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RESEARCH ARTICLE

SAMPLE POPULATION GENOMIC STUDY OF RAPD PCR-DNA ANALYSIS IN *SCYLLA SERRATA AND SCYLLA TRANQUBARICA* COLLECTED FROM TWO NICHES OF CHENNAI COAST

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ABSTRACT

Among the four species of mud crab two species viz., *Scylla serrata* and *Scylla tranqubarica* were found distributed in Kovalam and Pulicat estuary regions of Bay of Bengal east coast (Chennai). They represent genetically distinct sympatric populations as revealed by RAPD PCR. Among the two populations *S. tranqubarica* revealed conserved DNA similarity between them. Two species *S. serrata* and *S. Tranqubarica* represent genetically isolated sympatric non-congeners. The two populations of *S. tranqubarica* viz., Kovalam and Pulicat represent congeners with genetic exchanges and inbreeding potential. The Pulicat crabs also constitute the ideal forms for culture activities for environmental reasons.

INTRODUCTION

Conservation of biodiversity especially the species of economic value has been considered worldwide an ecological Jargon. Towards this line, besides enumeration of the species diversity by numerical counts and/census and traditional taxonomy, modern biotechnology devices especially genome studies are of value to find out the vulnerable and endangered categories of species subjected to the human action of depredation. Next to shrimp and fish production, crab industry has become a field of aquaculture importance.

The mud crabs belonging to genus *Scylla* represents the most economically important category of edible food species. Generally the mud crabs inhabit the mangrove and estuarine areas. They are widely distributed over vast geographic areas ranging from south-eastern and Eastern Africa to Southeast Asia and Indo-pacific regions (Fuseya and Watanabe, 1996). Taxonomical details reveal that the mud crabs represent more than one species. Previous studies have revealed that though the mud crabs are differentiated morphometrically and by the colouration, they all belong to the Scylla genus in the four geographical regions of Southeast Asia viz., Ranong and Surat, Thai in Thailand, Cangio in Vietnam and Sarawak in eastern Malaysia.

Genetic analysis using allosome electrophoresis and sequencing of cytochrome oxidase subunit 1 and 16s RNA genes in the mitochondrial genome have prompted revision of taxonomy in the mudcrab of the genus *Scylla*. Studies by Keenan *et al.,* (1998) revealed that mudcrabs could be divided into atleast four different species viz., *S. serrata*, *S. tranqubarica*, *S. paramamosain*, *S. olivacea*. Besides taxonomic oriented divisions among different species, the genetic diversity of them is an essential prerequisite to construct an appropriate management scheme regarding their culture of mud crabs in South India is in its insipient stage. The conventional practice of its culture involves the seed collection from the intertidal flats and mangroves and stocking them in the impoundments to reach the market sizes. The culture and farming of mud crabs is based on certain criteria such as a) quality of meat, b) availability of seeds, c) growth rates, d) agnostic behaviour e) disease resistance, f) absence of microbial contamination etc. The productivity could be enhanced in farms through species with better quality of meat, greater growth rate and which is less aggressive and whose seeds are available all the time. Previous studies have also

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revealed that identification of the correct brood stock species using molecular genetic markers is necessary for the successful culture of any species viz., *S. serrata, S. tranqubarica* alongside the niche variation on DNA pattern evaluated using Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD PCR). The RAPD PCR technique was selected as it is a simple and rapid method for determining genetic diversity in various organisms with the advantage that no prior Knowledge of the genome under study is needed.

MATERIALS AND METHODS

Sampling

Different individuals of the mud crabs, both *Scylla tranqubarica* (Figure 1a) and *Scylla serrata* (Figure 1b) were collected from Pulicat Lake and Kovalam Estuary, located at the eastern coast of Tamil Nadu. Kovalam is located nearby Kalpakkam, Chengalpet Taluk, While Pulicat lake is far located from the above in the same coastal line.

DNA Extraction

DNA extraction was done using the procedure followed by (Klinbunga *et al.*, 2000). Approximately 100µg of hemolymph of each mud crab was transferred to microfuge tube containing 4 volumes of a lysis solution (100 mMTris HCL, 100 mM EDTA, 250 mM NaCl, pH 8.0, 1% Sodium dodecyle sulphate-SDS and 200µg/ml proteinase K). Genomic DNA was extracted using Phenol-Choloform-SDS method according to (Klinbunga *et al.*, 1996). The concentration of the extracted DNA was determined using spectrophotometer and further adjusted using a mini-gel method (Maniatis *et al.*, 1982). DNA was stored at 4˚C until required.

PCR amplification and agarose gel electrophoresis

Three selected olignuleotide primers, UBC 456(5'-GCGGAGGTCC-3'), UBC457 (5'-CGACGCCCTG-3') and YNZ22 (5'-CTCTGGGTGTCGTGC-3') (Fristsch *et al.*, 1993 and Health *et al.*, 1993) were used to analyse the genetic diversity and identify species specific markers in two mud crabs *S. tranqubarica* and *S. serrata*. The amplification reaction was carried out in a 25 µL reaction volume 10 mM Tris-HCL, pH 8.3, 50 mM KCL, 2 mM $Mgcl_2$, 0.001% gelatin, 100µM each of dATP, dCTP, dGTP, dTTP, 0.2 µM of each primer, 1 U of Taq DNA Polymerase, and 25ng of high molecular weight genomic DNA. RAPD-PCR was performed in Eppendorf thermocycler for 35 cycles consisting of denaturation at 94˚C for 15 seconds (or 30 seconds for YNZ 22), annealing at 36˚C (50˚C for YNZ 22) for 60 seconds, and extension at 72˚C for 90 seconds. The final Extension was carried out at the same temperature for 7 minutes.The resulting products were electrophoretically analysed through 1.6% agrose gel, stained with ethidium bromide, and visualized using a UV transilluminator (Maniatis *et al.*, 1982). The similarity index between all possible comparisons of individuals was calculated by

