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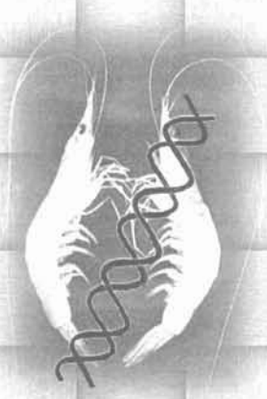
POPULATION GENETIC STRUCTURE OF THE MARINE PENAEID PRAWN - *Penaeus indicus* H. Milne Edwards 1837



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COCHIN-682 022

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By

BINDHU PAUL

भा कृ अनु प
I C A R

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
PB. No. 1603, KOCHI - 682 014, INDIA

NOVEMBER 2000

CERTIFICATE

Certified that the thesis entitled "**Population genetic structure of the marine penaeid prawn-*Penaeus indicus* H. Milne Edwards 1837**"is a bonafide record of the work carried out by **Ms.Bindhu Paul** under my guidance and supervision and that no part thereof has been presented for the award of any other degree, diploma or any other similar title.

Dr. M.K. George, M.Sc., Ph.D.,
(Supervising Guide)
Senior Scientist (Retd.),
Mangaly,
Konthuruthy lane,
Deshabhimani Rd.,
Kaloor, Kochi - 682 017.

Kochi,
November, 2000

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Introduction

The human population all over the world is increasing more and more rapidly. This has compelled the man to explore and exploit the resources around him, to meet his increasing food requirements. The water bodies in the form of oceans, rivers, lakes etc., have been exploited by man since time immemorial for the augmentation of food production. The heavy and sometimes ruthless exploitation has even caused extinction of many of the aquatic flora and fauna. There was an urgent need for the development of apt management strategies to exploit these resources judiciously. One of the management strategies, thus developed for the scientific management of these resources, was to identify the natural units of the fishery resources under exploitation (Altukhov, 1981). These natural units of a species can otherwise be called as 'stocks'. A stock can be defined as "a panmictic population of related individuals within a single species that is genetically distinct from other such populations" (Shaklee *et al.*, 1990).

In the past, taxonomists have traditionally dominated the systematics of fishery management, a group that was generally concerned with species or sub-species differences but not between individuals of a species. On the other hand, the modern stock concept demands genetic characterisation of the individuals of a species. Such studies at the genetic level can reveal the differences at the intra- and inter- populational levels of that species. If the population genetic structure of a species is known, the distribution of subpopulation in mixed fisheries can also be estimated easily. This can certainly help in the conservation of genetic variations between and within natural populations, as genetics and fishery management can interact in several ways. The genotype- phenotype relationships in the aquatic species are different from those of other non-aquatic species. This difference is compounded by the fact that the former live in the water, making it more difficult to observe them and to obtain even rough estimates of the basic genetic parameters usually available for the breeding and management of the latter. No other major food resource of man is captured from wild populations as heavily as

that of the fishery resources. The lack of knowledge about the genetic structure of these populations may end in the differential harvests of the populations. Hence, the genetic changes that occur due to differential harvests are also to be identified and regulated because of the drastic and long-term effect they may have on a population. Such regulations to protect the weaker populations, if there is any, can be made only if the distribution of subpopulations, stocks or strains of a species, is known accurately (Allendorf *et al.*, 1979). This necessitates the need for investigations encompassing the genetic variations at the intra- and inter- populational levels as well as at the intra- and inter-specific levels of the fish and shellfish resources of any nation.

For the accomplishment of the above objectives, scientists all over the world, developed different methodologies and are still in the look out for new techniques. One of the traditional methods of distinguishing fish stocks has been the comparative examination of morphological characters based on a set of measurements of the body form and different meristic characters (Hubbs and Lagler, 1947). The investigations on the morphological characters could reveal the relationship between one or two body measurements and the body weight in some of the decapod crustaceans. This aspect is of great use in the breeding programmes for the selection of brood stock. Taking the weight of a live individual is otherwise next to impossible. On the contrary, taking some of the length measurements of the body form is much easier.

But the conventional morphometric measurements have been graded as inefficient and biased, as they often produced uneven areal coverage of the body form. Most of the landmarks were repetitive and unidirectional lacking information of depth and breadth of the body forms (Strauss and Bookstein, 1982; Sathianandan, 1999). This led to the discovery of a new method called as truss network analysis, where the shape of the body forms of the fish or shellfish also was taken into account along with the size (Humphries *et al.*,1981). The areal coverage of the body form is even and the forms may be standardised to one or more common reference sizes by representing measured

distances on some composite measures of body size and reconstructing the form using the distance values predicted at some standard size (Sathianandan, 1999). Truss network analysis of three naturally occurring populations of Chinook salmon has revealed significant shape differences among them(Winans, 1984).

Even then, the application of truss network analysis for the identification of stocks is as complicated as the morphometric measurements. The reason for this is the role of non-genetic factors in determining the variability of morphological characters. As the distance between the characters studied and their informational source (DNA) grows, the genetic information decreases and the probability of error in precise stock identification increases. Therefore, assessment of stocks or estimation of the amount and distribution of genetic variation cannot be based exclusively on analysis of phenotypic characteristics, like, body size, shape, colouration or number of vertebrae or gill rakers. Though the morphological studies could explain the diversity within the species, the phenotypic variation has been statistically inadequate. This lack of reliable data has often prevented identification of subunits, or has overemphasised the genetic control of morphological characters that are also influenced by the environment.

These misgivings of the morphological studies of different kinds have turned the attention of the researchers to a more reliable and powerful technique developed as a result of the discovery of protein-DNA relationship. Thus the development of protein electrophoresis (Smithies, 1955) and the application of histochemical staining methods (Hunter & Markert, 1957) gained advantage over morphological studies by providing rapidly collected genetic data. This method is capable of unveiling the invisible differences at the molecular level as visible biochemical phenotypes. These variants are essentially unaffected by the environment and stable through time. Usually, a single gene whose alleles are co-dominantly expressed and inherited in a Mendelian fashion causes the observed variation (Ayala, 1975). Since the time of its discovery, it

has been extensively used by various researchers for the analysis of population structure, identification of different species and hybrids and as species - specific markers. Nevertheless, the later workers were sceptic about the potential of the electrophoretic techniques, as only one percent of the total genome could be studied with the use of them.

In retrospect, the question that aroused was that whether the electrophoretic techniques were potentially competent in revealing the actual genetic diversity at the DNA level through the screening of proteins and enzymes? They might not, as proteins were still two steps removed from the source of information needed for their production. Obviously, this put limits to the genetic information that could be gathered through electrophoretic analyses of proteins and enzymes. The amino acid substitutions of proteins detected by electrophoresis are indirect reflections of the actual base substitutions in base sequences. Further more, all the base substitutions do not necessarily result in changes of amino acids and all the amino acid substitutions do not result in protein changes that are electrophoretically detectable. It has been estimated that only about a third of the amino acid substitutions are detected under the conditions used to collect electrophoretic data in most laboratories (Lewontin, 1974).

It is apparent from the above facts that the electrophoretic identity of proteins does not necessarily mean identity of base sequences in DNA. The vast majority of DNA within the nucleus do not code for protein products and therefore, probably does not affect the fitness of an individual fish. Thus, these non-coding DNA sequences are under relaxed selective constraints and may be freer to evolve much more rapidly than the coding sequences. The multitude of DNA sequences available for analysis of heterogeneity among units of interest at or below the species level has increased the potential number of diagnostic features exponentially. A standard reference for basic meristic and morphometric analyses, Hubbs and Lagler (1947), lists approximately 40 features or dimensions as potential discriminatory characters, and Murphy *et*

al., (1990), presents close to 75 isozyme systems (representative of several hundred genetic loci) that may potentially be analysed in fishes whereas the DNA sequences offer more than three billion possible characters among which variations may be detected. These advanced informations have diverted the attention of the genetic workers to the techniques based on genomic as well as mitochondrial DNA. These modern techniques can reveal the restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD). The RFLP of mt DNA has been successfully used by so many researchers for the analysis of genetic diversity at the intergeneric as well as intra- and inter-specific levels. As it is maternally inherited, the analysis of maternal lineage can be done with ease. RFLP of mt DNA revealed great differences between the populations of many species that were earlier considered as homogeneous, as revealed by allozyme studies.

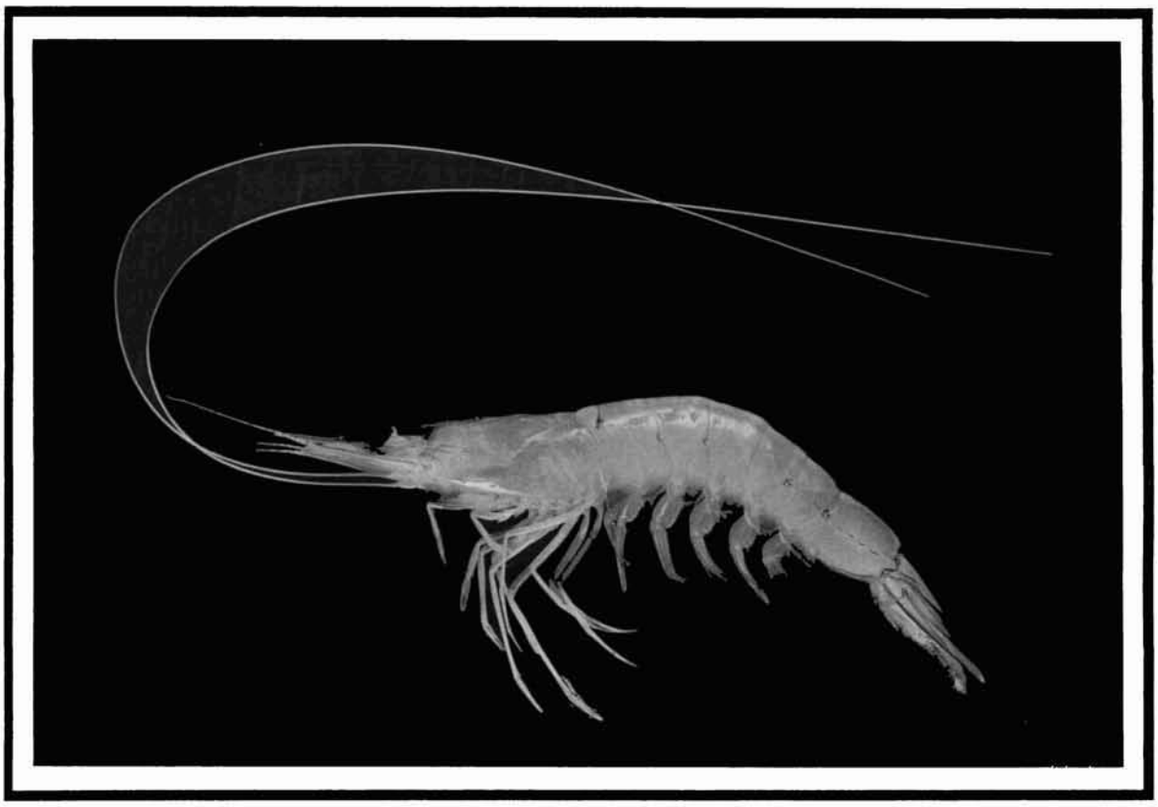
Recently, another technique is gaining popularity, with the advent of thermocyclers. The amplification of the DNA through polymerase chain reaction (PCR) requires only a small amount of DNA. This enabled the users to screen the polymorphism in the DNA of the individuals without sacrificing them. One such technique (developed by Williams *et al.*, (1990) and Welsh and McClelland (1990)), was random amplified polymorphic DNA (RAPD) based on the polymerase chain reaction using short arbitrary oligonucleotides that amplified random segments of the genome. Compared to RFLP and DNA finger printing, RAPD is feasible, less expensive, and more rapid and it does not require any previous knowledge of the targetted genomic region. Like allozyme markers, the amplified fragments are also inherited in Mendelian fashion (Williams *et al.*, 1990).

In brief, the techniques available to screen the variability at different levels of the species organisation are many ranging from simple morphometrics to molecular genetic methods that can reveal polymorphism at the DNA level. The species that was selected in the present investigation for applying these techniques was the Indian marine penaeid prawn, the *Penaeus indicus* H.

Milne Edwards 1837(Plate-1). Though it has been renamed as *Fenneropenaeus indicus*, in order to avoid confusion, the present study will be using the older name, *Penaeus indicus* H. Milne Edwards 1837. The major reasons for selecting this particular species are given below.

The *P.indicus,* popularly known as the Indian white prawn, is distributed widely in the Indo-Pacific, starting from New South wales in Australia in the east to the east coast of Africa in the west. However, the commercial fishery for *P. indicus* was reported only from India and Africa. In India, the species is distributed between Puri in the east coast and Mangalore in the west coast. Because of its heavy demand in the export market, the species has been exploited intensively from all along its areas of distribution in Indian waters (Rao *et al.*, 1993). Though the biology and fishery aspects of *P. indicus* have been studied extensively (Panikkar& Menon, 1956; Menon & Raman, 1961; George, 1961, 1962; George & Mohammed, 1967; George *et al.*, 1963; Jhingran & Natarajan, 1969; Kurup & Rao, 1974: Subrahmanyam, 1965, 1966, 1967; Suseelan, 1975; Rao, 1975 , 1987, 1988 a , 1988 b, MS 1, MS 2 ; Rao *et al.*, MS; Lalitha devi, 1986, 1988; Manisseri, 1988; Manisseri & Manimaran, 1981; Rajamani & Manickaraja, 1990), studies on the genetic stock structure of the species are largely lacking, except for the investigations made by Philip Samuel (1987). He concluded that the *P.indicus* populations distributed along the south-east and south-west coasts of India belong to a single stock. But the works on the size composition and abundance of the populations (Manisseri & Manimaran, 1981), the mark - recapture investigations made under the National Tagging Programme (Mar. Fish. Infor. Serv. T& E Ser., No. 45 : 1982) and the stock assessment studies (Rao *et al.*, 1993) were all suggesting the occurrence of two separate stocks along the east and west coasts of south India. The mark- recapture investigations proved the migratory nature of *P. indicus*, but it also found that beyond Manappad the prawns were not migrating towards north. This suggests that the populations of the west and east coasts may be maintained separately.

PLATE-1



Penaeus indicus

The literature that has been gone through revealed a very low level of genetic variation among the penaeids. But these earlier works were all based on morphological characters (Lester & Pante, 1992) and allozyme markers (Lester, 1979; Marvin *et al.*, 1977; Mulley & Latter, 1981; Richardson, 1982; Lester, 1983; Sunden & Davis, 1991) which were more distant from the source of genetic information (DNA). Some later works demonstrated that the penaeids are more structured than reported earlier. Their conclusions were based on the study of the polymorphism at the mt DNA (Palumbi, 1991; Benzie *et al.*, 1993) and total DNA (Garcia *et al.*, 1994; Garcia and Benzie, 1995).

In view of the above facts and reasons, a re-investigation of the population genetic structure of the Indian white prawn *Penaeus indicus* became essential. Naturally, the objective of the present study was to test the hypotheses that have emerged from the earlier studies on the species stock structure. For an in-depth testing of these hypotheses, three independent methods, namely, morphometrics, isozyme electrophoresis and the advanced molecular genetic techniques, like, RFLP and RAPD were applied to the extent possible.

Review of Literature

The organisms are incessantly undergoing micro and macro evolutionary processes both at molecular and organismal levels. Actually, the process of evolution starts at the molecular level, more precisely from a single base of the DNA molecule and ends up in variations at the organismal level. Genes are the factors which determine the phenotypic characters of any organism. Thus the variations that happen to the genes, in turn produce individuals which are different either at the molecular level or at the organismal level. These individuals may form separate groups within the species itself and such groups are the fundamental genetic units of evolution. These intraspecific groups were called as 'stocks' and fishery biologists started using these stocks as a basis to manage commercially important marine organisms. Shaklee *et al.*(1990) defined a stock as "a panmictic population of related individuals within a single species that is genetically distinct from other such populations". Therefore, in any management regime, the identification of stock becomes a critical element (Ihssen *et al.*, 1981a; Fetterolf, 1981). For the identification of these putative stocks at the practical level, Ihssen *et al.* (1981a) suggested that the study of the population parameters and physiological, behavioural, morphometric, meristic, calcareous, biochemical and cytogenetic characters are useful.

Of these, the morphometric investigations are based on a set of measurements of the body form (Hubbs and Lagler, 1947). The study on the life history, morphology and electrophoretic characteristics of five allopatric stocks of lake white fish showed that morphometry can be used to distinguish the individuals of different stocks (Ihssen *et al.*, 1981b), though the branching patterns for the morphometrics versus the biochemical variation were different. For the selection of the brood stock in genetic improvement programmes of certain penaeids, one or two morphometric variables could be identified, giving accurate estimate of the tail weight (Lester, 1983; Goswami *et al.*, 1986). A study on the Pacific white shrimp, *Penaeus vannamei* from different commercial hatcheries could find significant differences in all the morphometric

traits between sites, indicating that the environmental differences affected growth as well as shape of the shrimps (Chow & Sandifer, 1991). But in a study on the use of canonical discriminant analysis of morphometric and meristic characters to identify cultured tilapias, the results did not support the use of morphometric characters for differentiating the tilapia strains and introgressed hybrids (Pante *et al.*, 1988). These conventional data sets are biased and they have got several weaknesses too. (i) They tend to be in one direction only (longitudinal) lacking information of depth and breadth, (ii) they often produce uneven and biased areal coverage of the body form, (iii) repetition of landmarks often occur, (iv) many measurements extends over much of the body and (v) the amount of distortion due to preservation cannot be easily estimated in case of soft bodied organisms (Sathianandan, 1999). To overcome these problems, a new method called the truss network was developed in which an even areal coverage over the entire fish form was possible (Humphries *et al.*, 1981). This method can discriminate stocks of fishes and prawns on the basis of size free shape derived from distance measures. Here, the forms may be standardised to one or more common reference sizes by representing measured distances on some composite measure of body size and reconstructing the form using the distance values predicted at some standard body size. The composite mapped forms are suitable for biorthogonal analysis of shape differences between forms (Sathianandan, 1999). Truss network analysis on Chinook salmon demonstrated shape differences among the three naturally occurring populations (Winans, 1984). This method was introduced among prawns to study the shape differences among them (Lester and Pante, 1992) and a machine vision system was developed for the selection of brood stock by using the truss network (Perkins and Lester, 1990). A comparison of the conventional morphometrics and truss network analysis done on the blunt snout bream, finally described the truss network analysis as the better tool than the former for probing evolutionary processes or elucidating relationships among populations (Li *et al.*, 1993).

But the application of the above said techniques in stock identification,

however, is complicated by the fact that phenotypic variation in these characters are often influenced by environmental factors and has not been directly related to particular differences in the genome always (Clayton, 1981).

An innovative method that could reveal genetic variations while avoiding the effect of the environmental factors became a necessity. Meanwhile, the starch gel electrophoresis developed by Smithies (1955) helped to study the biochemical variation at the protein level. The proficiency of the electrophoretic techniques was enhanced by the application of histochemical staining methods of Hunter and Markert (1957). These methods could uncover a wealth of genetic variation at the molecular level which were reflected either as multilocus isozymes or allelic isozymes. The isozyme is considered as advantageous over the morphological and classical variables as (i) the biochemical phenotype is essentially unaffected by the environment, (ii)the biochemical phenotype of each individual is stable through time and (iii) the observed genetic variation is usually caused by a single gene whose alleles are co-dominantly expressed and inherited in the Mendelian fashion(Ayala, 1975). A comprehensive review by de Ligny (1969, 1972) shows that the use of isozyme or allozyme study has become essential for the analysis of the population genetic structure of many fishes. Using the isozyme analysis, six genetically distinct groups were identified among the samples of witch flounder from three different management areas(Fairbairn, 1981). Studies were successfully carried out using allozyme/ isozyme electrophoresis for the last two decades, to differentiate different populations of some teleost species, shark and copepods (Sidell *et al.*, 1980; Richardson, 1982; Utter,1989; Sevigny, 1989; Ferguson, 1991; Naish, 1993 ; Heist, 1995 ; Giaever and Stien,1998) and as species-specific markers to identify different species of sparids as well as hybrids at various stages of growth (Alarcon and Alvarez, 1999). The efficiency of electrophoretic and biochemical techniques in the study of population genetics thus have even proven as expected.

