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

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Certificate

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INTRODUCTION

1. INTRODUCTION

The fishery and genetic resources of India are 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 – popularly known as the **jumbo tiger prawn** – of Indian waters. Sea fishes and shell fishes like the penaeid prawn, *P. monodon* are a major source of 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 (Wright, 1978; Mac Lean and Evans, 1981; Shaklee and Salini, 1985; Ward *et al.*, 1994; Shaklee

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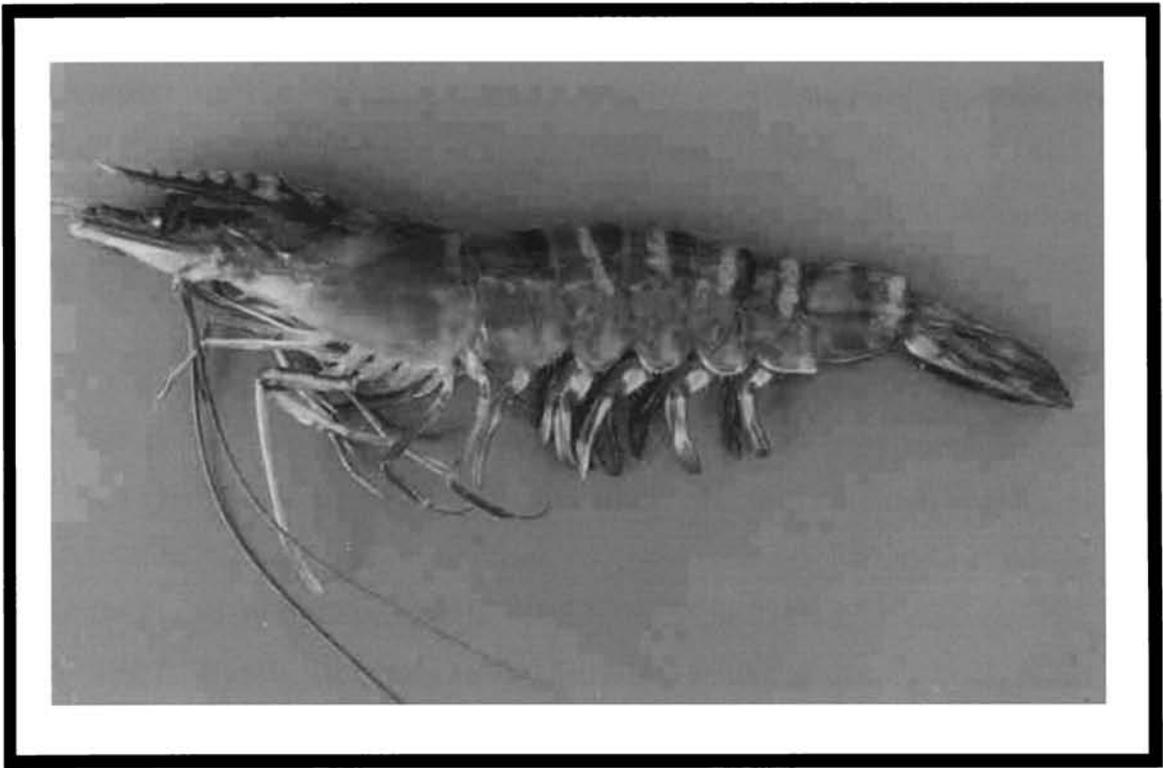
Penaeus monodon

and Bentzen, 1998; Booke, 1999). 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 (Booke, 1981, 1999). Nevertheless, defining and identifying distinct units of fisheries management remained as a problematic concept (Marr, 1957).

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, 1999; 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, 1997; 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 and genetic resources of any nation.

The populations of *P. monodon* which were exploited along the Indian coast were from Karwar, Mangalore, Calicut, Kochi (West coast) as well as from Chennai and Kakinada (East coast). The species, *P. monodon* selected 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. Its contribution is comparatively poor along the west coast, it being about 0.2% at Kochi, 0.4% at Calicut and 0.3% at Mangalore. At Karwar, *P. monodon* caught only in stray numbers in trawl operations (Anon, 2000, 2001).

