GENETIC STUDIES OF THE MARINE PENAEID PRAWN *Penaeus monodon* Fabricius, 1798

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APRIL 2002

Dedicated To My Family

DECLARATION

I hereby declare that this thesis entitled **"Genetic studies of the marine penaeid prawn *Penaeus monodon* Fabricius, 1798"** has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

Kochi,
April, 2002.

VINCENT TERRENCE REBELLO

CERTIFICATE

Certified that the thesis entitled "Genetic studies of the marine penaeid prawn ***Penaeus monodon*** Fabricius, 1798" is a bonafide record of the work carried out by Mr. Vincent Terrence Rebello 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.

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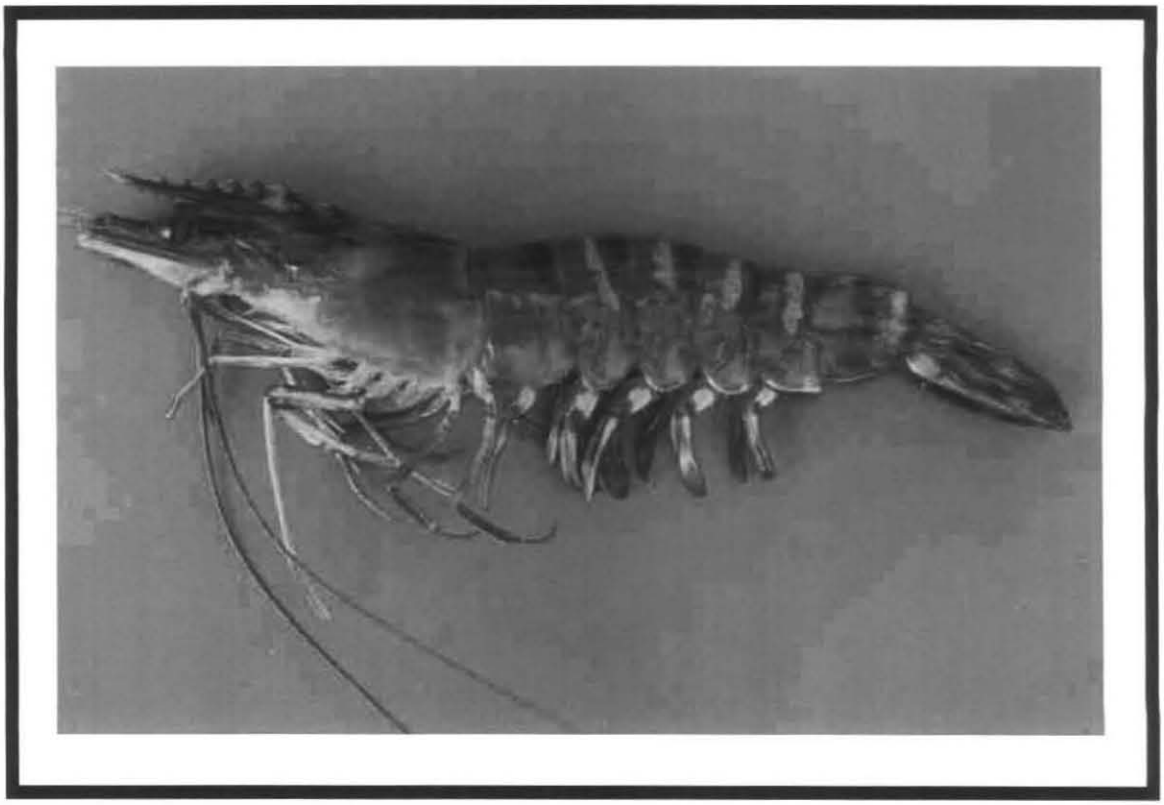
Introduction

1. INTRODUCTION

Sea fishes and shell fishes are a major source of cheap protein food for human consumption. Hence, from time immemorial, these fisheries resources have been subjected to worldwide commercial exploitation. Uncontrolled commercial exploitation of a resource may lead to its over exploitation or even its total loss as a fishery. To prevent over exploitation of some of these valuable sea fishery resources, many maritime nations have been forced to introduce fishing regulatory measures like, ban on trawling, reduction in fishing efforts, gear restrictions and seasonal restrictions on fishing of the species suspected as being overexploited or threatened or even endangered. Such restrictive management measures are essential not only for renewing the commercially over exploited fishery resources throughout its range of distribution but also for protection and conservation of a species or its populations with unique biological and genetic resource characteristics (Utter, 1981).

Taxonomically, the species concept has always remained as the largest unit of any fishery resources. The identification of a taxonomic species based on visible common morphological, anatomical and even biological characteristics is easier throughout its known areas of distribution. Now it is well known that a species may exist as geographically isolated populations or reproductively isolated stocks with their own fishery and biological characteristics. Hence, the ultimate success of any regulatory measures intended for protection against over exploitation and conservation of the basic fishery resources will depend on the identification of the natural units of each fishery resources and their protection and conservation to the desired extent and period of time. Nevertheless, defining and identifying distinct units of fisheries management remained as a problematic concept (Marr, 1957).



Penaeus monodon

The progressive refinement of the morphological, biochemical and molecular concept of the units of fishery management as well as the methods of their identification has further complicated the issue of conservation of the valuable fishery resources throughout the world (Booke, 1968, 1981; de Ligny, 1969, 1971; Busack *et al.*, 1980; Casselman *et al.*, 1981; Winans, 1984; Chow and Fujio, 1985; Allendorf *et al.*, 1987; Campton and Utter, 1987; Ovenden, 1990).

As a result, morphologically, biochemical genetically and even molecular genetically defined sub units (stocks) of fisheries management have been identified and reported within many fish and shellfish species of the maritime nations including that of India (Lester, 1980; Altukhov, 1981; Berglund and Lagercrantz, 1983; Berg and Gall, 1988; George, 1994; Santh Begum, 1995; Bindhu Paul, 2000). Such vital discoveries of the hitherto unknown subunits or stocks within a species should help in planning and formulating suitable management strategies for the scientific exploitation and conservation of valuable fishery as well as the genetic resources of any nation.

The fishery and genetic resources of India is enormously rich and diverse (Jhingran, 1984). Almost all these fishery resources are being exploited and managed under the traditional concept that each fishery is supported by wild populations having homogeneous characteristics. A typical example is the commercially very valuable penaeid prawn, *Penaeus monodon* Fabricius, 1798 of South India, popularly known as the jumbo tiger prawn. Its populations are caught from Karwar, Mangalore, Calicut, Kochi (West coast) as well as from Chennai and Kakinada (East coast). The species *P. monodon* for the present investigation ranks foremost in its fishery and aquaculture importance in India and overseas. Along the east coast, *P. monodon* contributes to nearly 3 – 3.5% of the total trawl landings at Chennai and 0.6-0.8% of the landings at Kakinada.

When compared to the east coast, contribution of the same species in the trawl operations is poor along the west coast. Its contributions in the west coast is about 0.1% at Kochi, 0.4% at Calicut and 0.3% at Mangalore. At Karwar, *P. monodon* is caught only in stray numbers in trawl operations.

It is also a commercially important species of South-East Asian countries. The distribution of the postlarvae of the species has been reported long back by Kemp (1915) from the Chilka lake and Ennur backwaters, from Adayar backwaters (Chennai) along with postlarvae during all months (Panikkar and Aiyar, 1939) and observed the fry of the species from the coastal areas, tidal rivers and estuaries. Kemp (1915) stated that the species is migratory in habit, the adults migrating out to sea during the breeding season. On the Kerala coast the species occurs both in the sea and in the backwaters in relatively smaller quantities. In the trawler catches of the region, it is seen that the larger sized prawns are obtained from the deeper waters. In Bombay, the catches mostly consist of immature specimens. *P. monodon* has a broad geographic range in tropical and subtropical waters, including east and west coast of South India.

In view of the modern concept of the units of fisheries management (Lester, 1980; Altukhov, 1981), it is essential to clearly know that whether all the wild populations of *P. monodon* being exploited in India are morphologically, biochemical genetically or molecular genetically homogeneous or not. Since, it is an ideal species for commercial aquaculture and selective breeding programmes (Lester and Pante, 1992), the questions of its stock structure become more meaningful and significant. Though, detailed informations on the biology and fishery of *P. monodon* of South India are available (Kemp, 1915; Panikkar and Aiyar, 1939; Delmedo and Rabanal, 1956; Hall, 1962; Rao, A.V.P., 1967; Rao *et al.*, 1993), the important questions mentioned above were

remaining almost unanswered. Hence, a doctoral problem entitled **"Genetic studies of the marine penaeid prawn, *Penaeus monodon* Fabricius, 1798"** was selected to answer the above questions to the possible extent. To achieve these objectives, three independent and refined methods - multivariate analysis of truss morphometrics (Humphries, 1981; Strauss and Bookstein, 1982; Winans, 1984; Lester *et al.*, 1990), isozyme related biochemical genetics (Ihssen *et al.*, 1981; Lester and Pante 1992) and molecular genetics based on random amplified polymorphic DNA (RAPD) analysis (Hallerman and Beckman, 1988; Williams *et al.*, 1990., Ovenden, 1990; Jayasankar and Dharmalingam, 1997) were applied to study and detect individual variations within each population samples of the species. The data obtained on the genetic variabilities between the population samples were statistically compared to measure the significance of the interpopulation differences with a view to finding the answers being sought. The present thesis contains the detailed results of the investigation as well as the significant conclusions drawn form the results.

Review of literature

2. REVIEW OF LITERATURE

The concept of species has been known from time immemorial. Its basis was clearly defined in the book of bible about 3500 years ago by saying that each organism will produce its own kind (Genesis 1:24, 25). The taxonomic description of the species based on phenotypes and their variability was developed during the classical periods of Linnaeus, Lamarck, Darwin and post-Darwinian periods (George, 1964). Later phenotypic and genotypic analysis of the species and its populations revealed the existence of subspecies or races within the species (Dobzhansky, 1967).

In Fisheries research and management the concept of species, subspecies, races, stocks or even strains have theoretical as well as practical applications. Therefore, detection of such subspecies levels of species organization that sustains a fishery is essential for planning scientific exploitation and conservation of dynamic fishery resources. Interestingly, phenotypic races were recognised in herring and cod fish species as early as 1898 and 1930 by Heinke and Schmid respectively (George, 1964). During sixties and seventies, species and its units of fishery management were defined mainly based on population dynamics and population genetics (Muzimic and Marr, 1960; Marr and Sprague, 1963). A popular definition of taxonomic species was "Species are groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr, 1970). Meanwhile, considering Mendelian principles of genetics, the definition was modified as "a reproductive community of sexual and cross-fertilized individuals among whom mating regularly occur and who consequently have a common genepool (Dobzhansky, 1967).

Meanwhile, the conventional methods and techniques applied for detection of species and its isolated populations were also got refined or elaborated. The methods subjected to refinement were morphometrics, biochemical genetics and molecular genetics. Application of these refined methods have revealed hitherto unknown levels of variabilities in species of fishes and shell fishes of the world. As a result, the earlier concepts of species and the possible boundaries of units of fisheries management were compared and modified in the light of clear evidences of biochemical genetic diversity between populations of the cod fish – *Gadus morhua* which was exploited and managed as homogeneous populations or suspected as having morphologically heterogeneous stocks (Sick, 1965; Moller, 1966, '68, '70, 71). More important, morphometrically and genetically heterogeneous populations within a species or on the contrary, homogeneity of populations in a wide ranging areas of its distribution have also been reported.

The present investigation was carried out applying the refined morphometric method (truss network), biochemical genetic method (electrophoretic analysis of isozymes) and molecular genetic methods (random amplified polymorphic DNA). Since, these methods were independent, the review of the literature concerning the present investigation has been arranged under separate headings. Besides, the review is restricted to those which ae more relevant to the present investigation.

MORPHOMETRICS

Species of penaeus differ significantly in a variety of morphological characteristics that are the expressions of species-specific polygenes (Ayala and Keiger, 1980). Hence, these can be used for taxonomic distinctions. The studies generally focussed on the structures of genitalia, appendages,

rostra, or sculpturing of the carapace. Studies of the variation among species in morphological characters related to size, shape or other commercial characters are quite limited except for correlating these variations to the tail weight of the species (Lester 1983; Goswami *et al.*, 1986). Such correlation helps to select breeders of desired tail weight without sacrificing the specimens. Quick measurements of the body parts that have the highest known correlation can be taken, avoiding weighing the whole live specimens by conventional method using a balance. The method helped to detect second abdominal segment depth (SAD) as having the highest correlation in *P. indicus* (Bindhu Paul, 2000).

The morphological variations detected in morphometric studies are of a continuous nature. Hence, these can not be directly used for differentiating genetically different populations. The significance of morphological variations measured for two populations can be estimated only by statistical analysis of measurements and counts and not by the frequency of morphotypes. Morphometrics clearly has something to do with the assignment of quantities to biologic shapes (Bookstein, 1982). The popular statistical method applied to identify and differentiate morphometrically distinct populations within a species is multivariate analysis (many sets of morphological measurements). The method of multivariate analysis of morphometric variability helped to distinguish morphometrically distinct stocks in chinook salmon (Winans, 1984). Multivariate analysis of morphometric variations in the milkfish, *Chanos chanos* also indicated stock differences between two population samples (Winans, 1984). Multivariate morphology was used to investigate the distinctness and inter relationships of six stocks of the common carp, *Cyprinus carpio* (Corti *et al.*, 1988). A 'size' component was clearly identified by multiple group principal component analysis. Corti *et al.* (1988) suggested that multivariate morphometrics could represent an appropriate and convenient tool to detect variation between strains in carp

culture. Riddel and Leggett (1981) have studied the body morphology for geographic variation in juvenile Atlantic salmon.

Most of the morphometric variations are due to size differences, both within and among species. There is usually very little variation among members of a species in shape. Size differences are sometimes difficult to measure accurately because of measure error. Multivariate analysis of shrimp body dimensions had proven to be useful as a very accurate method of size measurement (Lester *et al.*, 1990). Both principal component and discriminant analysis techniques were used in attempts to find a methodology that would allow the description of shape differences in a biologically meaningful way. The results expressed as a graph of the first principal component versus the sheared second principal component with each species shown by an ellipse representing the 95% confidence interval of the species centroid. The sheared second principal component should define the differences among the species in shape with size removed as a factor (Humphries *et al.*, 1981) where as principal component I expresses the variation in size.

Dimensions collected from photographs of shrimp in either lateral or dorsal view, either alive or dead, have been used in recent studies. The photographs are used in concert with a computer and a digitizing tablet to determine the location of landmark points around the periphery of the shrimp. Entry of a scale permits the use of these co-ordinates to calculate the distances between landmarks of a form may serve characters for morphometric analysis. Collections of landmarks and distances among them must be homologous from form to form for comparisons to be meaningful and an adequate character set should at least permit the full reconstruction of the original configuration of landmarks (Strauss and Bookstein, 1982). Reconstruction of the form from truss measures provides Cartesian co-ordinates for landmarks and allows estimation of, and compensation for, measurement error. (Lester and Pante, 1992).

The first known attempt to apply multivariate morphometrics to differentiate geographically distinct stocks within penaeid species was that of Horton (1982). Geographic populations of *Penaeus stylirostris* and *P. vannamei* showed significantly different principal component scores and UPGMA clustering. However, these findings were not corroborated by other methods (Lester and Pante, 1992). The multivariate analysis of body shape variables measured by truss network method (Strauss and Bookstein, 1982) was recommended as an accurate method for selective breeding programmes of prawns (Lester *et al.*, 1990; Li *et al.*, 1993). For the same reasons geographically differentiated morphometric stocks of prawn species can be detected by multivariate and principal component analyses of morphological variations detected by truss network method (Lester and Pante, 1992). The application of the multivariate analysis of conventional morphometric measurements in the Indian penaeid prawns, *Penaeus indicus* populations from Cochin, Tuticorin and Madras and that of *P. stylifera* from Cochin and Bombay indicated overall morphological homogeneity (Philip Samuel, 1987). The study of multivariate and Principal component analysis of truss data from *P. indicus* populations of South India also showed lack of morphological heterogeneity in its population (Bindhu Paul, 2000). *Penaeus monodon* has higher potential growth rate, which may be correlated to genetic stock structure. However, a study to separate geographically distinct stocks, if any, across the range of *P. monodon* was not attempted by morphometric methods (Lester and Pante, 1992). Interestingly, differences in the stock assessment values of the Indian *P. monodon* from east and west coasts of India suggested that these may be separate stocks (Rao *et al.*, 1993).

In spite of all the refinements in the morphometric methods, it suffers from an inherent problem. The morphological variations present in fishes and shell fishes are always affected to certain extent by environmental parameters like, temperature, salinity, food etc. These

variations are not directly controlled by the genes, though, these are expressions of polygenes (Clayton, 1981; Ayala and Keiger, 1980). In other words, the morphometric variations are less genetic than the variations detected by the biochemical genetic method. Therefore, the practice of applying biochemical genetic techniques alone or along with the morphometric methods became the most popular approaches in the study of population genetics of fishes and shell fishes.

BIOCHEMICAL GENETICS

The application of zone electrophoresis techniques (Smithies, 1955) and zymogram techniques (Hunter and Markert, 1957) revealed gene controlled co-dominant allelic variations in every type of organism ranging from Drosophila to Man (Lewontin and Hubby, 1966; Harris, 1966). The application of these techniques in fisheries science also revealed low or high levels of genetic variability in all the species of fishes and shell fishes (de Ligny, 1969). Significant differences in the allelic frequencies between populations of a species clearly indicated that these are not interbreeding but isolated populations of the species. Soon genetically distinct stocks were detected and reported in the cod fish (Jamieson, 1967, '70; Jamieson and Jones, 1967), tunas (Fujino and Kang, 1968), molluscs (Koehn *et al.*, 1973). The significance of similar worldwide reports in fishes and shell fishes was well evaluated in the international symposiums held in 1971 (de Ligny, 1971). Later, the special significance of the genetic stock concept at various levels of fisheries management and various techniques for detection of genetic stocks were re-evaluated in the international symposiums held in 1981 (Special issue, *Can. J. Fish. Aquat. Sci.* Vol., 38 (12), 1981). The co-dominant allelic forms of proteins detectable were described as the genetic tags present in every organisms (Jamieson, 1974). The genetic tags, serum transferrin, esterase and superoxide dismutase variants were applied to test the unit stock

hypothesis of European hake populations (Mangaly and Jamieson, 1978). Using isozyme genetic tags, six genetically heterogeneous stocks were detected in the flounder populations of Newfoundland region (Fairbairn, 1981). The worldwide reports of biochemical genetic polymorphism, biochemical genetic differences or lack of it are too many to review here. However, the application of biochemical genetics in the Indian fishes, *Mugil cephalus* (Vijayakumar, 1992), oil sardine, *Sardinella longiceps* (Venkita Krishnan, 1992) are important. Several heterogeneous stocks were detected in these two species which were not known earlier.

Literature review also shows that the efficiency of biochemical genetic techniques in revealing the intraspecies allozyme polymorphism and existence of heterogeneous or homogeneous stocks in various crustacean species is equally evident as in the case of fishes. Enzyme polymorphism in 44 species of decapod crustacea including five species of penaeidae (Nelson and Hedgecock, 1980). The first report of single enzyme polymorphism in a penaeus species was that of Procter *et al.* (1974). This was followed by similar reports in other penaeid species (Lester and Pante, 1992). The enzyme they screened was phospho-glucomutase (PGM) and the heterozygosities in these species varied from 0.14 to 0.37. However, testing of increased number of enzyme loci revealed comparatively low level of biochemical genetic variation in many of species penaeus and the heterozygosity was as low as 0.006 in *P. longistylus* or 0.008 in *P. monodon* (Lester and Pante, 1992., Redfield et al., 1980), 0.023 to 0.037 in *P. californiensis*; 0.038 to 0.086 in *P. Stylirostris* (de la Rosa Velez *et al.*, 2000) and as high as 0.092 (Lester, 1979). Contrary to many reports of significant biochemical genetic stock differences revealed by allozyme frequencies, most penaeid species populations had insignificant genetic distance values [D] throughout their geographic range (Lester and Pante, 1992). The reported exemptions were stock differences between population of *P. stylirostris* from the Gulf of California and other populations; significant genetic differences between populations of *P. indicus* tested from

coasts of Philippines and Kenya. Similarly, when four Australian *Penaeid* and one *Metapenaeus* species were genetically homogeneous, *P. latisulcatus* showed significant stock differences between its two geographic areas (Lester and Pante, 1992). Genetic polymorphism in four isozyme loci and significant differences in the allele frequencies of several populations of the lobster *Homarus gammarus* from Norway coast were reported (Jorstad and Fairstiveit, 1999).

Though the recent biochemical investigations of populations of *P. indicus* from South India (Philip Samuel, 1987; Bindhu Paul, 2000) also revealed genetic polymorphism at several enzyme loci, genetic distances among populations of each species were not significant. On the contrary, high biochemical genetic variability and significant genetic stock differences were detected in the populations of *Metapenaeus dobsoni* of South India (Santh Begum, 1995).

Literature review also shows that biochemical analyses of allozymes in populations of *P. monodon* from Australia, Thailand and Andaman sea revealed intraspecies polymorphism at many loci but also genetic isolation of some of its populations (Benzie *et al.*, 1992, 1993; Sodsuk *et al.*, 1992). The above review of reported findings of intraspecies allozyme polymorphism, levels of genetic variability, the phenomenon of significant differences in the genetic distances of populations or genetic homogeneity among populations in spite of their wide ranging geographic distributions suggests that each of these informations has its own significant implications in fisheries management, conservation of genetic resources and various aspects of aquaculture including selective breeding and brood stock management programmes (Lester *et al.*,1990; Lester and Pante, 1992).

The phenomenon of the very low level biochemical genetic variability and close genetic similarities of even distant geographic populations in species of penaeidae was re-examined by many curious investigators using

mitochondrial or nuclear DNA samples. Because, the electrophoretic techniques used for separation of proteins have their own limitations in revealing the actual genetic variability present in the species and besides, the number of loci examined are always much less than estimated in each species. Hence, the analysis of the very base sequences of the DNA was the best alternative in the study of population genetics. The results reported by the investigators have greater implications in fisheries management and conservation of the genetic resources than that provided by biochemical genetic method. A brief review of these reports relevant to the present investigation is given below under the heading "molecular genetics".