The decapod crustaceans, especially lobsters and penaeid shrimps are

an important part of the aquaculture industry in many areas of the world and hence the identification of their stocks or strains is equally important as that of fishes. Lester (1979) carried out a study on the penaeid shrimps from the Gulf of Mexico in order to find out the population substructure, if any, among them. He concluded that the entire Gulf of Mexico could be considered as a single management area for the species studied. Such studies are essential for the effective management of the cultured individuals through genetic improvement as the founder individuals are taken from the wild. The studies on the enzyme polymorphisms of *Penaeus setiferus* Linnaeus, *P.duorarum duorarum*, *P.brasiliensis* and *P.aztecus subtilis* enunciate the use of allozyme electrophoresis in population structure analysis of the penaeidae(Lansford *et al.*, 1976; Marvin *et al.*, 1977;). The correlation between different physiological and environmental adaptive strategies and the mean heterozygosity increases or reductions were elaborately explained by the studies on 44 species of decapod crustaceans(Nelson and Hedgecock, 1980) and on *Penaeus kerathurus* and *P.japonicus*(De Matthaeis *et al.*, 1983). The overall degree of divergence between populations of the penaeids were found to be quite small(Mulley and Latter, 1981 ; Richardson, 1982 ; Lester, 1983 ; Sunden and Davis, 1991). Later, the allozyme studies on *Metapenaeus bennettae* and *Penaeus monodon* suggested that the penaeid populations could be more structured than has been thought of (Salini, 1987; Benzie *et al.*, 1992,1993). The isozyme markers were also used in the studies of ontogenic changes of *Penaeus* species(Lester and Cook, 1987), for the identification of penaeid prawn postlarvae belonging to different species(Lavery and Staples, 1990), for studying the bottleneck effects and the depression of genetic variability in hatchery stocks of *Penaeus japonicus*(Sbordoni *et al.*,1986) and for deducing the phylogenetic relationships between some species of *Penaeus* and *Metapenaeus*(Tam and Chu,1993).

Though the authenticity of the results of the electrophoretic analyses are many fold when compared to that of the morphometric analyses, they often underestimate the extent of genetic variability arising from changes in the

isopolar amino acids and in the nucleotides that are not reflected in the corresponding protein sequences(Powers, 1993). The potency of the molecular tools like RFLP of mtDNA and RAPD is much more than the allozyme electrophoresis. These well advanced methods permit analysis of a broader scope of genetic diversity. They are so sensitive that even the study of egg and juvenile stages, tissue biopsies of adults and single cells are much easier. The results of a study done on the genus *Peromyscus* (Avise *et al.*,1979) indicated that the restriction analysis of mtDNA may become the most sensitive and powerful technique for reconstructing evolutionary relationships among conspecific organisms. The mtDNA sequence divergence study in two forms of *Salmo gairdneri* and cut throat trouts revealed detectable divergence between all geographic populations though there were exceptions (Wilson *et al.*,1985). Length and restriction site heteroplasmy were observed in the mtDNA of American shad collected from 14 rivers ranging from Florida to Quebec(Bentzen *et al.*,1988). The results of a survey made on the restriction site variation in mtDNA of the horseshoe crab(Saunders *et al.*, 1986) demonstrate that geographically, at least some continuously distributed marine organisms can show considerable mtDNA genetic differentiation. Tzeng *et al.*(1990) could propose to recognise two previously named species, *Crossostoma lacustre* and *C.tengi* of Taiwan as one valid species, *Crossostoma lacustre* by re-evaluating their identity using mtDNA. Furthermore, mtDNA polymorphism was studied to measure genetic variability among the individuals of two species of sea urchins(Palumbi, 1990) and no geographic structure was found in *Strongylocentrotus purpuratus*, while *S.droebachiensis* showed a small divergence between Atlantic and Pacific populations. In a study on morphologically similar penaeid shrimps, the data collected showed large mtDNA differences among them (Palumbi, 1991). The morphological similarity among the penaeids actually masks large genetic differences. An analysis of mtDNA of three laboratory strains of *Penaeus monodon* from Malaysia, Australia and Fiji and one farm population of *P. japonicus*, showed genetic divergence between Fiji strain and the Australia /Malaysia strains (Bouchon *et al.*, 1994). The other studies to analyse the population structures using

mtDNA include the study of the American plaice in the Gulf of St. Lawrence (Stott *et al.*, 1992), the comparison between *Penaeus notialis* and *P.schmitti* for sequence variability (Garcia *et al.*, 1993), the confirmation of the results revealed by allozymes regarding the population substructuring in *P.monodon* (Benzie *et al.*, 1993), the analysis of mtDNA variation and phylogenetic relationships of *Jasus* spp. (Brasher *et al.*, 1992) and checking of the mtDNA diversity in jackass morwong from Australian and New Zealand waters (Grewe *et al.*, 1994) . Lavery *et al.* (1996) studied the Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro* using mtDNA and the results were consistent with those from allozymes.

It is evident from the above reports that mitochondrial DNA is definitely a powerful genetic marker. Its other advantage is that it is inherited maternally only. But, it becomes difficult to trace an individual's or a population's paternal origin with mtDNA. Data collected from the nuclear DNA analysis only can reveal both the maternal and paternal lineages. Random Amplified Polymorphic DNA is one such technique developed by Williams *et al.* (1990) and Welsh and McClelland (1990). It is based on the polymerase chain reaction using short arbitrary oligonucleotides that amplify random segments of the genome. Compared to RFLP and DNA finger printing, RAPD is feasible, less expensive and more rapid (Penner *et al.*, 1993). RAPD technique does not require any previous knowledge of the targetted genomic region. As allozyme markers, the amplified fragments are also inherited in the Mendelian fashion (Williams *et al.*, 1990). The pioneering works which demonstrated the use of RAPD in the analysis of natural populations include, the study of the systematics of grasshoppers (Chapco *et al.*, 1992), the separation of different *Aedes* species as well as different populations of *Ae. albopictus* (Kambhampati *et al.*, 1992), the evaluation of intra-specific variability among plants (Balakrishna, 1995) and the discrimination of selected *Sargassum* species (Ho *et al.*, 1995). Studies done on schizostomes proved RAPD technique's potential in sex determination and analysis of genetic variation among the parasites (Simpson *et al.*, 1993; Neto *et al.*, 1993). Using this technique the

isolates of the crayfish plague fungus *Aphanomyces astaci* could be assigned to different groups successfully (Huang *et al.*, 1994). In a recent study the phylogenetic relationships among six populations of corn borers in China were analysed using RAPD (Xu *et al.*, 1998). The three molecular genetic techniques, RFLPs, RAPD and allozyme variability were used to evaluate the genetic diversity of cultured *Penaeus vannamei* (Garcia *et al.*, 1994) and found that mitochondrial DNA and molecular DNA techniques were more useful for the examination of genetic diversity. The study suggested that one of the key uses of RAPD is its ability to identify family or population specific markers and these markers can be related to specific traits of economical importance which can aid in selective breeding programmes. Garcia and Benzie (1995) also supported the above view after studying the RAPD markers' use in *Penaeus monodon* breeding programmes. Four wild forms and five cultivated varieties of *Discus* were tested for genetic diversity with RAPD and found that the present phenotypic classification has no genetic basis (Koh *et al.*, 1999).

The above studies suggested that RAPD could be a powerful genetic marker though it has so many disadvantages also. The main disadvantage is its lack of reproducibility. But that can be overcome if the temperature settings are done accurately. The method can not discriminate the heterozygotes from the dominant homozygotes. In spite of these misgivings, the technique has gained wide popularity among the fishery biologists, as the animals need not be sacrificed for analysis. In India, so far no work has been reported with RAPD as a molecular tool to analyse the population genetic structure of the penaeid prawn, *Penaeus indicus*.

Allozyme electrophoresis, RFLP of mtDNA, morphometrics and truss network, reviewed above were used along with RAPD to approach the problem taken for this thesis work, in order to obtain a comparative detailed picture of the population structure of the *P. indicus* distributed along the south east and south west coasts of India.

Material & Methods

3.1. Materials

Peneaus indicus of different size groups (TL - 100-200mm) were randomly collected from both the east and west coasts of South India.The collection centres were Kochi, Mangalore, Calicut, Chowghat and Neendakara along the west and Mandapam, Chennai and Kakinada along the east coasts (Fig. 1). The fresh samples purchased from the landing centres were transported to the laboratory in ice-boxes and stored at -20°C till used for further experimental analysis. A total of 1080 specimens were screened from 9 different centres.

3.2. Methods

Biochemical, molecular and morphometric methods were applied to study the genetic characteristics of the species.

3.2.1. Biochemical Genetics

3.2.1.1. Standardisation

It involved finding out suitable methods for extraction of proteins/ enzymes present in different tissues and their fine separation by gel electrophoresis.

a) Extraction

Adequate portions of muscle, hepatopancreas and eye were removed from the thawed specimens. Each tissue sample was first minced. Each minced sample was homogenised using a glass hand homogenizer under cold conditions. Tris-Glycine, Tris-NaCl-$MgCl_2$ and distilled water were used as homogenising media in selected proportions to the sample weight. The homogenates were then centrifuged at 4°C. The speed of revolution and time given also were varied. After the centrifugation, the supernatants were collected

Fig.1 Collection sites for *Penaeus indicus*

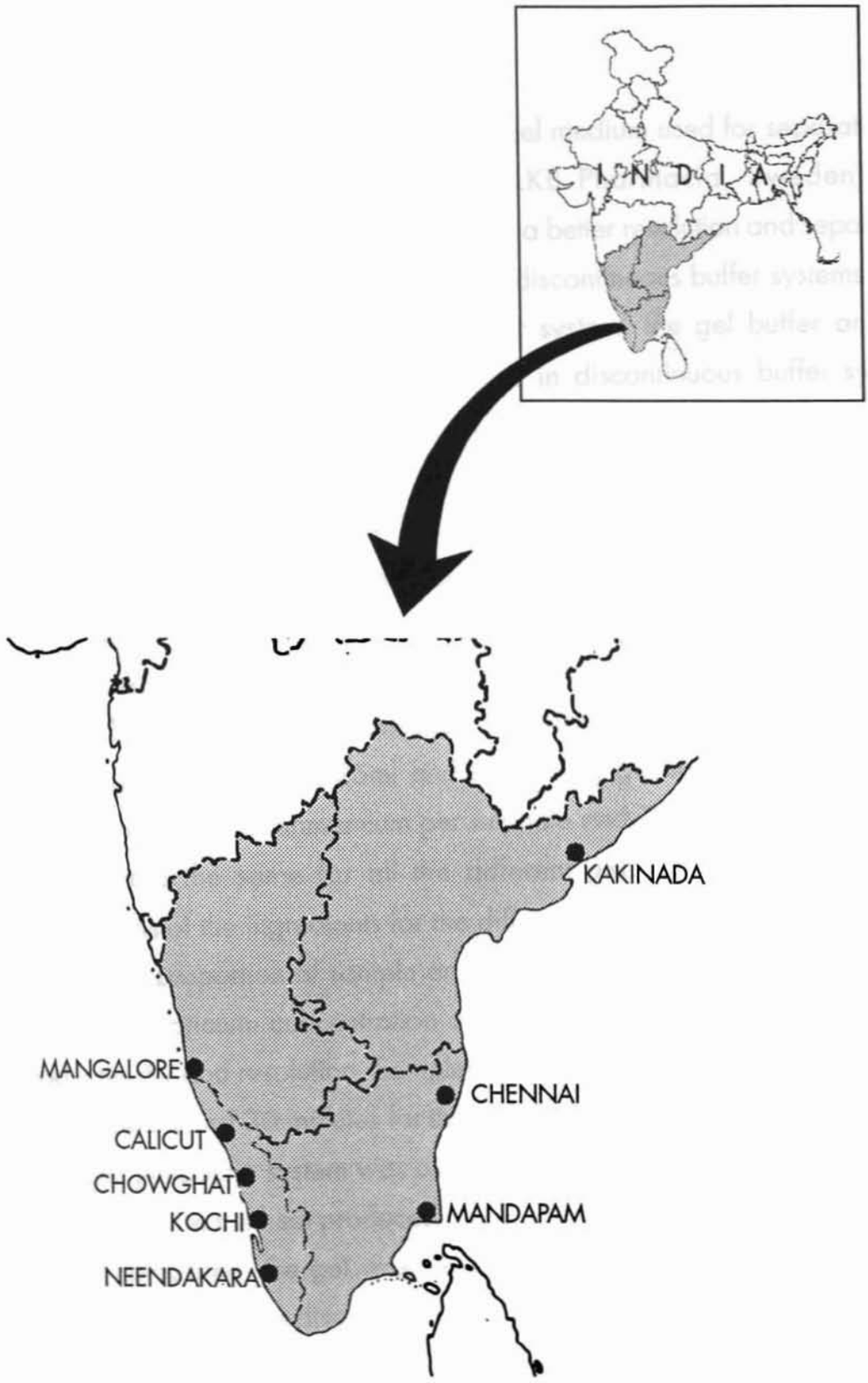


Table 1 Composition of the ingredients of buffers used for Electrophoresis

Buffers	Ingredients							
	Electrode buffer		pH	Gel buffer				
				Stacking		pH	Separating		pH
1. Tris-Citric-EDTA	0.135 M Tris	16.35g/1	7.0	0.009 M Tris	1.09g/1	7.0	0.009 M Tris	1.09g/1	7.0
(Ayala et al 1972)	0.045 M Citric acid	9.45g/1		0.003 M Citric acid	0.63g/1		0.003 Citric acid	0.63g/1	
	0.00125 M EDTA	0.47g/1		0.00125 M EDTA	0.47g/1		0.00125 M EDTA	0.47g/1	
2. Tris-Citric acid-Lithium Hydroxide-Boric acid	A								
	0.072 M Tris	8.7g/1	7.25	A	90ml	6.1	A	90 ml	8.2
	0.023 M Citric acid	4.8g/1							
	B								
	0.03 M Lithium Hydroxide	1.2/g1		B	42 ml		B	42 ml	
	0.19 M Boric acid	11.8g/1		DDH20	168 ml		DDH20	168 ml	
3. Tris-Citrate Lithium hydroxide - Boric acid	0.03 M Lithium hydroxide	1.2g/1	8.1	0.072 M Tris	8.7g/1	6.8	0.072 M Tris	8.7g/1	8.8
	0.19 M Boric acid	11.8g/1		0.023 M Citric acid	4.8g/1		0.023 M Citric acid	4.8g/1	
4. Tris-Glycine	2 M Tris	25 ml	8.3	1.8 M Tris	21.81g/100 ml	8.9	0.05 M Tris	6.06g/100 ml	6.8
Tris-HC1	0.2 M Glycine	15.04g/1		1 N HCI to adjust the pH			1 N HCI to adjust the pH		

Table 2 Composition of gels of various percentages used for horizontal slab gel

Components	Stacking gel			Separating gel				
	3.5%	4%	5%	7%	7.6%	8%	8.5%	10%
Distilled water	15.50 ml	15.29 ml	14.45 ml	7.00 ml	6.09 ml	5.38 ml	4.58 ml	2.05 ml
Buffer	6.25 ml	6.25 ml	6.25 ml	6.25 ml	6.25 ml	6.25 ml	6.25 ml	6.25 ml
Acrylamide and N,N' – Methylene Bisacrylamide (30%)	2.91 ml	3.33 ml	4.17 ml	11.70 ml	12.66 ml	13.34 ml	14.17 ml	16.66 ml
N,N,N',N'-Tetra-methyl ethylene diamine (TEMED)	25.00µl	25.00 µl	25.00 µl	**	**	**	**	**
Ammonium per sulphate (10%)	100.00 µl	100.00 µl	100.00 µl	25.00 ml*	25.00ml	25.00ml	25.00ml	25.00ml

- 400µl of 10% APS was taken in 25ml of DDH $_2$O.

** 125µl of TEMED was added to the separating gel buffer itself at the time of buffer preparation.

Finally, the standardised biochemical method included a combination of factors like a particular tissue, its extraction, suitable electrophoretic conditions and the staining procedures that produced visible variation in the protein/enzyme banding patterns between individuals of *P.indicus*.

The staining recipe used for the isozyme detection was that of Lester (1990). The enzymes studied are given in the table. The stock solutions used were also of the same concentration mentioned in the original recipe.

1.Adenylate kinase

0.2 M Tris HCl (pH 8.0)	50 ml
ADP	25 mg
Glucose	400 mg
Hexokinase	1 ml(20 units/ml)
0.1M $MgCl_2$	1 ml
1% NADP	1.5 ml
G-6-PDH	4 ml(1000 units/100ml)

When ready to stain, added :

1% PMS	0.6 ml
1% NBT	0.6 ml

Incubated at 37°C, till the bands were visible . (Agar was not used).

2. Aldehyde oxidase

Double distilled water	35 ml
0.2 M Tris-HCl (pH 8.0)	15 ml
Benzaldehyde	0.5 ml
1% NBT	2.0 ml

When ready to stain, added :

1% PMS	0.5 ml

Incubated at 37°C, till bands were developed.

3.Fructose - biphosphate aldolase

0.2 M Tris HCl (pH 8.0)	50 ml
Sodium arsenate	80 mg
Fructose 1,6-diphosphate	200 mg
G-3-PDH	2 ml (20 units/ml)
1 % NAD	1.5 ml
1 % NBT	1.5 ml

When ready to stain, added :

1 % PMS	0.5 ml

Incubated at 37°C till the bands were developed.

4.Glycerol -3- Phosphate dehydrogenase

0.2 M Tris HCl (pH 8.0)	50 ml
0.2 M MgCl2	1 ml
α-DL-glycerophosphate	250 mg
1 % NAD	2 ml
1 % NBT	1.3 ml

When ready to stain, added :

1 % PMS	0.5 ml

5. Octanol dehydrogenase

0.05 MTris HCl (pH 8.5)	50 ml
95 % Ethanol	1 ml
Octanol	0.2 ml
1 % NAD	1.25 ml
1 % NBT	1.0 ml

6. Alcohol dehydrogenase

0.2 M Tris-HCl (pH 8.0)	50 ml
0.1 M MgCl2	1 ml
95 % ethanol	3 ml
1 % NAD	2 ml
1 % NBT	1.3 ml

When ready to stain, added :

1 % PMS	0.5 ml

When ready for staining , added :

1 % PMS	500 ml

Incubated at 37° C till bands were visible.

7. L-Iditol dehydrogenase

0.2 M Tris-HCl (pH 8.0)	50 ml
0.1 M MgCl2	1 ml
Sorbitol	500 mg
1 % NAD	2 ml
1 % NBT	1.3 ml

When ready to stain, added :

1 % PMS	500 µl

I

Incubated at 37° C till bands appeared.

8. Glutamate dehydrogenase

0.2 M phosphate b Na2HPo4	25 ml
Double distilled water	16 ml
L-glutamic acid	0.18 g
1 % NAD	4 ml
1 % NBT	1.75 ml

When ready to stain, added :

1 % PMS	0.5 ml

9. 6-Phosphogluconate dehydrogenase

0.2 M Tris-HCl (pH 8.0)	12.5 ml
0.1 M MgCl2	8.75 ml
6-Phosphogluconic acid	25 mg
1 % NADP	0.125 ml
1 % PMS	0.125 ml
1 % MTT	0.5 ml

The stain was painted on the gel and incubated at 37° C in the dark.

10.Isocitrate dehydrogenase

0.2 M Tris-HCl (pH 8.0)	40 ml
0.25 M MnCl2	0.3 ml
0.1 M Isocitric acid	4 ml
1 % NADP	1.5 ml

When ready to stain, added :

1 % PMS	0.5 ml
1 % MTT	0.5 ml

Incubated at 37°C.

11. Glucose-6-phosphate isomerase

0.2 M Tris-HCl (pH 8.0)	25 ml
0.1 M $MgCl_2$	7.5 ml
18mM Fructose-6-phosphate	1.75 ml
G-6-PDH	10 ml
1 % NADP	0.625 ml

When ready to stain, added :

1 % PMS	0.5 ml
1 % MTT	1 ml

Incubated in the dark at 37°C.

12.Phosphoglucomutase

Double distilled water	25 ml
0.2 M Tris-HCl (pH 8.0)	5 ml
0.1 M $MgCl_2$	5 ml
Glucose-1-phosphate	5 ml
Glucose-1,6-diphosphate	2.5 ml
G-6-PDH	4 ml
1 % NADP	0.5 ml

When ready to stain, added :

1 % PMS	0.5 ml
1 % MTT	1 ml

Incubated at 37°C in the dark.

13.Lactate dehydrogenase

0.2 M Tris-HCl (pH 8.0)	40 ml
0.5 M Lithium lactate	9 ml
1 % NAD	1.3 ml
1 % NBT	300 µl

When ready to stain, added :

1 % PMS	500 µl

Incubated at 37°C.

14.Malate dehydrogenase

0.2 M Tris-HCl (pH 8.0)	40 ml
2 M D-L-Malic acid (pH 7.0)	6 ml
1 % NAD	2 ml
1 % NBT	2 ml

When ready to stain, added :

1 % PMS	0.6ml

Incubated at 37°C in the dark.