The species, *P. monodon* has a broad geographic distribution in tropical and subtropical waters of South-east Asian countries including east and west coast of South India (Anon, 1969; Annon, 1978). The species is migratory in habit, the adults migrate out to sea during the breeding season (Kemp, 1915). The species also occurs in the backwaters of the Kerala coast in relatively smaller quantities. The larger adults are caught by trawlers from offshore deeper waters. The distribution of the postlarvae of the species has been reported from the Chilka lake and Ennur backwaters (Kemp, 1915), from Adayar backwaters (Chennai) during all months and the fry of the species from the coastal areas, tidal rivers and estuaries months (Panikkar and Aiyar, 1939). The postlarvae and juveniles occur in nearshore/estuarine habitats with some vegetative structure. In Bombay, the catches mostly consist of immature specimens. Thus larger adults occur in offshore deeper waters and postlarvae/juveniles occur in nearshore/estuarine habitats with some vegetative structures.

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 exploited wild populations of *P. monodon* from east and west coast of India are homogeneous or not, in terms of (a) morphology, (b) biochemical genetics and (c) molecular genetics. Since it is an ideal species for commercial aquaculture and selective breeding programmes (Lester and Pante, 1992), the determination of its stock structure become more meaningful and significant. Though, detailed informations on the

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biology and fishery of *P. monodon* of South India are available (Kemp, 1915; Panikkar and Aiyar, 1939; Hall, 1962; Rao, A.V.P., 1967; Mohamed, 1979; Rao *et al.*, 1993), the important questions on its stock structure, mentioned above were remaining almost unanswered. Hence, the present doctoral topic entitled, **"Genetic studies of the marine penaeid prawn, *Penaeus monodon* Fabricius, 1798"** was undertaken to determine the population structure of *P. monodon* of the East and West coasts of India. Three independent refined methods: (1). Morphology - multivariate analysis of truss morphometrics (Humphries *et al.*, 1981; Strauss and Bookstein, 1982; Winans, 1984; Lester *et al.*, 1990); (2). Biochemical genetics (allozymes) (Ihssen *et al.*, 1981; Lester and Pante 1992) and (3). Molecular genetics based on random amplified polymorphic DNA (RAPDs) analysis (Hallerman and Beckman, 1988; Williams *et al.*, 1990; Ovenden, 1990; Jayasankar and Dharmalingam, 1997a, 1997b; Gopalakrishnan *et al.*, 2003) were applied to study and detect individual variations within each population samples of the species. The genetic data recorded were statistically analysed for measuring the degree of differences between populations. The present thesis contains the detailed results of the investigation as well as the significant conclusions drawn from the results.

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REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The concept of species has been known from time immemorial. 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 (Mangaly, 1974). Later, phenotypic and genotypic analysis of the species and its populations revealed the existence of subspecies or races within the species (Simpson, 1964, 1965; Dobzhansky, 1967, 1971; Ayala and Keiger, 1980, 1984).

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, the earliest report of phenotypic races may be that of herring (Heinke, 1898) and cod fish (Schmid, 1909) species as mentioned by Mangaly (1974). 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).

The conventional methods and techniques applied for detection of species and their isolated populations were also refined or elaborated. For example, instead of simple length measurements of body parts, the multivariate truss network analysis giving emphasis on body shape was

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adopted in morphometrics (Lester and Pante, 1992; Goswami *et al.*, 1996). In the electrophoretic techniques, tube gel electrophoresis of protein/enzymes were replaced by slab gel electrophoresis. Techniques for analysis of DNA molecule (RAPD, mtDNA, RFLP and microsatellite) were developed and preferred over the methods of analysis of DNA controlled gene-loci of enzymes. Application of any one or more of these renovated methods have revealed hitherto unknown levels of variabilities in species of fishes and shell fishes of the world. A typical example is that of the Atlantic cod, *Gadus morhua*. 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*. This species was exploited and managed as homogeneous populations or suspected as having morphologically heterogeneous stocks (Sick, 1965; Moller, 1966, '68, '70, '71; Jamieson, 1967). The electrophoretic study of its haemoglobin and serum transferrin variants showed that the cod species is composed of genetically distinct stocks. Later, morphometrically and genetically heterogeneous populations within a species or homogeneity of populations in a wide ranging areas of its distribution have also been reported. For example, stock difference among the populations of the Pacific hake, *Merluccius productus* (Utter, 1981) and biochemical genetic homogeneity among that of Atlantic hake, *Merluccius merluccius* (Mangaly and Jamieson, 1978). The methods/techniques applied for the present investigation were truss network analysis of body shape, multiphore slab gel electrophoresis of allozymes and random amplified polymorphic DNA (RAPD) analysis of DNA extracts (detailed report on page 19-26). Since, these methods were independent, the review of the literature relevant to the present investigation has been arranged under separate headings.