MOLECULAR GENETICS

The application of restriction enzymes to study mitochondrial DNA sequence polymorphism in natural populations was recommended by Avise *et al.* (1979). The superiority of mitochondrial DNA (mtDNA) variability and its applications in genetic stock assessment was reviewed by Ovenden (1990). The restriction fragment length polymorphism (RFLP) detected by mtDNA analysis revealed higher amount of genetic diversity between populations and between species where lower diversity was shown by biochemical genetic analyses. The application of molecular markers in fish population studies was highlighted by Ferguson *et al.* (1995).

Meanwhile, the recent introduction of thermocyclers for amplification of the DNA segment through polymerase chain reaction (PCR) and the techniques developed by Welsch *et al.* (1990) and Williams *et al.* (1990) enabled to detect and observe DNA base level polymorphism in microsamples of the organisms.

The application of DNA techniques permits detection and observation of genetic diversity unknown before. Hitherto unknown

genetic divergence was detected between species of *Salmo* and between regional populations of some species (Wilson *et al.*, 1985) between river populations of American shad (Bentzen *et al.*, 1988), geographic differences in horseshoe crab (Saunders *et al.*, 1986) and genetic differentiation of morphologically similar species (Palumbi, 1991).

The reports of hitherto unknown level of genetic polymorphism in species of penaeid prawns as well as genetic diversity between populations are not rare. The very low level or insignificant diversity among geographic populations of *Penaeus vannamei* rose to twenty fold when same populations were examined by mitochondrial DNA techniques and six fold in *P. stylirostris* (Lester and Pante, 1992).

The published reports on the application of RFLP and RAPD techniques in studying the genetic polymorphism in fishes and shell fishes of India are almost nil. A preliminary attempt to apply these two techniques was attempted very recently by Bindhu Paul, 2000. Restriction fragment length polymorphism was detected in *P. indicus*. The RAPD profiles of its samples from east and west coast of South India showed significantly different pattern whereas these samples had shown morphometric and biochemical genetic homogeneity.

There are interesting reports on the results of application of RFLP and RAPD techniques in the study of population genetics of *P. monodon* from Australia, Philippines, Fiji and Thailand. Populations of *P. monodon* from Fiji showed significantly different mitochondrial DNA profile from that of Australia/Malayasia strains (Bouchon *et al.*, 1994). Genetic variations and genetically distinct stocks within the Thailand populations of *P. monodon* were detected by RAPD techniques (Tassanakajon *et al.*, 1997, 1998). The recent review of genetic structure of penaeid prawns reveals low allozyme variability, higher mtDNA variability and the highest nuclear DNA (nDNA) variability, especially at microsatellite

region (Benzie, 2000). For the same reasons, RAPD techniques were most efficient in differentiating genetic stock diversities in these penaeid species especially in *P. monodon*. The RFLP and RAPD techniques also helped to differentiate distinct stocks from Australia and Indo-Pacific region (Benzie; 1993; Benzie *et al.*, 2000). Genetic stock differences can occur between areas of short distance ranging from 250 – 600 kms. (Benzie, 2000).

The above review on the concept of unit stock in fisheries management, on the methods of detection of such units and the results of application of these methods by different investigations worldwide reaffirm the efficiency of these techniques and the necessity of investigating the genetics of *Penaeus monodon* of India by morphometric, biochemical genetic and molecular genetic methods.

Materials and methods

3. MATERIALS AND METHODS

3.1 Materials

Population samples of *Penaeus monodon* were randomly collected from selected landing centres of west (Karwar, Mangalore, Calicut, Kochi) and east (Chennai, Kakinada) of South India (Fig.1). These samples captured by trawl net purchased from landing centres were first frozen and transported to the laboratory in wet ice and then stored at -20° C until used for experiments. A total of 627 samples of *P. monodon*, with a size range of 100-300 mm were collected for Morphometric, Biochemical and Molecular genetic analysis during 1996-2001.

3.2 Methods

Basic informations required for genetic studies were collected by the morphomertric, biochemical and molecular methods. The procedures for collection of basic data were standardized as detailed below.

3.2.1. Morphometrics

The morphometric data analysed were measurements of the body parts, body shape and counting of meristic character.

3.2.1.a Length measurements

First, the thawed samples were weighed individually using the electronic weighing balance (Sartorius). Then a set of 7 variables was measured as follows (Fig: 2). Length measurements were taken using a dial caliper (0.05 mm accuracy) (Lester, 1983; Goswami *et al.*, 1986; Lester, *et al.*, 1990; Lester and Pante, 1992). The variables measured

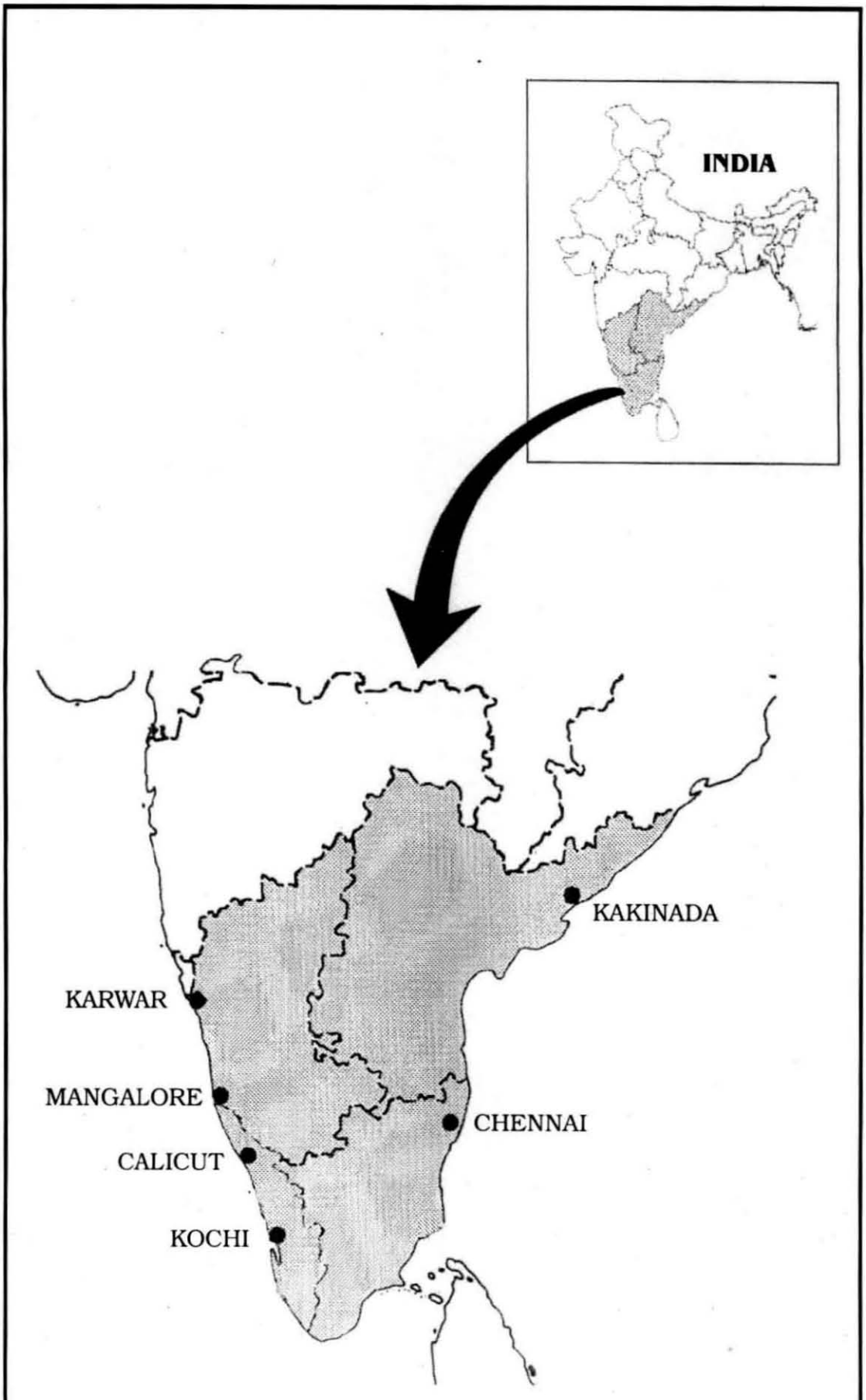
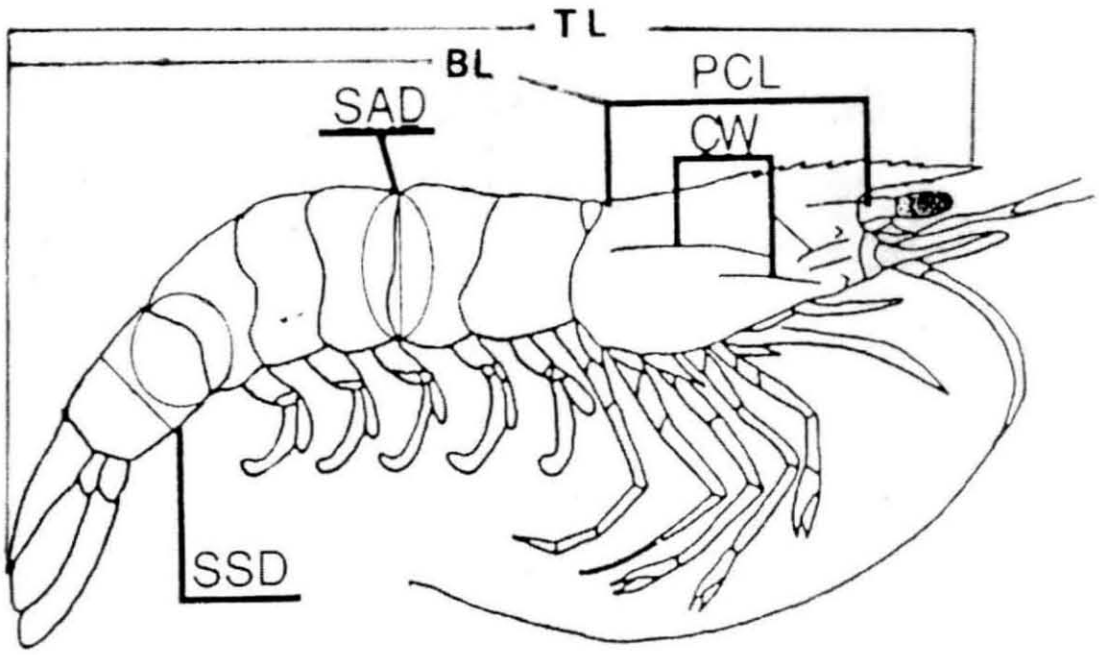


Fig. 1 Collection sites for ***Penaeus monodon*** of South India

were the length of the first abdominal segment along the mid-dorsal line (FSL) the length of the sixth abdominal segment along the mid-dorsal line with shrimp extended (SSL); partial carapace length (PCL) from the posterior margin of the orbit to the posterior edge of the carapace; width of the carapace (CW) at the point of the last dorsal rostral tooth; length of the fifth abdominal (FLF) segment when the shrimp is flexed ventraly; depth of the abdomen (SSD) at the mid-point of the sixth segment; depth of the abdomen (SAD) at the intersection of the second and third segments, circumference of the abdomen (AAC) at the intersection of segments five and six; the weight of the abdomen (TW) severed along the posterior edge of the carapace was also recorded. The rostral teeth number was also counted as a meristic character of the populations. The length variables selected for the present study are listed below (Table 1).

3.2.1.b Truss measurements

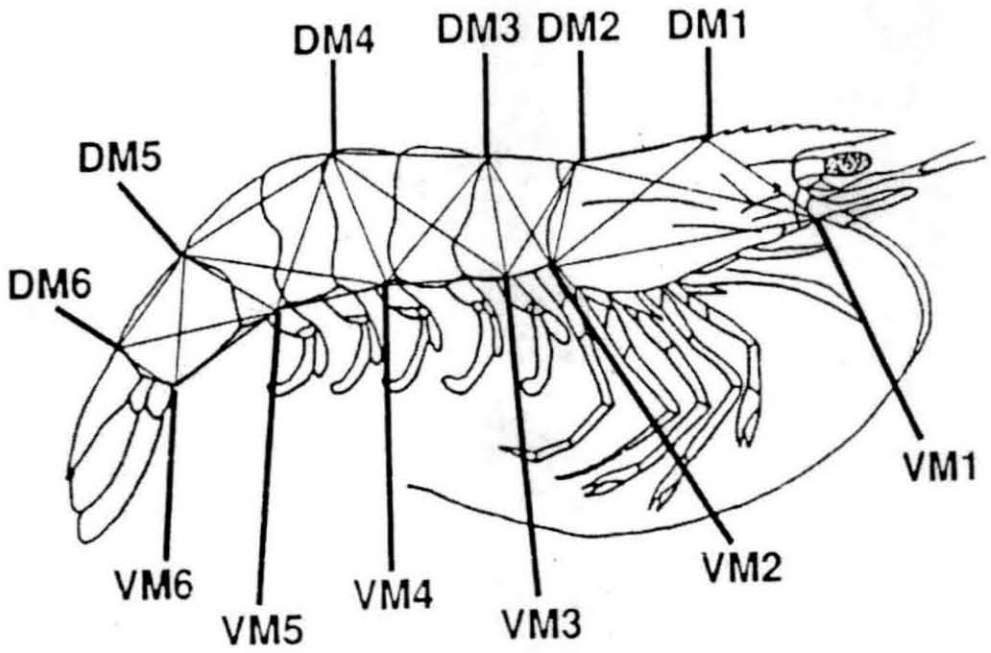
The body shape of each sample specimen was measured by truss network method (Lester *et al.*, 1990; Lester and Pante, 1992). The thawed specimen was positioned on a water-resistant drawing sheet, head towards the RHS, and body posture and appendages were teased into a natural position. Positioning of specimens in this fashion is a precise process, as evidenced by low measurement error (Winans, 1984). Distinctive and homologous landmarks were selected around the outline of the prawn. Each landmark along the body was indicated and recorded by making a hole with a dissecting needle in the water resistant paper alongside its respective location. Data such as specimen number, body weight, and colour were recorded alongside each specimen. After the landmark informations from a set of specimens were recorded (pinned), the paper was placed on an X-Y coordinate of a graph paper to establish a reference set of X and Y axes to view interlandmark distances (Lester *et al.*, 1990; Lester and Pante, 1992). The Euclidean or morphometric distances between pairs of landmarks were then calculated by computer (using the pythagorean theorem).



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)	:	

Fig.2 Shows the length variables measured in *P. monodon*.



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)

Fig. 3 Shows the truss network landmarks made for measuring the body shape of *P. monodon*.

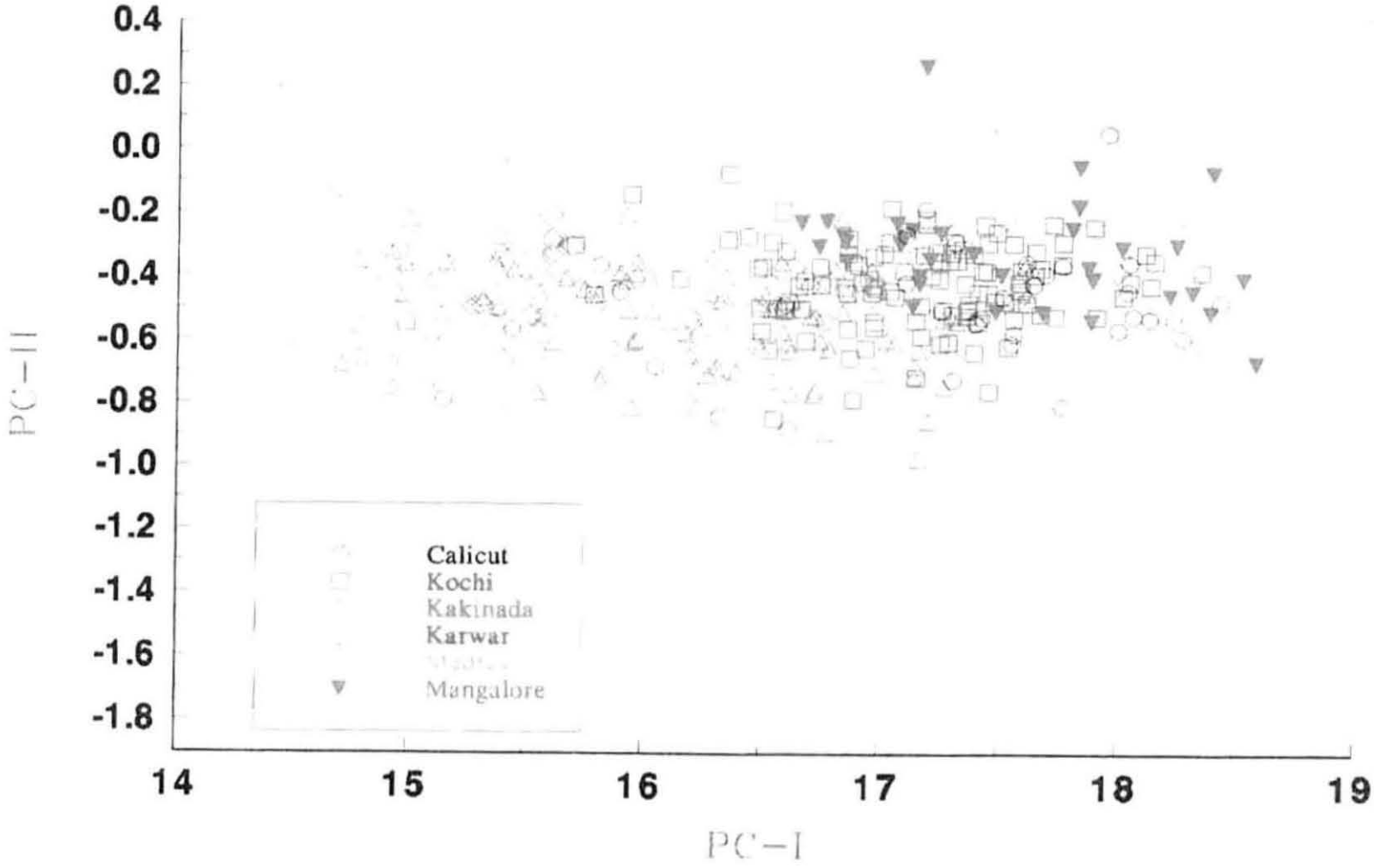


Fig.4 Morphometric profiles (truss measurements) of six populations of *P. monodon* of South India

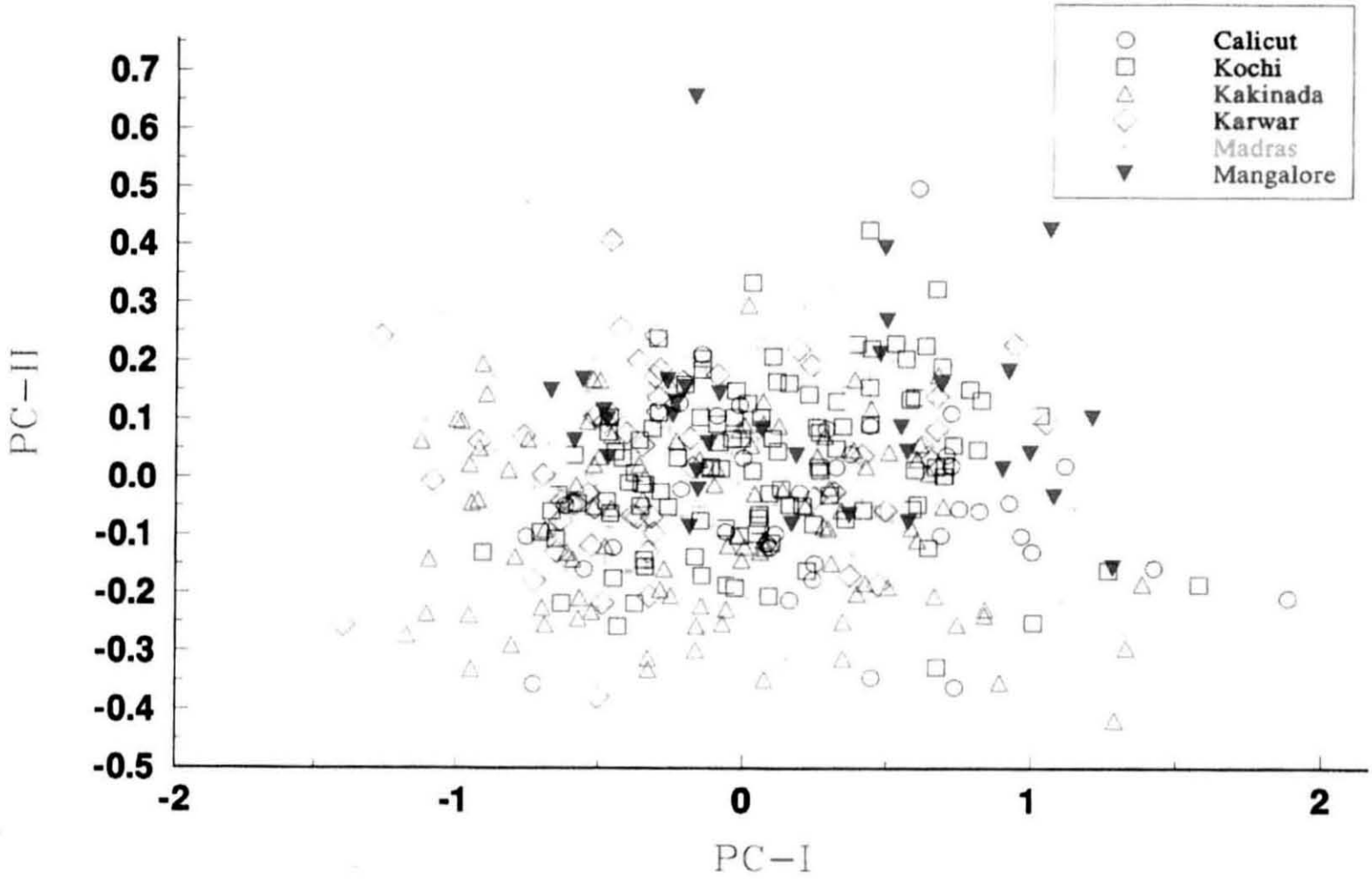


Fig.5 Morphometric profiles (sheared PC scores of truss measurements) of six populations of *P. monodon* of South India

Principal component analysis computes a set of uncorrelated composite variables called principal components (PCs) from a variance-covariance (or correlation) matrix (Dunn and Everitt, 1982). The first principal component (referred as PC I) explains the most of the variance in the data set. Geometrically, PC I is thought to lie parallel with the largest axis in the hyperdimensional cloud of data (Green, 1976; Campbell and Atchley, 1981). PC II is independent of PC I, that is, it lies perpendicular to the axis of PC I, and explains the second largest component of variation in the data set. Each PC is a linear combination of the variables and is defined by a vector (an eigen vector) of coefficients and an eigenvalue. The coefficients are essentially a measure of covariance of the character on that PC. The eigenvalue is a measure of variability explained by a particular PC; the sum of the eigenvalues equals the total variability in a data set. Since on any component only a few characters have large coefficients, the biological interpretation of a component is based on the magnitude and signs of these so-called important characters. The details of the parameters considered for the truss network analysis are given in figure 3.