15. Glucose-6-Phosphate dehydrogenase		16.Hexokinase	
0.5 M Tris-HCl (pH 7.1)	4 ml	0.2 M Tris-HCl (pH 8.0)	50 ml
Double distilled water	30 ml	0.1 M MgCl2	1 ml
Na2 Glucose-6-phosphate	180 mg	α-D-glucose	50 mg
NaCn	25 mg	ATP	12 mg
1 % NADP	1.1 ml	1 % NADP	1.75 ml
1 % NBT	0.75 ml	G-6-PDH	2 ml
When ready to stain, added :		When ready to stain, added :	
1 % PMS	0.2 ml	1 % PMS	0.2 ml
Incubated at 37°C.		1 % NBT	0.4 ml
		Incubated at 37°C.	

17.Acid Phosphatase		18. Esterase	
0.1 M Acetate buffer	50 ml	Fast Blue RR salt	50 mg
Na-a-napthyl acid phosphate	50 mg	a-b-naphthyl acetate	1.5 ml
When ready to stain, added :		0.5 M Tris-HCl (pH 7.1)	5 ml
Black K salt	20 mg	Double distilled water	43.5 ml
Incubated at 37°C.		Incubated at 37°C in the dark.	

3.2.2. Molecular Genetics

This includes methods that can reveal finer variations existing at the structural level of nucleic acids that are undetectable by electrophoretic analysis of proteins/ enzymes produced by the DNA molecule. Both mt DNA and genomic DNA can be screened for such polymorphism. One way for the detection of this polymorphism is the use of restriction enzymes that cut mt DNA and genomic DNA molecule at specific sites of their lengths, separation of the cut fragments by electrophoresis and its detection by staining process. Another technique used for the detection of the same is the amplification of the DNA using arbitrary primers. Both these methods were applied to detect polymorphism at the molecular level.

3.2.2.1. Restriction Fragment Length Polymorphism of mt DNA

a) Standardisation

Mature ovaries were removed from the *P.indicus* specimens as

soon as they were brought to the laboratory and kept under -20°C till further use. Mitochondrial DNA present in the ovarian tissue of each specimen was isolated by alkaline lysis. The procedure adopted for isolation was as follows:

ISOLATION OF mt DNA

FIRST DAY

↓

Minced the tissue to small pieces

↓

Homogenised in Sol.I (SET buffer) in the ratio of 1gm: 10 ml

↓

Centrifuged the homogenate at 3000 rpm for 5 min. at 4°C in a refrigerated centrifuge

(Savant)

↓

Collected the supernatant and recentrifuged at 10,000 rpm, for 15 min. at 4°C.

↓

Decanted the supernatant and dissolved the pellet in sol.II (TEN buffer) in the ratio 1: 1. Kept on ice for 5 minutes.

↓

Double the volume of alkaline lysis buffer was added and mixed thoroughly by inversion. Kept on ice for 10-15 minutes.

↓

1.5 volumes of 3M Potassium acetate was added and mixed by inversion. Kept on ice for 10-15 minutes

↓

The lysed sample was centrifuged at 10,000 rpm for 10 minutes at 4°C

↓

The supernatant was collected and an equal volume of neutral phenol was added. Mixed well by inversion and allowed to stand for 5 minutes.

Centrifuged at 10,000 rpm for 15minutes at 4°C

↓

The aqueous layer was drawn off and the extraction with the neutral phenol was repeated.

↓

After centrifugation, the aqueous phase was collected and an equal volume of chloroform : isoamyl alcohol (24 : 1) was added.

↓

Centrifuged at 10,000 rpm for 5 minutes at 4°C

↓

Collected the aqueous phase and 1/10th of 3M sodium acetate was added. Mixed well and 2 volumes of chilled ethanol were added. Kept overnight for precipitation of mtDNA at -20°C

↓

SECOND DAY

↓

Centrifuged at 10,000 rpm for 20 minutes at 4°C

↓

Decanted the supernatant and the pellet was washed with 70 % ethanol. Centrifuged as above.

↓

Pellet was air dried and dissolved in Tris-EDTA buffer.

b) Electrophoresis of mt DNA

The isolated sample was tested for the presence of mtDNA. A 0.8 % agarose gel electrophoresis of 3-4 µl of isolated mtDNA sample using 1X TEB as electrode buffer was conducted for 3 hours at constant voltage. Lambda DNA, double digested with ECORI/Hind III was also run along with the sample as marker to find out the size of the sample DNA. The gel was stained with ethidium bromide for 20 minutes and viewed on 260 nm UV light to confirm the presence of the isolated mtDNA in the sample.

c) Digestion with restriction enzymes

The individually checked samples were digested with individual restriction enzymes viz., EcoR I, Hind III, Bam H I, Kpn I, Pst I etc. The reaction mix included 12 µl of sample, 10 % of the specific buffer for the enzyme used, 2 µl of enzyme and 1 µl of nuclease free distilled water. The reaction mix was incubated for 4 hours at 37°C. After 4 hours the reaction was stopped by keeping the eppendorf tubes with the reaction mixes on ice. These digested samples were subjected to electrophoresis as above. The stained gels were viewed under UV light and photographed to detect naturally existing RFLPs in the samples.

The compositions of the reagents used for mtDNA isolation are given below.

1.Solution I - SET	100ml	2.Solution II-TEN (pH 8.0)	100 ml
(Homogenising buffer pH 7.5)		10 mM Tris	0.12 g
0.25 M sucrose	8.56 g	10 mM EDTA	0.372 g
10mM EDTA	0.373 g	0.15 M NaCl	0.88 g
30 mM Tris	0.364 g		
3. NaOH	**10 ml**	**4. SDS**	**10 ml**
1.8 M NaOH	0.72 g	10 % SDS	1 g
5.Potassium acetate	**100 ml**	**6. Sodium acetate**	**50 ml**
3 M Potassium acetate	29.45 g	3 M Sodium acetate	12.30 g
7. Tris-EDTA (pH 8.0)	**50 ml**	**8. Neutral phenol**	**100 ml**
10mM Tris	0.06 g	Melted phenol	30 ml
1mM EDTA	0.02 g	0.1 % 8-hydroxy quinoline	0.1 g

3 % NaCl	30 ml
Chloroform	30 ml
2 M Tris- HCl (pH 7.0)	6.4 ml

9.Alkaline lysis buffer2ml	
1.8 M NaOH	0.2 ml
Sterile distilled water	1.6 ml
10 % SDS	0.2 ml

10. 10X TEB	150 ml
0.89 M Tris	16.2 g
0.02 M EDTA	1.12 g
0.89 M Boric acid	8.25 g

3.2.2.2 Random Amplified Polymorphic DNA

a) Protocol

ISOLATION OF TOTAL DNA

FIRST DAY

↓

10 mg of muscle tissue was minced in cold TEK buffer

↓

500 µl of TEK buffer, 100 µl 10 % SDS, and 1 µl of 10 mg/ml proteinase K were added to the minced tissue taken in an eppendorf tube. Mixed well.

↓

Incubated at 60°C in a water bath for 1-2 hours,till the tissue was completely dissolved in the buffer

↓

After incubation, an equal volume of 25:24:1 Phenol:Chloroform:Isoamylalcohol was added. Kept for 5 minutes

↓

Spun at 8,000 rpm for 5 minutes at 4°C

↓

The aqueous layer was collected and the phenol : chloroform : isoamylalcohol (25:24:1) extraction was repeated

An equal volume of chloroform : Isoamylalcohol was added to the aqueous phase, drawn. Kept for 5 minutes

↓

Centrifuged at 8,000 rpm for 5 minutes, at 4°C

↓

The aqueous phase was drawn off and 2.5 volumes of chilled absolute ethanol was added. Left overnight at -20°C

↓

SECOND DAY

↓

Centrifuged at 10,000 rpm for 20 minutes at 4°C

↓

Decanted the supernatant and the pellet was washed with 70 % chilled ethanol. Centrifuged as in the previous step.

↓

The pellet was air dried and dissolved in sterilised distilled water.

b) Polymerase Chain Reaction

The DNA was quantified and about 55 ng of sample DNA was used for amplifications. The reaction mix contained 0.2 µl of Taq polymerase (0.6 U), 3 µl of dNTPs, 2.5 µl of assay buffer (10X), 2.5 µl of primer (0.2mM), 15.55 µl of millipore water and 55 ng of sample DNA. The samples were set for reaction in duplicates in the thermocycler (MJ Research Inc.,USA) in two separate blocks and the reactions were cycled through two different temperature regimes. Block 'A' was run for forty cycles as follows: denaturation at 94°C for 1 min., annealing at 37°C for 1 min., extension at 72°C for 2 min., final extension at 72°C for 7 min.,. The initial denaturation was at 94°C for 5 min. For block 'B', forty cycles were run as follows : denaturation at 92°C for 1 min., annealing at 35°C for 1 min. 30 sec., extension at 72°C for 1 min. The final extension was at 72°C for 7 min. An additional

denaturation at 92°C for 5min. was given initially. The reaction was then cooled to 4°C. The reality of the PCR products were tested by using controls, one without primer, second without Taq DNA polymerase and the third without genomic DNA. No amplification occurred in any of these controls.

c) Analysis of PCR products

Following amplification, the reaction mixtures were run on a 1.5% agarose gel for one and a half hours using 1X TBE buffer. Ethidium bromide was added to the gel solution itself before solidification. After completion of the run, the gels were viewed under UV and photographed.

The sequences of the primers (Operon technologies, Inc., USA) used are AGGGGTCTTG(A5), TCCCAGCAGA(AC1), CCTGGGTCAG (AC11), GGCGAGTGTG(AC12), GACCCGATTG(AC13), AGTCCGCCTG(AC19).

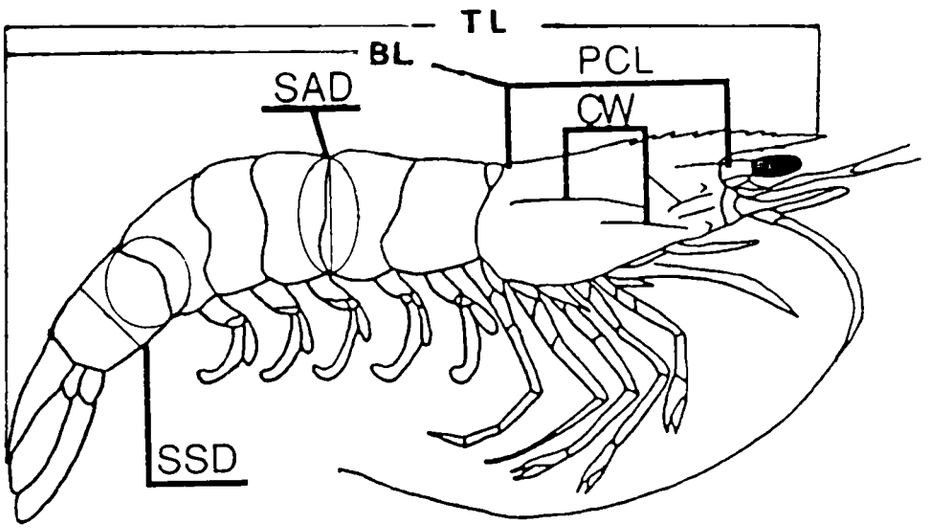
Composition of reagents

1. TEK buffer (pH 7.5)	100ml
50mM Tris	0.61 g
10mM EDTA	0.37 g
1.5 % KCl	1.5 g

3.2.3.a. Morphometrics

Each specimen was measured linearly using a scale and dial caliper (0.05mm accuracy). Initially a total of 19 metric measurements and 1 meristic count were taken and from these finally 9 metric and 1 meristic measurements were selected. The selected measurements were subjected to regression analysis to find out the correlation between each measurement and the tail weight. Only four variables were taken for the analysis finally and the other variables that involved the extremities were excluded, as they were prone to

Fig. 2. Morphometric variables of *Penaeus indicus*.



Variables

1.	Total length (TL)	Tip of the rostrum-tip of telson.
2.	Body length (BL)	Postorbital border of the carapace –tip of telson.
3.	Sixth segment Depth (SSD)	Depth at the mid-point of the 6^{th} segment.
4.	Second abdominal segment depth (SAD)	Depth at the mid-point of the 2^{nd} and 3^{rd} segment.
5.	Partial carapace length (PCL)	Posterior margin of orbit-posterior edge of carapace.
6.	Carapace width (CW)	At the point of the last dorsal tooth.
7.	Rostral length (RL)	Tip of the rostrum-last dorsal tooth.
8.	Total body weight (TW)	:
9.	Tail weight (TLW)	:
10.	Rostral teeth number (RTN)	:

easy damage. The correlation matrix thus obtained was further subjected to the path coefficient analysis to remove the indirect effect, of the inter related variables on the tail weight through the variable concerned. Thus the direct effects of each variable on tail weight were obtained. The details of the variables selected are given in figure-2.

3.2.3.b. Truss Network Analysis

Individual *P. indicus* was weighed to the nearest milligram using an electronic balance (Sartorius). For truss network analysis, each specimen was positioned on a drawing sheet and the landmarks were marked along the body surface of the sample, on to the paper with a needle. These landmarks were again copied to a graph sheet on which both X and Y co-ordinates for each of the landmarks were marked. A computer programme was written in dBase to convert these co-ordinates to distance measurements. The converted distance measurements were transformed as sheared principal components. PC-I, which denotes growth, and PC-II, which denotes shape, were plotted on X and Y-axes of the graph respectively, to find out whether there was any tendency for clustering.

The details of the parameters considered for the truss network analysis are given in figure-3.

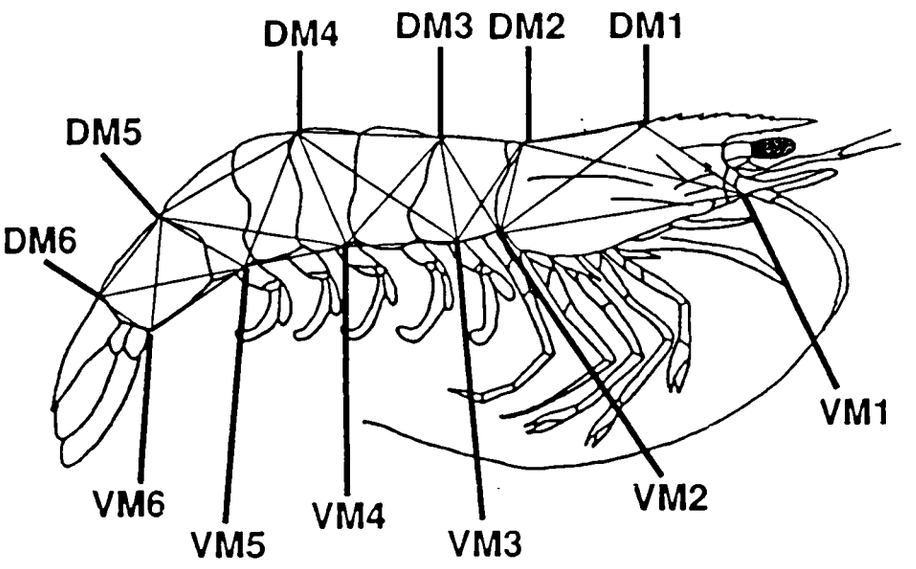
3.3. Statistical Analyses of the Data

3.3.1. Biochemical Genetics

Source: Zymograms (Fig. 4 & 5)

The stained gels showing visible banding patterns of the specific enzymes/ proteins belonging to the specimens subjected to electrophoresis are known as zymograms. The zymograms were drawn as a record or

Fig. 3. Truss Network Landmarks of *Penaeus indicus.*



Truss network landmarks

1.	DM 1	-Epigastric tooth (anterior)
2.	VM 1	-Base of the antennal flagellum
3.	DM 2	-Posterior dorsal median edge of carapace
4.	VM 2	-Posterior ventral corner of the carapace
5.	DM 3	-Posterior dorsal edge of tergum of the 1st abdominal segment
6.	VM 3	-Mid ventral point of the 1st abdominal segment
7.	DM 4	-Posterior dorsal edge of tergum of the 3rd abdominal segment
8.	VM 4	-Mid ventral point of the 3rd abdominal segment
9.	DM 5	-posterior dorsal edge of tergum of the 5th abdominal segment
10.	VM 5	-Mid ventral point of the 5th abdominal segment
11.	DM 6	-Posterior ventral edge of the tergum of the 6th abdominal segment
12.	VM 6	-Posterior ventral edge of the 6th abdominal segment

(DM –Dorsal measurement; VM – Ventral measurement)

photographed if necessary for further analysis. The homozygotic and heterozygotic banding patterns at assumed loci for each individual were recorded as follows. The banding patterns of an individual may consist of zones of fast, medium and slow moving bands and these zones were designated as 1, 2, 3 starting from the fast zone. A comparative analysis of each of these zones between individuals may have revealed variable phenotypic patterns such as either slow or fast moving single banded phenotypes or a combination of slow and fast moving phenotypes. Such phenotypic patterns were assumed as products of a particular locus and such genes were designated as loci 1, 2, 3 starting from the fastest zone. The zones with constant variant phenotypes were considered as products of polymorphic loci whereas zones with non-variant phenotypes were scored as that of non-polymorphic loci. The zones that showed inconsistent banding patterns were not considered as loci. Again, fast and slow moving phenotypes were scored as slow and fast homozygotes while a two-banded phenotype which was a combination of these slow and fast phenotypes, was called as heterozygote. However, when a three-banded heterozygote appeared instead of two banded, it was assumed that the enzyme structure was dimeric whereas the former that produced only two-banded heterozygote was monomeric in nature.

Since the electrophoretic mobility differences between slow and fast phenotypes at one locus was, most of the times, negligible and as also there occurred slower or faster phenotypes, their mobilities were actually measured to decide the class to which they belonged. For practicality, the commonest single banded phenotype was given a value of 100 while others depending on the comparative position, were given higher or lower values than 100. Thus, in genetic terms, there were slow, slower, fast, faster single banded homozygotes or two/three banded heterozygotes which were products of any of these two alleles. A counting of the number of phenotypes belonging to each class of mobilities in each sample/population was obtained. These

counts formed the basic genetic data on the population tested/compared. The data on phenotype counts were used to estimate frequencies of alleles, observed and expected frequencies of genotypes, heterozygosities, proportion of polymorphic loci, mean effective and average number of alleles, the degree of allele frequency differences between populations and the genetic distance between populations. The goodness of fit between the expected and the observed genotype frequencies was assessed by the Chi-square test. The degrees of freedom was found out using the formula d.f = n(n-1)/2, where 'n' is the number of alleles observed at a locus. The level of polymorphism was measured by the parameters like, heterozygosities, proportion of polymorphic loci, mean effective number of alleles and average number of alleles.

The following appropriate statistical formulae were applied.

a) Genetic Identity Analyses

Nei's (1972) formula was used to find out the mean genetic identity and distance between the populations.

Mean genetic identity $I = I_{XY}/\Sigma I_X I_Y$

Where , I_{XY}, I_X and I_Y are the arithmetic means of $X_i Y_i$, X_i^2 and Y_i^2 respectively, over all the loci.

Genetic distance was taken as -ln I.

b) F-analyses

Applying the methodolody suggested by B.S.Weir and C.Clark Cockerham (1984), did a co-ancestry assessment between the populations. F_{ST} values were estimated at each individual locus and the values were interpreted as showing little, moderate, great or very great levels of genetic

differentiation according to the qualitative guidelines suggested by Wright (1978).

3.3.2. Molecular Genetics

Two methodologies were adopted for the collection of the data for the genetic analysis.These methods were i) Restriction Fragment Length Polymorphism of mtDNA and ii) Random Amplified Polymorphic DNA, where the total DNA polymorphism was studied. In both the methods the stained gels were photographed and these photographs were used to analyse the banding patterns, i.e., the number of bands were counted as seen in the photographs. For the estimation of similarity coefficients, the banding pattern of each individual was compared with each of the remaining individuals. The number of bands common to both the individuals compared was counted first, and the number of bands present in each of them also was taken.

a) Data Analysis

The formula, suggested by Nei (1987), was used for the estimation of the genetic similarity, in both RFLP and RAPD.

The similarity, $S = 2N_{XY}/(N_X+N_Y)$

Where,

N_{XY} - the number of bands shared by x and y

N_X - the number of bands seen in x

N_Y - the number of bands seen in y

The genetic distance, D, was calculated by subtracting the similarity value from 1. The similarity values were averaged over all the primers used, to find out the mean similarity between the populations and within the populations.

3.3.3.a) Morphometric analysis

The values were fed into the computer and the correlation of the variables, viz., SSD, SAD, PCL & CW with tail weight was deduced by regression analysis. These variables were all interrelated and hence there was a chance for each of them to influence the other's correlation with the tail weight. In order to remove such indirect effects on the tail weight, the correlation matrix was further subjected to path coefficient analysis. Thus direct effects of the 4 different variables on the tail weight was assessed.

b) Truss Network Analysis

The x and y co-ordinate values for each landmark was fed into the computer and a programme was written in dBase III+ to convert these co-ordinates to the distance measurements between the landmarks. The distance measurements were further subjected to sheared principal component analysis and the PC scores got from the analysis were plotted on a graph (Excel or Axum) with PC I and PC II on X and Y axes respectively.