Morphometrics

Species of *Penaeus* differ significantly in a variety of morphological characteristics that are the expressions of species-specific polygenes (Ayala and Keiger, 1980, 1984). Hence, these are used for taxonomic distinctions. The studies generally focussed on the structure of genitalia, appendages, rostra, or sculpturing of the carapace. Investigations on 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, and thus 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 within a species. 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 biological 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* and whiter fish, *Lactarius lactarius* also indicated stock

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differences between two population samples (Winans, 1984; Gopalakrishnan *et al.*, 2003). 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 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 within 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 trussnetwork studies. The photographs in concert with a computer and a digitizing tablet were used 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 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 Indian white prawn, *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 its genetic stock structure. However, a study to separate geographically distinct

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stocks, if any, across the range of jumbo tiger prawn, *P. monodon* of India was not attempted by morphometric methods in the past. Interestingly, differences in the stock assessment values of the *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, the morphometric data collected may have been affected by environmental parameters. 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, 1984; Lester and Pante, 1992a). In other words, the morphometric variations are less genetic than the variations detected by the biochemical genetic method. Therefore, the practice of applying biochemical and molecular 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 a wide range of genetic variability in all the species of fishes and shell fishes (Ligny, 1969). The studies on the biochemical genetics of fish/shellfish populations evolved from early discriptions of simple polymorphism at one or a few general protein/enzyme loci as reported in the haemoglobin polymorphisms in fishes (Sick, 1965); esterase polymorphism in the Atlantic herring (Ridgeway *et al.*, 1970); phosphoglucomutase

polymorphism in brown shrimp, *Penaeus aztecus* (Procter *et al.*, 1974) to detailed analysis of scores of isozyme loci as in the case of green tail prawn, *Metapenaeus bennettae* (Salini, 1987), barramundi, *Lates calcarifier* (Shaklee and Salini, 1985: Gopalakrishnan *et al.*, 2003) and *Metapenaeus dobsoni* (Santh Begum, 1995). Significant differences in the allelic frequencies between populations of a species clearly indicated that these are not interbreeding but isolated populations of the species (Ayala and Keiger, 1980, 1984). 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 of genetic diversity in fishes and shell fishes was well evaluated in the international symposia held in 1971 (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 symposia held in 1981, the proceedings of which were published as a 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 (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 stock differences or lack of it are too many to review here. The examples of important biochemical genetic studies in India are that of mullet, *Mugil cephalus* (Vijayakumar, 1992); oil sardine, *Sardinella longiceps* (Venkita Krishnan, 1992) and *Lactarius lactarius* (Gopalakrishnan *et al.*, 2003) and of prawn, *Metapenaeus dobsoni* (Santh Begum, 1995); *Penaeus indicus* (Bindhu Paul, 2000). The populations of each of these first three species though being exploited as homogeneous stocks, could be differentiated into heterogeneous stocks by

biochemical techniques. Thus significant allele frequency differences were detected at seventeen polymorphic loci in three *Mugil cephalus* populations compared. Significant allele frequency differences at fourteen enzyme loci differentiated in *S. longiceps* into five heterogeneous stocks. Allele frequencies at ten of fourty seven loci were significantly different in five populations of *M. dobsoni*. However, statistical analyses of fourteen loci in seven populations of P. *indicus* revealed a homogeneous biochemical genetic stock structure of the species. This is comparable with homogeneous stock structure reported in European hake, *Merluccius merluccius* (Mangaly and Jamieson, 1978). The above examples reveal that the biochemical genetic techniques are efficient in differentiating the natural stock structure of fish/shellfish species.