3.2.2 Biochemical Genetics

3.2.2.1 Sample preparation

Eyelens, hepatopancreas and abdominal muscle tissues were removed from the thawed specimens. Each tissue sample was first minced and then homogenized using a glass hand homogenizer under cold conditions. Tris-Glycine, Tris-NaCl-$MgCl_2$ and ice-cold 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 were varied. The supernatants were stored at –20°C examined for electrophoretic studies.

3.2.2.2 Electrophoresis of samples

The supernatants of the three tissues were analysed by Polyacrylamide gel electrophoresis (PAGE) using 7%, 7.5%, 8%, 8.5%, 9%, 9.5%, 10%, 10.5% and 11% gel (Table 9) the band patterns (zymogram) were detected by specific enzyme and protein staining procedures. Since the abdominal muscle tissue produced a better banding patterns, it was selected for further studies. Abdominal muscle tissue samples (1g/1ml) were homogenized in ice-cold DDW. After centrifuging at 10,000 Xg for 50 minutes (-4°C), the supernatants were collected and stored in refrigerated condition (-20°C) till used for further analysis.

Electrophoresis was carried out in horizontal slab gel apparatus – Multiphore II (Pharmacia, LKB). Continuous and discontinuous buffer systems were used for the present study. Electrophoresis was performed using gel buffers of different compositions (Table 8). Banding patterns were scored by designating loci 1, 2 etc. in order of descending anodal mobility and allozymes. Nearly fourteen enzyme systems were tested (Table 6) in abdominal muscle tissue of *P. monodon* and from them only the following enzyme systems were selected for the investigation: (1) Aldolase (FBALD-8%), (2) Aldehyde Oxidase (AO-8%), (3) Adenylate Kinase (AK-7.5%), (4) Esterase (EST-10%), (5) Glyceraldehyde-3-Phosphate dehydrogenase (GPDH-8%), (6) Octanol dehydrogenase (ODH-10%), (7) Malate dehydrogenase (sMDH-10%) and (8) General protein (PROT-10%).

Buffer systems with different pH and molarity were tried for the present study, as given in the Table 8. Stock solutions of acrylamide and bis-acrylamide were prepared with different concentrations and various gel percentages also were tried in order to maximize the resolution of the protein bands. For the continuous buffer systems, 75 ml of gel solution was needed, while the discontinuous system needed a stacking gel solution of 25

Table 8 Composition of the buffer systems selected for electrophoresis of enzymes in *P. monodon* of South India

Buffers	Ingredients							
	Electrode buffer		pH	Gel buffer				
				Stacking		pH	*Separating*		pH
1.Tris- Citric- EDTA (Ayala et al., 1972)	0.135 M Tris	16.35g/l	7.0	0.009 M Tris	1.09g/l	7.0	0.009 M Tris	1.09g/l	7.0
	0.045 M Citric acid	9.45g/l		0.003 M Citric acid	0.63g/l		0.003Citric acid	0.63g/l	
	0.00125 M EDTA	0.47g/l		0.00125 M EDTA	0.47g/l		0.00125 M EDTA	0.47g/l	
2.Tris- Citric acid - Lithium Hydroxide - Boric acid	**A**								
	0.0472 M Tris	8.7g/l	7.25	**A**	90ml	6.1	A	90ml	8.2
	0.023 M Citric acid	4.8g/l							
	B								
	0.03 M Lithium Hydroxide	1.2g/l		**B**	42ml		B	42ml	
	0.19 M Boric acid	11.8g/l		DDH_20	168ml		DDH_20	168ml	
3.Tris- Citrate Lithium Hydroxide - Boric acid	0.03 M Lithium Hydroxide	1.2g/l	8.1	0.072 M Tris	8.7g/l	6.8	0.072 M Tris	8.7g/l	8.8
	0.19 M Boric acid	11.8g/l		0.023 M Citric acid	4.8g/l		0.023 M Citric acid	4.8g/l	
*4.Tris- Glycine Tris- HCl	2 M Tris	25ml	8.3	1.8 M Tris	21.81g/100ml	8.9	0.05 M Tris	6.06g/ 100ml	6.8
	C140.2 M Glycine	15.04g/l		1N HCl to adjust the pH			1N HCl to adjust the pH		

*** Buffer system selected**

Table 9 Gel compositions tested for electrophoretic separation of enzymes in *P. monodon* of South India

	Separation gel			Stacking gel		
	7 %			**3.5 %**		
	DDW	-	6.57 ml	DDW	-	15.5 ml
*	Tris/HCl	-	6.25 ml	± Tris/HCl	-	6.25 ml
**	Acrylamide	-	12.18 ml	** Acrylamide	-	3.04 ml
***	APS	-	25 ml	± ± Temed	-	25 µl
				*** APS	-	100 µl
	7.5 %			**3.5 %**		
	DDW	-	5.71 ml	DDW	-	15.5 ml
*	Tris/HCl	-	6.25 ml	± Tris/HCl	-	6.25 ml
**	Acrylamide	-	13.04 ml	** Acrylamide	-	3.04 ml
***	APS	-	25 ml	± ± Temed	-	25 µl
				*** APS	-	100 µl
	8 %			**4 %**		
	DDW	-	5.42 ml	DDW	-	15.3 ml
*	Tris/HCl	-	6.25 ml	± Tris/HCl	-	6.25 ml
**	Acrylamide	-	13.33 ml	** Acrylamide	-	3.33 ml
***	APS	-	25 ml	± ± Temed	-	25 µl
				*** APS	-	100 µl
	10 %			**5 %**		
	DDW	-	2.05 ml	DDW	-	14.34 ml
*	Tris/HCl	-	6.25 ml	± Tris/HCl	-	6.25 ml
**	Acrylamide	-	16.70 ml	** Acrylamide	-	4.16 ml
***	APS	-	25 ml	± ± Temed	-	25 µl
				*** APS	-	100 µl

	DDW	-	Double Distilled Water
*	**Tris (1.8 M)**	-	weighed and pH adjusted with HCl (8.9). Added 125 µl Temed (gel buffer).
**	**Acrylamide**	-	(29.1g) weighed and added N, N'- Methylene bisacrylamide (0.9g) and dissolved it in 50 ml DDW.
***	**APS**	-	(0.1g/1ml) weighed and dissolved it in 1 ml DDW. Taken 400 µl of 10% APS and added to 25 ml of DDW.
±	**Tris (0.5 M)**	-	weighed and pH adjusted with HCl (6.9).
± ±	**Temed**	-	Tetramethylene ethylene diamine

ml and separating gel solution of 50 ml. The amounts of gel buffer, ammonium per sulphate (APS) and TEMED (N,N,N',N'- Tetramethyl ethylene diamine) added to the mixture were the same for all the different gel percentages used. The proportion of sample and loading buffer were also changed to find out the optimum concentration of the sample that can give bands with good intensity and resolution. Samples were loaded at the cathodal end and it took two hours and thirty minutes for the indicator dye to reach the anodal end of the gel. A cooling system was connected to the electrophoretic apparatus so as to minimise the heat produced during the electrophoretic run of the enzymes. After each run was completed, the gel was stained either for protein or for a specific isozyme using standardised protocols. Some isozymes that needed a further standardisation which was done subsequently.

Thus, the standardised biochemical method composed of a combination of factors like a particular tissue, its extraction, electrophoretic conditions and the staining procedures that produced visible variation in the protein/enzyme banding pattern between individuals of *P. monodon*.

The staining recipe used for the isozyme detection were modified from that of Shaw and Prasad (1970) and Shaklee *et al.* (1990). The enzymes studied are given in the table 6. The stock solutions used were also of the same concentration mentioned in the original recipe.

1. Adenylate kinase (AK)

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

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 (AO)

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(FBALD)

0.2 M Tris-HCl (pH 8)	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. Glyceraldehyde-3-Phosphate dehydrogenase (α GPDH)

0.2 M Tris-HCl (pH 8)	50 ml
0.2 M $MgCl_2$	1 ml
α-DL-glycerophospahte	250mg
1 % NAD	2 ml
1 % NBT	1.3 ml

When ready to stain, added:

1 % PMS	0.5 ml

5. Octanol dehydrogenase (ODH)

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

When ready to stain, added:

1 % PMS	0.5 ml

6. Alcohol dehydrogenase (ADH)

0.2 M Tris-HCl (pH 8.0)	50 ml
0.1 M $MgCl_2$	1 ml
95 % ethanol	3 ml
1 % NAD	2 ml
1 % NBT	1.3 ml

When ready to stain added:

1 % PMS	500 µl

Incubated at 37°C till bands were visible.

7. L-Iditol dehydrogenase (IDDH)

0.2 M Tris-HCl (pH 8.0)	50 ml
0.1 M $MgCl_2$	1 ml
Sorbitol	500 mg
1 % NAD	2 ml
1 % NBT	1.3 ml
When ready to stain, added:	
1 % PMS	500 µl

8. Glutamate dehydrogenase (GLUDH)

0.2 M Phosphate βNa_2HPO_4	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. Malate dehydrogenase (sMDH)

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	0.6 ml

Incubated at 37°C in the dark.

10. Esterase (EST)

Fast Blue RR salt	50 mg
α -β naphthyl acetate	1.5 ml
0.5 M Tris-HCl (pH 7.1)	5 ml
Double distilled water	43.5 ml

Incubated at 37°C in the dark.

11. Glucose-6-Phosphate dehydrogenase (G-6PDH)

0.5 M Tris-HCl (pH 7.1)	4 ml
Double distilled water	30 ml
Na_2 Glucose-6-phosphate	180 mg
NaCn	25 mg
1 % NADP	1.1 ml
1 % NBT	0.75 ml
When ready to stain, added:	
1 % PMS	0.2 ml

Incubated at 37°C.

12. Hexokinase (HK)

0.2 M Tris-HCl (pH 8.0)	50 ml
0.1 M $MgCl_2$	1 ml
α -D-glucose	50 mg
ATP	12 mg
1 % NADP	1.75 ml
G-6-PDH	2 ml
When ready to stain, added:	
1 % PMS	0.2 ml
1 % NBT	0.4 ml

Incubated at 37°C.

13 Isocitrate dehydrogenase (mIDHP)

0.2 M Tris-HCl (pH 8.0)	40 ml
0.25 M $MnCl_2$	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.

14. Acid phosphatase (ACP)

0.1 M Acetate buffer	50 ml
Na- α -naphthyl acid phosphate	50mg
When ready to stain, added:	
Black K salt	20mg

Incubated at 37°C.

15. General Protein (PROT)

Coomassie Brilliant Blue	1.25 mg
Methanol	230 ml
Double distilled water	230 ml
Glacial acetic acid	40 ml

Mix well, filter the solution. Stain the gel in dark
For 90 minutes and wash. Transfer to destainer.

Destainer

Acetic acid	500 ml
Methanol (95 %)	1500 ml

Make up to 5 % with DDW, mix well.

3.2.3 Molecular Genetics

The molecular genetic method can reveal finer variations existing at the structural level of nucleic acids (DNA) that are undetectable by electrophoretic analysis of proteins/enzymes produced by the DNA molecule. The molecular techniques standardized in the present study consisted of three major procedures viz., isolation of total DNA, polymerase chain reaction (PCR) and analysis of PCR products. The methodical procedures adapted here have been described below (Maniatis, 1982; Garcia and Benzie, 1995).

3.2.3.1 Random Amplified Polymorphic DNA (RAPD)

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:Isoamyl alcohol was added. Kept for 5 minutes

↓

Centrifuge at 8000 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 8000 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 10000 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 sterilized distilled water

3.2.3.1b Polymerase Chain Reaction

The DNA was quantified and about 55 ng of sample DNA was used for amplifications. The reaction mix contained 0.33 µl of Taq polymerase (1.0 U), 1 µl of dNTPs (300 µM), 3.3 µl of assay buffer (2mM $MgCl_2$), 2 µl of primer (10 picomoles), 17.37 µl of millipore water/ deionised DDW and 1 µl template DNA – a total of 25 µl.. The samples were set for reaction in duplicates in the thermocycler (Perkin Elmer Geneamp PCR system, 2400) in the block and the reactions were cycled through different temperature regimes. It was run for fourty five cycles as follows: denaturation at 92° C for 1 min, annealing at 35° C for 1 min. 30 seconds, 72° C for 1 minute and an extension at 72° C for 7 minutes. An additional denaturation at 92° C for 3 minutes was given initially. The reaction was then cooled to 4° C. The length of the run was nearly 4 hours and 30 minutes. The presence of the PCR products were confirmed by using controls, one without primer, second without Taq DNA polymerase and the third without genomic DNA. No amplifications occurred in any of these controls. Amplification is also carried out with different concentration of template DNA in order to optimise the template DNA which give the best amplification products (1µl to 100, 30, 15 µl respectively), annealing temperatures (34,35 and 36° C), the number of cycles(40 & 45) and finally by the duration of each step (30 seconds to 2 minutes). Finally, the dilution of 1 µl to 100 µl with 55 ng of DNA was selected for screening of the sample. The primers, OPA-1 to OPA-10 were used for polymerase chain reactions.

3.2.3.1c PCR amplifications and electrophoresis of PCR products

Following the amplification, the reaction mixtures (loaded 7 µl product mixed with dye) were run on a 1.5% or 2 % agarose gel for one and a half hours using 1**X** TAE buffer. The gel was stained in ethidium

bromide for 20 minutes. After completion of the run, the gels were washed in double distilled water and viewed under UV and photographed. All the OPA primers used amplified the isolated DNA samples. However only OPA-2 and OPA-4 revealed DNA variations in the samples.

The sequences of the primers used: TGCCGAGCTG - OPA 2 and AATCGGGCTG - OPA 4.

Composition of Reagents

1. TEK buffer (pH 7.5)	100 ml	2. TAE buffer (pH 8.0)	100ml
50 mM Tris	0.61 g		
10mM EDTA	0.37 g		
1.5% KCl	1.5 g		

3.3. Statistical Analyses of the Data

3.3.1.a Morphometric analysis

The length measurement on four variables of 428 samples were fed into the computer and the correlation of the variables, viz., SSD, SAD, PCL and CW with the tail weight was deduced by correlation matrix and path-coefficient (direct effect) 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 (direct effect) analysis. Thus direct effects of the 4 different variables on the tail weight was assessed.

3.3.1b Truss Network analysis

For the analysis of truss data on body shape, the X and Y co-ordinate values for each landmark of 428 samples were 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.

3.3.2 Biochemical Genetics

Source: Zymograms : (Fig 6, 7, 8)

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 photographed if necessary for further analysis. The homozygotic and heterozygotic banding patterns of an individual may consist of zones of fast medium and slow moving bands and these zones were designated as 1, 2 starting from the fast zone. A comparative analysis of bands of each individuals in each of these zones 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 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 non-consistent banding patterns were not considered as loci. Again, fast and slow moving single

banded phenotypes were scored as slow and fast homozygotes, while 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 is dimeric whereas the former that produced only two banded heterozygote was monomeric in nature.

Since the difference in the electrophoretic mobility between slow and fast phenotypes at one locus was, 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 the electrophoretic mobility value 100. All other bands moving faster than 100 were given actually measured additional values whereas that moving slower than 100 were given lesser values. 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. The number of different phenotypes observed at each locus was counted in each specimen of each sample. These counts of the phenotype 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 to estimate the significance of biochemical genetic variability between the populations.

3.3.2.1 F- analyses

A co-ancestry assessment of the individuals between the populations was done by applying the methodology suggested by B.S. Weir and C. Clark Cockerham (1984). F_{ST} values were interpreted as showing little, moderate, great or very great levels of genetic differentiation according to the qualitative guidelines suggested by Wright (1978).

3.3.2.2 Genetic Identity Analyses

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

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

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 **-1n I.**

3.3.3 Molecular Genetics

The methodology adopted for the collection of the data for the genetic analysis of the present study was Randomly Amplified Polymorphic DNA (RAPD), where the total DNA polymorphism studied. 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.

3.3.3.1 Data analysis of RAPD

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

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

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 (D = 1-S). The similarity values were averaged over all the primers used, to find out the mean similarity between the populations and within the populations.

Results

4. RESULTS

4.1 Morphometrics

4.1.1 Correlation with tail weight

Ten metric and only one meristic variables were measured to detect variation among the random samples from different locations (Table 1). Since six of the ten measurements involved the body extremities, only the other four viz., sixth segment depth (SSD), second abdominal depth (SAD), partial carapace length (PCL), and carapace width (CW) were considered for the study. Among these four variables, PCL showed the highest correlation with the tail weight (TLW) in both males (0.30971299) and females (0.48813414) of *P. monodon* from the six locations studied. From the path coefficient analysis, it was found that the partial carapace length (PCL) was the variable having highest correlation with the tail weight irrespective of sex (Table 3 & 4).

4.1.1.2 Truss network analysis

Truss data of *P. monodon* (428 specimens) from six centres (Karwar, Mangalore, Calicut, Kochi, Chennai and Kakinada) collected during 1996-2001 were used for the analysis. The 26 truss measurements made on each sample specimen of *P.monodon* from Kochi, Calicut, Mangalore, Karwar, Chennai and Kakinada were log transformed and subjected to principal component analysis. The first principal component accounted for 85.80% and the second accounted for 3.35% (Table 5) of the total variations in the truss data. These two principal components accounted for 89.15% of the variations in truss measurements data and were used to explain the variations. The PC-I and PC-II scores were computed for each of the samples and PC-I scores were plotted against PC-II scores to see morphometric changes between stations. From the plot it was found that samples from Mangalore formed a separate cluster

Table 1 Morphometric Variables

1.	TL	:	Tip of the rostrum to the tip of the telson
2.	BL	:	Postorbital border of the carapace to tip of the telson
3.	SAD	:	At the intersection of the 2^{nd} and 3^{rd} segment
4.	SSD	:	Depth at the mid-point of the 6^{th} segment
5.	PCL	:	Posterior margin of orbit to posterior edge of carapace
6.	CW	:	At the point of the last dorsal tooth
7.	RL	:	Tip of the rostrum to the last dorsal tooth
8.	RT	:	Number of teeth on the dorsal and ventral side
9.	TW	:	Total weight
10.	TLW	:	Tail weight (without peeling)

Table 3 Correlation Matrix between different morphometric characters of *P. monodon* (Male)

Variables	SSD	SAD	PCL	CW	TLW
SSD	1.0000	0.9400	0.9583	0.9473	0.9482
SAD	0.9400	1.0000	0.9595	0.9357	0.9457
PCL	0.9583	0.9595	1.0000	0.9691	0.9605
CW	0.9473	0.9357	0.9691	1.0000	0.9496
TLW	0.9482	0.9457	**0.9605**	0.9496	1.0000

Table 3 (a) Path-Coefficient (Direct Effects) on TLW (Male)

Vars	SSD	SAD	PCL	CW
Effects	0.2326	0.2274	**0.3097**	0.2153

Table 4 Correlation Matrix between different morphometric characters of *P. monodon* (Female)

Variables	SSD	SAD	PCL	CW	TLW
SSD	1.0000	0.9609	0.9645	0.9543	0.939
SAD	0.9609	1.0000	0.9715	0.9616	0.9568
PCL	0.9645	0.9751	1.0000	0.9718	0.9639
CW	0.9543	0.9616	0.9718	1.0000	0.953
TLW	0.939	0.9568	**0.9639**	0.9530	1.0000

Table 4 (a) Path-Coefficient (Direct Effects) on TLW (Female)

Variables	SSD	SAD	PCL	CW
Effects	-0.0214	0.3087	**0.4881**	0.2022

Table 2 Truss Network Landmarks

1.	DM1	:	Epigastric tooth (anterior)
2.	VM1	:	Base of the antennal flagellum
3.	DM2	:	Posterior dorsal median edge of carapace
4.	VM2	:	Posterior ventral corner of the carapace
5.	DM3	:	Posterior dorsal edge of the tergum of the first abdominal segment
6.	VM3	:	Mid-ventral point of the first abdominal segment
7.	DM4	:	Posterior-dorsal edge of tergum of the third abdominal segment
8.	VM4	:	Mid-ventral point of the third abdominal segment
9.	DM5	:	Posterior-dorsal edge of tergum of the fifth segment
10.	VM5	:	Mid-ventral point of the fifth abdominal segment
11.	DM6	:	Posterior-dorsal edge of tergum of the sixth abdominal segment.
12.	VM6	:	Posterior-ventral edge of the sixth abdominal segment

(DM : Dorsal measurement ; VM : Ventral measurement)

PLATE – 3

(a) Zymogram patterns of AO enzyme in abdominal muscle tissue in *P.monodon*

AO-1 100/100* : *Homozygote*

AO-1 100/110* : *Heterozygote*

AO-2 50/100* : *Heterozygote*

AO-2 100/100* : *Homozygote*

(b) Zymogram patterns of FBALD enzyme in abdominal muscle tissue in *P.monodon*

FBALD-1 100/100:* *Homozygote*

FBALD-1 100/111:* *Heterozygote*

Table 6 List of enzymes tested in *P. monodon* of South India

Name of enzymes	Enzyme number (IUBNC 1984)*	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**
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	**mIDHP**
L – Iditol dehydrogenase	1.1.1.14	**sIDDH**
Malate dehydrogenase	1.1.1.37	**sMDH**
Octanol dehydrogenase	1.1.1.73	**ODH**

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

Though 14 enzyme systems were tested, only 7 (AK, AO, EST, FBALD, αGPDH, sMDH and ODH) could be detected by specific enzyme activity in *Penaeus monodon*. (Table 6). The rest showed poor (GDH), or no activity (ACP) or inconsistent activity (mIDHP) or poor resolution (G6PDH). sIDDH, ADH, HK and AK showed same zymogram pattern. The enzymes EST, FBALD and ODH were single locus enzymes. The AK, AO, αGPDH and sMDH-1 had two loci each.