Results

4.1. Biochemical Genetics

4.1.1. Standardization of methodology

Discontinuous buffer system was found to be suitable for the electrophoretic analysis of proteins and allozymes. Among the various electrode buffers tried, 0.2M Tris - Glycine, pH 8.3 gave the best results. Likewise, 1.8M Tris-HCl, pH 8.9 and 0.5M Tris-HCl, pH 6.8, as separating and stacking gel buffers respectively, worked excellently in combination with Tris-Glycine. Maximum number of bands with better resolution and separation were produced when the tissues were homogenized in chilled double distilled water in the ratio 1.5g tissue : 1ml double distilled water. Muscle tissue produced the optimal banding patterns. The hepatopancreas was not giving consistent and reproducible banding patterns,while eye lens did not show any variation in the banding pattern. Hence, these tissues had to be excluded from further use. Tris-Glycine (electrode buffer) and Tris-HCl(gel buffer) buffer systems produced the best resolution of the six polymorphic enzymes. The gel percentages varied with different enzymes and the optimal gel percentage for each enzyme was found out empirically. An 8% gel was preferred by AK, AO, FBALD and HK, when αGPDH and ODH chose 7 and 7.6% respectively.

Though tests were conducted to detect eighteen enzymes, only thirteen showed their presence(the names, enzyme commission numbers, and abbreviations are given in table-3).The enzymes that could not be detected were, glutamate dehydrogenase(GLUDH), 6-phosphogluconate dehydrogenase(PGDH), isocitrate dehydrogenase(sIDHP), glucose-6-phosphate dehydrogenase(G6PDH) and acid phosphatase(ACP). Of the twelve enzymes that were detected, nine showed phenotypic variations while three were monomorphic in nature. Out of the nine enzymes that showed variations, two, namely, malate dehydrogenase(MDH), and esterase(EST) exhibited inconsistent phenotypic patterns. Again, out of the eight enzymes that produced expected genotypic variations, three, alpha glycerol phosphate dehydrogenase(αGPDH),

Table 3 List of enzymes used, with their numbers (IUBNC 1984*) and abbreviations

Name of enzymes	Enzyme number	Enzyme abbreviation
Acid phosphatase	3 . 1 . 3 . 2	ACP
Adenylate kinase	2 . 7 . 4 . 3	AK
Alcohol dehydrogenase	1 . 1 . 1 . 1	ADH
Aldehyde oxidase	1 . 2 . 3 . 1	AO
Esterase	3 . 1 . 1 . -	EST
Fructose biphosphate aldolase	4. 1 . 2 . 13	FBALD
Glucose – 6 – phosphate dehydrogenase	1 . 1 . 1 .49	G6PDH
Glucose - 6 – phosphate isomerase	5 . 3 . 1 . 9	GPI
Glutamate dehydrogenase	1 . 4 . 1 . -	GLUDH
Glycerol-3-phosphate dehydrogenase	1 . 1 . 1 . 8	αGPDH/G_3PDH
Hexokinase	2 . 7 . 1 . 1	HK
Isocitrate dehydrogenase	1 . 1 . 1 . 42	sIDHP
L - Iditol dehydrogenase	1 . 1 . 1 . 14	IDDH
Lactate dehydrogenase	1 . 1 . 1 . 27	LDH
Malate dehydrogenase	1 . 1 . 1 . 37	sMDH
Octanol dehydrogenase	1 . 1 . 1 . 73	ODH
Peptidase	3 . 4. - . -	PEP
6 - Phosphogluconate dehydrogenase	1 . 1 . 1 . 44	PGDH
Phosphoglucomutase	5 . 4 . 2 . 2	PGM

* Shaklee, J.B. *et al.* (1990)

alcohol dehydrogenase(ADH) and L-iditol dehydrogenase(IDDH) produced identical zymograms. Hence, out of these three, only αGPDH was selected for screening the populations. Thus, the six enzymes that showed consistent phenotypic variations and useful for genetic analysis of *P.indicus*, were adenylate kinase(AK), aldehyde oxidase(AO), fructose biphosphate aldolase(FBALD), glycerol-3-phosphate dehydrogenase(αGPDH), octanol dehydrogenase(ODH) and hexokinase(HK). The banding patterns of these six enzymes were controlled by 11 loci in total, out of which 6 were polymorphic. A locus was considered as polymorphic only if its most common allele had a frequency not higher than 0.95. The polymorphic loci detected were *AK-2**, *AO-2**, *FBALD-2**, *αGPDH-2**, *HK-2** and *ODH-1**.

A detailed description of the six polymorphic enzymes used for genetic analysis and observations on the other enzymes are given below.

a) Polymorphic enzymes with consistent banding patterns

1. Adenylate kinase (AK)

AK was tested only in samples from Kochi in the west coast and in Mandapam and Chennai in the east coast. Two zones of enzymatic activity were observed, of which the first, fast migrating locus, was monomorphic and the second, slow migrating locus was polymorphic. The first locus from the populations of Mandapam and Chennai showed two banded patterns also. The second locus showed single banded and double banded patterns which were regarded as homozygotes and heterozygotes respectively (Plate-2, Fig.4). The double-banded pattern of the heterozygotes suggests that the structure of the enzyme is monomeric. Each population showed three types of phenotypes,viz., the slow homozygotes (*AK-2 * 81/81*), the slow-fast heterozygotes (*AK-2* 81/100*) and the fast homozygotes (*AK-2* 100/100*).

The locus was under the control of a fast moving allele, *AK-2*100* and a slow moving allele, *AK-2* 81*. Fifty five percent of the individuals tested were heterozygous.

2. Aldehyde oxidase (AO)

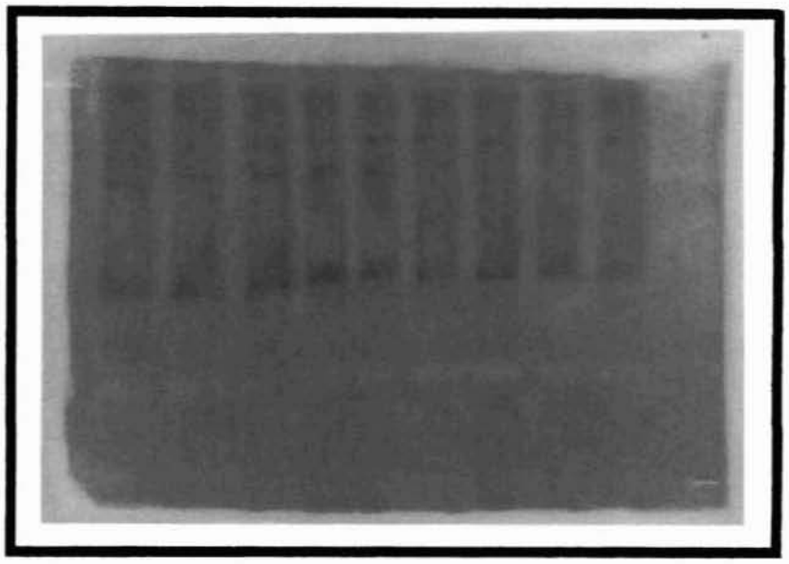
AO was tested in 4 populations in the east and 3 populations in the west coast. The enzyme was found to be under the control of 2 loci, the monomorphic *AO-1** and the polymorphic *AO-2**. The fast migrating monomorphic locus produced only a single type of homozygote (Plate-2 , Fig. 4). The second polymorphic locus showed 3 phenotypes, viz., the slow homozygote (*AO-2 * 71/71*), the slow-fast heterozygote (*AO-2* 71/100*), the fast homozygote (*AO-2* 100/100*) in all the populations. The two alleles at the locus were the slow moving *AO-2*71* and the fast moving *AO-2*100*. The heterozygotes showed two bands, one slow and the other fast, suggesting, the enzyme structure is monomeric. Sixty six percent of the sample was fast homozygotes.

Additional bands were seen between the first and second loci in some of the populations. But these phenotypes were not taken into account due to their inconsistent nature.

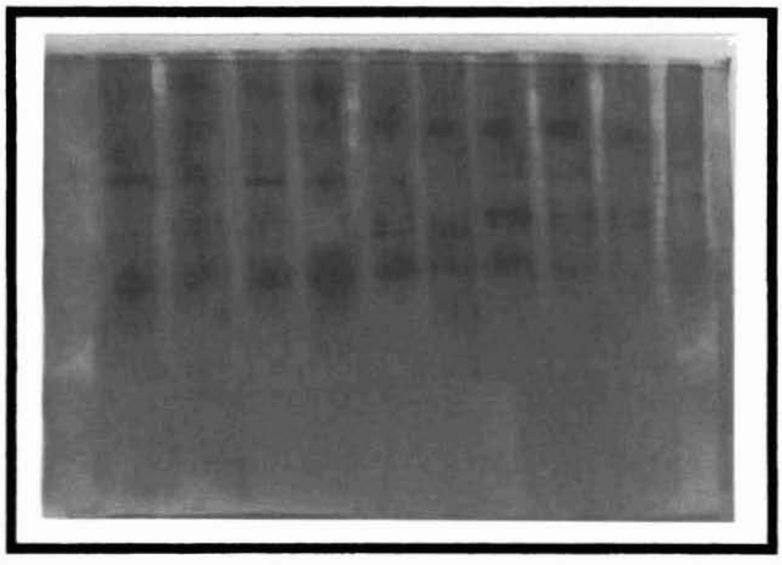
3. Fructose biphosphate aldolase (FBALD)

The zymograms of FBALD showed a fast moving zone, an intermediate zone and a slow moving third zone. The third zone was excluded from scoring, because of its inconsistent nature. The allele of the first locus, *FBALD-1*100* produced only a single phenotype (Plate-3, Fig.5). The second locus produced single banded fast and slow homozygotes and double banded heterozygotes indicating that the enzyme is polymorphic and monomeric in structure. The phenotypes scored were, slow homozygotes (*FBALD-2*100/100*), slow- fast heterozygotes (*FBALD-2*100/119*) and fast homozygotes (*FBALD-2*119/*

PLATE-2



a) Adenylate kinase



b) Aldehyde oxidase

119). The slow moving allele, *FBALD-2*100* and the fast moving *FBALD-2*119* controlled the genotypes of the locus. Fifty five percent of the individuals screened were heterozygous. But the locus was later excluded from the statistical analysis as it deviated very significantly from the Hardy-Weinberg equilibrium in 4 out of the 6 populations tested. This can be due to the typing errors that might have occurred at the time of scoring of the zymograms.

4. Glycerol-3-phosphate dehydrogenase (αGPDH)

Glycerol-3-phosphate dehydrogenase gave two zones of enzymatic activity. The first zone, fast in migration was monomorphic. The allele, α*GPDH-1*100* was found to be controlling the locus. The comparatively slow moving second locus was polymorphic in nature and produced three phenotypes, viz., the slow homozygotes (α*GPDH-2*52/52*), the slow-fast heterozygotes (α*GPDH-2*52/100*) and the fast homozygotes (α*GPDH-2*100/100*) (Plate-3, Fig.4). The heterozygotes had two minor bands and one major intermediate band. This three banded pattern of the heterozygotes revealed the dimeric structure of the enzyme. The locus α*GPDH-2** was regulated by α*GPDH-2*100* and α*GPDH-2*52* alleles. Fifty seven percent of the sample was homozygous and most of the fast homozygotes were males. Out of the 6 populations tested, 5 showed dominance of fast homozygotes while seventy five percent of the Mandapam population was heterozygotes.

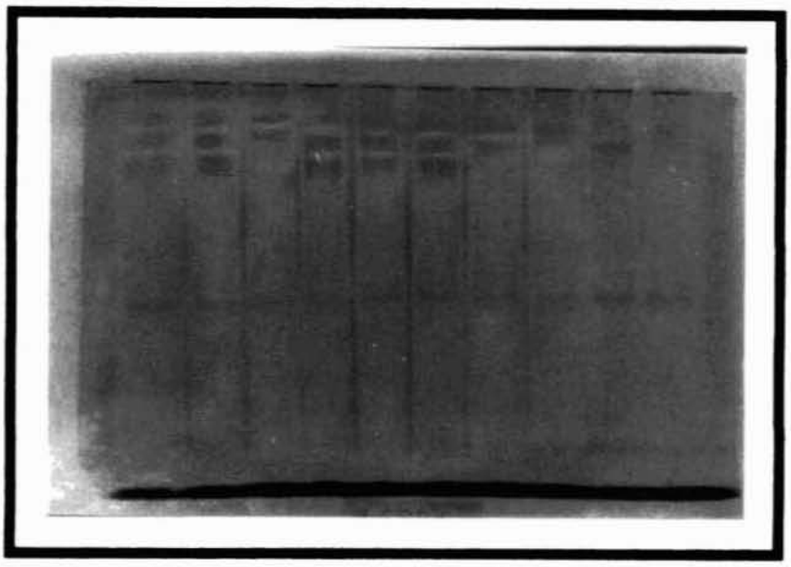
5. Alcohol dehydrogenase(ADH)

The zymogram patterns of ADH were identical to that of the αGPDH. Hence it was not used for further analysis.

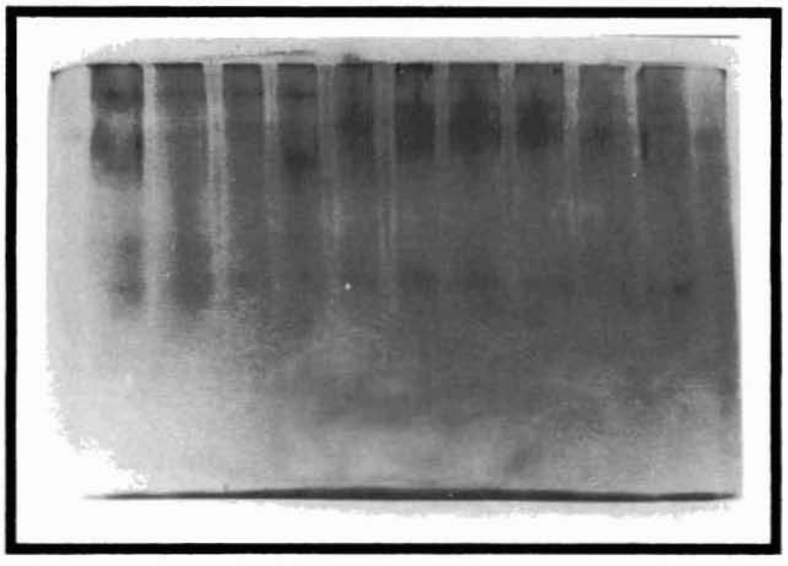
6. L-Iditol dehydrogenase(IDDH)

The zymogram patterns were identical to that of αGPDH and ADH. Hence the enzyme was not used for analysis.

PLATE-3



a) Alpha glycerophosphate dehydrogenase



b) Fructose biphosphate aldolase

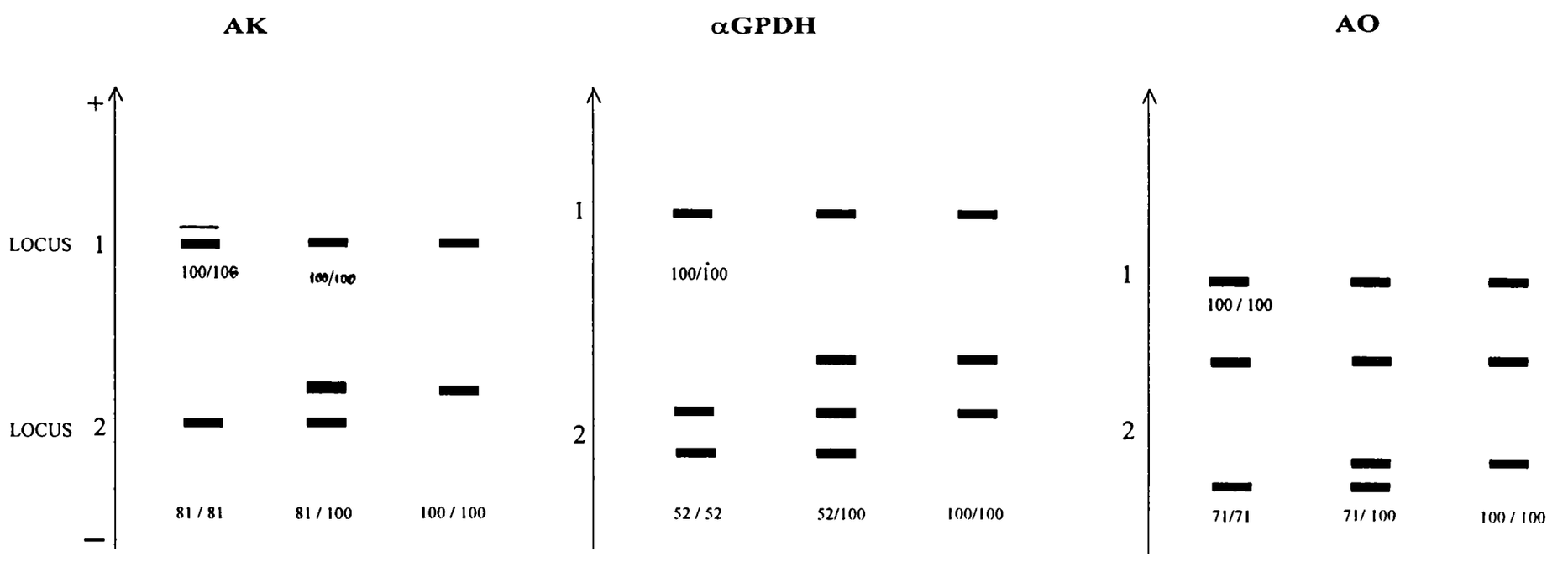


Fig.4 Zymogram patterns of AK, αGPDH, AO in muscle tissue of *P. indicus*

Band with 4x intensity

Band with 2x intensity

Band with 3x intensity

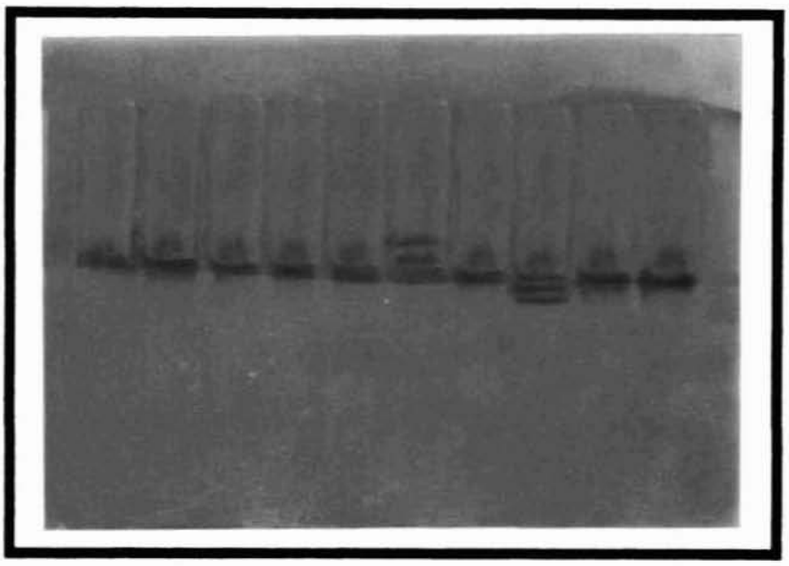
7. Octanol dehydrogenase (ODH)

Octanol dehydrogenase was tested in all the 7 populations. A single locus was found to be responsible for the enzyme activity. The zymograms of the enzyme exhibited polymorphism. Three types of individuals were seen, a single type of homozygote and two types of heterozygotes (Plate-4, Fig.5). Both the heterozygotes showed the typical banding pattern of a dimeric enzyme. Each heterozygote showed a prominent intermediate band and two minor bands above and below it. The occurrence of the two different heterozygotes and their band positions suggested that the locus produced three alleles. The slow band of the first type of heterozygotes shared its position with that of the fast band of the second type of heterozygotes. The slow and fast alleles, *ODH-1*100* and *ODH-1*119* expressed the first type of slow-fast heterozygotes (*ODH-1*100/119*). A slower allele, *ODH-1*81* and the slow allele, *ODH-1*100* produced the second type of heterozygotes (*ODH-1*100/81*). The homozygotes were all the slow type (*ODH-1*100/100*). Out of the 7 populations tested (Calicut, Chowghat, Kochi, Neendakara, Mandapam, Chennai, Kakinada), 3(Calicut, Neendakara, Chennai) had only homozygotes, another 3(Chowghat, Kochi, Mandapam) had both homozygotes and only one type of heterozygotes. Both types of heterozygotes along with the homozygotes were observed only in the population from Kakinada.

