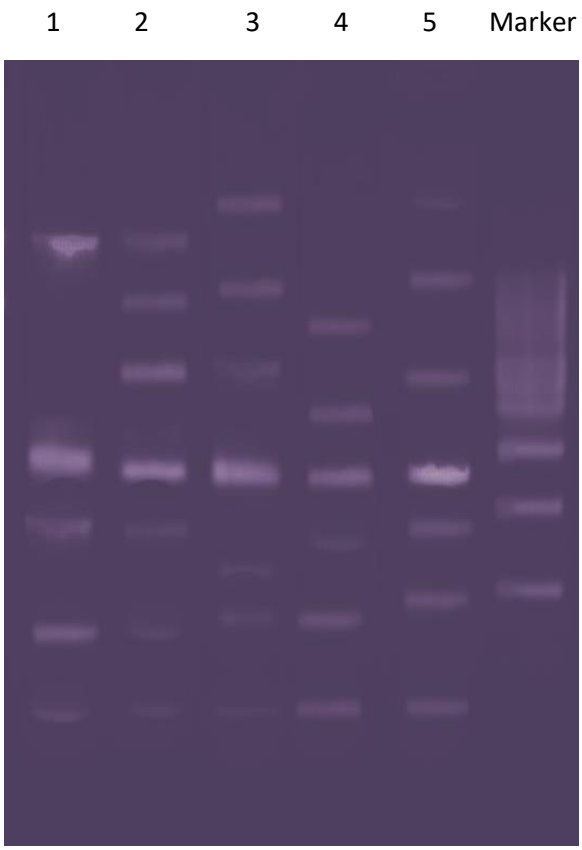

Fig 1. RAPD banding patterns of primer AGCATTAGGG (1 – Vizhinjam, 2 – Cochin, 3–Muttom, 4–Chinnamuttom, 5 – Tuticorin, M – 100 bp ladder Marker).

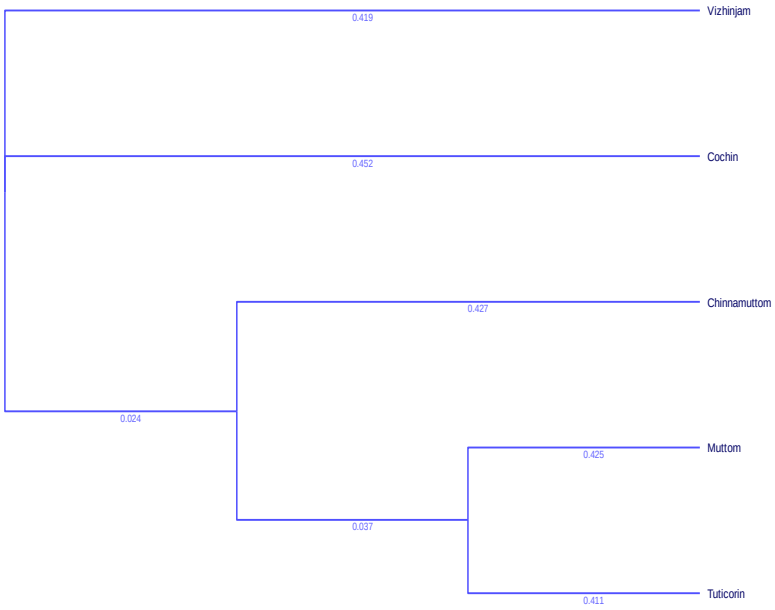

Fig 2. Phylogenetic tree constructed from RAPD electrogram.

Table 1. Distance Matrix generated from RAPD data

	'Vizhinjam'	'Cochin'	'Muttom'	'Chinnamuttom'	'Tuticorin'
'Vizhinjam'	0	0.87097	0.88136	0.86567	0.92537
'Cochin'	0.87097	0	0.93103	0.90909	0.92063
'Muttom'	0.88136	0.93103	0	0.92063	0.83636
'Chinnamuttom'	0.86567	0.90909	0.92063	0	0.84375
'Tuticorin'	0.92537	0.92063	0.83636	0.84375	0

REFERENCES

[1] Pollock, D. E. Speciation in spiny lobsters – clues to climatically-induced changes in ocean circulation patterns. Bulletin of marine science, 53(3): 937-944, (1993).

[2] Radhakrishnan, E. V. Deshmukh, V. D. Mary, K. Manisseri, M. Rajamani, Joe, K. Kizhakudan and Thangaraja, R. Status of the major lobster fisheries in India New Zealand Journal of Marine and Freshwater Research, Vol. 39: 723–732, (2005)

[3] Holthuis, L. B. Marine Lobsters of the World. An Annotated and Illustrated Catalogue of Species of Interest to Fisheries Known to Date, FAO Fisheries Synopsis 125. FAO, Rome, (1991).

[4] George, W. Tectonic plate movements and the evolution of Jasus and Panulirus spiny lobsters (Palinuridae). Marine freshwater research, 48: 1121-1130, (1997).