The enzyme loci, AK-1, AK-2, AO-1, AO-2, EST, FBALD, αGPDH-1, αGPDH-2, sMDH-1 and ODH were polymorphic in all the tested samples. The details of the results under each category are described below.

a) <u>Polymorphic enzymes</u>

1. FBALD (Fructose-biphosphate aldolase)

The enzyme showed three zones of enzyme activity - the fastest moving zone (bluish red) alone showed consistent banding patterns. The other two zones were inconsistent and hence were discarded from further analyses. The first zone (locus) produced two and single banded polymorphic phenotypes. The alleles, *FBALD-1*100* and *FBALD-1*111* accounted for the two banded heterozygous phenotype (100/111) and allele *FBALD-1*100* for the single banded homozygous phenotype (100/100). FBALD is monomeric in structure in *P. monodon* (Fig. 6 and Plate 3b).

2. AO (Aldehyde oxidase)

Though the enzyme showed three zones of enzyme activity only two zones showed consistent banding patterns. The fastest moving zone (AO-1 locus) appeared as bluish red. Two and single banded polymorphic phenotypes were observed at this first zone. The allele *AO-1*100/110* accounted for the heterozygous phenotype and allele AO-1 for homozygous phenotype (100/100), at the first locus. The alleles *AO-2*50* and *AO-*

from samples of other stations though there is mixing up of samples. Further analysis was attempted by shearing the principal components of all the size samples. The sheared PC analysis was then carried out. The first two sheared principal components accounted for 89.15% of the total variation in the data. The sheared PC scores were then computed and plotted for the samples from these six stations. There was no separate cluster formation in the plot of sheared PC scores and hence the morphometrics of the samples from the six stations were not significantly different.

4.2 Biochemical Genetics

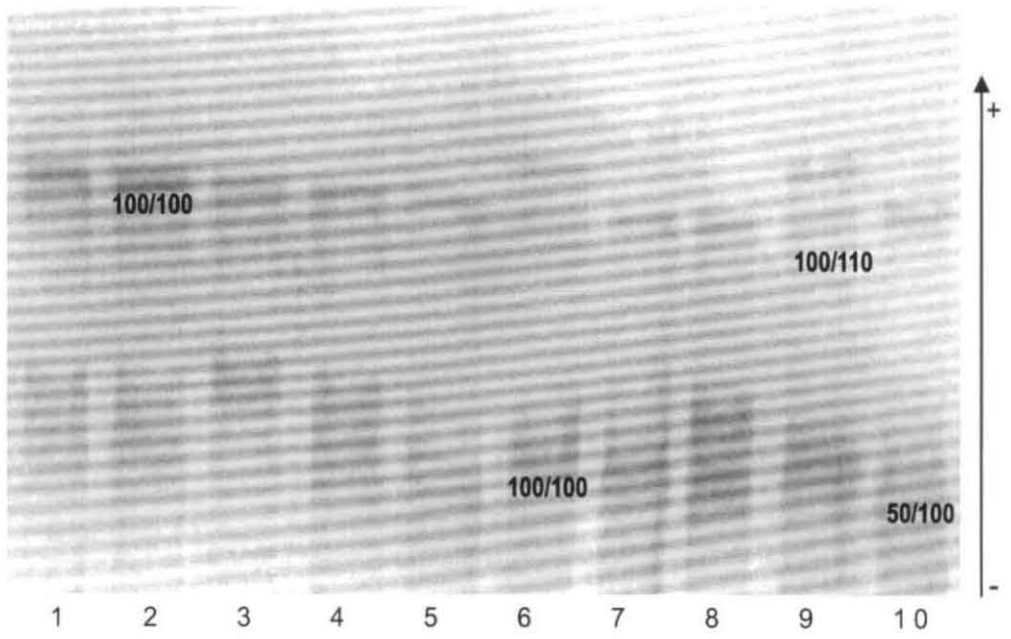
4.2.1 Standardisation of Methodology

The discontinuous buffer system was found suitable for the electrophoretic analysis of proteins and allozymes. The system composed of tris-glycine (0.2M), pH 8.3 (electrode buffer). 1.8M Tris-HCl, pH 8.9 as separating gel and 0.5M Tris-HCl, ph 6.8, as stacking gel buffer produced better resolution of the banding patterns. Maximum number of bands with better resolution and separation were produced when the tissues were homogenized in chilled double distilled water in the ratio 1g tissue : 1ml double distilled water. Muscle tissue produced the optimal banding patterns. The hepatopancreas was not giving consistent banding pattern probably due to storage effect. The eye lens did not show any variation in the banding pattern. Hence, only muscle tissue was used for the analysis of the biochemical genetics of the species. Tris-Glycine (electrode buffer) and Tris-HCl (gel buffer) buffer systems also produced the best resolution of the six polymorphic enzymes. However, the gel percentages required for different enzymes varied and the optimal gel percentage for each enzyme was found out empirically. 10% gel for ODH, EST, sMDH and General protein, 8% for AO, FBALD, αGPDH and 7.5% for AK were selected for electrophoresis.

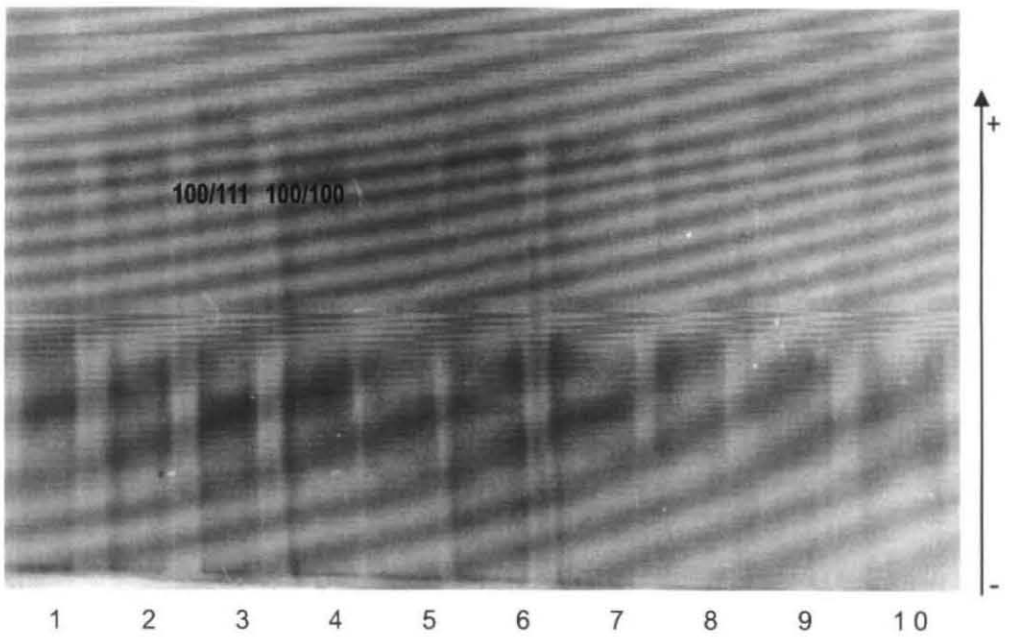
Table 5 Percentages of principal component analysis in *P. monodon* of South India

PC#	Eigen Value	Percentage	Cum. Percentage
1	342.8323	85.80	85.80
2	13.3903	3.35	89.15
3	6.2296	1.56	90.71
4	5.7940	1.45	92.16
5	4.7872	1.20	93.36
6	4.0971	1.03	94.38
7	3.7836	0.95	95.33
8	3.2311	0.81	96.14
9	2.8502	0.71	96.85
10	2.2864	0.57	97.42
11	1.9486	0.49	97.91
12	1.7772	0.44	98.36
13	1.4752	0.37	98.73
14	1.2093	0.30	99.03
15	0.8541	0.21	99.24
16	0.7057	0.18	99.42
17	0.6435	0.16	99.58
18	0.6064	0.15	99.73
19	0.4010	0.10	99.83
20	0.3214	0.08	99.91
21	0.2418	0.06	99.97
22	0.0402	0.01	99.98
23	0.0377	0.01	99.99
24	0.0158	0.00	100.00
25	0.0129	0.00	100.00
26	0.0032	0.00	100.00

PLATE 3



(a) Aldehyde oxidase



(b) Fructose Biphosphate Aldolose

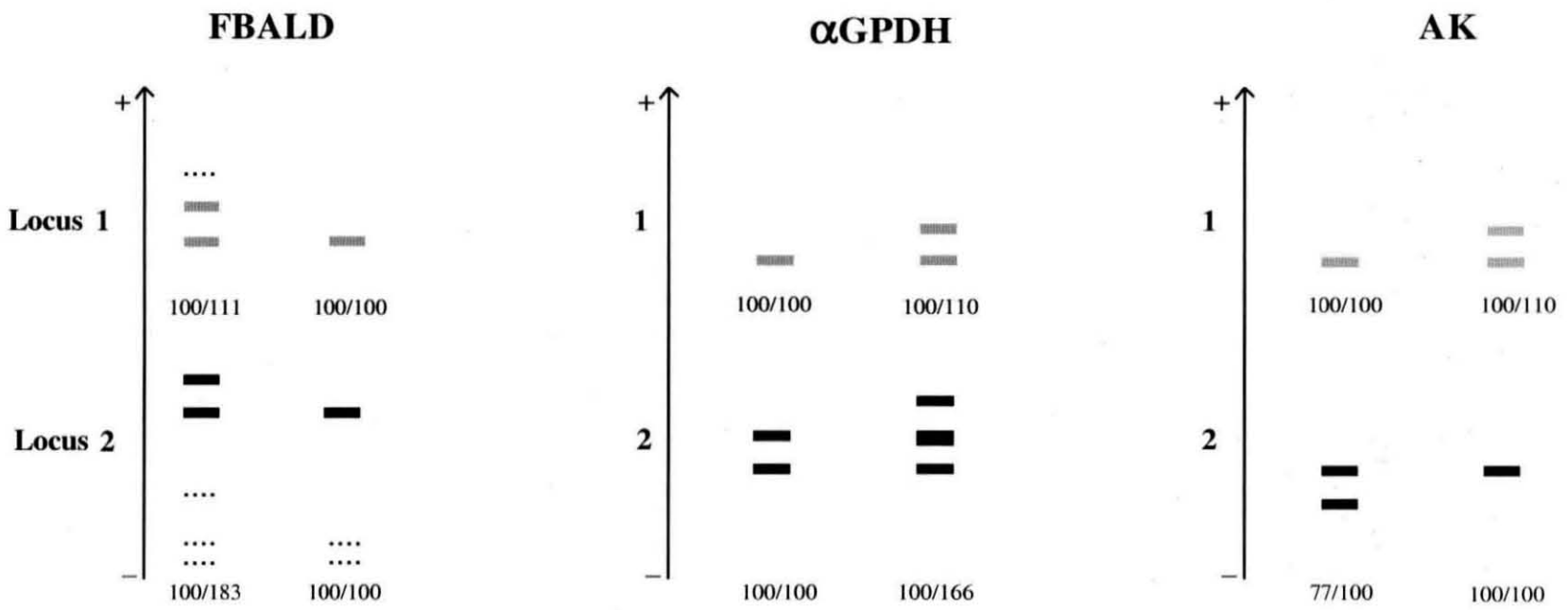


Fig. 6 Zymogram patterns of FBALD, αGPDH, AK in muscle tissue of *P. monodon* of South India

*2*100* accounted for the heterozygous phenotype (50/100). The allele *AO-2*100* produced the homozygous phenotype (100/100) is second locus. The third zone with inconsistent banding patterns were not scored. The first zone also showed minor bands in some individuals. The enzyme is monomeric in *P. monodon* (Fig. 8 and Plate 3a).

3. αGPDH (Glycerol-3-phosphate dehydrogenase)

It also showed three zones of enzyme activity, but only two zones were scored. The fastest moving bluish red zone produced single and double banded phenotypes. The alleles *αGPDH-1*100* and *αGPDH-1*110* produced the two banded heterozygous and the allele *αGPDH-1*100* represented the single banded and for homozygotes (100/100). Interestingly the second zone produced double-banded homozygotes and triple-banded heterozygotes. The alleles that produced two and three banded phenotypes here were *αGPDH-2*100* and *αGPDH-2*166*. The second band of the two banded homozygote (100/100) had migration rate equal to that of the middle band of the three banded heterozygote. The αGPDH enzyme is dimer in *P. monodon* (Fig. 6 and Plate 4a).

4. EST (Esterase)

The enzyme esterase also expressed three zones of activity (Fig. 8 and Plate 2c). The fastest and slowest moving bands were formed of inconsistent bands which were not scored. The banding pattern in the middle zone only were scored. Though, minor bands appeared at the cathodal and anodal ends, they were also not considered for the present study. Its patterns consisted of single banded homozygotes and double banded heterozygotes. The alleles *EST-1*100* and *EST-1*117* produced heterozygous phenotype. The homozygous was formed of *EST-1*100* allele. Esterase is monomeric in *P. monodon*.

PLATE – 4

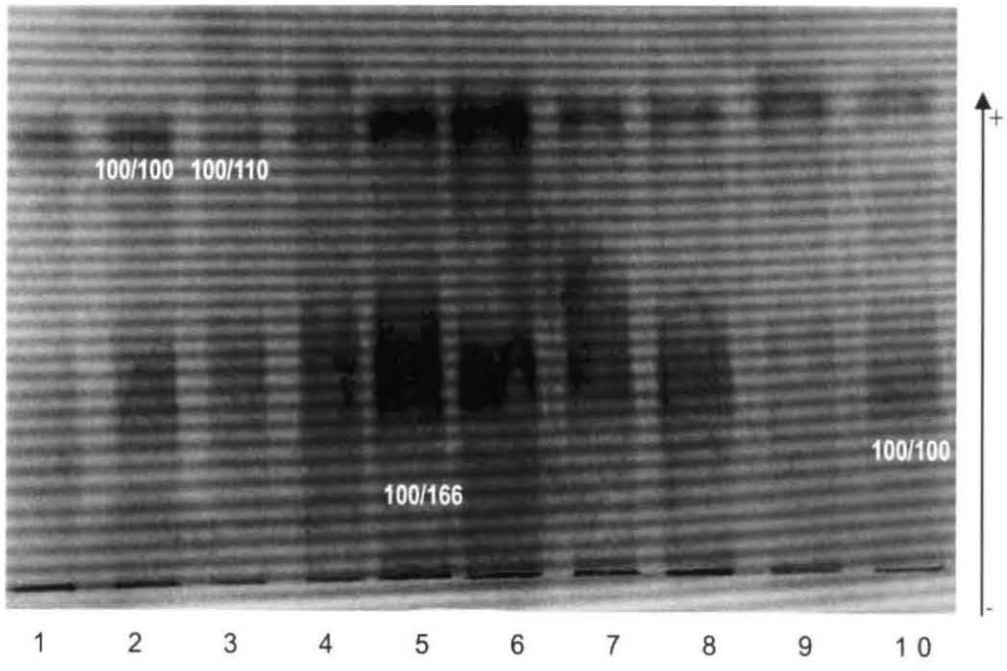
(a) Zymogram patterns of αGPDH enzyme in abdominal muscle tissue in *P. monodon*

αGPDH -1 100/100*	:	*Homozygote*
αGPDH -1 100/110*	:	*Heterozygote*
αGPDH -2 100/100*	:	*Homozygote*
αGPDH-2 100/166*	:	*Hetrozygote*

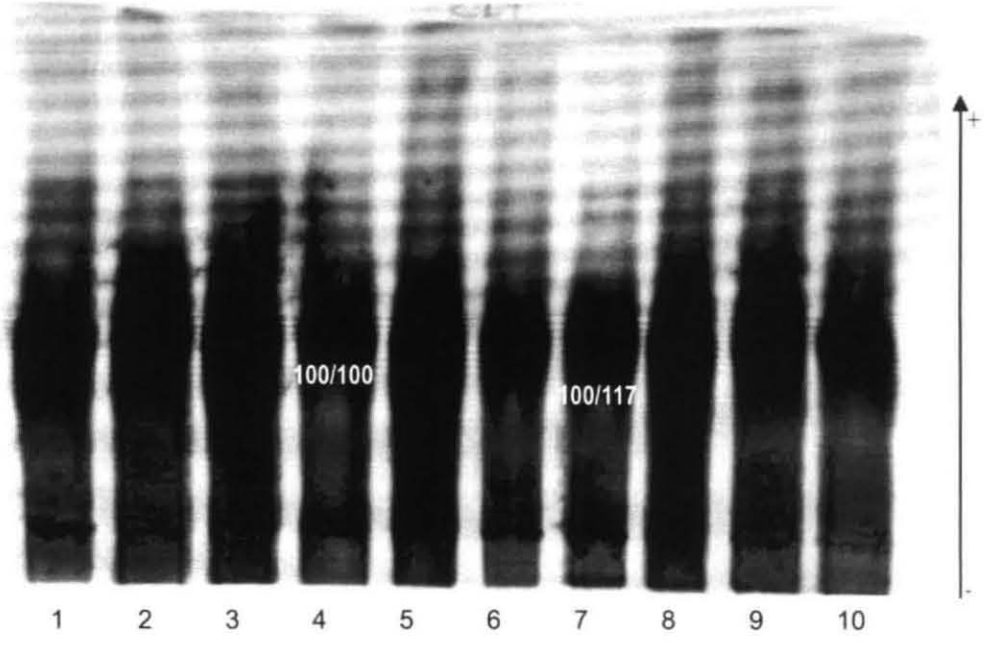
(b) Zymogram patterns of General Protein in abdominal muscle tissue in *P. monodon*

*PROT–1 to 5 & 7*100/100*	:	*Homozygote*
*PROT-6*100/100*	:	*Homozygote*
*PROT – 6*100/117*	:	*Heterozygote*

PLATE 4



(a) Glycerol-3-phosphate dehydrogenase



(b) General Protein

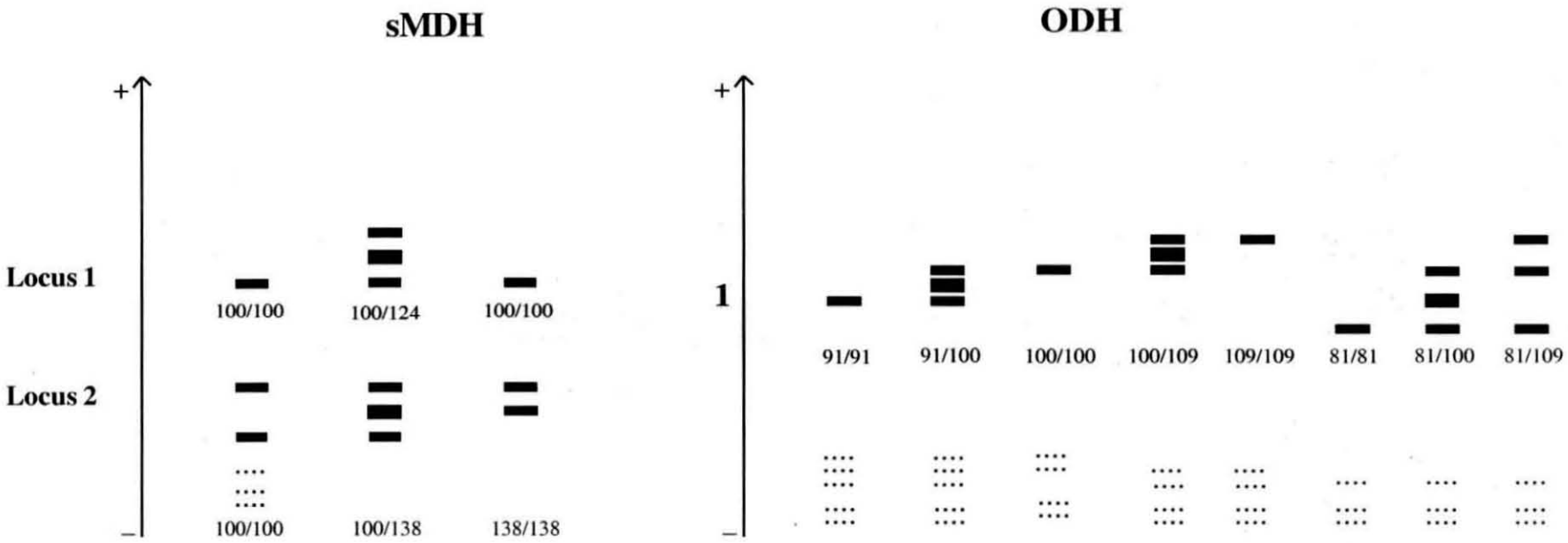


Fig.7 Zymogram patterns of sMDH, ODH in muscle tissue of *P. monodon* of South India

5. sMDH (Malate dehydrogenase)

It also showed three zones of enzyme activity. However, the fastest and slowest moving zones produced only inconsistent patterns in some samples. Hence, these two zones were not scored. The middle zone showed one and three banded consistent phenotypes which were scored. The alleles *sMDH-1*100* and *sMDH-1*124* produced the homozygote, 100/100 and the heterozygote 100/124 patterns. The enzyme is appeared in some specimens at the cathodal end (Fig. 7 and Plate 2b).