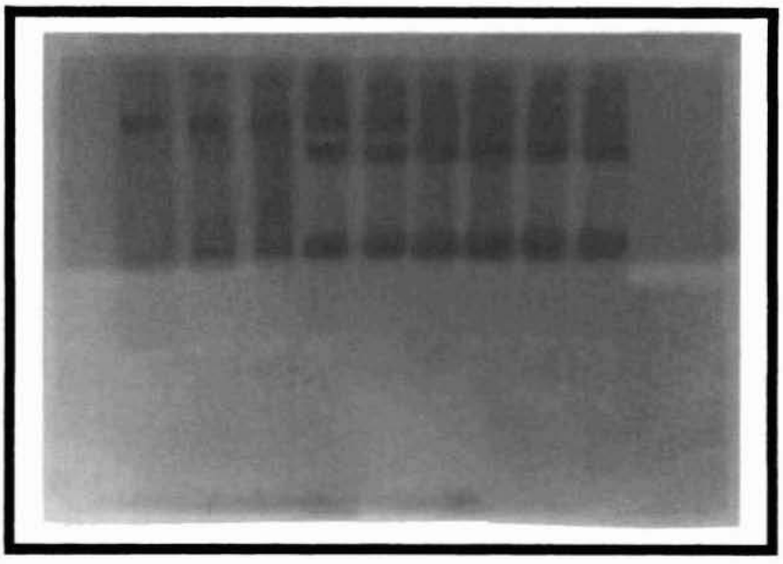
8. Hexokinase (HK)

Hexokinase was regulated by 2 loci.. The first locus expressed only a common slow band whereas an additional fast band was observed in few samples. As the frequency of the most common allele, *HK-1*100* was more than 0.95, the locus was not treated as polymorphic. The second locus showed single banded slow and fast homozygotes *HK-2*53/53* and *HK-2*100/100* respectively and two banded slow-fast heterozygotes, *HK-2*53/100*(Plate-4, Fig. 5). The locus was found to be regulated by the slow moving allele, *HK-*

PLATE-4



a) Octanol dehydrogenase



b) Hexokinase

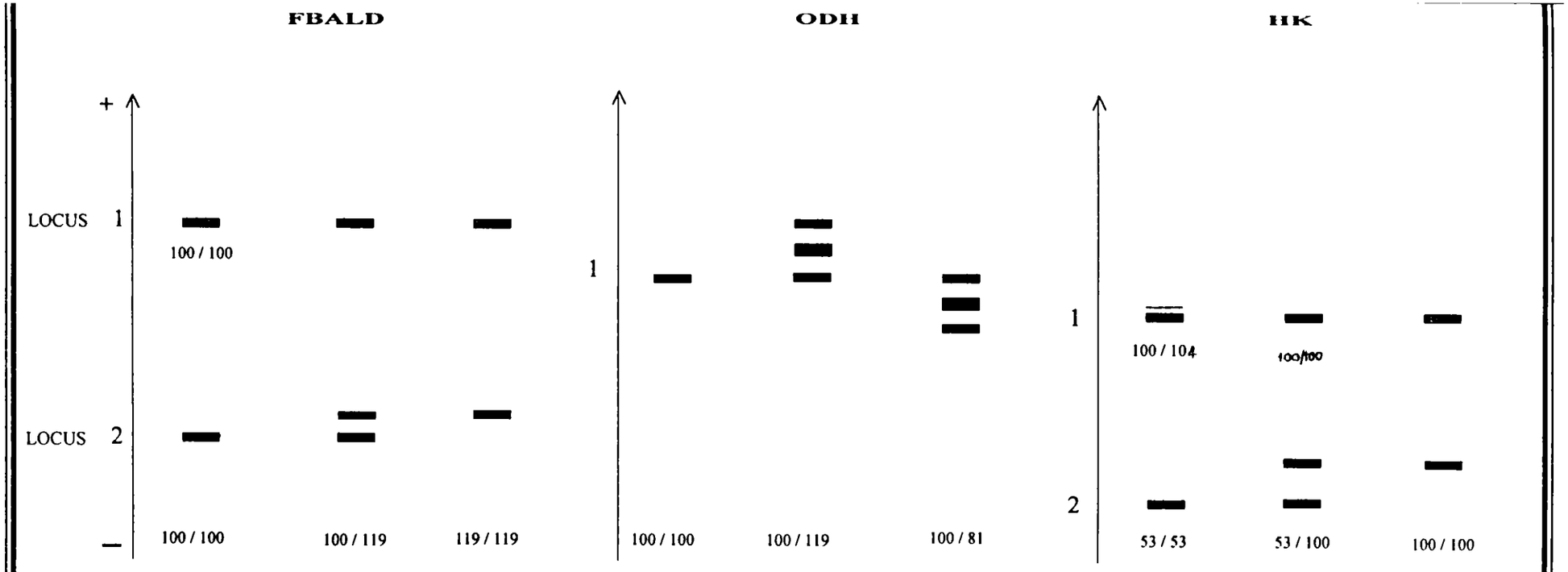


Fig. 5 Zymogram patterns of FBALD, ODH, HK in muscle tissue of *P. indicus*

Band with 4x intensity Band with 2x intensity Band with 3x intensity

*2*53* and a fast moving allele, *HK-2*100*. The slow homozygotes were seen only in Chowghat in the west and Chennai in the east. Fifty six percent of the sample population was homozygous.

b) Monomorphic enzymes

1. Glucose-6-phosphate isomerase (GPI)

Glucose-6-phosphate isomerase was found to be controlled by a single locus. It showed a single banded pattern invariably in all the individuals tested.

2. Gly, Leu, Peptidase

A single locus controlled the enzyme and the locus showed single banded pattern in all the individuals tested.

3. Lactate dehydrogenase (LDH)

Two zones of activity were observed in LDH of which the second zone was present only in some individuals. The first locus had 3 bands without any variation in all the individuals tested. Hence it was not screened further. As all the individuals were alike phenotypically, the genotype was considered as *LDH-1* 100/100*.

c) Enzymes with inconsistent banding patterns

1. Phosphoglucomutase (PGM)

Phosphoglucomutase gave both four banded and three banded patterns which was controlled by a single locus. The variation observed may be due to non-genetic reasons.

2.Malate dehydrogenase (MDH)

Though zones of enzyme activity were present, the resolution of bands was very poor. The banding pattern was also inconsistent. Due to these problems, the scoring of the zymograms of MDH was difficult and often misleading to wrong conclusions. One or two individuals only showed the typical dimeric pattern of expression in the heterozygotes with three bands. From this, the structure of MDH in *P.indicus*, can be assumed as dimeric. Due to the inconsistent and non-reproducible pattern of expression, it was not found as suitable to analyse the population structure.

3. Esterase (EST)

Esterase was found to be controlled by 4 loci, of which only *EST-1** was polymorphic. The enzyme failed to produce any activity in some of the other populations, obviously due to non-genetic reasons. So EST was not counted as a candidate enzyme for the comparative analysis of the population structure.

d) Undetectable enzymes

1. Glutamate dehydrogenase (GLUDH)

The enzyme was tested repeatedly with all the four buffer systems(Table-1), in the muscle tissue.The substrate used was L-glutamic acid.None of the attempts produced any result. So it was removed from the work plan.

2. 6-Phosphogluconate dehydrogenase (PGDH)

6-Phosphogluconate dehydrogenase could not be detected in the muscle tissue with any of the four buffer systems given in the table-1. 6-Phosphogluconic

acid was used as the substrate.

3.Isocitrate dehydrogenase (sIDHP)

Isocitrate dehydrogenase also did not show any enzymatic activity in any of the buffer systems (Table-1), in muscle tissue. The substrate used was isocitric acid.

4. Glucose-6-phosphate dehydrogenase (G6PDH)

The tests for its presence produced no results in any of the four buffer systems. It was tested in the muscle tissue only and the substrate used was Na_2 glucose-6-phosphate.

5.Acid Phosphatase (ACP)

Acid phosphatase could not be detected in the muscle tissue, in any of the buffer systems tried. Na-a-naphthyl acid phosphate was the substrate used.

4.2. Molecular Genetics

4.2.1. RAPD of total DNA

a) Polymerase chain reaction

Standardisation: The samples in the 'B' block, which were run for forty cycles with 1 min denaturation at 92°C, followed by the annealing of the primer at 35°C for 1 min. 30sec. and extended at 72°C for 1min., got amplified. Only 3 of the six 10mer primers,viz., AC12, AC13 and AC19 were found to amplify the total DNA of the species. Polymorphism was observed in all the three amplifications with the above primers. Details of the amplification profile are given below.

1. AC19 (AGTCCGCCTG)

Sixteen samples, 8 each from Chennai and Kochi were analysed with AC19. The number of fragment or the bands ranged from 2 to 3 in Chennai and 2 to 5 in Kochi in different individuals. Thus AC19 produced 7 bands in total. These were of the sizes, 3.5Kb, 2Kb, 1.8Kb, 1.6Kb, 1.5Kb and two fragments of <1.5Kb(Plate-5). The 3.5Kb and 2Kb fragments were seen only in Kochi. It means that the RAPD structure of Kochi and Chennai is heterogenous. The AC19 primer may be a potential primer to differentiate the genetic stock differences in the species. The statistical significance of this has been explained in the appropriate section.

2. AC12 (GGCGAGTGTG)

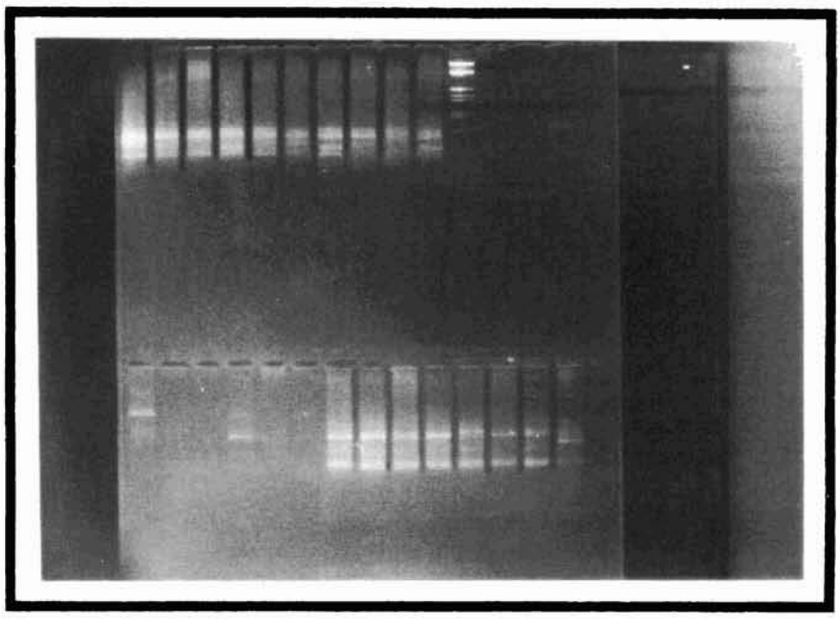
Ten individuals from Chennai(east coast) and eight individuals from Kochi(west coast) were screened. The number of bands varied between 1 and 3 in different individuals of both regions. The sizes of the fragments were all below 1.5kb(Plate-5). The AC12 primer, though produced polymorphic bands, did not reveal any difference between the two samples.

3. AC13 (GACCCGATTG)

A total of 19 individuals were screened, 9 from Kochi and 10 from Chennai. This primer produced 4 to 5 bands in Kochi of the sizes 3.5Kb, 1.7Kb, 1.5Kb and the rest below 1.5Kb(Plate -5). But as the amplification was not proper with the Chennai samples, the data provided by this primer in Kochi could not be used for comparison.

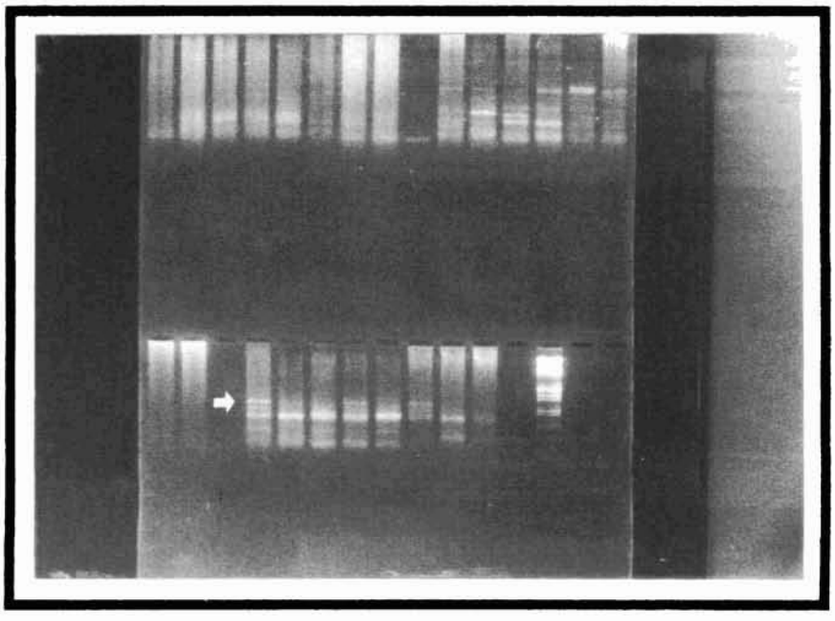
Due to limitations in the experimental facilities for RAPD analysis, the AC19 profile of the other populations could not be studied and compared.

PLATE-5



a) Amplified DNA fragments of *P.indicus* from Chennai

Lanes 1-10: AC12, 11: DNA marker, 12-21: AC13, 22-29: AC19



b) Amplified DNA fragments of *P.indicus* from Kochi

Lanes 1-9: AC12, 10-17: AC13, 19-26: AC19,28 DNA marker

Arrow shows the population specific fragment

4.2.2. RFLP studies with the mitochondrial DNA

a) Standardisation: The alkaline lysis method for the isolation of mtDNA by removing the nuclear DNA completely was successfully carried out with 3 individuals from Kochi. Later, due to an irreparable damage happened to the refrigerated centrifuge (Savant), the mtDNA isolation could not be done successfully any further. As more individuals could not be screened for the restriction fragment length polymorphism of mtDNA, the method was excluded from the analysis part.

b) Restriction digestion

The 3 samples from Kochi were digested with the restriction enzymes,viz., HindIII, PstI, EcoRI, BamHI and KpnI. Among the above restriction enzymes, PstI had only one cutting site, while EcoRI, BamHI and KpnI showed 2 cutting sites each. HindIII had 6 sites in sample # 1 and only 2 sites in sample # 2 and 3. From the restricted pattern of the mtDNA, it was clear that the mtDNA of *P.indicus* is of ~21Kb size.

1.HindIII

It produced 6 fragments of the sizes, 5Kb, 4.5Kb, 4Kb, 3.5Kb, 3Kb and 1Kb in sample # 1. Sample # 2 and 3 showed only 2 fragments of the sizes 11.5Kb and 9.5Kb. This enzyme seems to be a potential marker to reveal RFLP in the species.

2.EcoRI

EcoRI cut both the samples at 2 sites producing 2 fragments of 11.5Kb and 9.5Kb.

3.PstI

PstI produced only a single band of 12Kb. As the size of the mtDNA of *P.indicus* was found to be of ~21Kb, there could be some smaller fragments which might have either run out or was not visible as feebly stained.

4.BamHI

BamHI was tested only in one sample. It produced 2 fragments of the sizes
12Kb and 9Kb.

5. KpnI

Two fragments of the sizes, 12.5Kb and 8.5Kb were produced by KpnI.

Due to the permanent breakdown of the high-speed centrifuge, application of the potential Hind III restriction enzyme could not be accomplished.

4.3. Statistical Analyses of the Data

4.3.1. Biochemical Genetics

The parameters viz., average number of alleles per locus (Na), effective number of alleles per locus (Ne), the percentage of polymorphic loci and heterozygosity were estimated to measure the degree of genetic variation in the species. The Na values for different populations from Calicut, Chowghat, Kochi, Neendakara, Mandapam, Chennai and Kakinada were 1.22,1.30,1.38,1.14,1.46,1.46 and 1.44 respectively. Like wise, the respective Ne values estimated for Calicut, Chowghat, Kochi, Neendakara, Mandapam, Chennai and Kakinada were 1.07,1.09,1.21,1.10,1.20,1.26 and 1.14. The

Table - 4 Measures of genetic variation

Measures of genetic variation		CCT	CHGT	KCH	NDKRA	MDM	CHNI	KKNDA
Ne		1.07	1.09	1.21	1.10	1.20	1.26	1.14
Na		1.22	1.30	1.38	1.14	1.46	1.46	1.44
H	He	0.05	0.07	0.12	0.06	0.12	0.14	0.09
	Ho	0.06	0.06	0.15	0.08	0.15	0.12	0.10

For the species - He = 0.09 ; Ho = 0.10

Proportion of polymorphic loci in the species - 0.39

percentage of polymorphism observed in the species was 39%(Table-4). The above values suggest that the species, *P.indicus* has high biochemical genetic variability.

a) Allelic frequencies and goodness of fit

Allelic frequencies were estimated at the 14 loci belonging to 9 different enzymes, viz., AK, AO, FBALD, αGPDH, GPI, HK, LDH, ODH and PEP (Table-5). Noticeable allele frequency variations were observed only at *AO-2** and *αGPDH-2** , between certain populations. Allele frequency differences were observed between Chowghat and Chennai and between Chowghat and Neendakara at *AO-2** and the frequencies of the most common allele for these places were 1.00, 0.68 and 0.71 respectively. The allele frequency of Chowghat and Kochi also varied which being 0.889 and 0.56 respectively. The frequency of the allele *αGPDH-2*52* in the Mandapam samples was 0.63, while the same allele's frequency ranged between 0.20 and 0.36 in the rest of the populations. The other difference noticed was that the locus *ODH-1** was polymorphic in Chowghat, Kochi, Mandapam and Kakinada, while it was not polymorphic in Calicut, Neendakara and Chennai. Though the allele *ODH-1*119* was observed in Chowghat, Mandapam and Kakinada, it was absent in other stations. The significance of the above described allele frequency differences between populations was examined by F_{ST} test. The results of the test are given in the next page.

Another interesting type of allele frequency difference occurred was at the *AK-1** locus. Though the *AK-1** locus showed its allele *106* in the east coast sample stations Mandapam and Chennai in very low frequencies, which being 0.02 and 0.01 respectively, it was absent in the west coast station, Kochi. Its absence here could not be due to sample size in Kochi, because the sample size was comparatively higher than that of the east coast stations where the rare allele occurred. These facts, therefore, suggest that the rare

Table 5 Allele frequencies of the isozymes of *P.indicus* from South East & South West coasts of India

Locus	Allele	Calicut	Chowghat	Kochi	Ndkra	Mandapam	Chennai	Kakinada
AK	(N)			(46)		(26)	(36)	
1	100	NT	NT	1.00	NT	0.98	0.99	NT
	106			0.00		0.02	0.01	
2	81			0.46		0.31	0.38	
	100			0.54		0.69	0.62	
AO	(N)	(40)	(9)	(89)	(17)	(55)	(103)	(35)
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1	71	0.05	0.00	0.13	0.29	0.13	0.32	0.26
2	100	0.95	1.00	0.87	0.71	0.87	0.68	0.74
FBALD	(N)	`(40)		‘(89)	‘(18)	`(55)	`(104)	`(9)
1	100	1.00	NT	1.00	1.00	1.00	1.00	1.00
2	100	0.79		0.74	0.53	0.72	0.58	0.89
	119	0.21		0.26	0.47	0.28	0.42	0.11
αGPDH	(N)	`(40)	`(10)	`(38)		`(20)	`(88)	`(33)
1	100	1.00	1.00	1.00	NT	1.00	1.00	1.00
2	52	0.23	0.25	0.20		0.63	0.36	0.20
	100	0.77	0.75	0.80		0.37	0.64	0.80
GPI	(N)	`(20)	`(10)	`(20)	`(20)	`(20)	`(20)	`(20)
1	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
HK	(N)		`(9)	`(26)		`(18)	`(33)	
1	100	NT	1.00	1.00	NT	1.00	0.96	NT
	104		0.00	0.00		0.00	0.04	
2	53		0.111	0.44		0.25	0.35	
	100		0.889	0.56		0.75	0.65	
LDH	`(N)	`(20)	`(10)	`(20)	`(20)	`(20)	`(20)	`(20)
1	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ODH	(N)	`(40)	`(10)	`(89)	`(20)	`(67)	`(86)	`(35)
1	100	1.00	0.95	0.98	1.00	0.99	1.00	0.93
	81	0.00	0.00	0.02	0.00	0.00	0.00	0.04
	119	0.00	0.05	0.00	0.00	0.01	0.00	0.03
PEP	(N)	`(20)	`(10)	`(20)	`(20)	`(20)	`(20)	`(20)
1	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00

(N) – Number of Samples NT – Not Tested

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allele *AK-1*106* may be a characteristic feature of the east coast populations. It means that the east coast populations may be genetically heterogeneous and they do not mix with that of the west.