Reports on the efficiency of biochemical genetic techniques in revealing the intraspecies allozyme polymorphism and existence of heterogeneous or homogeneous stocks in various crustacean species are also many. The first report of enzyme polymorphism was that of phosphoglucomutase in the brown shrimp, *Penaeus aztecus* (Procter *et al.*, 1974). Later, enzyme polymorphism was detected in 44 species of decapod crustacea, including five species of penaeidae (Nelson and Hedgecock, 1980) and other penaeid species (Lester and Pante, 1992). The polymorphic enzyme they screened was phosphoglucomutase (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 species of penaeus. The range of 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 in fishes, most penaeid species populations had insignificant genetic distance values [D] throughout their geographic range (Mulley and

Latter, 1980, 1981a, 1981b; Lester and Pante, 1992). The reported exemptions were stock differences in populations of *Penaeus latisulcatus*, *Metapeaneus bennettae, M. maclayei, M. endeavouri* of Australian waters (Mulley and Latter, 1981a, 1981b; Salini, 1987; Lester and Pante, 1992) and *P. stylirostris* from the Gulf of California and *P. indicus* from coasts of Philippines and Kenya (Lester and Pante, 1992).

The first attempt to study the population genetics of South Indian penaeid species from Indian coast was that of Philip Samuel (1987). Using disc electrophoretic method, he detected seven polymorphic enzyme loci in four populations of *P. indicus* and in two of six populations of *P. stylifera.* Allelic frequencies and genetic distances were not significantly different in the populations of these two species studied. Morphometrics of these populations also showed their homogeneous structure. Using the methods of flat slab gel electrophoresis, truss network analysis of body shape and random amplified polymorphic DNA (RAPD) analyses, Bindhu Paul (2000) made another attempt to study the genetic structure of the populations of *P. indicus* of South India. Here again, the morphometrics and allozyme frequencies were not significantly different. However, the results of the RAPD method revealed that the genetic structure of its east and west coast populations is heterogeneous. Interestingly, using allozyme markers, high biochemical genetic variability (H = 0.264) and significant genetic stock differences (D = 0.0902) were detected in the populations of *Metapenaeus dobsoni* of South India (Santh Begum, 1995).

The species, *Penaeus monodon* enjoys a wide ranging distribution along the coastal waters of India, Indonesia, Australia, Africa, Japan, Thailand and Andaman sea. The Australian species has been subjected to detailed population genetic investigation using electrophoretic techniques (Mulley and Latter, 1980; Lioe, 1984; Benzie *et al.*, 1992; Sugama *et al.*, 2002). Allozyme polymorphism in *P. monodon* from Australian waters

was first reported by Mulley and Latter (1980). Allozyme genetic polymorphism with rare alleles was later detected in geographic samples of *P. monodon* from Fiji, the Philippines and Taiwan by Lioe (1984). However, allele frequencies were not significantly different in the geographic samples examined by him as mentioned by Benzie *et al.* (1992). Later, Benzie *et al.* (1992) screened seventy five enzymes in the species of which only seven showed polymorphic loci. Allele frequency analyses at eight polymorphic loci (GPI, LGG, LT-1, MDH-1, 2, MPI, PGDH, PGM) in seven samples of eastern, western and northern coasts of Australia showed highly significant differences between western and northern/eastern populations, especially at GPI, PGM and MPI loci. Screening of thirteen allozymes, in seven samples of the species from waters of Indonesia (Sugama *et al.*, 2002) revealed six polymorphic loci and only one population showed significantly different allele frequencies. These above reports suggest that chances of detection of isozyme polymorphism and biochemical genetic stock differences in penaeid species may depend on screening of as many loci as possible in the investigation when one or more loci may emerge as suitable genetic markers for stock differentiation.

The above findings strongly suggest that when popular methods of population genetic studies fail to reveal inherent genetic stock differences present in some species, it is most desirable to apply more efficient techniques methods before drawing any conclusions on the population structure of the species. That will also help to confirm the stock differences already shown by the popular methods.