6. ODH (Octanol dehydrogenase)

The ODH enzyme showed only one major zone of activity in *P. monodon*. Eight different phenotypes were expressed by the enzyme (Fig. 7 and Plate 1 & 2a). Four of these (81/100, 81/109, 91/100, 100/109) were three banded and others were single banded. The alleles responsible for the eight phenotypes were *ODH-1*81,*91,*100* and **109*. The observed three banded phenotypes were (81/81), (81/100), (91/91), (91/100), (100/100) and (100/109). *ODH*100* allele accounted for homozygous phenotype (100/100). ODH showed dimeric pattern in *P. monodon*. The slowest moving minor bands also formed inconsistant phenotypes.

7. AK (Adenylate kinase)

The enzyme resolved into two zones. The fast moving zone was scored as locus-1 and the slow moving zone as locus-2. Both the zones expressed one and two banded polymorphic phenotypes. Single banded phenotype at the first locus were formed by the allele *AK-1*100* (100/100) and two banded by the alleles (100/110).

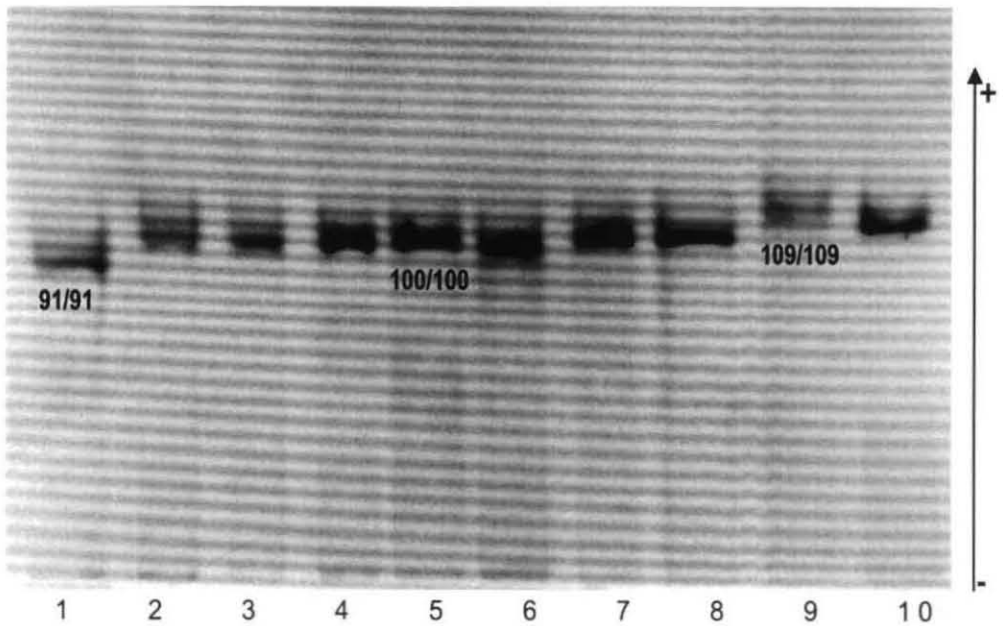
The second locus also having single banded and double banded phenotypes which were homozygous and heterozygous respectively. The

PLATE – 1

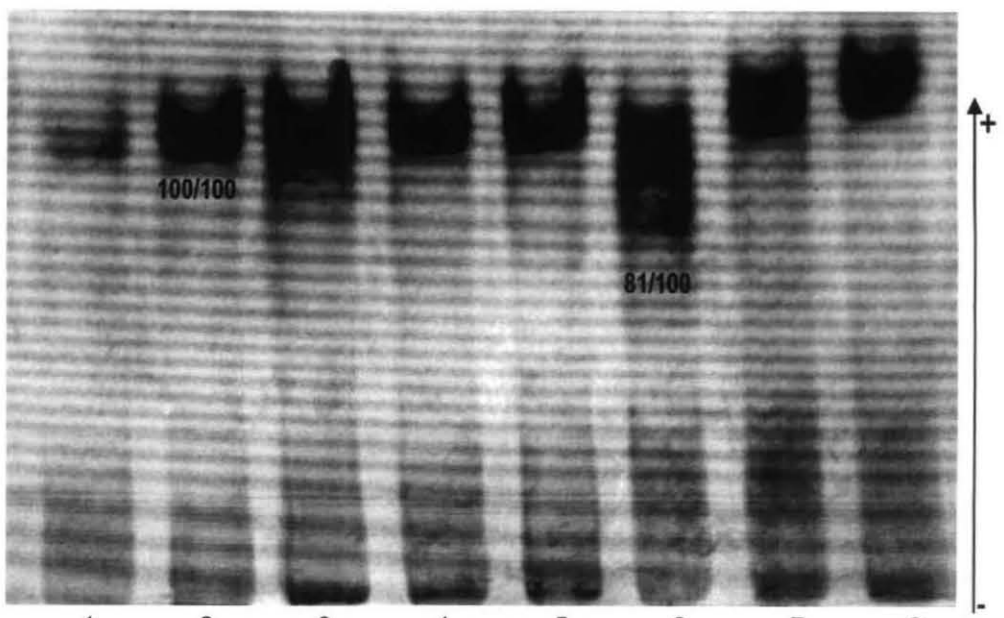
Zymogram patterns of ODH enzyme in abdominal muscle tissue in *P. monodon*

ODH-1 81/100*	:	*Heterozygote*
ODH-1 81/109*	:	*Heterozygote*
ODH-1 91/91*	:	*Homozygote*
ODH-1 91/100*	:	*Heterozygote*
ODH-1 100/100*	:	*Homozygote*
ODH-1 109/109*	:	*Homozygote*

PLATE I



1 2 3 4 5 6 7 8 9 10



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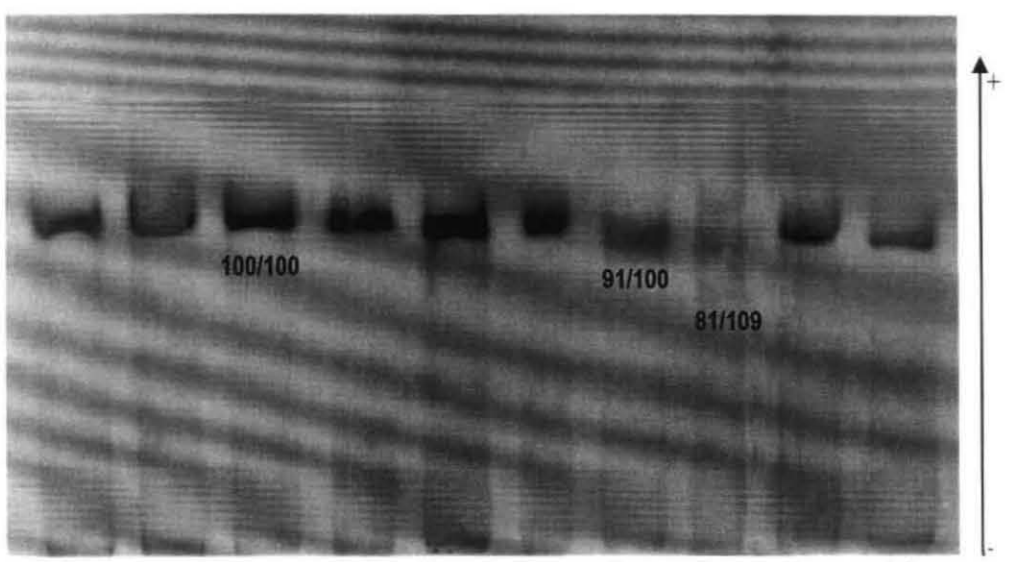


1 2 3 4 5 6 7 8 9 10

Octanol dehydrogenase

PLATE – 2

(a) Zymogram patterns of ODH enzyme in abdominal muscle tissue in *P. monodon*

ODH-1 81/81* : *Homozygote*

ODH-1 100/100* : *Homozygote*

(b) Zymogram patterns of MDH enzyme in abdominal muscle tissue in *P.monodon*

sMDH-1 100/100* : *Homozygote*

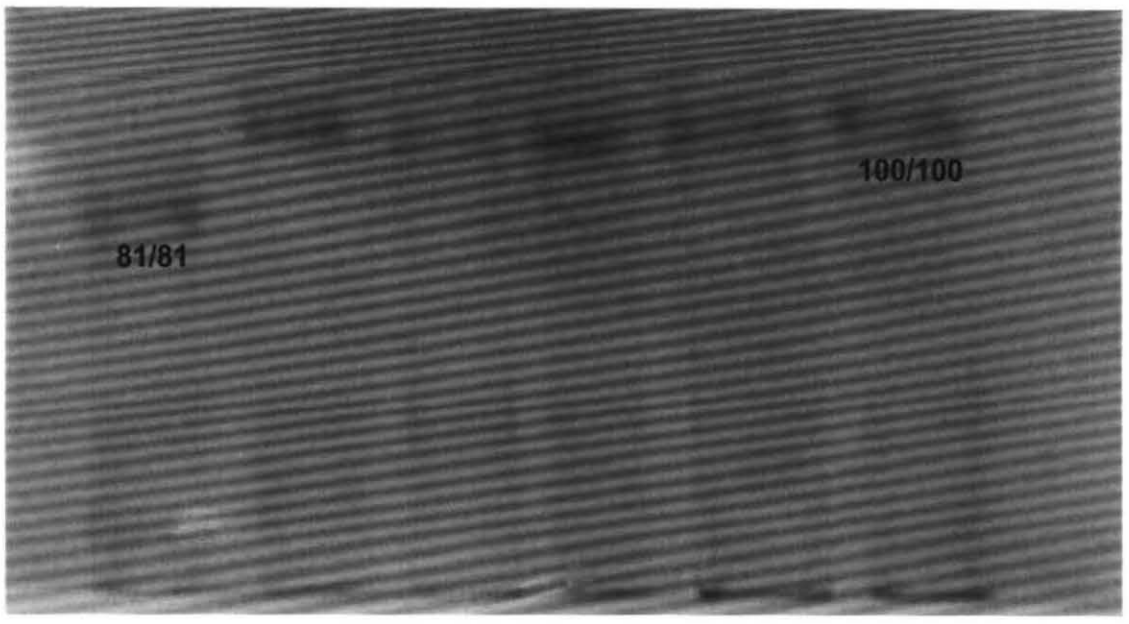
sMDH-1 100/124* : *Heterozygote*

(c) Zymogram patterns of Esterase enzyme in abdominal muscle tissue in *P.monodon*

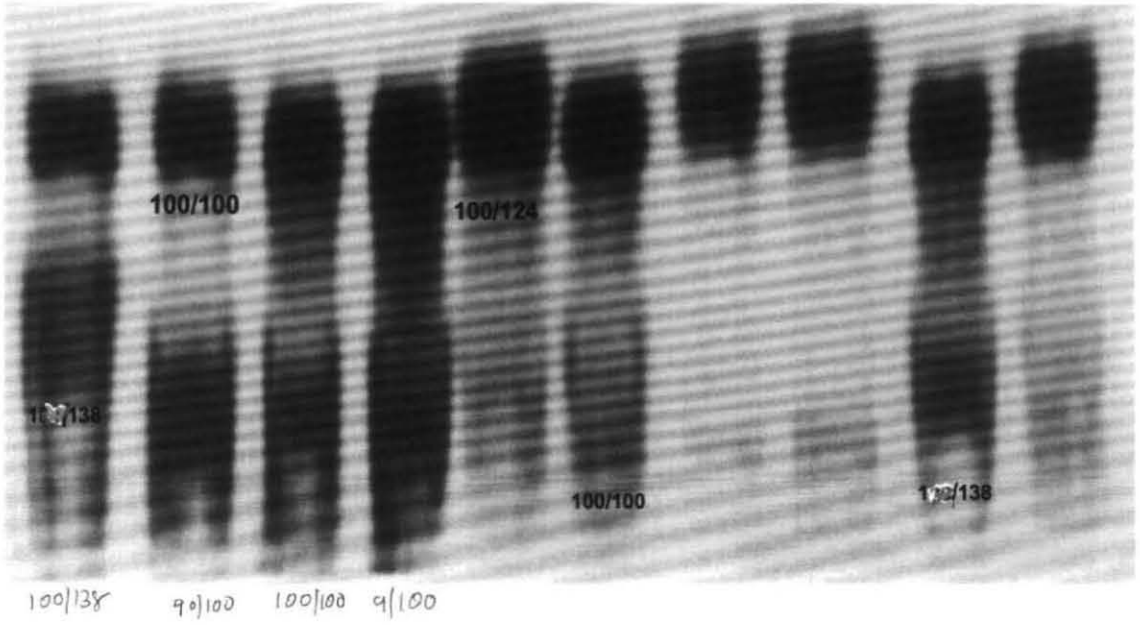
EST-1 100/100* : *Homozygote*

EST-1 100/117* : *Heterozygote*

PLATE 2

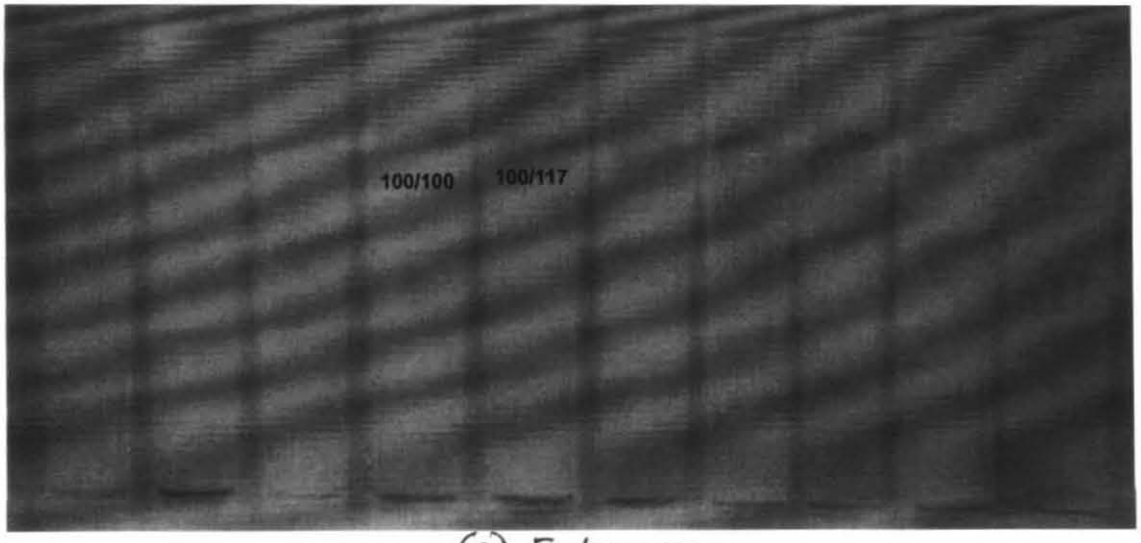


(a) Octanol dehydrogenase



100/138 90/100 100/100 9/100

(b) Malate dehydrogenase



(c) Esterase

(C) Esterase

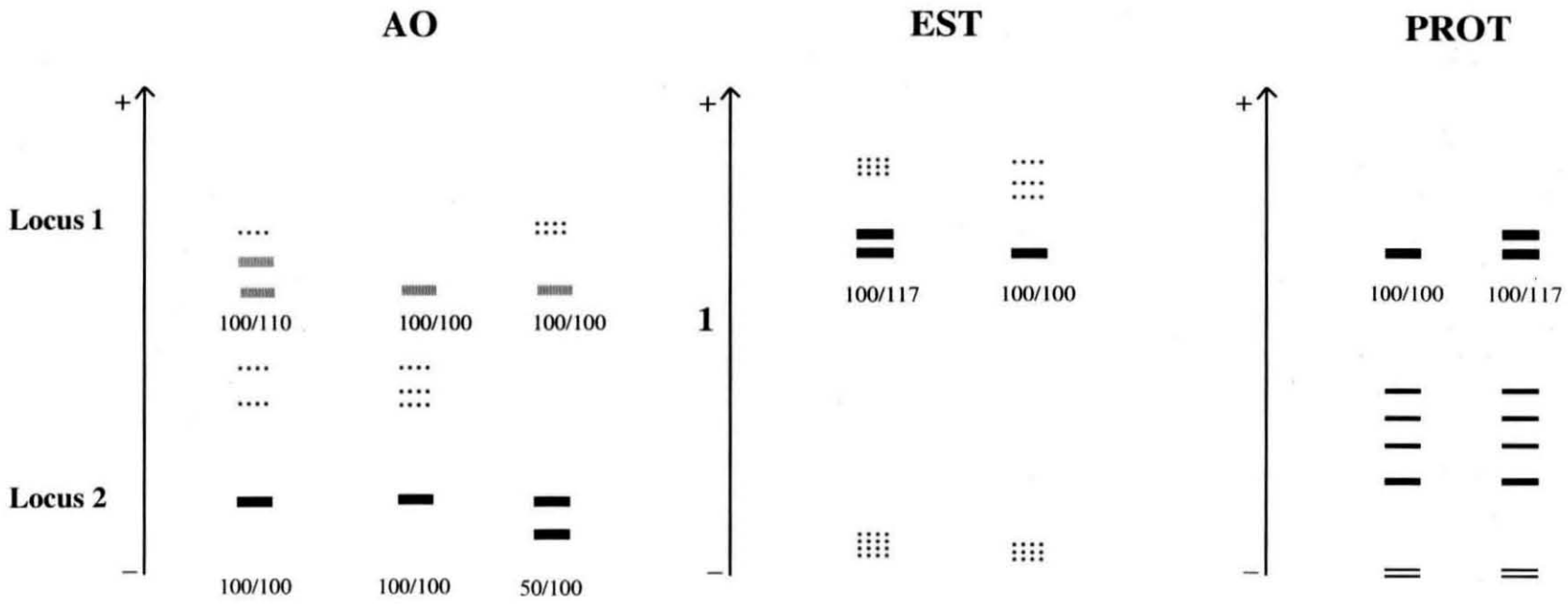


Fig.8 Zymogram patterns of AO, EST, PROT in muscle tissue of *P. monodon* of South India

Table 7 Enzyme loci examined for genetic variation in the populations of *P. monodon*

Loci	Enzyme number	Polymorphism	No.of alleles
FBALD-1	4.1.2.13	Polymorphic	2
AO-1	1.2.3.1	Polymorphic	2
AO-2		Polymorphic	2
αGPDH-1	1.1.1.8	Polymorphic	2
αGPDH-2		Polymorphic	2
EST-1	3.1.1.-	Polymorphic	2
sMDH-1	1.1.1.37	Polymorphic	2
ODH-1	1.1.1.73	Polymorphic	4
AK-1	2.7.4.3	Polymorphic	2
AK-2		Polymorphic	2
PROTEIN			
1-5 & 7		Monomorphic	1
6		Polymorphic	2

alleles of locus-2 were *AK-2*100* and *AK-2*77* accounted for heterozygous phenotype (77/100) and *AK-2*100* for homozygous (100/100). The enzyme is monomeric in the species (Fig. 6).

b) Enzymes with homologous pattern

The zymogram patterns of the major zones identified as first and second loci in Adenylate kinase (AK), Hexokinase (HK), Alcohol dehydrogenase (ADH) and L-Iditol dehydrogenase (SDH) were homologous in their phenotypic expressions. Hence, only one of these (AK) was considered for screening in the sample populations.

c) Enzymes showing poor or no activity

The enzyme mIDHP (Isocitrate dehydrogenase) showed poor activity and resolution in TG buffer. The activity/resolution of G6PDH (Glucose- 6-phosphate dehydrogenase) and GLUDH (Glutamate dehydrogenase) was very poor in different buffers tried for their detection. The enzyme Acid phosphatase (ACP) could not be detected in any of the buffers tried.

d) General Protein (PROT)

The general proteins present in hepatopancreas, eyelens and abdominal muscle tissues of *P. monodon* were separated and resolved well in TG discontinuous system. The number and position of major and minor bands differed among all the tissues showing tissue-specific nature of the proteins (Fig. 8 and Plate 4b). All tissues showed about ten bands, which may be controlled by seven or more loci. Though hepatopancreas and eyelens showed best resolution of the bands, only abdominal muscle showed polymorphic phenotypes. Hence, general proteins were tested only in the abdominal muscle tissue.

4.3 Molecular Genetics (DNA)

4.3.1 RAPD profiles of total DNA

4.3.1a Polymerase chain reaction

To detect random amplified polymorphic DNA (RAPD), ten primers that are known to amplify the DNA were used. The primers OPA 1-10 amplified the total DNA of the species. However, only OPA-2 and OPA-4 revealed polymorphic DNA profiles in the samples tested. Hence, samples were analysed using only these two primers. The RAPD profiles revealed by these two primers are described below.

1. OPA 2

Twenty specimens, 10 each from Chennai and Kochi were screened with OPA 2 (Fig.9, 10 & Plate 6a, b). A total of 16 bands of different sizes were detected in the species (Table 12). Thus OPA 2 produced 16 RAPD fragments from Kochi and Chennai. The kilobases of the OPA-2 fragments considered for comparison varied from 0.1 to 1.9 kb (0.1, 0.125, 0.2, 0.56, 0.8, 0.9, 0.95, 1.0, 1.1, 1.15, 1.2, 1.3, 1.4, 1.5, 1.6, 1.9 kb). The significant aspects of the results are that the number of fragments present in the two samples of the species is significantly different. Eight RAPD fragments (0.1, 0.2, 0.8, 0.9, 0.95, 1.3, 1.9 Kb) produced by this primer were unique to Kochi population where as no fragments were unique to Chennai population (Table 14).

2. OPA 4

Twenty specimens, 10 each from Kochi and Chennai were screened with OPA 4. The total number of fragments or the bands produced OPA 4 was 15 (Fig. 11,12 and Plate 5a, b). A total of 15 fractions were produced by OPA 4 from Kochi and Chennai populations. The sizes of the fragments were 0.0025, 0.003, 0.005, 0.009, 0.1, 0.3, 0.125, 0.56, 0.8, 0.9, 0.95, 1.0, 1.1, 1.4 and 1.6 Kb size in Kochi and Chennai populations.