The results of the statistical test of goodness of fit between the observed and expected genotype frequencies are shown in table-6. The expected genotype frequencies were estimated from the estimated allelic frequencies at each polymorphic locus. The Chi-square test involved 5 polymorphic loci and 7 population samples. Of the 27 chi-square values obtained, only 2 were significant at $P<0.05$. These significant values were produced at *AK-2** locus in Kochi and *αGPDH-2** in Mandapam. However, the distribution of the genotypes at these two loci in all other samples showed a high goodness of fit. It means, the results considered here are of genetic nature and are useful for the analysis of population genetic structure.

b) Heterozygosity

The average observed and expected heterozygosities were estimated for each population. The average expected heterozygosity values for Calicut, Chowghat, Kochi, Neendakara, Mandapam, Chennai and Kakinada were 0.05, 0.07, 0.12, 0.06, 0.12, 0.14 and 0.09 respectively, while the respective observed heterozygosities for the above populations were 0.06, 0.06, 0.15, 0.08, 0.15, 0.12 and 0.10. The average expected and observed heterozygosities for the species were found to be 0.09 and 0.10 respectively(Table-4).

These values of heterozygosity and average number of polymorphic loci and alleles indicate that *P.indicus* examined here has high genetic variability or level of polymorphism at the loci considered.

… Observed and expected (within the parentheses) genotype frequencies of *P.indicus* from South east and South west coasts of India

Loci	Genotype	Calicut	χ^2	Chowghat	χ^2	Kochi	χ^2	Ndkra	χ^2	Mandapa	χ^2	Chennai	χ^2	Kakinada	χ^2
AK	(N)	NT		NT		(46)		NT		(26)		(36)		NT	
2	81/81					4(9.7)				2(2.5)		7(5.2)			
	81/100					34(22.8)				12(11.1)		13(17)			
	100/100		--		--	8(13.4)	11.0274*		--	12(12.4)	0.1859	16(13.8)	1.9150		--
AO	(N)	(40)		(9)		(89)		(17)		(55)		(103)		(35)	
2	71/71	0(0.1)		0(0.00)		1(1.5)		0(1.4)		2(0.93)		10(10.5)		2(2.4)	
	71/100	4(3.8)		0(0.00)		21(20.1)		10(7.0)		10(12.4)		45(44.8)		14(13.5)	
	100/100	36(36.1)	0.1108	9(9.00)	0.0000	67(67.4)	0.2093	7(8.6)	2.9834	43(41.6)	1.7427	48(47.6)	0.0281	19(19.2)	0.0873
αGPDH	(N)	(40)		(10)		(38)		NT		(20)		(88)		(33)	
2	52/52	1(2.11)		1(0.625)		0(1.52)				5(8)		12(11.4)		0(1.32)	
	52/100	16(14.17)		3(3.75)		15(12.2)				15(9.3)		40(40.6)		13(10.6)	
	100/100	23(23.72)	0.8421	6(5.625)	0.4000	23(24.3)	2.2322		--	0(2.7)	7.3185*	36(36.04)	0.0405	20(21.1)	1.9207
HK	(N)	(NT)		(9)		(26)		NT		(18)		(36)		NT	
2	53/53			0(0.111)		4(5.03)				0(1.13)		6(4.4)			
	53/100			2(1.776)		15(12.8)				9(6.8)		13(16.4)			
	100/100		--	7(7.113)	0.1410	7(8.2)	0.7646		--	9(10.1)	1.9616	17(15.2)	1.4999		--
ODH	(N)	(40)		(10)		(89)		(20)		(67)		(86)		(35)	
1	100/100	40(40)		9(9.025)		85(85.5)		20(20)		65(65.7)		86(86)		30(30/3)	
	100/81	0(0)		0(0)		4(3.5)		0(0)		0(0)		0(0)		3(2.6)	
	81/81	0(0)		0(0)		0(0.04)		0(0)		0(0)		0(0)		0(0.06)	
	81/119	0(0)		0(0)		0(0)		0(0)		0(0)		0(0)		0(0.08)	
	100/119	0(0)		1(0.95)		0(0)		0(0)		2(1.3)		0(0)		2(2)	
	119/119	0(0)	0.0000	0(0)	0.0027	0(0)	0.1144	0(0)	0.0000	0(0.01)	0.3944	0(0)	0.0000	0(0.03)	0.2345

(N) = Number of samples NT = Not tested * Significant at $P<0.05$

c) Standardised variance in allele frequencies(F_{ST})

The standardised variance in the allele frequencies (F_{ST}) was estimated for pair wise comparisons of the populations, using the equations formulated by Weir and Cockerham(1984) for single locus and multiple loci. Table-7 summarizes the F_{ST} values for 22 pair wise comparisons done on locus by locus (only polymorphic) basis. There were 71 F_{ST} values in total , of which 15 were negative (according to Waples(1987), F_{ST} can be negative , when finite sample sizes are used). The values were interpreted as showing nil, little, moderate, great or very great levels of genetic differentiation based on the guidelines put forward by Wright(1978). Out of the 71 pairwise comparisons involving 7 samples and 5 polymorphic loci, only 15 values differed significantly. Interestingly eight of these differences were due to *AO-2**, four due to *αGPDH-2**, one due to *HK-2** and two due to *ODH-1**. It means that a total of 12(out of 15) significantly different F_{ST} values were just due to two loci, namely *AO-2** and *αGPDH-2**. Besides, six of these occurred in Chowghat and Neendakara whereas four of these occurred in Mandapam alone. The reason for the differences at *AO-2** in Chowghat and Neendakara may be due to the very small sample size from these two regions. The major reason for the difference at *αGPDH-2** in Mandapam may be due to a correlation between large size of the specimens and the enzyme itself. Above all, the allele frequencies never differed at two or more loci between any two centres. However, ten out of these fifteen significant differences were between east and west coast locations but correlated to *AO-2** and *αGPDH-2** loci alone, as explained above. In brief, the differences may not be due to genetic reasons.

d) Genetic Identity

Nei's genetic identity values of 21 pair wise comparisons between 4 populations along the west coast and 3 populations along the east coast, ranged between 0.97865 to 0.99986 (Table-8) indicating high levels of genetic similarity among the populations.

Table 7 F_{ST} Values for the pairwise comparisons between sampling locations of *Peneaus indicus*

Comparisons	AK-2	AO-2	αGPDH-2	HK-2	ODH-1
Calicut/Chowghat	--	-0.00134	-0.02976	--	0.08991[m]
Calicut/Kochi	--	0.024883[l]	-0.00815	--	-0.007434
Calicut/Neendakara	--	0.221785[g]	--	--	0.00000
Calicut/Mandapam	--	0.024007[l]	0.281034[v]	--	0.0029004
Calicut/Chennai	--	0.169582[g]	0.029387[l]	--	0.00000
Calicut/Kakinada	--	0.14971[m]	-0.00908	--	0.05194[m]
Chowghat/Kochi	--	0.049279[l]	-0.02113	0.180985[g]	0.334107[v]
Chowghat/ Neendakara	--	0.21838[g]	--	--	0.03778[l]
Chowghat/Mandapam	--	0.04412[l]	0.230553[g]	--	0.027630[l]
Chowghat/Chennai	--	0.182497[g]	-0.00247	0.08806[m]	0.182872[g]
Chowghat/Kakinada	--	0.15366[g]	-0.02264	--	-0.003416
Kochi/Neendakara	--	0.07747[m]	--	--	0.0000038
Kochi/Mandapam	0.035992[l]	-0.00782	0.325246[v]	0.05753[m]	0.0092349
Kochi/Chennai	0.002743	0.451924[v]	0.049109	0.001183[l]	0.015594[l]
Kochi/Kakinada	--	0.04967[l]	-0.01118	--	0.024756[l]
Ndkra/Mandapam	--	0.795699	--	--	0.0015931
Neendakara/Chennai	--	-0.01437	--	--	0.000000
Neendakara/Kakinada	--	-0.01652	--	--	0.034516[i]
Mandapam/Chennai	-0.00809	0.08394[m]	0.12482[m]	0.000213[i]	0.0080478
Mandapam/Kakinada	--	0.600381[v]	0.322013[v]	--	0.043134[l]
Chennai/Kakinada	--	-0.00106	0.047615[l]	--	0.084434[m]
All Sites	0.010965	0.05847[m]	0.07833[m]	0.040600[l]	0.028452[l]

-- Comparisons which were not possible
l Little genetic differentiation (0-0.05)
m Moderate genetic differentiation (0.05-0.15)
g Great genetic differentiation (0.15-0.25)
v Very great genetic differentiation (above 0.25)

Table 8 Mean genetic similarities (above the diagonal) and genetic distances (below the diagonal) of *P. indicus* between different locations

	Calicut	Chowghat	Kochi	Ndkra	Mandapam	Chennai	Kakinada
Calicut	--	0.99928	0.99836	0.98834	0.98034	0.98957	0.99444
Chowghat	0.00072	--	0.98606	0.98530	0.98029	0.98126	0.99060
Kochi	0.00164	0.01404	--	0.99616	0.97865	0.99316	0.99785
Ndkra	0.01173	0.01481	0.00385	--	0.99622	0.99986	0.99933
Mandapa	0.01986	0.01991	0.02158	0.00379	--	0.98897	0.98168
Chennai	0.01048	0.01892	0.00686	0.00014	0.01109	--	0.99596
Kakkinad	0.00558	0.00944	0.00215	0.00067	0.01849	0.00405	--

4.3.2. Molecular Genetics

a) Random Amplified Polymorphic DNA

The bands were counted from the photographs and the band sharing indices between the individuals were estimated using Nei's formula, for each primer. These index values were averaged over the primers in order to find out the mean genetic similarities among the individuals within the populations and between the populations. The similarity values in the total population ranged between 0 and 1(Table-9). The mean percentage similarity among the individuals of Chennai population was 76%, whereas that of Kochi population was 66%. A mean percent similarity of 66% was observed between the Chennai and Kochi populations. This indicate the large changes at the base pair level, reflecting the difference in the population structures of two different coasts.

b) RFLP of mtDNA

From the restriction digestion, it was found that the size of the mtDNA of *P.indicus* is around ~21Kb. As the experiments could not be repeated with more number of individuals, the data collected was insufficient for the subsequent statistical analysis.

4.3.3. Morphometrics

1. Correlation with tail weight.

From the path coefficient analysis, it was found that the second abdominal depth was the variable having highest correlation with the tailweight irrespective of sex(Table-10). Therefore, it can be used as the best alternative parameter for the tail weight in the breeding programmes of *P.indicus*. The

Table 9 Genetic similarities (above the diagonal) and distances (below the diagonal) between individual prawn samples

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	K1	K2	K3	K4	K5	K6	K7	K8
C1	-	0.9	0.9	0.85	0.75	0.75	0.85	0.60	0.80	0.40	0.65	0.75	0.75	0.60	0.90	0.60	0.70	0.70
C2	0.10	-	1.00	0.90	0.85	0.80	0.90	0.70	1.00	0.50	0.75	0.85	0.85	0.70	1.00	0.45	0.60	0.60
C3	0.10	0.00	-	0.90	0.85	0.80	0.90	0.70	1.00	0.50	0.75	0.85	0.85	0.70	1.00	0.45	0.60	0.60
C4	0.15	0.10	0.10	-	0.75	0.75	1.00	0.60	0.80	0.80	0.65	0.75	0.75	0.60	0.90	0.40	0.55	0.55
C5	0.25	0.15	0.15	0.25	-	0.60	0.75	0.50	1.00	0.50	0.75	0.65	0.70	0.50	0.85	0.25	0.20	0.20
C6	0.25	0.20	0.20	0.25	0.40	-	0.75	0.65	0.80	0.40	0.55	0.80	0.65	0.65	0.80	0.65	0.75	0.75
C7	0.15	0.10	0.10	0.00	0.25	0.25	-	0.60	0.80	0.80	0.65	0.75	0.75	0.60	0.90	0.40	0.55	0.55
C8	0.40	0.30	0.30	0.40	0.50	0.35	0.40	-	0.70	0.70	0.25	0.85	0.85	0.85	0.70	0.55	0.45	0.45
C9	0.20	0.00	0.00	0.80	0.00	0.20	0.20	0.30	-	0.50	0.70	1.00	0.70	0.70	1.00	0.50	0.80	0.80
C10	0.60	0.50	0.50	0.80	0.50	0.60	0.20	0.30	0.50	-	0.00	0.50	0.70	0.70	0.50	0.50	0.40	0.40
K1	0.35	0.25	0.25	0.65	0.25	0.45	0.35	0.75	0.30	1.00	-	0.60	0.40	0.40	0.75	0.30	0.40	0.40
K2	0.25	0.15	0.15	0.75	0.35	0.20	0.25	0.15	0.00	0.50	0.40	-	0.70	0.70	0.85	0.45	0.60	0.60
K3	0.25	0.15	0.15	0.75	0.30	0.35	0.25	0.15	0.30	0.30	0.60	0.30	-	0.85	0.85	0.55	0.45	0.45
K4	0.40	0.30	0.30	0.40	0.50	0.35	0.40	0.15	0.30	0.30	0.60	0.30	0.15	-	0.70	0.75	0.45	0.45
K5	0.10	0.00	0.00	0.10	0.15	0.20	0.10	0.30	0.00	0.50	0.25	0.15	0.15	0.3	-	0.70	0.60	0.60
K6	0.40	0.55	0.55	0.60	0.75	0.35	0.60	0.45	0.50	0.50	0.70	0.55	0.45	0.25	0.30	-	0.65	0.65
K7	0.30	0.40	0.40	0.45	0.80	0.25	0.45	0.55	0.20	0.60	0.60	0.40	0.55	0.55	0.40	0.35	-	1.00
K8	0.30	0.40	0.40	0.45	0.80	0.25	0.45	0.55	0.20	0.60	0.60	0.40	0.55	0.55	0.40	0.35	0.00	-

C - Chennai K - Kochi

Table 10 Correlation values between four metric variables and tail weight

VARIABLES	TAIL WEIGHT	
	Males	Females
SSD	-0.01021038	0.00241461
SAD	0.49067448	0.43102502
PCL	0.08363850	0.28772450
CW	0.39556304	0.23359922

weighing of the live shrimps was rather difficult, while SAD can be measured easily and accurately. Hence, the present finding of the above correlation has its own significance.

2. Truss Network Analysis

A total of 1080 specimens collected from 6 stations (Mangalore, Calicut, Kochi, Mandapam, Chennai & Kakinada) during 1994-1997 were pooled and PC analysis was done after log transformation. The first principal component accounted for 86.54% and the second accounted for 2.60% (Table-11) of the total variations in the truss data. These two principal components accounted for 89.14% of the variation. To check the existence of any clustering the PC-I scores were plotted against PC-II scores for samples from the 6 locations (Fig.6). There was no specific clustering of samples from any of the stations indicating that there is no significant difference in morphometric characters between the populations. The six populations compared are morphometrically homogeneous.

Table 11 Principal component analysis

PC #	Eigen Value	Percentage	Cum. Percentage
1	740.3977	86.54	86.54
2	22.2554	2.60	89.14
3	17.3617	2.03	91.17
4	11.0282	1.29	92.46
5	8.9284	1.04	93.50
6	8.7742	1.03	94.53
7	7.6321	0.89	95.42
8	7.2263	0.84	96.26
9	5.4942	0.64	96.61
10	4.5514	0.53	97.44
11	3.9971	0.47	97.90
12	3.3299	0.39	98.29
13	3.1265	0.37	98.66
14	2.7844	0.33	98.98
15	1.6705	0.20	99.18
16	1.5816	0.18	99.36
17	1.3690	0.16	99.52
18	1.2253	0.14	99.67
19	1.1111	0.13	99.80
20	0.9133	0.11	99.90
21	0.6005	0.07	99.97
22	0.1552	0.02	99.99
23	0.0316	0.00	100.00
24	0.0132	0.00	100.00
25	0.0107	0.00	100.00
26	0.0086	0.00	100.00

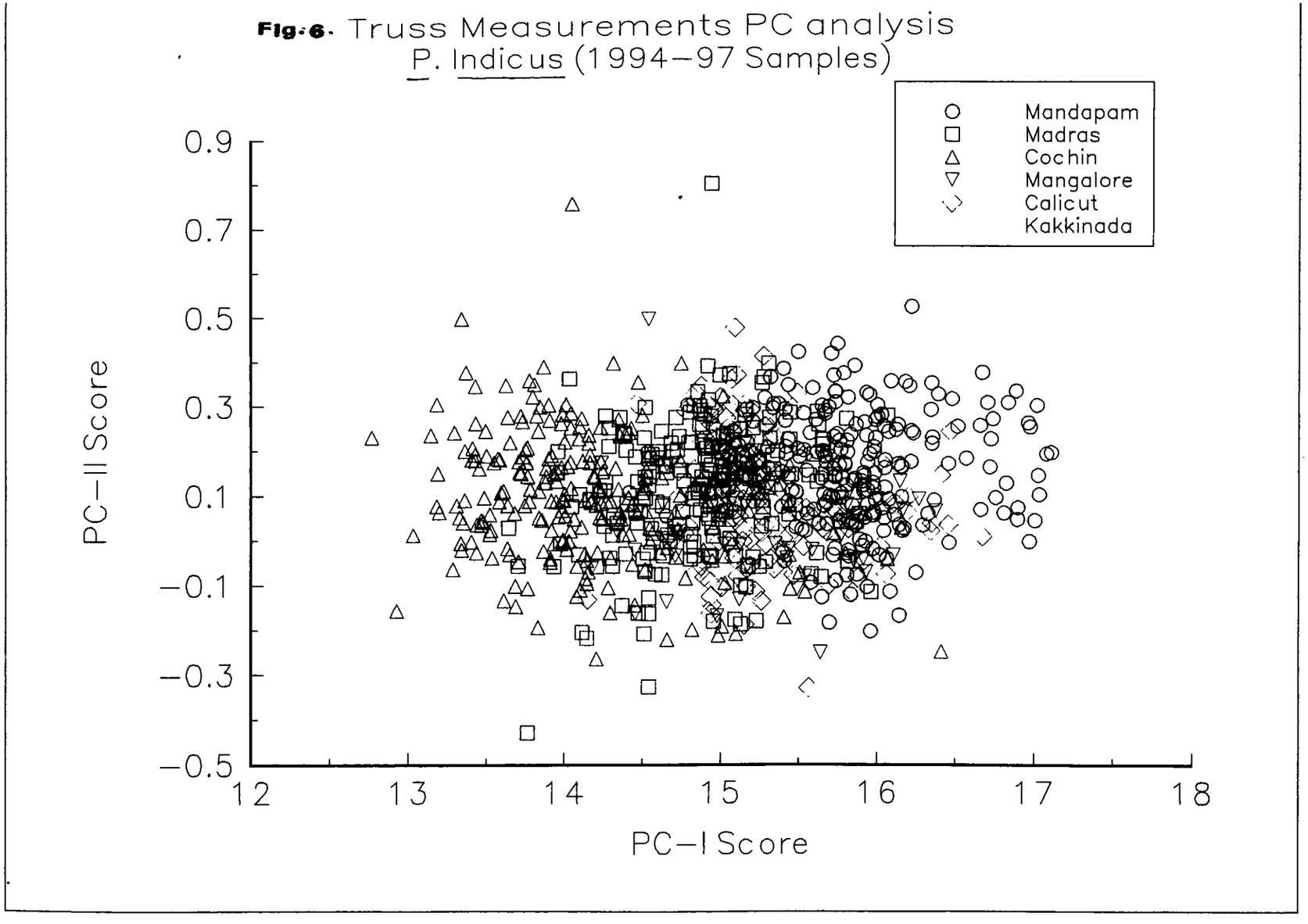
Fig.6. Truss Measurements PC analysis
P. Indicus (1994–97 Samples)
Mandapam
Madras
Cochin
Mangalore
Calicut
Kakkinada
0.9
0.7
0.5
0.3
0.1
−0.1
−0.3
−0.5
PC–II Score
12
13
14
15
16
17
18
PC–I Score

Discussion

The scientific exploitation and conservation of commercially important fish and shellfish resources is of fundamental importance to any nation. Naturally a basic question arises: what is the natural unit of the resources that is to be recognised, managed and conserved so as to enable it to replenish while it is continuously exploited? An accurate answer to that question is also of basic importance for planning successful aquaculture breeding programmes (Bye, 1983; Lester & Pante, 1992). Sincere efforts to answer the above question have been initiated many decades ago (Schmidt, 1909). However, different forms of scientific definitions for the units of the resources to be managed and conserved were well established during some recent international symposia held on the topic (deLigny, 1972). The major themes discussed in these two symposia were concerning the units of fishery resources to be managed/conserved and various methods for its identification.