$$S_{\chi Y} = 2n_{\chi Y}/(n_\chi + n_Y)$$

Where n_χ and n_γ are the numbers of RAPD bands in individuals x and y, and $n_{\chi\gamma}$ is the number of shared bands between those individuals (Nei and Li), 1979). The average similarity index within populations (S) and between pairs of populations (S_ij) were also calculated using the same equation.

Genetic distances between paired individuals ($D_{\chi\gamma}$) or populations (D_ij) were converted from inter individual and inter population similarity indices using equations $D_{\chi\gamma}=1-S_{\chi\gamma}$ or $D_ij=1=S_ij$ respectively.

RESULTS

The number of polymorphic bands of *Scylla tranquebarica*, seen in each primer was 11, 9 and 9 respectively in UBC 456, UBC 457 and YNZ 22. The similarity index between two populations was seen prominently in primer UBC 457, whereas in other two primers, the index was 1 (100% similarity). The similarity index of *Scylla tranquebarica* with that of *Scylla seratta* was 0.77 in UBC 456, 0.8 in UBC 457 and 0.57 in YNZ 22(Figure 2).

Figure 1a *S. serrata.*

Fig 2b *S. Tranquebarica*

Legend for Figure2
Agarose Gel
Lane 1: Molecular weight marker
Lane 2: Primer 1 *S. Tranquebarica* Pulicate Lake specimen
Lane 3: Primer 1 *S. tranquebarica* Kovalam specimen
Lane 4: Primer 1 *S. serrata*
Lane 5: Primer 2 *S. Tranquebarica* Pulicate Lake specimen
Lane 6: Primer 2 *S. pulicate* Lake specimen
Lane 7: Primer 2 *S. serrata*
Lane 8: Primer 3 *S. tranquebarica* Pulicate Lake specimen
Lane 9: Primer 3 *S. Tranquebarica* Kovalam specimen
Lane 10: Primer 3 *S. serrata.*

DISCUSSION

Though *S. serrata, S. tranquebarica, S. oceanica* represent the sympatric species, whether genetic exchanges between different mudcrab species exists or not is crucial in their phylogenetic consideration as well as for their culture programmes. An analysis of mud crab species in Thailand conducted in the Chandapuri and Trat ecological niches revealed that there exists a high level genetic diversity in the three Scylla species. They have also revealed that the three species did not share RAPD genotypes between them. The apparent lack of genetic exchanges between these sympatric species was thus evidenced. In the present study also RAPD analysis using DNA markers revealed the shuttle difference between the two species *S. serrata* and *S. tranquebarica* collected from the same niche. However among the *S.tranquebarica* individuals, one population collected from Pulicat and another population collected from Kovalam revealed no difference and variability in DNA patterns.

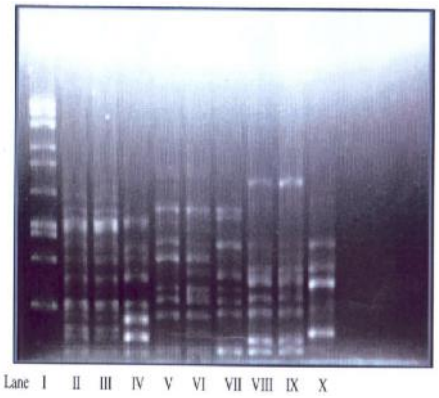

Fig. 2 Agrose gel of RAPD PCR

The above data also revealed that though the two populations are separated by niche orientation, they still retain the genic homology, suggesting thereby the possibility of inbreeding potential within them. The above pattern similarity of DNA between two populations of *S. tranquebarica* is also of significance in view of their seed collection, culture and management. Both the niches in the Bay of Bengal represent therefore ideal centres for the seed collection and for further rearing. Moreover, considering the contamination potential and pollution point of view, the populations showing difference genomic composition will be at loss since genetic makeup attributes for their metabolic scope and immunologically, the disease resistance capacity. Hence, the present study, from the point of view of imunogenetics reveals that both *S. serrata* and *S. tranquebraica* sharing the common niche represent sympatric but non-congeners without any genetic exchanges, whereas the population of *S. tranquebraica* represent a congener species with genic similarity irrespective of the niche variation and separation by distance. The study also reveals that the Pulicat niche represents the ideal and potential source for the culture of both the species considering the inadvertent and impending contamination and thermal pollution that may arise due to nuclear reactor processing activities at Kalpakkam in the surrounding coastal areas especially at Kovalam. The sustainable maintenance of biodiversity at Pulicat Lake has also been enumerated by Sanjeevaraj (2006). Considering the export value of *S. tranquebarica* and the diagnostic immunological characteristics such as higher total haemocyte count (THC), phenoloxidase activity (PO) and antibacterial peptide (Ramalingam and Bharathi Rajan, 2007). (Personal observation) synthetic capacity consequent to antigenic challenge, the present finding is of significance towards its conservation and management.

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