[5] Graves, J. E. Molecular insights into the population structures of cosmopolitan marine fishes. The Journal of Heredity, 89: 427-437, (1998).

[6] Tassanakajon, A. Pongsomboon, S. Rimphanitchayakil, V. Jarayabhaud, P and Boonsaeug, V. Random amplified polymorphic DNA (RAPD) markers for determination of genetic variation in wild population of the black tiger prawn (*Penaeus monodon*) in Thailand. Molecular Marine Biology and Biotechnology, 6: 110–115, (1997).

[7] Diniz, F. M. Maclean, N. Ogawa, M. Cintra, I. H. A and Bentzen, P. The hypervariable domain of the mitochondrial control region in Atlantic spiny lobsters and its potential as a marker for investigating phylogeographic structuring. Marine Biotechnology, 7: 462-473, (2005).

[8] Garcia, D. K and Benzie, J.A.H. RAPD markers of potential use in penaeid prawn *(Penaeus monodon)* breeding programs. Aquaculture, 130: 137-144, (1995).

[9] Vignal, A. Milan, D. San Cristobal, M. and Eggen, A. A review on SNP and other types of molecular markers and their use in animal genetics. Gen Sel Evol, 34: 275-305, (2002).

[10] Utter, F. M. Biochemical genetics and fishery management: an historical perspective. Journal of Fish Biology, 39: 1-20, (1991).

[11] Kikuchi, M. Ikeda, M and Fujio, Y. Genetic variability and di¡erentiation within and among populations of Japanese char: a comparison between isozyme and RAPD polymorphism. Fish Genetics and Breeding Science, 25: 27-36, (1997).

[12] Koh, T. L. Khoo, G. Fan, L. Q amd Phang, V. P. E. Genetic diversity among wild forms and cultivated varieties of Discus (*Symphysodon spp*.) as revealed by Random Amplified Polymorphic DNA (RAPD) fingerprinting. Aquaculture, 173: 485-497, (1999).

[13] Williams, J. G. Kubelik, A. R. Livak, K. J. Rafalski, J. A and Tingey, S. V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research. 109: 39-43, (1990)

[14] Welsh, J and McClelland, M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research, 18: 7213–7218, (1990).

[15] De Jong Y. Van Der Wurff, A. G. Stam, W. T. Olsen, J. L. Studies on Dasyaceae: 3. Towards a phylogeny of the Dasyacean (*Ceramilaes, Rhodophyta*), based on comparative rbcL gene sequences and morphology. European Journal of Phycology, 33: 187-201, (1998).

[16] Nei, M. Genetic distance between populations. American Naturals, 106: 283-292, (1972).

[17] Yeh, F. C, Yang, R. C and Boyle, T. POPGENE version 1.31, Microsoft Windows-based freeware for population genetics analysis. University of Alberta and Centre for International Forestry Research, Research, (1999).

[18] Fuchs, H. Gross, R, Stein, H and Rottmann, O. Application of molecular genetic markers for the differentiation of bream (*Abramis brama* L.) populations from the rivers Main and Danube. Journal of Applied Ichthyology, 14: 49-55, (1998).

19. Postlethwait, J. H. Jhonson, S. L. Midson, C. N. Talbot, W. S. Gates, M. Ballinger, E. W. Africa, D. Andrews, R. Carl, T. Eisen, J. S. Horne, S. Kimmel, C. B. Hutchison, M. Johnson, M and Rodriguez, A. A genetic linkage map for the zebrafish. Science, 264: 699-703, (1994).

[20] Caccone, A. Allegrucci, G. Fortumato, C and Sbordoni, V. Genetic differentiation within the European Sea Bass (*D. labryxo* as revealed by RAPD PCR assays. Journal of Higher Education and Research Development, 88: 316-324, (1997).

[21] Liu, Z. J. P. Li, B. J. Argue, R. Dunham, A. Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish Aquaculture. 174: pp. 59-68, (1999).

[22] Lynch, M. The neutral theory of phenotypic evolution. pp. 86-108. In L. Real (ed.) Ecological Genetics, (1994).

[23] Einum, S and Fleming, I. A. Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. Journal of Fish Biology, 50: 634-651, (1997).

[24] Rejinie Mon T.S. M. Victor Joseph and V. A. J. Huxely. 18S rRNA gene polymorphisms of *Panulirus homarus* populations from different geographic regions of Peninsular India. Journal of Theoretical and Experimental Biology, 8 (1 and 2) 85-93, (2011)

[25] Pelc, R. A. Warner, R. R and Gaines, S. D. Geographical patterns of genetic structure in marine species with contrasting life histories. Journal of Biogeography, 36: 1881–1890, (2009).

***Corresponding Author:**
T. S. Rejinie Mon*
Biotech Research Laboratory, Department of Zoology,
Thiru.Vi.Ka Govt Arts College,
Thiruvarur, Tamilnadu-610003, India