A significant outcome of these two symposia was that the ultimate unit of populations of fishery resources to be conserved and exploited, is the one that is genetically discrete. The basic and significant role of population genetics in the concept of unit stock structure of fishery resources, was high lighted by Altukhov (1981), Allendorf and Phelps (1981) and Mclean and Evans (1981). Genetically, it may be defined as a discrete population which has its own gene pool significantly different from that of other populations (Moller, 1971; Jameison, 1974; Mangaly, 1974; Shaklee, 1990). An equally important outcome of these two symposia was the comparative evaluation of various techniques/procedures available for the identification of the units of fishery resources. Various available techniques and their merits were discussed and evaluated in the second symposium (Ihssen *et al.*, 1981a). Until recently, electrophoretic analysis of individual gene controlled proteins/ enzymes was considered as the best method to obtain the basic genetic data to study the population genetic structure (Altukhov, 1981).

More innovative techniques for its detection have been introduced in addition to the popular morphometric and biochemical genetic methods. The

recent innovative methods that are being applied to detect hitherto unknown subdivisions in the genetic structure of the populations are those, which reveal differences in the structure of mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) of the individuals in a population (Avise *et al.*, 1979; Ovenden, 1990; Chapco *et al.*, 1992).

In view of the significance of stock concept, its definition and the methods now available for its detection, an investigation was undertaken to critically evaluate the two opposing hypotheses on the stock structure of the commercially important marine penaeid prawn, *Penaeus indicus* which has been heavily exploited from south-east and south-west coastal waters of India. Manisseri & Manimaran (1981) have reported that the south-east and south-west populations maintain their separate status as shown by the size composition and abundance between Tuticorin fishery and Manappad fishery. According to them the seasonal fishery off Manappad was supported by the prawns from the west coast during the monsoon period when the phenomenon called upwelling occurred along the south-west coast (Ramamirtham & Jayaraman, 1960). They have ruled out the possibilities of recruitment from the northern side stating that the prawn fishery of areas further north, like Erwadi and Mandapam, was mostly contributed by *P.semisulcatus* (C.M.F.R.I, 1975). Though the above cited work was based on the landings, it was empirically proved a year later by the mark-recapture investigations done under the National Tagging Programme (Mar.Fish.Infor. Serv. T&E Ser., No. 45: 1982). The tagged prawns were recaptured from Ovari and Manappad in Tinnelvelli coast 330 and 380 km, respectively, away from Kochi. Significantly, there had been no recovery from north of Manappad, which seemed to be the end point of the migration of *P.indicus* from Kochi. This was in agreement with the above-cited studies on the fishery statistics along the south-east and south-west coasts. Further, in the stock assessment studies of the *Penaeus* spp. of the east coast, Rao *et al.*, (1993) considered the east coast stocks of the three species, viz., *P.indicus*, *P.semisulcatus* and *P. monodon* as separate from the west coast stocks. However, based on the electrophoretic studies, Philip Samuel (1987)

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proposed a unit stock structure for its populations from east and west coasts. The topic that is discussed here is the results of the present investigation. The significance of the results discussed here is that these were produced by three independent, but complementary methods, which were used to test the two hypotheses on the stock structure of the species. The three methods applied in the investigation were, the traditional morphometrics, the currently popular biochemical genetics and the more innovative, molecular genetic techniques.

A review of literature on various aspects of the stock concept and different methods/techniques applied to detect the stock differences shows that the first objective of the investigations on stock structure is to discover and measure intraspecies variability inherent among the populations of a species. Then the different sets of data thus collected are compared between populations with the view of measuring the level of statistical significance of the differences between any two populations tested (Ihssen, *et al.*, 1981b; Ayala & Kiger, 1980).

The intraspecies variability discussed here is that of the population samples of *P.indicus* from Mangalore, Calicut, Kochi (West coast) and Mandapam, Chennai and Kakinada (East coast). The truss network analysis of the body forms of 1080 specimens from these six locations, revealed the morphometric variability in the species. The final results of the principal component analysis of the truss data are graphically presented in figure-6. The profile of the graph is free of any specific cluster formations. It means that the measured body shape variability differences among the six populations are not significant. In other words, the sample populations of *P. indicus* from Mangalore, Calicut, Kochi, Mandapam, Chennai and Kakinada are morphologically homogeneous and therefore, the populations of *P.indicus* compared belong to a single morphological stock. It is also reported that the method of truss network measurements is superior to other morphometric methods for stock differentiation or brood stock selections (Humphries *et al.*, 1981; Strauss and Bookstein, 1982; Lester, 1983; Lester and Pante, 1992).

Thus, truss network analysis differentiated morphologically differentiated stocks in Chinook salmon (Winans, 1984) and blunt snout bream (Li, *et al.*, 1993). Does it mean that morphological homogeneity or heterogeneity can be equated with genetic homogeneity or heterogeneity respectively? It need not be, as the lack of morphometric differences may be due to the process of stabilising selection (Lester and Pante, 1992).

Moreover, like any other method to detect the morphometric parameters, namely, the body size used for the truss network analysis, is also subject to non-genetic influences (Clayton, 1981; Chow & Sandifer, 1992; Pante *et al.*, 1988; Lester & Pante, 1992). Therefore, any detected stock homogeneity or heterogeneity based on truss network data may not be, fully correlated to genetic homogeneity or heterogeneity (Clayton, 1981). In other words, though the morphological characteristics are also products of polygenes (Ayala & Kiger, 1980), the present finding of morphometric homogeneity in *P.indicus*, need not be fully due to lack of any genetic stock differences. Besides, earlier studies on the size composition/abundance of its populations (Manisseri & Manimaran, 1981), stock assessment studies (Rao *et al.*, 1993) and tagging experiments (Mar. fish. Info. Ser. T.&E Ser. no. 45: 1982) have suggested that its east and west coast populations have characteristics of two separate stocks. In an earlier study (Philip Samuel, 1987), a comparison of 11 length parameters in *P.indicus* showed that 9 of them differed significantly between Kochi and Madras and 8 of them differed significantly between Tuticorin and Madras, all at 5% level. The unsuitability of simple length measurements of different body parts, particularly of soft bodied animals like prawns, for stock differentiation studies was explained by Strauss & Bookstein(1982). However, the results of the present truss network analysis also supported the hypothesis that population of *P.indicus* belong to a single morphological stock irrespective of its east-west coastal distributions. Logically, the present finding of the morphometric homogeneity among *P.indicus* populations have to be tested using alternative methods before such a conclusion is finally accepted.

Before discussing the results of the genetic methods, it is important to report that there exists a significant morphological correlation with the tail weight in *P.indicus*. Lester(1983) has emphasised the importance of morphological variables that can be used as indicators for the estimation of tail weight in penaeids. It is why because; the weighing of the live specimens was quite difficult and always erroneous. Hence, he correlated different variables with the tail weight and found that SSD (sixth segment depth) and PCL (partial carapace length) were the estimators of the tail weight in *P.vannamei* and *P.stylirostris* respectively. In the present study on *P.indicus*, only four, out of the nine metric variables were used for the regression analysis. Others were excluded as they involved the extremities of the specimens. Among these, the second abdominal segment depth (SAD) was found to be having the highest correlation with the tail weight (Table-10). Though the morphological similarity was very high among the penaeids, the variables that can give highest correlation with the tail weight, were different in various species. Goswami *et al.*(1986), reported partial carapace length (PCL), exopod of uropod length (EUL) and posterior abdominal circumference (PAC) as giving accurate estimate of the tail weight in *P.merguiensis* (de Man, 1888). Lester (1983) opined that a comprehensive study with larger and more varied samples could prove whether these inter specific differences were simply a function of the data set or not. Any how, the highest correlation of SAD to tail weight in *P.indicus* found out in the present investigation can now be used as an indicator of the tail weight, which is a prominent commercial factor for the brood stock selection. It is easily measurable and the animals do not have to be sacrificed. This is for the first time such a correlation was found out in *P.indicus* of India.

Now let us evaluate the present results of the biochemical genetic studies of the species, *P.indicus*. The allozyme loci examined and electrophoretic method adopted in the present investigation is almost totally different from that of Phillip Samuel (1987). Hence, the results of the biochemical genetic

studies discussed here are significantly different from that of Phillip Samuel (1987). For example, except three of the 14 loci examined in the present investigation were different from that of Philip Samuel (1987). Consequently, the present investigation gives additional information on 11 more loci of the species. Besides, flat bed gel electrophoretic methods were applied in the present study, reducing the experimental and personal errors to the minimum, while disc gel electrophoretic method which consisted of independent tubular gels was used by Philip Samuel(1987). In other words, the chances of mistyping of electrophoretic fractions on a single flat gel are much less than that of independent tubular gels.

The measurement of natural genetic variability is the first step in the study of population genetics, especially in the differentiation of genetically discrete stocks. The estimated values for average number of alleles (Na), effective number of alleles (Ne) , percentage of polymorpic loci and above all, average heterozygosity (H) for the populations of a species are considered as indicators of the actual level of genetic variability in that species. The statistically significant differences in these values, particularly in heterozygosities and allele frequencies between any two populations of the species are evidences of their reproductive isolation. In other words, the two populations belong to genetically different stocks which do not interbreed (Allendorf, 1979; Ayala & Kiger, 1980; Bye, 1983; Altukhov, 1981).

In the present investigation, the estimated, comparatively high values of Na, Ne, and H in the populations of *P. indicus* suggest that the species has high genetic variability (Table-4). A detailed critical discussion on these points may help to evaluate the merits of the genetic variability in the species. The expected heterozygosity values for the seven populations tested here varied from 5% to 14% and the observed values for the same were between 6% and 15%. The expected and observed average heterozygosity values estimated for the species were 9% and 10% respectively. Generally, the penaeids show

very low heterozygosity values. Nelson & Hedgecock (1980), after studying a wide range of decapods, showed that the range of heterozygosity values for the decapods were between 0.8% and 12.5%. The works of Lester (1979), Mulley & Latter (1980) and Sbordoni *et al.* (1986), have specifically shown the heterozygosity values for the penaeids as 0.6% to 9%. The expected and observed heterozygosity values for the species *P.indicus* examined here also were within the above range. But some of the heterozygosity values at the populational level were higher. In the west coast, Kochi(12%/15%) and Mandapam(12%/15%) and Chennai (14%/12%) in the east coast, were found to be exceeding the range. Among these, the expected values for Kochi as well as Mandapam and the observed value for Chennai also were still within the range as reported by Nelson and Hedgecock (1980) for the species *Emertia anologa* (12.5%) and De Mathaeis *et al.*(1983) for the species, *P.japonicus* (12%/11.8%). But the observed value for Kochi and Mandapam(15% each) and the expected value for Chennai (14%) were higher than that reported for the decapods in general. This could be due to the high heterozygosity values at the individual loci *AK-2**, *AO-2**, *αGPDH-2** and *HK-2**(9.5%-73.91%). May be the species is highly heterozygous for these enzymes.

Thus, the type of enzymes selected for the analysis and the number of loci detected influence the heterozygosity values. In order to avoid a serious error in the estimation of average heterozygosity, a large number and wide range of isozyme loci should be examined. All the other works, where the heterozygosity values were reported as very low, have screened more than 20 loci among which the polymorphic loci were less in number (De Mathaeis *et al.*, 1983; Sunden & Davis, 1991; Benzie *et al.*, 1992). In the present investigation, only nine enzymes were selected which revealed fourteen loci, out of which six were polymorphic. There were reasons for selecting these enzymes. First of all, these were already found to be potentially polymorphic either in prawn species or in other crustaceans. The second reason has something to do with the objective of the present investigation, namely, detection

of as many polymorphic loci as possible, as the genetic markers for stock differentiation. The heterozygosity is mainly helpful to determine the degree of genetic variability within the species. Testing a larger number of enzymes in *P.indicus* may bring down the heterozygosity reported here. Interestingly, Philip Samuel(1987) has reported very low range of heterozygosity values (0.96% to 1.42%) for *P.indicus* from Kochi, Tuticorin, Madras and Waltair, The reasons for his reporting of much lower heterozygosity values in the above populations of *P.indicus*, which were also sampled for the present investigation, were many. The major reason was that he counted the loci from all the three different tissues. Thus the total number of loci for his heterozygosity calculations was twenty five of which only eight was polymorphic, whereas in the present study only the loci from muscle tissue were considered for the estimation of heterozygosity. The second reason was that majority of the enzymes tested in the two investigations were different. Thirdly, the electrophoretic techniques that were used by these two studies totally differed. All these differences in the two approaches naturally produced differences in the estimation of the heterozygosity values of the species.

It is interesting to note that the east coast populations of *P.indicus* were showing higher values of expected /observed heterozygosities than that of the west coast populations, as in the case of average number of alleles. This regional difference in heterozygosities and average number of alleles may imply that the populations of both the coasts keep separate profiles of genetic variability.

In order to test the above reasoning, the standardised allelic variance, F_{ST}, was estimated (Weir& Cockerham, 1984) at each polymorphic locus. But there were no coast specific differentiations in these values. Only twenty one percent of the F_{ST} values revealed great levels of genetic differentiation between any two populations irrespective of their coast-wise origin. Of these more than fifty percent of the significant values were due to a single locus, the *AO-2**. The allozyme, aldehyde oxidase belongs to the Group-II enzymes (enzymes

involved in the non-metabolic pathway) that act on externally derived substrates. This enzyme may probably have differential activity correlated to some other unknown factors. Such a possible correlation can produce differences in the estimated F_{ST} values. Smith (1979) has illustrated such a relationship between the esterase gene frequencies and temperature, in the New Zealand snapper, *Chrysophrys auratus*. He observed a decrease in the frequency of the Est-4^2 allele with the increase in temperature. A comparison of alleles and genotype frequencies between pairs of year classes showed differences between the warmest and coldest years. From the vast pool of Snapper larvae spawned each year, there was an apparent selection in cold years for those individuals carrying the Est-4^2 allele and in warm years for fishes carrying the Est-4^3 allele, suggesting differential mortality of larval genotypes. He has explained that the selective agent maintaining the Est-4 polymorphism might not be temperature, but rather an associated physical parameter such as salinity or pH, or even a biological variable such as food type available at the onset of larval feeding. Here it should be remembered that like aldehyde oxidase, esterases also act on externally derived substrates. He has observed that the west coast snapper from cooler waters has a lower Est-4^2 frequency than fish from warmer waters on the east coast. He considered this as an indication of different selective pressures and responses between east and west coast snapper.

As in the above case, some unknown factor may have got an influence over the enzyme AO of *P.indicus* and the alleles *AO-2*71* and *AO-2*100*. In the west coast the allele *AO-2*71* was having lower frequencies (0.05,0.00& 0.13) except in Neendakara(0.29). But in Neendakara the fishery was seasonal and the specimens were collected from the mudbank during the monsoon season. As mentioned earlier, the prawns move in the southern direction during the south-west monsoon in order to escape from the sudden physico-chemical changes due to up welling. In that context, with all the probability the migrating individuals might have been supporting the fishery at Neendakara. Unlike the other populations, here the number of heterozygotes

was in excess. In spite of that, the population was in Hardy-Weinberg Equilibrium. On the contrary, all the east coast populations showed higher frequencies of *AO-2*71* (0.13,0.32 & 0.26). Nelson & Hedgecock (1980) also had shown that the ecological parameters did influence gene frequencies and heterozygosity values. According to them, the factors like latitude and productivity have got a positive correlation with heterozygosity. A well planned critical study of the relationship between the enzyme AO and the ecological as well as biological parameters only can reveal the specific parameter that influences the gene frequencies at the *AO-2** locus in *P.indicus*.

Another non-metabolising enzyme, ODH acting on externally derived substrates was also observed to be showing high F_{ST} values indicating great levels of differentiation between Chowghat/Kochi and Chowghat/Chennai. But the allelic frequencies did not show many differences between the populations. Three alleles were controlling this locus. The east coast population, Kakinada expressed all the three alleles at *ODH-1* (100,81 &119)*. In Calicut, Neendakara and Chennai, the dominant slow allele, *ODH-1*100* was cent percent. The fast allele *ODH-1*119* was present in Chowghat, Mandapam and Kakinada (Table-5). A major reason for the high F_{ST} values between Chowghat and Kochi and between Chowghat and Chennai should be the small sample size from Chowghat (Table-7). The other enzyme that showed significant F_{ST} values was αGPDH. But this was only due to the differences seen in the frequency of the allele *αGPDH-2*52* at Mandapam. Obviously, the significant F_{ST} values were between the comparisons wherever Mandapam was involved (Table-7).

None of the other loci gave significant F_{ST} values for the sites compared. This indicates the absence of any sub-structuring within the species. Thus the F_{ST} differences shown were only of minor nature and due to either small sample size or loci that has apparent correlation with external factors. Nevertheless, as pointed out by Wright(1969), "a rather small value of F may be associated with a very considerable amount of differentiation among the sub-populations".

This has to be considered while drawing conclusions about the population structure of a species by interpreting the allele frequencies.

Allele frequencies at several loci in the sample populations are vital data for getting a closer view of the present genetic stock structure of the species, *P.indicus*. Genetically differentiated stocks are expected to possess significantly different allele frequencies at one or more loci in space and time (Allendorf & Phelps, 1981; Altukhov, 1981).

Interestingly, the overall allele frequencies estimated at 14 isozyme loci belonging to nine enzymes were not significantly different in seven population samples of *P.indicus* tested from east and west coasts of South India. Besides, an estimation of Nei's Genetic Identity and Distance (1972) using these allele frequencies was also made. The values ranged between 0.97865(Kochi/ Mandapam) and 0.99986(Neendakara/Chennai). None of the identity (I) or distance (D) values between any two populations as well as east and west coasts were significant (Table-8). Thus the frequencies of the major allele detected in the present investigation also support the hypothesis of Philip Samuel (1987) that populations of *P.indicus* have a homogeneous structure.

Naturally, another question arises that whether these values of genetic identity/distance, based on allele frequencies at a few protein loci, do really reveal basic molecular structural differences between any two populations or regions? They may not, because of some important reasons as explained by others. First of all, the allele frequencies involve only the structural proteins, which comprise approximately 1% of the total genome of an individual (Bye, 1983). The second reason is related to selection pressure that may hide the inherent genetic stock differences. Shaklee (1984) did not consider the observed biochemical uniformity of *S.fasciolatus* through out the Hawaiian Archipelago as an evidence for the existence of a single panmictic population. According to him, it was rather an evidence for a constant selection pressure regime through out the region, which prevented genetic differentiation. In general,

majority of the structural protein loci is conserved. As already opined by Lester & Pante (1992), in the case of morphological similarity, such evolutionary conservatism can not be associated with a lack of genetic variation. Probably, it could be the result of stabilising selection. Spieth (1974) has explained that migration at the rate of one migrant per loci per generation is generally sufficient to obscure any disruptive effects of drift. But according to Allendorf & Phelps (1981), though one migrant is sufficient to ensure that the same alleles are shared over long periods of evolutionary time, one migrant is not sufficient to maintain identical allelic frequencies between the populations. As described by Allendorf & Phelps (1981), the basic 'rule of thumb' was that very little genetic exchange between populations would prevent divergence in the absence of natural
variation .

Sevigny *et al.*,(1989) considered the significant differences among species, in degrees of heterozygosity and in frequencies of common alleles, even among sympatric species along with the presence of private alleles, as the consequences of barriers to gene flow among the morphological species. In *P.indicus*, most of the allele distributions were more or less identical in different areas, suggesting that there are no barriers to gene flow. But Marvin *et al.*(1977), argued that such a situation was not indicating the strong gene flow through out the areas, because small gene flow with large populations and an absence of strong selection pressure could maintain the allele frequencies at the same levels. Conversely, selective forces could be acting to maintain gene frequencies at the same levels in the absence of interchange between two populations.

On the contrary, there are many reports of significant stock differences detected by the biochemical genetic methods in fishers and shellfishes. Distinct stocks were detected in the Pacific hake, *Merluccius productus* (Utter 1969), Atlantic cod (Sick, 1965), lake white fish (Ihssen *et al.*, 1981b) , Chum

salmon and redfish (Altukhov,1981), Indian oil sardine *Sardinella longiceps* (Venkitakrishnan, 1993), Grey Mullet, *Mugil cephalus* (Vijayakumar,1992). The examples of reports on biochemical genetic stock differences in shellfishes are *Penaeus stylirostris* & *Penaeus indicus* from the coasts of Philippines & Kenya (Lioe, 1984) as cited by Lester & Pante (1992) and *Metapenaeus dobsoni* of Indian coastal waters (Santh Begum , 1995).