Table 14 The number and size (kb) of RAPD fractions detected in Kochi (West Coast) and Chennai (East Coast) samples of *P. monodon* of South India

A

Primer OPA – 2 fractions			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		Size	0.1	0.125	0.2	0.56	0.8	0.9	0.95	1.00	1.1	1.15	1.2	1.3	1.4	1.5	1.6	1.9
Sample	:	Kochi																
Sample	:	Chennai	-		-		-	-	-					-				-

B

Primer OPA – 4 fraction			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Size	0.0025	0.003	0.005	0.009	0.1	0.125	0.3	0.56	0.8	0.9	0.95	1.0	1.10	1.40	1.60
Sample	:	Kochi	-										-				
Sample	:	Chennai									-						-

'-' indicates that the fraction is absent in the sample and the blank columns without '-' indicate the fractions are present in the sample. The dash marks in one sample also imply that these fractions are unique to the other sample. Thus the fractions numbered 1, 3, 5, 6, 7, 12 & 16 are unique OPA-2 fractions to Kochi.

Similarly, the fractions 9, 15 are unique OPA-4 fractions to Kochi whereas fraction 1, 11 are unique OPA-4 fractions to Chennai.

Table 7 Enzyme loci examined for genetic variation in the populations of *P. monodon*

Loci	Enzyme number	Polymorphism	No.of alleles
FBALD-1	4.1.2.13	Polymorphic	2
AO-1	1.2.3.1	Polymorphic	2
AO-2		Polymorphic	2
αGPDH-1	1.1.1.8	Polymorphic	2
αGPDH-2		Polymorphic	2
EST-1	3.1.1.-	Polymorphic	2
sMDH-1	1.1.1.37	Polymorphic	2
ODH-1	1.1.1.73	Polymorphic	4
AK-1	2.7.4.3	Polymorphic	2
AK-2		Polymorphic	2
PROTEIN			
1-5 & 7		Monomorphic	1
6		Polymorphic	2

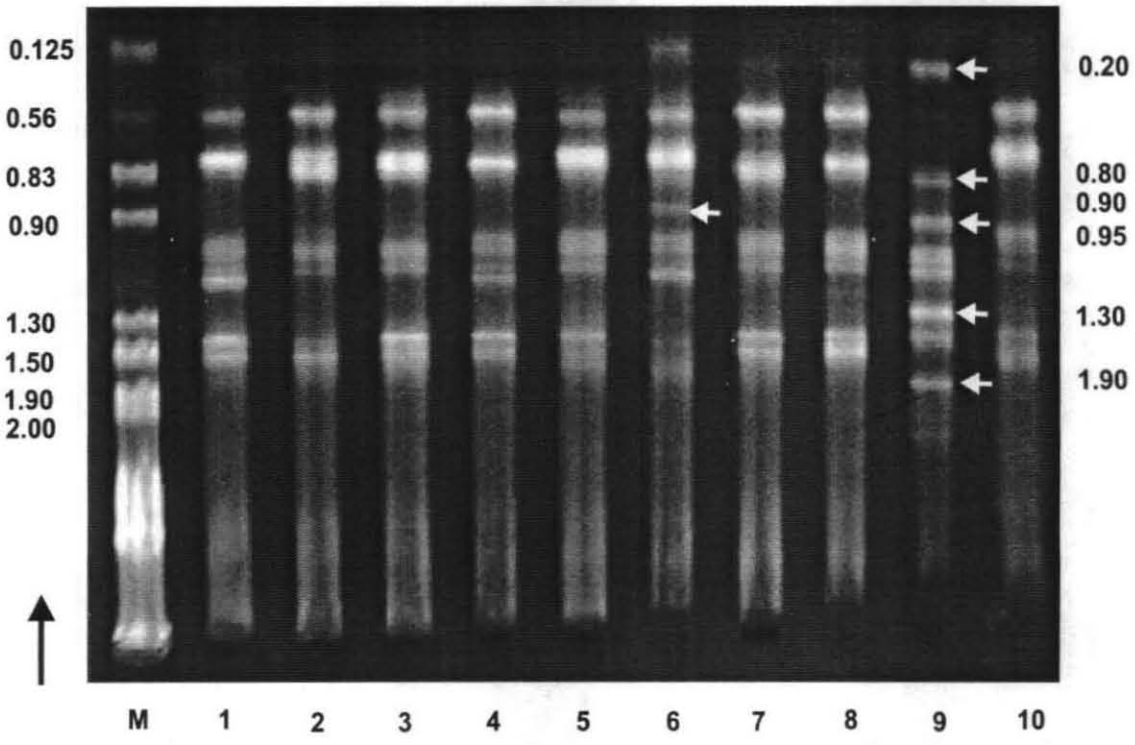


Fig.9 RAPD (OPA-2) banding pattern of *P. monodon* of South India

Columns 1 to 5 belong to specimens of Chennai
and 6 to 10 are that of Kochi.

Column **M** is the pattern of the Marker
(Lambda DNA Eco R1. Hind III Double Digest)
(Size: 0.125 to 2.0 kb)

→ **Shows unique bands in specimens from Kochi.**

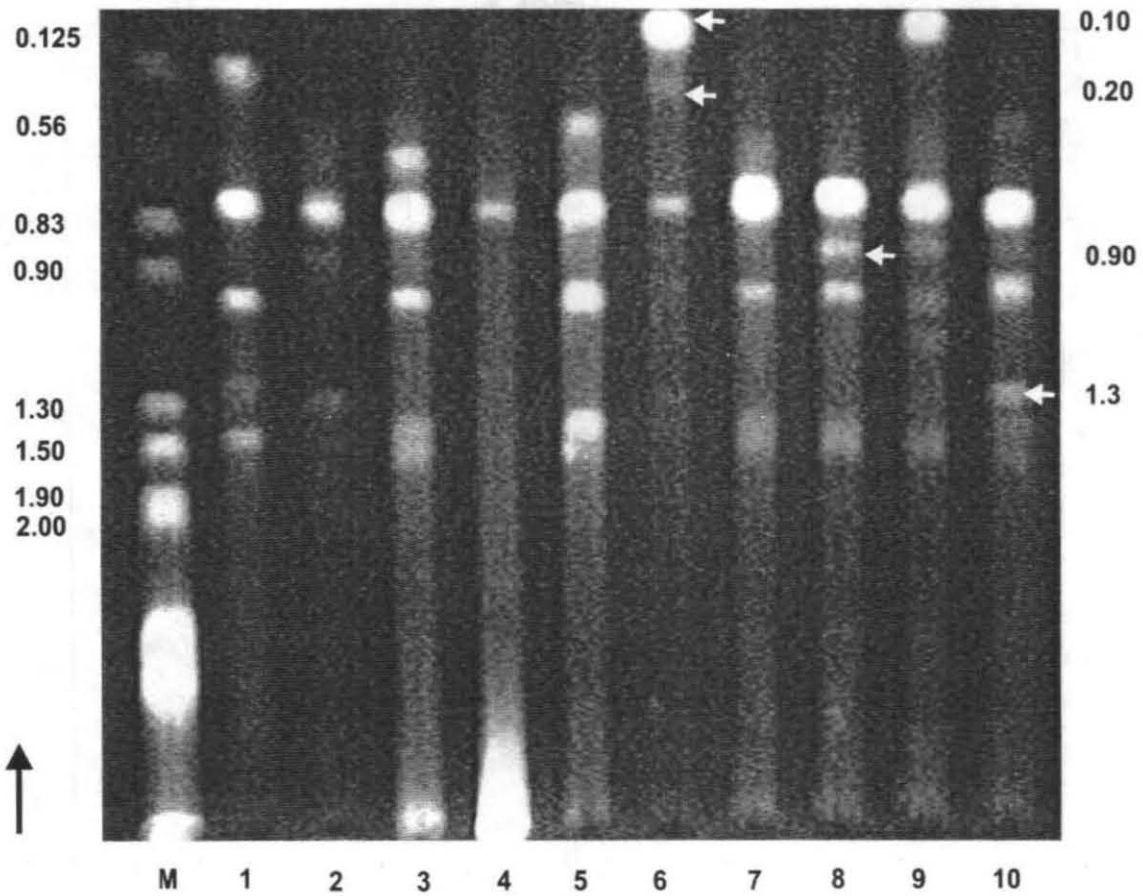


Fig.10 RAPD (OPA - 2) banding pattern of *P. monodon* of South India

Columns 1 to 5 belong to specimens of Chennai
and 6 to 10 are that of Kochi.

Column **M** is the pattern of the Marker
(Lambda DNA Eco R1. Hind III Double Digest)
(Size: 0.125 to 2.0 kb)

→ **Shows unique bands in specimens from Kochi.**

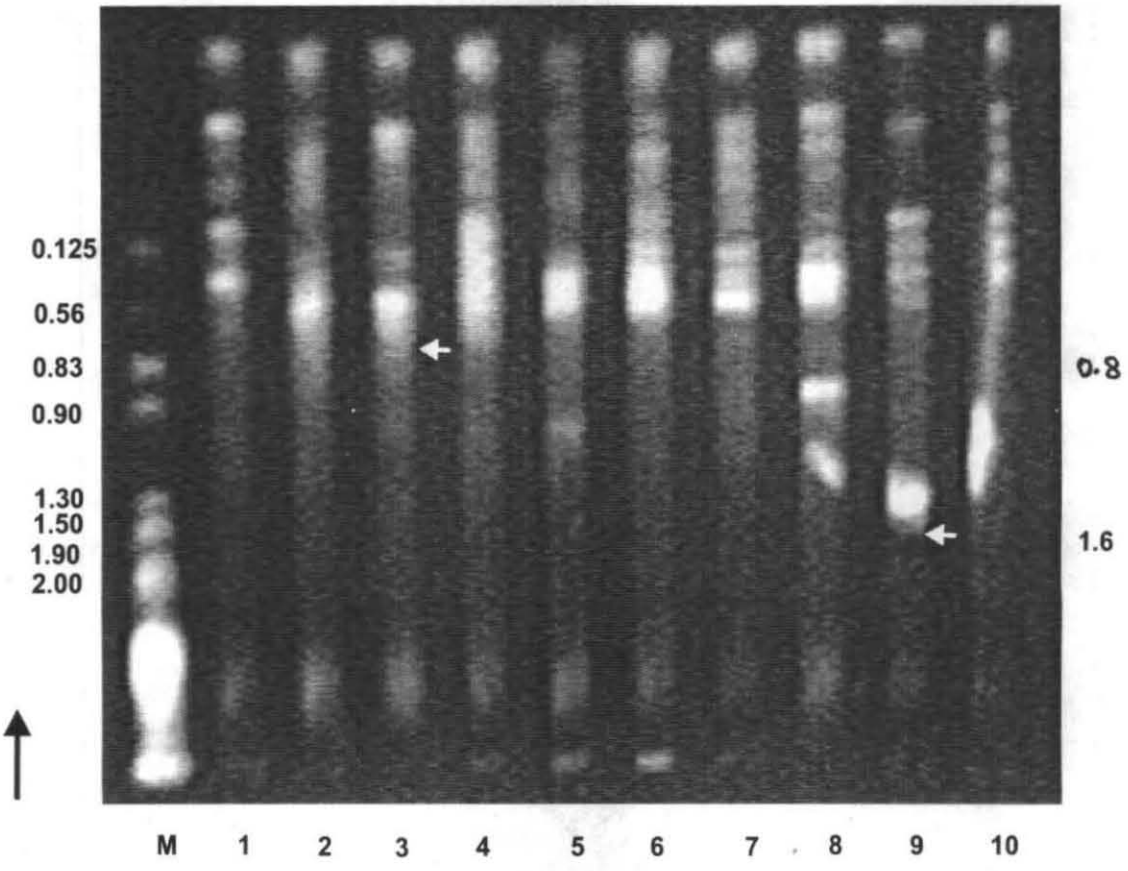


Fig.11 RAPD (OPA - 4) banding pattern of *P. monodon* of South India

Columns 1 to 10 belong to specimens of Kochi

Column **M** is the pattern of the Marker
(Lambda DNA Eco R1. Hind III Double Digest)
(Size: 0.125 to 2.0 kb)

→ Shows unique bands in specimens from Kochi.

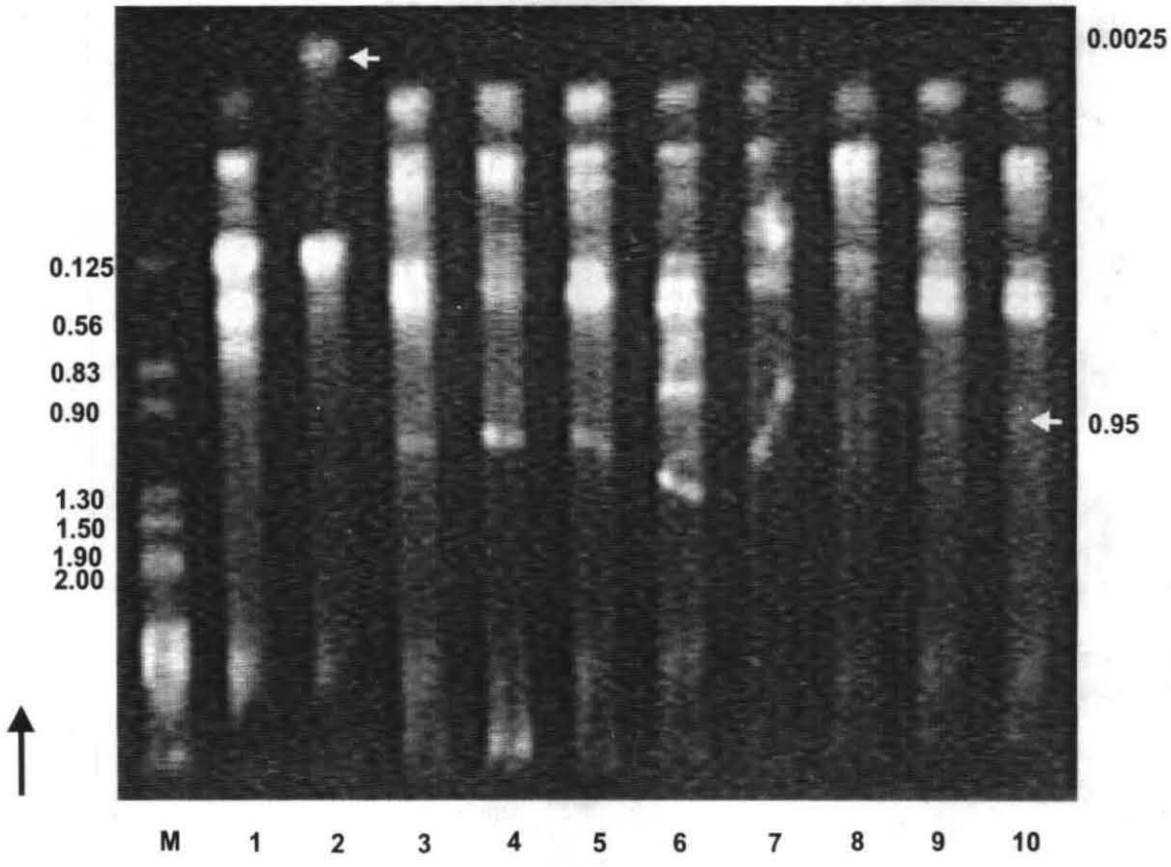


Fig.12 RAPD (OPA- 4) banding pattern of *P. monodon* of South India

Columns 1 to 10 belong to specimens of Chennai

Column **M** is the pattern of the Marker
(Lambda DNA Eco R1. Hind III Double Digest)
(Size: 0.125 to 2.0 kb)

→ **Shows unique bands in specimens from Chennai**

PLATE – 5

(a) RAPD (OPA-4) banding pattern of *P. monodon* from Chennai

Column M is the pattern of Marker (Eco R1. Hind III Double Digest)

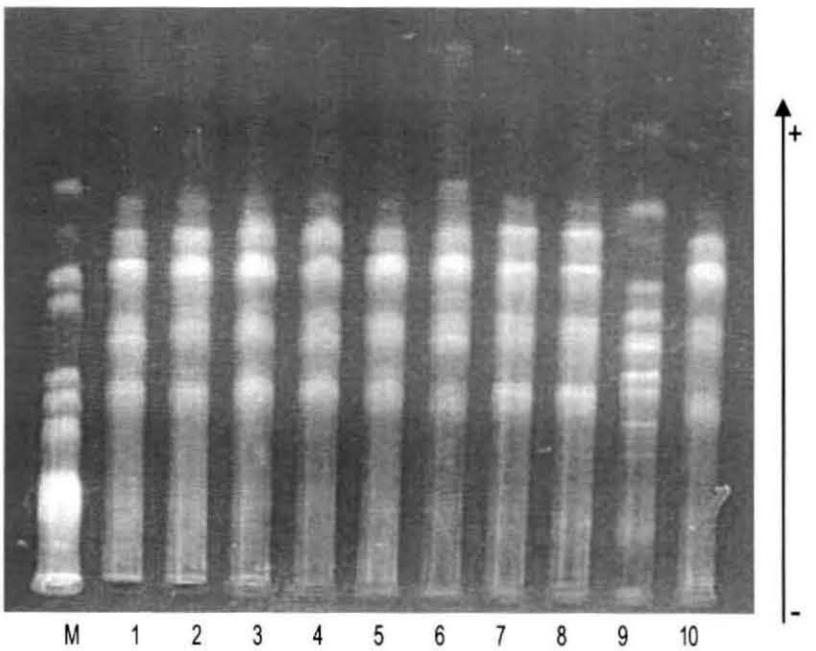
Lanes 1 to 10 : Chennai sample

(b) RAPD (OPA-4) banding pattern of *P. monodon* from Kochi

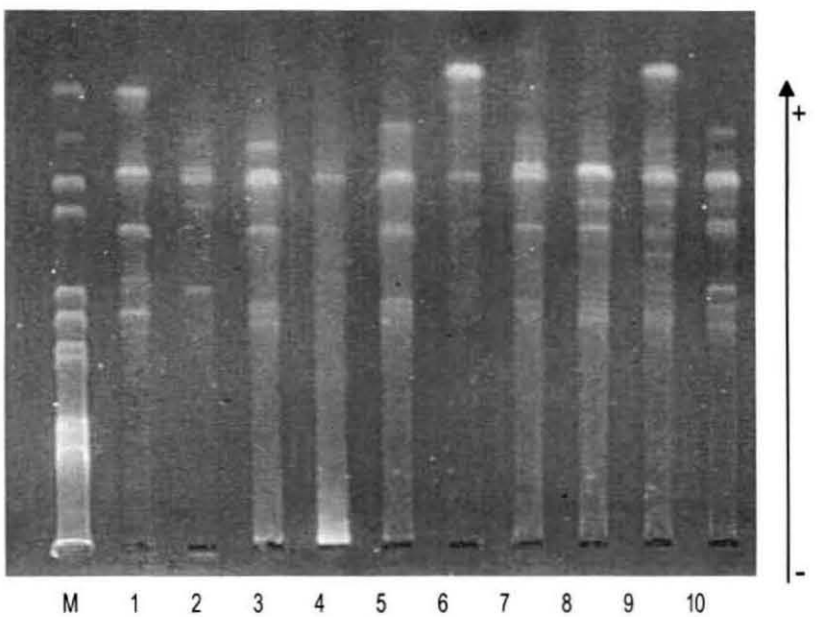
Column M is the pattern of Marker (Eco R1. Hind III Double Digest)

Lanes 1 to 10 : Kochi sample

PLATE 5



(a) RAPD (OPA-2) Banding pattern of *P. monodon* of Chennai and Kochi



(b) RAPD (OPA-2) Banding pattern of *P. monodon* of Chennai and Kochi

PLATE – 6

(a) RAPD (OPA-2) banding pattern of *P. monodon* from Chennai and Kochi

Column M is the pattern of Marker (Eco R1. Hind III Double Digest)

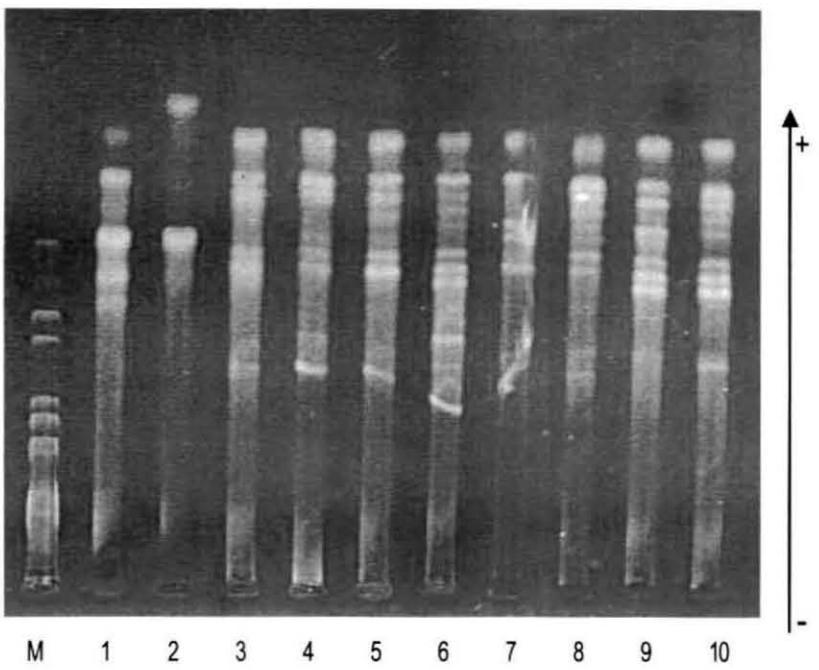
Column 1 to 5 belong to Chennai sample
and 6 to 10 are that of Kochi sample

(b) RAPD (OPA-2) banding pattern of *P. monodon* from Chennai and Kochi

Column M is the pattern of Marker (Eco R1. Hind III Double Digest)

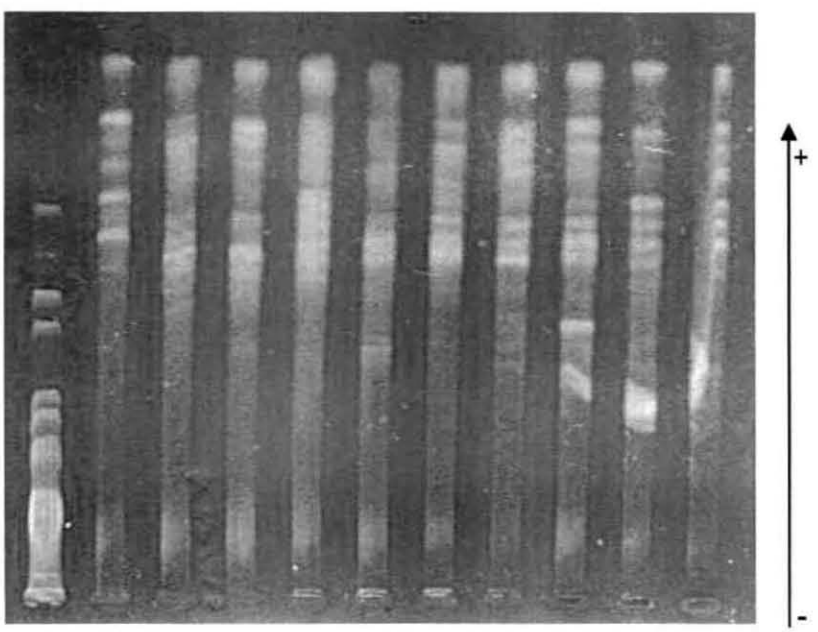
Column 1 to 5 belong to Chennai sample
and 6 to 10 are that of Kochi sample

PLATE 6



M 1 2 3 4 5 6 7 8 9 10

(a) RAPD (OPA-4) Banding pattern of *P. monodon* of Chennai



M 1 2 3 4 5 6 7 8 9 10

(b) RAPD (OPA-4) Banding pattern of *P. monodon* of Kochi

OPA 4 revealed two unique fragments each in both the sample populations. The two unique fragments in Kochi population (0.80, 1.6 Kb) whereas, the two unique fragments (0.0025, 0.95 Kb) were seen in Chennai (Table 14).

4.4 Analyses of the Data

4.4.1 Morphometrics

4.4.1a Correlation with tail weight

Ten metric and only one meristic variables were measured to detect variation among the random samples from different locations (Table 1). Since, six of the ten measurements involved the body extremities, the other four viz., sixth segment depth (SSD), second abdominal depth (SAD), partial carapace length (PCL) and carapace width (CW) were considered for the study. Among these four variables, PCL showed the highest correlation with the tail weight in both males (0.3097) and females (0.4881) of *P. monodon* from the six locations studied. From the path-coefficient analysis, it was also found that the partial carapace length (PCL) was the variable having highest correlation with the tail weight irrespective of sex (Table 3, 4).

4.4.1b Truss network analysis

Truss data of *P. monodon* from six centres (Karwar, Mangalore, Calicut, Kochi, Chennai and Kakinada) collected during 1996-2001 were used for the analysis. The 26 truss measurements (Fig. 3 and Table 2) made on each sample specimen of *P. monodon* from Karwar, Mangalore, Calicut, Kochi, Chennai and Kakinada were log transformed and subjected to principal component analysis. The first principal component accounted for 85.80% and the second accounted for 3.35% (Table 5) of the total variations in the truss data. These two principal components accounted for 89.15% of the variations in truss measurements data and were used to

explain the variations. The PC-I and PC-II scores were computed for each of the samples and PC-I scores were plotted against PC-II scores to see morphometric changes between centres. From the plot it was found that samples from Mangalore formed a separate cluster from samples of other localities though there is mixing up of samples. Further analysis was attempted by shearing the principal components of all the six samples. The sheared PC analysis was then carried out. The first two sheared principal components accounted for 89.15% of the total variations in the data. The sheared PC scores were then computed and plotted for the samples from these six centres. There was no separate cluster formation in the plot of sheared PC scores and hence the morphometrics of the samples from the six centres were not significantly different.

4.4.2 Biochemical Genetics

The zymograms of *P. monodon* revealed 11 polymorphic loci (AK-1, AK-2, AO-1, AO-2, EST-1, FBALD-1, αGPDH-1, αGPDH-2, sMDH-1, ODH, PROT-6) in all the samples tested. The frequencies of the observed genotypes (expected genotypes) and that of 22 alleles at the ten loci and eight alleles at the seven general protein loci were estimated in all the six sample populations tested (Table 11).

4.4.2.1 Allele frequencies

The frequencies of twenty alleles at ten enzyme loci and eight alleles at seven general protein loci were closely similar in all the six populations tested except at AK-2 locus between Kochi (1.00) and Chennai (0.67) (Table 10). The AK enzyme was not tested in other four populations. The enzymes that could not be tested in same populations were αGPDH (Mangalore) and EST (Karwar, Kakinada). Only one (PROT-6) of the seven general protein loci (PROT-1 to 7) was polymorphic from

Table 10 Allele frequencies of isozymes in *P.monodon* of South India

Locus	Allele	Kochi	Calicut	Mangalore	Karwar	Chennai	Kakinada
FBALD-1							
(N)		(40)	(20)	(10)	(19)	(60)	(30)
	100	0.93	0.90	0.90	0.87	0.92	0.85
	111	0.07	0.10	0.10	0.13	0.08	0.15
AO-1							
(N)		(40)	(20)	(10)	(19)	(60)	(30)
	100	0.95	0.90	1.00	0.87	0.92	0.85
	110	0.05	0.10		0.13	0.08	0.15
AO-2							
(N)		(40)	(20)	(10)	(19)	(60)	(30)
	50	0.01	0.05			0.20	0.03
	100	0.99	0.95	1.00	1.00	0.80	0.97
αGPDH-1							
(N)		(20)	(20)	0	(19)	(10)	(30)
	100	0.90	0.90	NT	0.87	0.90	0.85
	110	0.10	0.10		0.13	0.10	0.15
αGPDH-2							
(N)		(20)	(20)	0	(19)	(10)	(30)
	100	1.00	0.98	NT	0.82	0.95	1.00
	166		0.02		0.18	0.05	
EST-1							
(N)		(10)	(20)	(10)	(0)	(40)	(0)
	100	1.00	0.90	1.00	NT	0.95	NT
	117		0.10			0.05	
sMDH-1							
(N)		(30)	(20)	(10)	(19)	(50)	(30)
	100	1.00	1.00	1.00	1.00	0.99	1.00
	124					0.01	
ODH-1							
(N)		(40)	(20)	(10)	(19)	(58)	(30)
	81	0.00			0.03	0.03	0.00
	91	0.03			0.08	0.00	0.07
	100	0.97	1.00	1.00	0.84	0.97	0.90
	109	0.00			0.05	0.00	0.03
AK-1							
(N)		(10)	(0)	(0)	(0)	(20)	(0)
	100	1.00	NT	NT	NT	0.95	NT
	110					0.05	
AK-2							
(N)		(10)	(0)	(0)	(0)	(20)	(0)
	77					0.33	
	100	1.00	NT	NT	NT	0.67	NT
PROTEIN							
(N)		(60)	(20)	(20)	(20)	(60)	(30)
1-5 & 7	100	1.00	1.00	1.00	1.00	1.00	1.00
6	100	1.00	0.95	1.00	1.00	0.98	1.00
	117		0.05			0.02	

NT = Not Tested

(N) = Number of samples

two centres (Calicut-West coast; Chennai-East coast) and the allele frequencies at PROT-6 was closely similar in all the six populations.

4.4.2.2 Hardy-Weinberg equilibrium

The significance of the differences between the observed and expected genotype frequencies were calculated by the chi-square method. The values of chi-square were not significantly different at fourty Eight comparisons made. Significant deviations were observed only at two loci ie., AK-2 locus in Chennai, ODH-1 locus in Chennai and Karwar at $P < 0.05$ level (Table 11). The observed and the expected genotype frequencies were found to be in Hardy-Weinberg equilibrium condition in the six populations compared and hence, the observed phenotype variations at all the loci were considered as genetic variations controlled by the respective loci detected. The observed genetic variations were thus used to estimate the genetic variability in each sample populations and thus in the species.

4.4.2.3 Genetic variability

The indices of overall genetic variability, like average number of allele per locus, effective number of alleles and heterozygosity were estimated and the values are given below.

4.4.2.3a Average number of alleles/locus

Table 18 shows the average of twenty two alleles detected at ten enzyme and seven general protein loci in six sample populations. The average number of alleles varied in the six populations from 1.16 to 2.00. The averages between east and west coast populations were 1.7 and 2.00 respectively. The genetic variability according to average number of alleles was higher in the east coast populations.

Table 11 Observed and expected (within paranthesis) genotype frequencies of isozymes in *P.monodon* from South India

Loci	Genotype	Kochi	χ^2	Calicut	χ^2	Mangalore	χ^2	Karwar	χ^2	Chennai	χ^2	Kakinada	χ^2
(N)		40		20		10		19		60		30	
FBALD-1	100/100	34(34.6)	0.3302	16(16.2)	0.2469	8(8.1)	0.1234	14(14.38)	0.4440	50(50.78)	0.5470	21(21.60)	0.8600
	100/111	6(5.21)		4(3.6)		2(1.8)		5(4.3)		10(8.83)		9(7.65)	
	111/111	0(0.2)		0(0.2)		0(0.1)		0(0.32)		0(0.38)		0(0.6)	
(N)		40		20		10		19		60		30	
AO-1	100/100	36(36.1)	0.1108	16(16.2)	0.2469	10(10)	0	14(14.38)	0.4440	50(50.78)	0.5470	21(21.60)	0.8600
	100/110	4(3.8)		4(3.6)				5(4.3)		10(8.83)		9(7.65)	
	110/110	0(0.1)		0(0.2)				0(0.32)		0(0.38)		0(0.6)	
AO-2	50/50	0(0.004)	0.0608	0(0.05)	0.0554					0(2.4)	3.7500	0(0.03)	0.0676
	50/100	1(0.79)		2(1.9)						24(19.2)		2(1.75)	
	100/100	39(39.2)		18(18.05)		10(10)	0	19(19)	0	36(38.4)		28(28.23)	
(N)		20		20		0		19		10		30	
αGPDH-1	100/100	16(16.2)	0.2469	16(16.2)	0.2469	NT		14(14.38)	0.4440	8(8.1)	0.1234	21(21.60)	0.8600
	100/110	4(3.6)		4(3.6)				5(4.3)		2(1.8)		9(7.65)	
	110/110	0(0.2)		0(0.2)				0(0.32)		0(0.1)		0(0.6)	
αGPDH-2	100/100	20(20)	0	19(19.2)	0.0741	NT		12(12.77)	1.0164	9(9.03)	0.0327	30(30)	0
	100/166			1(0.78)				7(5.6)		1(0.95)			
	166/166			0(0.01)				0(0.62)		0(0.03)			
(N)		10		20		10		0		40		0	
EST-1	100/100	10(10)	0	16(16.2)	0.2469	10(10)	0	NT		36(36.1)	0.1108	NT	
	100/117			4(3.6)						4(3.8)			
	117/117			0(0.2)						0(0.2)			
(N)		30		20		10		19		50		30	
sMDH-1	100/100	30(30)	0	20(20)	0	10(10)	0	19(19)	0	49(49.01)	0.0051	30(30)	0
	100/124									1(0.99)			
	124/124									0(0.005)			
(N)		40		20		10		19		58		30	
ODH-1	81/81									1(0.05)			
	81/100									1(3.38)			
	81/109							1(0.06)					
	91/91	0(0.04)						1(0.12)					
	91/100	3(2.33)						1(2.55)				4(3.78)	
	100/100	37(37.64)	0.2035	20(20)	0	10(10)	0	15(13.41)	41.96*	56(54.57)	19.7638*	24(24.3)	0.1356
	100/109							0(1.60)				2(1.62)	
	109/109							1(0.05)				0(0.03)	
(N)		10		0		0		0		20		0	
AK-1	100/100	10(10)	0	NT		NT		NT		18(18.05)	0.0554	NT	
	100/110									2(1.9)			
	110/110									0(0.05)			
AK-2	77/77									0(2.18)	4.5742*		
	77/100									13(8.84)			
	100/100	10(10)	0	NT		NT		NT		7(8.98)		NT	
PROTEIN													
(N)		60		20		20		20		60		30	
1-5 & 7	100/100	60(60)	0	20/20	0	20(20)	0	20(20)	0	60(60)	0	30(30)	0
6	100/100	60(60)	0	18(18.05)	0.0554	20(20)	0	20(20)	0	58(57.62)	0.0746	30(30)	0
	100/117			2(1.9)						2(2.35)			
				0(0.05)						0(0.02)			

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

4.4.2.3b Effective number of alleles

The effective number of alleles estimated for the six populations and between east and west coast populations are shown in the Table 18.

4.2.2.3c Heterozygosity

Heterozygosity values of *P. monodon* are shown in Table 18. The observed heterozygosity values varied from 0.05 to 0.22. The maximum value of heterozygosity was shown by Chennai (0.22) in east coast and Karwar (0.21). The expected values showed great variations from different locations (0.05-0.18). It ranged from 0.05 to 0.08 in west coast samples (Kochi, Calicut and Mangalore) but showed increase from Karwar (0.15). In the east coast, it was 0.09 (Kakinada) and 0.18 from Chennai (18%), thus showed great variation from these two centres. The mean expected heterozygosity value per individual was 0.103 and observed heterozygosity was 0.126.

To estimate the statistical significance of the differences of genetic variabilities and that of allele frequencies between sample populations and between east and west coast samples, these differences were subjected to F- analyses and Nei's (1972, 73) genetic similarity analyses for paired populations. The results of these analyses are shown below.

4.2.2.4 F_{ST} analysis

The F_{ST} values showed the levels of genetic differentiation among the populations compared (Table 12). 15 pairwise comparisons produced 83 F_{ST} values. Of these, 82 were negative, hence, the F_{ST} values were not at all significant between any populations or between east and west coast samples. The only insignificant positive F_{ST} value (0-0.05) was shown between Kochi and Chennai at AK-2 locus alone.

Table 12 F_{ST} **values for the pairwise comparisons of isozyme genetic variabilities in *P. monodon of South India***

	FBALD-1	AO-1	AO-2	αGPDH-1	αGPDH-2	ODH-1	sMDH-1	AK-1	AK-2
Kochi/Calicut	-0.01617	-0.01822	-0.01331	-0.02339	-0.01899	-0.00246	--	--	--
Kochi/Mangalore	-0.02803	-0.03057	-0.02419	--	--	-0.02259	--	--	--
Kochi/Karwar	-0.01640	-0.01720	-0.01561	-0.02313	-0.00567	-0.01612	--	--	--
Kochi/Chennai	-0.00696	-0.00911	-0.00119	-0.64312	-0.03887	-0.00411	-0.01295	-0.03067	**0.03708**[l]
Kochi/Kakinada	-0.01057	-0.01082	-0.01374	-0.01780	--	-0.01167	--	--	--
Calicut/Mangalore	-0.03587	-0.03473	-0.03301	--	--	--	--	--	--
Calicut/Karwar	-0.02234	-0.02326	-0.02958	-0.02313	-0.01528	-0.02298	--	--	--
Calicut/Chennai	-0.01458	-0.01457	-0.00624	-0.03587	-0.03163	-0.02384	-0.01787	--	--
Calicut/Kakinada	-0.01780	-0.01780	-0.03298	-0.01780	-0.01566	-0.01704	--	--	--
Mangalore/Karwar	-0.03426	-0.03227	--	--	--	-0.03395	--	--	--
Mangalore Chennai	-0.02690	-0.02398	-0.00994	--	--	-0.03895	-0.02911	--	--
Mangalore/Kakinada	-0.02886	-0.02418	-0.03485	--	--	-0.02913	--	--	--
Karwar/Chennai	-0.01392	-0.01413	-0.00387	-0.03504	-0.02982	-0.01244	-0.01720	--	--
Karwar/Kakinada	-0.01842	-0.01842	-0.02088	-0.01842	-0.01074	-0.02015	--	--	--
Kakinada/Chennai	-0.00964	-0.00926	-0.00292	-0.02901	-0.03132	-0.01344	--	--	--

-- 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 13 Mean genetic similarities (above the diagonal) and genetic distances (below the diagonal) based on isozyme allelic frequencies in *Penaeus monodon* of South India

	Kochi	Calicut	Mangalore	Karwar	Chennai	Kakinada
Kochi	----	0.9956	0.9949	0.9869	0.9816	0.9961
Calicut	0.0044	----	0.9967	0.9942	0.9971	0.9977
Mangalore	0.0051	0.0033	----	0.9953	0.9923	0.9946
Karwar	0.0131	0.0058	0.0047	----	0.9866	0.9935
Chennai	0.0184	0.0029	0.0077	0.0134	----	0.9915
Kakinada	0.0039	0.0023	0.0054	0.0065	0.0085	----

Table 18 Summary of Genetic Variation data in six populations of *P. monodon*

		Locations					
		Kochi	Calicut	Mangalore	Karwar	Chennai	Kakinada
Sample size		60	20	10	20	60	30
Number of loci (enzyme & protein)		17	17	17	17	17	17
Number of loci (enzyme)		10	10	10	10	10	10
Proportion of polymorphic loci (protein & enzyme)		59%	59%	59%	59%	59%	59%
Proportion of polymorphic loci (enzyme)		90%	90%	90%	90%	90%	90%
Average heterozygosity per locus	He	0.07	0.08	0.05	0.15	0.18	0.09
	Ho	0.08	0.09	0.05	0.21	0.22	0.11
Effective number of alleles		1.09	1.13	1.04	1.22	1.22	1.19
Average number of alleles/locus		1.64	1.75	1.16	1.86	2.00	1.86

4.4.2.5 Genetic Identity

Nei's values of genetic similarity between paired populations obtained are presented in Table 13. The values ranged from 0.9721 to 0.9964. The corresponding values of genetic distance ranged between 0.0036 to 0.0279. The lowest distance value of recorded between Kochi/Chennai (0.0036) and the highest between Calicut/Mangalore (0.1299) were not significant. These values indicate that the six populations from Kochi, Calicut, Mangalore, and Karwar of west coast and Chennai and Kakinada of east coast are genetically identical.

4.4.3 Molecular Genetics

4.4.3.1 Random Amplified Polymorphic DNA

The number of bands was counted from the photographs and 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 16). The mean percentage similarity among the individuals of Kochi population for OPA 2 and 4 was 74%, whereas that of Chennai population was 69%. A mean percent similarity of 78% was observed between the Kochi and Chennai populations for the primers (Table 17). This indicate the large changes at the base pair level, reflecting the difference in the population structures of east-west coasts.

Table 15 Regional inter-specimen RAPD similarities (above the diagonal) and distances (below the diagonal) in *P. monodon* of South India

OPA 2/Kochi

	1	2	3	4	5	6	7	8	9	10
1	--	0.27	0.92	0.92	0.71	0.86	0.80	0.77	0.86	0.77
2	0.73	--	0.25	0.25	0.35	0.47	0.56	0.50	0.35	0.63
3	0.08	0.75	--	0.86	0.67	0.80	0.63	0.57	0.67	0.57
4	0.08	0.75	0.14	--	0.67	0.93	0.63	0.63	0.8	0.71
5	0.29	0.65	0.33	0.33	--	0.88	0.94	0.93	0.75	0.67
6	0.14	0.53	0.2	0.07	0.12	--	0.71	0.67	0.63	0.67
7	0.20	0.44	0.37	0.37	0.06	0.29	--	0.88	0.71	0.50
8	0.23	0.50	0.43	0.37	0.07	0.33	0.12	--	0.67	0.43
9	0.14	0.65	0.33	0.20	0.25	0.37	0.29	0.33	--	0.40
10	0.23	0.37	0.43	0.29	0.33	0.33	0.50	0.57	0.60	--

OPA 2/Chennai

	1	2	3	4	5	6	7	8	9	10
1	--	0.86	1.00	0.80	0.85	0.92	0.73	0.85	0.77	0.71
2	0.14	--	0.85	0.80	1.00	0.92	0.55	0.57	0.62	0.86
3	0.00	0.15	--	0.80	0.71	0.92	0.55	0.71	0.62	0.57
4	0.20	0.20	0.20	--	0.80	1.00	0.50	0.80	0.57	0.67
5	0.15	0.00	0.29	0.20	--	0.92	0.55	0.71	0.46	0.57
6	0.08	0.08	0.08	0.00	0.08	--	0.40	0.77	0.50	0.46
7	0.27	0.45	0.45	0.50	0.45	0.60	--	0.36	0.40	0.36
8	0.15	0.43	0.29	0.20	0.29	0.23	0.64	--	0.46	0.57
9	0.23	0.38	0.38	0.43	0.54	0.50	0.60	0.54	--	0.31
10	0.29	0.14	0.43	0.33	0.43	0.54	0.64	0.43	0.69	--

OPA 4/Kochi

	1	2	3	4	5	6	7	8	9	10
1	--	0.80	0.88	0.94	0.94	1.00	0.94	0.88	0.88	0.80
2	0.20	--	0.71	0.75	0.75	0.82	0.67	0.67	0.67	0.71
3	0.12	0.29	--	0.95	0.95	0.80	0.86	0.78	0.78	0.71
4	0.06	0.25	0.05	--	0.88	0.84	0.90	0.82	0.82	0.88
5	0.06	0.25	0.05	0.12	--	0.74	0.80	0.82	0.82	0.75
6	0.00	0.18	0.20	0.16	0.26	--	0.86	0.89	0.89	0.82
7	0.06	0.33	0.14	0.10	0.20	0.14	--	0.84	0.84	0.67
8	0.12	0.33	0.22	0.18	0.18	0.11	0.16	--	0.88	0.67
9	0.12	0.33	0.22	0.18	0.18	0.11	0.16	0.12	--	0.67
10	0.20	0.29	0.29	0.12	0.25	0.18	0.33	0.33	0.33	--