The above reports of genetic homogeneity in some species while heterogeneity in some other species detected by biochemical genetic techniques prove that the methods are definitely efficient for genetic stock differentiation. However, the success of detection of naturally existing discrete stocks may depend on the screening of large number of loci so as to discover a few loci which are specifically potential genetic markers for genetic stock differentiation in a particular species. As thousands of gene loci are present in any organism (Ayala & Kiger, 1980), one has to correctly detect those loci which are polymorphic as well as heterogenic with reference to its allele frequencies for detecting stock differences in a particular species.

The above reasoning on the efficiency of the biochemical genetic technique in the detection of the genetic stock differences implies that the present conclusion of biochemical homogeneity in the populations of *P.indicus* based on just fourteen loci need not be the final right conclusion on the population genetic structure of the species. Because the present, very interesting finding of the two rare alleles designated, *AK-1*106* and *HK-1*104* only in the east coast populations may suggest that an inherent genetic stock difference exists between east and west coast populations of *P.indicus*. These two rare alleles were observed in very low frequencies in Mandapam and Chennai alone. A phenomenon of persistent occurrence of rare alleles only in some loci in some populations and its absence in other populations may be considered as strong signs of genetic stock heterogeneity in the populations (Jameison, 1974). However, the present proposition of genetic stock differences between the east and west coast populations of *P.indicus*, based on the rare

alleles, *AK-1*106* and *HK-1*104*, are to be confirmed by screening for them in larger population samples of the species from both the coasts. Because, the faster rare alleles at the loci *AK-1*106* and *HK-1*104* were observed only in the east coast populations, Mandapam and Chennai, in very low frequencies (Table-5, Fig.4 & 5). The allele *AK-1*106* was present only in 2% individuals of Mandapam and 1% individuals of Chennai. Unfortunately, only the Kochi population could be tested for AK in the west where the allele was not observed. The absence of the *AK-1*106* allele in Kochi could not be due to sample size (46) which was larger than that of Chennai (36). It may be really absent in Kochi. Likewise, in the east coast, AK was not screened in the Kakinada population. The *HK-1*104* allele was the other rare allele which was present in 4% individuals of the Chennai population, whereas the Chowghat and Kochi populations were devoid of this allele. On the contrary, the Mandapam population in the east coast too did not express this allele. The probable reason for its absence in Chowghat, Kochi and Mandapam may be due to smaller sample size in these centres. HK also could not be tested in the Kakinada population. Interestingly, number of average alleles per locus (Na) was also comparatively higher in the east coast populations, as expected because of the rare alleles additionally present there. The Na values ranged between the coasts support the earlier assumptions made by Manisseri & Manimaran (1981), the mark-recapture investigations by the NTP (1982) and the stock assessment studies by Rao *et al.*(1993), that the east and west coast populations of *P.indicus* are heterogeneous stocks. According to them, Manappad is the northern limit up to which *P.indicus* dominates in the landings. Even if the planktonic larvae migrate northerly beyond Manappad, they may not be contributing to the gene pool of the east coast populations. Beyond Manappad the bottom is rockier which is not preferred by *P.indicus*. So it has to be assumed that the south east coast, beyond Manappad, is maintained as a separate stock from the west coast.

But such a stock difference was not indicated by overall allele frequencies, as discussed earlier. However, a close examination of the

individual loci revealed that the frequencies at the *αGPDH** locus were considerably high in Mandapm population. In all the populations, excluding Mandapam, the frequencies of the most common allele at the locus, *αGPDH-2*100* ranged from 0.64 to 0.80, whereas it was only 0.37 in Mandapam, while, *αGPDH-2*52* dominated here with a frequency of 0.63. Besides, the observed-expected genotype frequencies were significantly different at the *αGPDH-2** locus in Mandapam alone. The difference cannot be due to any errors in genotyping, because, the values were normal in other populations. Another possible reason can be the sudden disturbances in the gene frequencies due to physical mixing of genetically different populations. But the earlier reports on mark-recapture investigations do not support this view of mixing. The landings data and stock assessment studies also reached the conclusion that the east and west coast populations of *P.indicus*, were maintained as separate stocks. Above all, if mixing had occurred, the other two populations of the east coast, Chennai and Kakinada also would have displayed such differences in the allele frequencies.

Consequently, the allele and genotype frequencies were re-examined sex-wise so as to find out whether the values were sex dependent. Very surprisingly, the genotypic frequencies differed between males and females. It was quite interesting to note that when majority (70%) of the females in the total samples, were heterozygotes (*αGPDH-2*52/100*), only 19 of the males were heterozygotes. Among the rest of the males, 72% were fast homozygotes (*αGPDH-2*100/100*) and 9% were slow homozygotes (*αGPDH-2*52/52*). Among the females 8% were slow homozygotes (*αGPDH-2*52/52*) while the remaining 22% were fast homozygotes (*αGPDH-2*100/100*).

None of the other enzymes tested had shown such an unexpected difference. Hence, the question is, what could be the possible reasons for the observed differences at the *αGPDH-2** locus in Mandapam alone? The enzyme αGPDH belongs to the class of Group-I enzymes (Gillespie & Langely, 1974) involved in the metabolic pathway. The food consumption of the females of

the species is comparatively more than the males at the stage of maturity and immediately after spawning. The adult mature females are larger than the males of the same age class. Size and weight are two important factors that can influence the process of maturation. Moreover, the fecundity of each female is directly proportional to the length and weight of that individual. The heterozygosity values at the locus *αGPDH-2** were also higher in the females of *P.indicus* from Mandapam. What could be the possible explanation for the higher heterozygosities in the females of Manadapam?

Various studies conducted by others have suspected a relationship between the biochemical polymorphism and variations in the physiological state of the aquatic animals or their habitat. A review on such works by Ponniah(1989) shows some interesting aspects of the phenomenon. Johnson *et al.*(1970), reported an abyssal population of the fish, *Sebastodes alutus* as having excess heterozygosity when compared with its shallow water population, at the loci Pgm and glycerophosphate dehydrogenase. Another work demonstrated the differential in catalytic efficiency of the two alleles of the enzyme Lap, in different habitats. In oceanic waters the frequency of the allele Lap94 was higher than the frequency of the same in estuarine waters. Some other works showed a positive correlation between heterozygosity and shell length in bivalves. The body weight of each individual *C.virginica* had been positively correlated with average heterozygosity. The faster growth rate of the triploid oysters, in comparison with the diploids, had been attributed to the increased heterozygosity of the triploids (Stanley *et al.*, 1984). The physiological mechanism responsible for the higher growth rate of the heterozygotes was also established. In adult *M.edulis* when gamete production exceeded the somatic growth, a positive correlation between heterozygosity and fecundity was seen, which was absent in younger individuals (Rodhouse *et al.*, 1986).

In the present investigation a positive correlation between heterozygosity at *αGPDH** locus and larger size/high fecundity rate in females is suspected.

This hypothesis has to be tested by detailed breeding experiments to prove the correlation between the enzyme αGPDH and factors like size, weight and fecundity. It has to be checked whether the individual alleles of αGPDH has got any specific activity with respect to the physiological mechanisms at various stages of maturity. Interestingly, the Mandapam population of *P.indicus* had the largest sized females with ripe ovaries and all these females were heterozygotes at the *αGPDH-2** locus. Fast homozygotes were totally absent in the Mandapam population and only 5 individuals, out of 20, were slow homozygotes. The rest were all heterozygotes. Thus the higher heterozygosity and the absence of homozygotes, naturally, caused the significant deviation in the chi-square values at the locus *αGPDH-2** as well as higher frequency for the slow allele in the Mandapam sample (Table-5 & 6). On the contrary, no correlation between αGPDH phenotypes and sex or maturity stages was found in Pacific Ocean perch *Sebastodes alutus* (Johnson *et al.*, 1970). A detailed study of various enzyme polymorphisms and adaptive strategies in decapods (Nelson & Hedgecock, 1980) found out positive or negative correlation between heterozygosities in Group I and Group II enzymes and wide ranging niche or organismic conditions. This phenomenon of adaptive strategies in heterozygosities appears to be more prominent in invertebrates. The enzymes PGI, PGM (Group I), and AO, EST (Group II) were more vulnerable to the adaptive strategies in decapods.

The apparent lowest frequency of the allele *AO-2*71* in Chowghat and its higher frequency in Chennai (0.32) and that of similar conditions for *HK-2*53* allele in some regions and Kochi must be the consequences of considerable differences in the sample size between the regions. Out of the 27 Chi-square tests only two showed significant deviations at $P<0.05$. This is not unusual. Ihssen *et al.*(1981b), has stated that the magnitude of the two deviations found is expected at 5% level for 25 independent tests. Hence, the *P.indicus* populations can be considered as panmictic in terms of isozyme allele frequencies.

The above elaborate discussion on the present findings of biochemical genetic homogeneity among the populations of *P.indicus* based on allelic frequencies at protein loci alone, leads to some important conclusions: (1) statistically significant differences detected in the allele frequencies among populations of a species are to be considered as positive proofs for the existence of genetically different stocks within that species; (2) on the contrary, statistically insignificant differences detected in the allele frequencies among the populations of a species may not be positive proofs for genetic homogeneity unless it is also proved by some alternative and more efficient methods at the molecular genetic level. It is because; the electrophoretic technique has its own limitations. Natural protein variations due to insignificant molecular weight differences cannot be detected by ordinary gel electrophoresis. It is estimated that only less than 25% of amino acid substitutions are detectable by conventional gel electrophoresis (Bye 1983; Powers, 1993). Many genetic variants, which are potential markers for stock differentiation, are likely to be missed by electrophoretic techniques. Therefore, a preliminary attempt was made to crosscheck the present findings of morphometric and biochemical genetic homogeneity in the populations of *P.indicus* by more efficient techniques. The rechecking was carried out using techniques for the analyses of mitochondrial and nuclear DNA variability within the species. The results of the restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) analyses of selected samples of *P.indicus*, are very promising.

Due to technical constraints, RFLP studies could not be completed. However, the results obtained are informative. The first informative aspect of the mt DNA study is that ovary was found as the best tissue that gave good yields when compared with muscle tissue. The size of the mt DNA of the species was ~21Kb. The experiments with five restriction enzymes helped to identify a potential restriction enzyme marker for mt DNA variability study in the species *P.indicus*. The five restriction enzymes used were Hind III, Pst I, EcoRI, BamH I and Kpn I. The number of fragments produced by these enzymes also varied in the three specimens tested. It was, one by Pst I, two each by

BamH I, EcoR I and Kpn I, whereas Hind III produced two to six fragments. Hence, Hind III can be considered as the most informative among the endonucleases used, for the purpose of discovering mt DNA diversity in the species *P.indicus*, as it could produce more than one haplotype. This basic information is essential and vital for planning a successful study on the genetic stock assessment using mt DNA as the source of genetic variability in a species (Ovenden, 1990). The example of recent reports on discovery of hitherto unknown genetic diversity and genetic stock discreteness based on the analysis of mt DNA in invertebrate and vertebrate species are as many as detailed in the review of literature. The examples of discovery of new genetic stocks or strains, as a result of mt DNA analysis of the genome in the penaeid species are, *P.monodon* and *P.japonicus* (Lioe, 1984; Stott *et al.*, 1992; Benzie, 1993). Now, considering the high efficiency of the mt DNA techniques in detecting and identifying genetically different stocks within the penaeid species, it is essential to conduct an elaborate investigation on the mt DNA diversity among the *P.indicus* populations of India. In this respect it is hoped that the basic information reported in this study, about the successful method of isolation of mt DNA from *P.indicus* and the identification of Hind III as a potential restriction enzyme marker, could be a useful contribution.

But the most promising and significant information of the present investigation is the results from Random Amplified Polymorphic DNA (RAPD) analysis on the species. Due to many constraints like the cost of the chemicals and lack of special laboratory facilities, the number of RAPD analysis were restricted to two population samples, namely, Kochi and Chennai, which represented the west and east coast populations of the species respectively. Though three primers, AC12, AC13 and AC19 were used for RAPD, only the results of the amplifications with AC12 and AC19 could be used for the evaluation of the stock structure. These two primers produced polymorphic bands of varying lengths. However, only the operon AC19 produced significantly different number of bands in the Chennai and Kochi samples. Of

the seven fragments produced, the two bands of the sizes 3.5 Kb and 2 Kb were present only in Kochi population. Thus these two bands distinguished the Kochi population from the Chennai population of *P.indicus*.

Like the rare isozyme alleles, which were described as specific to the east coast, the above said unique DNA bands are specific to the Kochi population (Plate-5). Moreover, the mean percent similarity in the DNA profile among the individuals of Chennai was 76%, whereas that of Kochi was only 66%. In other words, DNA variability was higher in Kochi than that of Chennai. This is contradictory to the conclusions made on the basis of heterozygosity, average number of alleles etc. That is, biochemical genetic variability is higher in Chennai than that of Kochi. A comparable situation was also reported in *P.vannamei* using 3 different molecular techniques (Garcia *et al.*, 1994). There, the population that showed a lower percentage of polymorphism using RAPD had a higher degree of variation for allozyme analysis. This apparent contradictory phenomenon may be the consequences of differential selection pressures for allozymes (Ben-Shlomo & Nevo, 1988). Thus the differential selection pressures may also substantiate the comparable differences observed between the levels of the allozyme variability and DNA variability, between the east and west coast populations of *P.indicus*.

In spite of the selection pressures at various levels of species organisation, the highest efficiency of the biochemical and molecular genetic techniques in differentiating hitherto unknown genetic stocks of penaeid species was well demonstrated by very recent population genetic studies. Benzie *et al.*(1992) could find significant allozyme frequency differences among Australian populations of *P.monodon* and later, he was able to find higher levels of population genetic differences by mt DNA analyses (Benzie *et al.*, 1993). By random amplified polymorphic DNA (RAPD) analyses, Garcia and Benzie(1995) proved that the penaeids were, actually, more highly structured than has been thought. The biochemical genetic studies by Lioe (1984) on

P.indicus from Philippines and Kenya have shown that these were genetically different stocks and were separated by a genetic distance of 0.193. An analysis of the population structure of *Portunus trituberculatus* in the coastal waters of Okayama prefecture by RFLPs in the whole region of mt DNA, could detect genetic heterogeneity among the populations even in a small area, which were previously reported as panmixial based on allozyme research (Imai *et al.*, 1999).

However, since the data of the present RAPD analysis are only limited, the genetic stock difference observed here between Kochi and Chennai does not conclusively support the earlier hypothesis of stock differences between east and west coast populations of *P.indicus* (Manisseri and Manimaran, 1981; Rao *et al.*, 1993). Due to a time bound work plan and certain constraints like, laboratory facilities, chemicals etc., only a single population from each of the coasts could be analysed by RAPD. Hence, it is advisable to plan a comprehensive study using a wide range of primers and population samples from both the coasts to confirm the present findings regarding the Kochi-Chennai stock difference as well as the genetic structure of the other populations that were not tested here. Besides, due to lack of time, the reproducibility of the amplification reactions could not be checked in the present investigation. This is another factor that demands further confirmation of the present hypothesis that the RAPD structure of *P.indicus* of east and west coasts of South India is heterogeneous.

5.1.Management implications

For the judicious exploitation of the aquatic resources, apt management strategies are highly needed. One of the management strategies, thus developed for the scientific management of these resources, was to identify the natural units of the fishery resources under exploitation(Altukhov, 1981). These units are otherwise known as 'stocks' which can be defined as "a panmictic population of related individuals within a single species that is genetically distinct from other such populations"(Shaklee *et al.*, 1990). This stock

concept demands genetic characterisation of the individuals of a species. If the population genetic structure of a species is known, the distribution of sub-populations in mixed fisheries also can be estimated easily. The genetic changes that occur due to differential harvests are also to be identified and regulated because of the drastic and long-term effect they may have on a population. Such regulations to protect the weaker populations, if there is any, can be made only if the distribution of sub-populations, stocks or strains of a species, is known accurately (Allendorf *et al.*, 1987). Here is the relevance of the results of the present investigation lies. Already there are reports based on morphological studies that the east and west coast populations of *P.indicus* are different(Manisseri and Manimaran, 1981; Rao *et al.*, 1993). The preliminary results of the molecular genetic analyses done in the present study also are indicating the existence of two stocks along the east and west coasts of South India. It may really be useful in designing a suitable management strategy to maintain the integrity and heterogeneity of these two separate stocks. But the fidelity of these results has to be confirmed by doing a detailed study encompassing tagging experiments, study of morphological as well as biological aspects, biochemical and molecular genetic analyses. This may definitely help to save the populations from the presently going on ruthless exploitation which may lead to the extinction of the *P.indicus* population. Such a study may also help in understanding the distribution of the stocks along the east and west coasts of South India.

Conclusion

In brief, the above discussion on the results of the population genetic studies on *P.indicus* leads to the following two major conclusions:

1. The morphometric as well as biochemical genetic results support the hypothesis that the east and west coast populations of *P.indicus* belong to homogeneous stock. However, the presence of the rare alleles, *AK-1*106* and *HK-1*104* in Chennai/Mandapam and their absence in populations of west coast suggests that the east and west coast populations of the species may be genetically heterogeneous stocks.

2. The significant differences in the number of DNA fragments (obtained from RAPD analysis) between Kochi and Chennai populations support the hypothesis that the east and west coast populations of *P.indicus* are genetically heterogeneous stocks.

Summary

The thesis contains the results of an investigation on the " Population Genetic Structure of the *Penaeus indicus* " from southeast and southwest coasts of India. The population genetic characteristics of the species were examined by three independent but complementary techniques, namely, morphometrics (truss network), biochemical genetics (isozyme electrophoresis) and molecular genetics (RFLP and RAPD). A statistical comparison of the morphometric data variability present in population samples from Mangalore, Calicut, Kochi, Mandapam, Chennai and Kakinada revealed morphologically homogeneous structure of the species. Among the seven length variables correlated to tail weight of the species, Second Abdominal segment Depth (SAD) has the highest correlation. The electrophoretic analysis of nine isozymes (AK, AO, FBALD, aGPDH, GPI, HK, LDH, ODH and PEP) showed all these isozymes except GPI, LDH and PEP as polymorphic in nature. A total of 14 loci, comprising six polymorphic and eight monomorphic loci, were used to estimate genetic variability and genetic stock differences in the species. The values of average number of alleles (1.14 to 1.46), effective number of alleles(1.07 to 1.26) and average values of heterozygosity (0.06 to 0.15) and percentage of polymorphic loci (39%) suggest that the species has comparatively high genetic variability. The frequency of heterozygotes at *aGPDH-2** locus is significantly different in mature females of the species. A correlation between aGPDH locus and larger size/high fecundity in females of the species is suspected. A statistical comparison of allele frequencies, heterozygosity values and genetic distances estimated from the six polymorpic loci in the sample populations from Calicut, Chowghat, Kochi, Neendakara, Mandapam, Chennai and Kakinada again revealed that these seven populations of the species tested are biochemically homogeneous. However, the presence of the rare alleles *AK-1*106* in Mandapam and Chennai and *HK-1*104* in Chennai and their absence in the west coast populations suggest that the east and west coast populations of the species may be genetically different. Due to certain constraints, the results obtained from the studies of restriction fragment length

polymorphism (RFLP) were limited. Though five restriction enzymes were tested to detect mt DNA variability in three specimens from Kochi, only Hind III was found as a potential marker to reveal the polymorphic fragments in the mt DNA. It produced six fragments in two of the specimens, while only two in the third specimen. The total length of mt DNA is around ~21Kb.The results of Random Amplified Polymorphic DNA (RAPD) analysis of one sample each from Kochi and Chennai were very promising and significant. Three primers, AC12, AC13 and AC19 were used to detect genetic variability in the DNA of the above samples. AC12 produced similar number of fragments in both populations, while the number of fragments produced by AC19 was 2 to 3 in Chennai and 2 to 5 in Kochi. The significant difference in the number of bands in these two sample populations strongly suggests that these two populations have considerably different population genetic structures. Thus, though the results of the present morphometric and biochemical genetic studies support the earlier hypothesis of Philip Samuel (1987) that the populations of *P. indicus* has a unit stock structure, the results of the present RAPD analysis of DNA support the opposing hypothesis of Manisseri & Manimaran (1981) & Rao *et al.*,(1993) which described the east and west coast populations of *P.indicus* as having distinct stock characteristics. Considering the proven merits of biochemical & molecular genetic techniques in revealing the genetic stock structure differences, as evidenced by the differences in presence/absence of rare alleles as well as the number of DNA fragments between Kochi and Chennai populations of *P.indicus*, it is recommended that a final detailed analysis of the genetic stock structure of the east and west coast populations of the species should be carried out before conclusively accepting the genetic stock discreteness of east and west coast populations of *P.indicus*, reported here.

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