OPA 4/Chennai

	1	2	3	4	5	6	7	8	9	10
1	--	0.88	0.80	0.86	0.82	0.82	0.71	0.82	0.36	0.88
2	0.12	--	0.80	0.71	0.82	0.82	0.71	0.82	0.36	0.75
3	0.20	0.20	--	0.77	0.75	0.75	0.63	0.75	0.40	0.67
4	0.14	0.29	0.23	--	0.80	0.80	0.80	0.67	0.44	0.86
5	0.18	0.18	0.25	0.20	--	0.78	0.89	0.89	0.33	0.82
6	0.18	0.18	0.25	0.20	0.22	--	0.89	0.89	0.33	0.71
7	0.29	0.29	0.37	0.20	0.11	0.11	--	0.78	0.33	0.71
8	0.18	0.18	0.25	0.33	0.11	0.11	0.22	--	0.33	0.71
9	0.64	0.64	0.6	0.56	0.67	0.67	0.67	0.67	--	0.36
10	0.12	0.25	0.33	0.14	0.18	0.29	0.29	0.29	0.64	--

Table 16 Inter regional RAPD similarities in *P. monodon* of South India

OPA 2/Kochi-Chennai

	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
C1	0.92	0.38	0.86	0.86	0.93	0.93	0.63	0.86	0.80	0.57
C2	0.76	0.38	0.86	0.86	0.93	0.93	0.75	0.86	0.80	0.57
C3	0.76	0.38	1.00	1.00	0.93	0.93	0.75	0.86	0.80	0.57
C4	0.86	0.35	0.80	0.93	0.75	1.00	0.71	0.80	0.86	0.53
C5	0.76	0.38	0.86	0.860	0.80	0.93	0.63	0.86	0.67	0.71
C6	1.00	0.40	0.92	0.92	1.00	0.71	0.67	0.92	0.86	0.62
C7	0.60	0.46	0.55	0.55	0.50	0.50	0.62	0.55	0.67	0.72
C8	0.77	0.38	0.71	0.71	0.67	0.80	0.63	0.86	0.67	0.71
C9	0.67	0.40	0.62	0.62	0.57	0.57	0.67	0.62	0.71	0.62
C10	0.62	0.38	0.57	0.57	0.67	0.67	0.75	0.71	0.67	0.86

OPA 4/Kochi-Chennai

	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
C1	0.88	0.67	0.78	0.82	0.82	0.89	0.84	0.75	0.88	0.80
C2	0.88	0.67	0.78	0.82	0.94	0.89	0.95	0.75	0.88	0.80
C3	0.93	0.71	0.82	0.88	0.88	0.82	0.78	0.80	0.80	0.86
C4	0.86	0.77	0.88	0.80	0.93	0.75	0.71	0.71	0.86	0.77
C5	0.94	0.75	0.95	0.780	0.77	0.95	0.90	0.82	0.82	0.75
C6	0.94	0.75	0.95	0.89	0.89	0.84	0.80	0.82	0.82	0.75
C7	0.94	0.75	0.84	0.89	0.89	0.95	0.70	0.82	0.94	0.75
C8	0.94	0.75	0.95	0.89	0.89	0.95	0.80	0.75	0.94	0.75
C9	0.73	0.60	0.46	0.50	0.50	0.46	0.43	0.55	0.73	0.60
C10	0.88	0.80	0.78	0.82	0.82	0.89	0.74	0.88	0.88	0.80

Table17 Average of inter regional RAPD similarities in *P. monodon* of South India

OPA 2/OPA 4/ -	Kochi -	Chennai	Average
K1/C1	0.92	0.88	0.90
K2/C2	0.38	0.67	0.53
K3/C3	1.00	0.82	0.91
K4/C4	0.93	0.80	0.87
K5/C5	0.80	0.77	0.79
K6/C6	0.71	0.84	0.78
K7/C7	0.62	0.70	0.66
K8/C8	0.86	0.75	0.81
K9/C9	0.71	0.73	0.72
K10/C10	0.86	0.80	0.83
	0.71	**0.78**	**0.78**

1. Primer OPA 2/4 - 'S' values of individuals of Kochi = 0.74 **(74%)**
2. Primer OPA 2/4 - 'S' values of individuals of Chennai = 0.69 **(69%)**
3. Primer OPA 2/4 - 'S' values of individuals of Kochi/Chennai = 0.78 **(78%)**

Discussions

5. DISCUSSION

An accurate knowledge of the natural units that sustain a fishery is of fundamental importance for its scientific exploitation, conservation and for its modern aquaculture practices through selective breeding programmes. Such knowledge can be gained by accurate measurement of genetic characteristics of the sample populations of the species in question (Altukhov, 1981; Lester and Pante, 1992). The topic of the present discussion here is the results of the study of the population genetic characteristics of *Penaeus monodon* of South India. The genetic characteristics of the species were measured by applying modern techniques of morphometrics, biochemical genetics and molecular genetics. The significant results produced by these three independent methods are discussed below separately.

MORPHOMETRICS

Twenty six truss measurements of body variations present in 428 specimens representing of six sample populations collected from Karwar, Mangalore, Calicut, Kochi (West coast), Chennai and Kakinada (East coast) were used to compute Principal component scores for each sample. The plotting of PC I scores against PC II scores of each sample on a graph produced a single clustering which indicated that the morphological profiles of all these six populations are homogeneous. It means that populations of *P. monodon* of South India, irrespective of east and west coasts may belong to a single morphological stock (Fig.4 & 5). So the results of morphometric study did not support the hypothesis of stock differences suggested by stock assessment results of Rao *et al.* (1993).

The reports of stock differentiation of penaeid species are not known except the first attempt of Horton (1982). He detected significantly different morphometric variations in population samples of *P. stylirostris* and *P. vannamei*. However, he could not conclude these differences as basis for genetic stock differences (Lester and Pante, 1992). The reports of stock separations by multivariate analysis of morphometrics of fishes are many (Ihssen *et al.*, 1981; Winans, 1984).

However, lack of significant morphological differences even between east and west coast samples of the species need not always mean these coastal populations are not interbreeding and hence, morphologically similar. The phenomenon of stabilizing selection in different geographical areas may suppress the potential for significant morphological differences which are also expressions of polygenes (Ayala and Keiger, 1980; Lester and Pante, 1992).

Therefore, it was necessary to test the above hypothesis of morphometric homogeneity of these six populations of *P. monodon* by an alternative and more efficient biochemical genetic method. The results of biochemical genetic analysis of the same sample populations have been discussed below.

BIOCHEMICAL GENETICS

Surprisingly, the results of the biochemical genetic analysis also support the morphometric homogeneity of these six populations. The biochemical genetic variations of east and west coast samples were also not significantly different. The above second hypothesis of genetic homogeneity was based on variations of allele frequencies estimated at about ten enzyme and seven protein loci (Table 10), F_{ST} values estimated for fifteen pairwise comparisons of genetic variabilities at nine enzyme loci (Table 12) and fifteen values of genetic similarities made for six population

samples (Table 13). Interestingly, comparable reports of insignificant biochemical genetic variations among geographical populations of other penaeid species with reference to the allozyme loci examined in each species. For example, distant populations of *P. setiferus*, *P. aztecus* and *P. duorarum* from Gulf of Mexico and the Atlantic coast of Florida showed biochemical genetic similarity at several enzyme loci examined (Lester, 1979, 1983). Allozyme frequencies were closely similar in the six populations of South Indian *P. indicus* (Philip Samuel, 1987; Bindhu Paul, 2000). The phenomenon of closely similar allozyme frequencies has also been reported in fishes (Mangaly and Jamieson, 1978; Shaklee, 1984).

On the other hand, the examples of reports of significant different allozyme frequencies and hence genetic stock differences in the species of prawns and fishes are not rare. The examples are genetic stock differences in *P. indicus* (Lioe, 1984), *P. latisulcatus* and *Metapenaeus endeavouri* (Mulley and Latter, 1981b), *M. dobsoni* (Santh Begum, 1995). The earliest examples of detection of reproductively isolated stocks in fishes are that of cod fish (Sick, 1965; Moller, 1968; Jamieson, 1967, '70; Jamieson and Jones, 1967). Significantly, different allele frequency differences indicating reproductively isolated stocks were clearly detected in red fish - *Sebastes mentella*, anchovy (*Engrulis encarasicholus*), sockeye salmon (*Oncorhynchus nerka*) and the reproductive isolation of some of these stocks were demonstrated by repeated sampling for several years (Altukhov, 1981).

The chances of geographic isolation of stocks due to evolutionary forces like migration, genetic drift and natural selection are more than genetic stabilization. Then why allele frequencies at many loci examined here in *P. monodon* and in many other penaeid species and fishes are not significantly different even between distant geographic locations ?. The debatable reasons are many and may suit to specific cases. A major reason for showing lack of differences is that the number of loci examined

in most cases are too less compared to hundreds of gene loci present in the species (Murphy *et al.*, 1990). Equally important another reason is that the potential loci having significantly different allele frequencies have not been examined.

This is clearly evident in the report of Benzie *et al.* (1992). The examination of three enzyme loci-PGM, GPI and MPI was sufficient to detect stock differences of the populations in *P. monodon* of Australia. Avoiding these three enzyme loci and comparing allele frequencies of other loci would have shown probably a different genetic stock structure. Another important theoretical reasoning for the observation of closely similar allozyme frequencies is that gene flow between two distant populations caused by migration of fifty individuals per generation (Allendorf and Phelps, 1981). The probable reason for the lack of significant allele frequency differences, especially between the east and west coast samples of *P. monodon* of South India is that the number of potential polymorphic loci examined were insufficient or in other words the potential loci like PGM, GPI and MPI that would have shown significant allele frequency differences were not tested in the present investigation.

Since, preliminary tagging experiments of prawns showed that mixing of prawn populations of east and west coast of South India may not be taking place (MFIS, No.45; Vijayaraghavan *et al.*, 1982). The mixing of these two populations by migrations probably prevented by the hydrographical barrier (Ramamirtham and Jayaraman, 1960). The closely similar allele frequencies estimated here between east and west coast samples of *P. monodon* do not support the hypothesis of non-mixing of these two populations. However, a critical analysis of distribution of specific alleles at ODH-locus appears to support non-mixing hypothesis of east and west coast populations of the species. For example, the allele, *ODH-1*91* was absent in Chennai and Kochi, though

sample size was sufficiently large in both areas. Another allele *ODH-1*81* was absent in Kochi population but present in Chennai. The presence or absence of such rare or private alleles in populations may imply their separate stock identity (Jamieson, 1974). Comparable rare allele phenomenon was also reported in *P. indicus* (Bindhu Paul, 2000). If the rare alleles are indicative of stock isolation, the present observation of absence of the allele *ODH-1*81* in Kochi and its presence in Karwar also suggests that these two local populations in the west coast are isolated populations. On the same reasoning, presence of the allele *ODH-1*91* in Kakinada samples and its absence in Chennai samples should mean these two east coast populations are also isolated. If any of these two populations are proved to be really reproductively isolated, these rare alleles can be utilized as genetic markers for selection for controlled breeding programmes from desired stock for selective breeding programmes (Lester, 1983; Goswami *et al.*, 1986; Lester and Pante, 1992). The very significant difference in the allele frequencies at AK-2 locus between Kochi (1.00) and Chennai (0.67) may also point out some inherent stock difference between these two populations (Table 10); though allele frequencies at other nine enzyme loci were not significantly different.

The genetic variability in penaeid prawns is comparatively low, ranging from 0.008 to 0.092 (Lester, 1979) or 0.006 to 0.033 (Mulley and Latter, 1980), 0.023 to 0.086 (de la Rosa-Velez *et al.*, 2000). The estimated heterozygosity for *P. monodon* from South India was 0.126 (Table 18) which is comparatively much higher than that of other penaeids and the value shows that the species has high genetic variability at the allozyme loci examined here. The genetic variability reported for the species from South east Asia is also lower level (Sodsuk, 1996). The recent review (Benzie, 2000) of allozyme variability in twenty seven species of penaeus and metapenaeus showed allozyme variability range as low as

0.008 in two populations of *P. monodon* to 0.089 in *P. aztecus*. Interestingly, the genetic variability indicated by average heterozygosity was significantly higher in *P. monodon* of South India, it being 0.126 which is more than thirty percent than the highest reported (0.089) in penaeids (Benzie, 2000) and more than forty percent (0.083) reported for *P. monodon* from South-east Indian Ocean (Forbes *et al.*, 1999; de la Rosa-Velez *et al.*, 2000). The logical reason for such significant differences in the genetic variability reports by different investigators must be due to differences in the allozymes examined or differences in the number of loci examined or differences in the techniques followed.

The above critical analysis of biochemical genetic results lead us to conclude and make suggestions as follows. The overall close genetic similarities at the ten allozyme loci indicate that all the six populations of *P. monodon* of South India and its east and west coast populations have homogeneous stock structure. Since, the presence or absence of the rare alleles - *ODH-1*81, 91, 109* (Table 10) may indicate some inherent genetic stock structural differences, a detailed screening of the ODH locus in larger samples along with the highly potential genetic stock-marker loci such as PGM, GPI and MPI should be attempted in the future to test the above hypothesis of east-west biochemical genetic stock homogeneity. Before making final conclusions on the present investigation let us discuss the more interesting results of the molecular genetic method.

MOLECULAR GENETICS

A total of sixteen RAPD kilobased fractions were produced by the primer, OPA-2 and fifteen fractions by OPA-4. However, the number of fractions present in Kochi and Chennai were significantly very different.. For example, Chennai had only nine out of sixteen fractions, while Kochi had all the sixteen fractions. In other words, seven fractions (0.1, 0.2, 0.8,

0.9, 0.95, 1.3, 1.9 Kb) of the primer OPA-2 were unique to Kochi sample (Table 14). The OPA-4 primer produced a total of fifteen fractions. However, Kochi and Chennai had only 13 fractions. In other words, each sample was short of two but different fractions. For example, the fractions 0.0025 and 0.95 Kb were absent in Kochi, while the fractions 0.8 and 1.6 Kb were absent in Chennai. That is, each sample had its own two unique OPA-4 fractions (Table 14). In short, the above significant differences in the number of fractions strongly suggest that the east and west coast samples may be genetically distinct stocks. Besides, the significant differences in the number of DNA fractions (Plate 5a,b & 6a,b) between individuals of each sample also mean that level of DNA variability is significantly higher in Kochi than that of Chennai (Table15). Comparable reports of molecular genetic stock differences were reported in Melvin trout populations (Ferguson *et al.*, 1995). There, the unique DNA fractions (alleles) present enabled to separate Ferox, Gillaroo and Sonaghen populations. A single RAPD fraction present in one of the two populations of *Macrobrachium borellii* was considered as genetic marker for stock identification (D' Amato and Corach, 1996). The recent review of the genetic structure of penaeids (Benzie, 2000) reveals that the RAPD technique is the most efficient technique for detection of natural genetic diversities in penaeid prawns, especially in *P. monodon* which fact is significant. This, thus strongly reports the present conclusion of genetic stock structure differences detected only by RAPD method and not by morphometric and biochemical genetic methods in *P. monodon* of South India.

The present finding of higher level RAPD genetic variability in Kochi and the unique RAPD fractions present in the east and west coast samples support the earlier non-genetic hypothesis based on stock assessment results (Rao *et al.*, 1993) that east and west coast samples of *P. monodon* are separate fishery stocks. Besides, these unique DNA fractions can be used as genetic markers to select the desired east and west coast breeders

for the purpose of selective breeding programmes and to monitor the level of DNA variability in the wild or cultured populations of the species. Besides, these stock-specific unique alleles can be used to detect any possible mixing of these two stocks especially during selective breeding programmes or larval rearing period.

The present findings and conclusions are based on only a few specimens and a single test. Considering the sensitivity of the RAPD procedures, the reproducibility of the present results should be confirmed before drawing a final conclusion on the east-west stock diversity of *P. monodon*.

Finally, the highlights and the significance of the present investigation on the genetics of the *P. monodon* may be summarised as follows. The body shape variations measured truss network method and allozyme variations and protein detected by electrophoresis were not significantly different in six populations (Karwar, Mangalore, Calicut, Kochi, Chennai, Kakinada) of *P. monodon*. However, the presence or absence of ODH-rare alleles between east and west coast populations may indicate inherent stock differences in the species. The species has high biochemical genetic variability. The unique RAPD alleles detected in Kochi and Chennai samples alone suggest that these two populations may be distinct genetic stocks.

The significance of the present investigation may be further emphasized. The morphometric, biochemical genetic and molecular genetic informations detected in the species *Penaeus monodon* of South India and reported here are original.

Conclusions

6. CONCLUSIONS

1. The morphometric variations compared between populations of *Penaeus monodon* from Karwar, Mangalore, Calicut, Kochi (West coast) and Chennai, Kakinada (East coast) were not statistically significant and hence, it is concluded that these six populations have homogeneous stock structure (Fig. 4, 5).

2. Partial carapace length (PCL) has the highest correlation to the tail weight (TLW) in *P. monodon* (Table 3,3a & 4,4a).

3. The estimated average number of alleles per locus (1.71), percentage of polymorphic loci (90%) and the average heterozygosity (0.126) reveal that the species, *P. monodon* has high genetic variability (Table 18).

4. The statistical comparisons of the biochemical genetic variations between the six populations of the species did not produce significant values and hence it is concluded that these six populations have homogeneous biochemical genetic stock structure.

5. The results of molecular genetic method were contrary to that of the other two methods. The random amplified polymorphic DNA (RAPD) profiles (Plate 5a,5b & 6a,6b) studied in the specimens from Kochi and Chennai were significantly different and hence it is concluded that the molecular genetic stock structure of these two populations is heterogeneous (Fig. 9, 10, 11, 12 , Table 16&17).

Suggestions

7. SUGGESTIONS

1. A detailed biochemical genetic screening of all the populations of *Penaeus monodon*, using especially the most potential stock market such as enzymes Glucose phosphate isomerase (GPI). Phospho-glucomutase (PGM) and Mannose-6-phosphate isomerase (MPI) is essential (Lester and Pante, 1992; Benzie *et al.*, 2000) and Adenylate kinase (AK) which showed significantly different allozyme frequencies in the present investigation.

2. The RAPD analysis, using OPA-2 and OPA-4 was done only for Kochi and Chennai samples of *P. monodon*. Hence, a detailed analysis of the RAPD profiles of all the other populations, using these two primers are essential to draw a final conclusion on the population genetic stock structure of the species. Since the highest genetic variability present in *P. monodon* is shown by microsatellite techniques, this is also to be applied in the future investigations (Supungul *et al.*, 2000).

Summary

8. SUMMARY

The thesis contains the detailed aspects of an investigation entitled **"Genetic Studies of the Marine Penaeid prawn *Penaeus monodon* Fabricius, 1798".** The reasons for selecting this particular research problem, the three research methods for investigating the problem and the reasons for selecting *Penaeus monodon* as the candidate species are highlighted in the introductory chapter.

The published informations relevant to the topic of the present investigation and the methods of investigation were reviewed separately under the sub headings: Morphometrics, Biochemical genetics and Molecular genetics.

The standardized procedures followed for collection of morphometric, biochemical genetic and molecular genetic data and the statistical procedures for interpretation of these separate data were given under the chapter Materials and Methods.

The data obtained under each method were objectively analyzed using figures and tables prepared for the purpose. The detailed aspects of the results were presented under the chapter Results. The highlights of the results are given below.

Of the four morphometric variables (Fig. 2) correlated to the tail weight (TLW) of the species, partial carapace length (PCL) showed the highest correlation to tail weight (Table 3, 4).

The morphometric body shape variations measured by truss network method (Fig.3) and converted into sheared principal component score PC I and PC II (Fig. 5). The plotting of PC I against PC II scores of

each sample from Karwar, Mangalore, Calicut, Kochi (West coast), Chennai and Kakinada (East coast) produced a single cluster (Fig.4), indicating measured morphometric variations are not significantly different. The populations of the species have a homogeneous morphometric structure, irrespective of the east and west coast source of the samples.

The estimated values of allozyme frequencies at ten enzyme loci and allele frequencies at seven protein loci were closely similar in all the population samples from Karwar, Mangalore, Calicut, Kochi (West coast), Chennai and Kakinada (East coast). The only significant exemption was between Kochi and Chennai, the values being 1.00 and 0.67 respectively. The *AK-2*77/100* locus was not tested in other populations (Table 10).

The estimated values of average number of allozyme alleles per locus (1.71), heterozygosity (0.126) and percentage of polymorphic loci (90%) indicate that the species has comparatively high biochemical genetic variability (Table 18). The statistical significance of differences in the allele frequencies or genetic variabilities between populations was measured by F_{ST} and Nei's genetic similarities tests. None of these values were significant (Table 12). Hence it was concluded that all these six populations of the species have a homogeneous biochemical genetic stock structure.

Though ten primers (OPA – 1 to 10) were tried to polymerize DNA extracts from specimens from Kochi and Chennai, only OPA-2 and OPA-4 proved to be potential RAPD markers. A total of sixteen fractions of different kilobases were produced by OPA-2 and fifteen by OPA-4. Of these sixteen fractions of OPA-2, seven were absent in the Chennai sample. Of the fifteen OPA-4 fractions, two different fractions were absent in Kochi and Chennai samples. In other words, seven OPA-2 fractions were unique to Kochi while two each of OPA-4 fractions were also unique to Kochi as well as Chennai samples (Table 14, Fig. 9, 10, 11, 12).

Since the RAPD profiles of Kochi and Chennai were significantly different (Plate 5, 6), the molecular genetic stock structure of Kochi and Chennai populations of the species are significantly different.

The morphometric, biochemical and molecular genetic results were critically evaluated and discussed separately in the last chapter of the thesis. The discussions of the present results by comparing or contrasting with the relevant results reported by others enabled to draw the final conclusions from the present investigation and to make appropriate suggestions for future investigation.

The final major conclusions drawn are :

1. The morphometric and biochemical genetic stock structure of the sample populations of *P. monodon* tested from Karwar, Mangalore, Calicut, Kochi (West coast) Chennai, Kakinada (East coast) is homogeneous.

2. Contrary to the above conclusion, the molecular genetic stock structure of population samples of the species from Kochi and Chennai was significantly different, suggesting that the east and west coast populations of the species may be of separate genetic stocks.

3. The potential applications and implications of the present significant findings in the management and culture programmes of this commercially very important species were mentioned.

To draw a final accurate conclusions on the population genetics of the species *P. monodon* of India, specific suggestions made to future workers were listed at the end of the thesis.

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