The phenomenon of the very low level biochemical genetic variability and close genetic homogeneity of even distant geographic populations in species of penaeidae was re-examined by many curious investigators using mitochondrial or nuclear DNA samples. The electrophoretic techniques used for separation of proteins have their own limitations. First of all, the

number of enzyme loci examined are always much less than hundreds of protein loci present in each species. Probably, less than 25% of estimated amino acid substitutions are detectable by gel electrophoresis (Bye and Ponniah, 1983; Powers, 1993). Besides, all protein variants can not be detected by electrophoresis unless such variants also produce electrophoretically detectable level of electric charge differences. Moreover, all the differences in the DNA sequences are not translated directly to protein polymorphism detected by electrophoretic methods. On the other hand, modern DNA techniques can reveal and measure even variations in nucleotide sequences in very subsamples of DNA fragments (Ayala and Keiger, 1984). Hence, the analysis of the very base sequences of the DNA was the best alternative in the study of population genetics. Thus, DNA results may have greater implications in fisheries management and conservation of the genetic resources than that provided by biochemical genetic method. A brief review of reports on DNA studies relevant to the present investigation is presented below.

Molecular Genetics

More than 175 restriction enzymes have been extracted from different species of bacteria. These have characteristic ability to break apart the DNA strand at its own specific sites. Hence, these enzymes are powerful tools for structural analysis of genomes. DNA fragments cleaved by these enzymes have characteristic number and unique sizes. These fragments can be detected by prescribed agarose gel electrophoretic techniques. The length variations of the fragments can be measured on comparison with specific molecular markers (Ayala and Keiger, 1984). The application of restriction enzymes to study mitochondrial DNA length polymorphism in natural populations was recommended by Avise *et al.* (1979).

As a result, the application of mtDNA techniques permitted detection and observation of genetic diversity unknown before in many species of commercial or scientific interest. Thus 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). New levels of genetic polymorphism in different species of penaeid prawns as well as genetic diversity between their populations were detected. Thus, the very low level of genetic variability rose to twenty fold in geographic populations of *Penaeus vannamei* and six fold in *P. stylirostris* (Lester and Pante, 1992).

The superiority of mitochondrial DNA (mtDNA) variability over protein loci variability and its applications in genetic stock assessment was reviewed by Ovenden (1990). The restriction fragment length polymorphism (RFLP) detected by mtDNA analysis have revealed higher amount of genetic diversity between populations of a species and also 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 thermo-cyclers for amplification of the DNA segment through polymerase chain reaction (PCR) and the techniques developed by Welsch and McClleland (1990) and Williams *et al.* (1990) enabled to detect and observe DNA base level polymorphism in microsamples of the organisms.

There are a few published reports on the application of molecular genetic tools such as RAPD, RFLP and microsatellite in studying the genetic polymorphism in fishes and shell fishes of India (Padhi and Mandal, 1995; Jayashankar and Dharmalingam, 1997a, 1997b; Padhi *et al.*, 1998; Mohindra *et al.*, 2001; Gopalakrishnan *et al.*, 2003). An attempt was made to apply RAPD technique to study the population

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genetics of *Penaeus indicus* (Bindhu Paul, 2000). The RAPD profiles of its samples from east and west coast of South India were significantly different, though, these population samples had shown morphometric and biochemical genetic homogeneity. Three Operon primers (AC-12, AC-13, AC-19) were used to compare the RAPD profiles in *P. indicus* samples from east and west coasts of South India. The number of DNA fragments produced by AC-12 varied from 1 to 3 and the size was below 1.5 kb in the samples. However, the number and size of the fragments produced by AC-19 primer were significantly different in both samples. The number varied form 2 to 3 in east coast and 2 to 5 in the west coast. The fragment sizes varied from 1.5 kb to 3.5 kb and two fragments below 1.5 kb. The 3.5 and 2 kb sized fragments were present only in the west coast sample, indicating that east and west coast populations have significantly different RAPD structures.

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/Malaysia 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 revealed low allozyme variability while, higher mtDNA and nuclear DNA (nDNA) variability, especially at microsatellite region (Benzie, 2000). The RAPD techniques were most efficient in differentiating genetic stock diversities in these penaeid species, especially, in *P. monodon*. The RFLP and RAPD techniques differentiated distinct stocks of the species from Australia and Indo-Pacific region (Benzie *et al.*, 1993; Benzie *et al.*, 2000). Genetic stock differences can occur even between areas of short distance ranging from 250 (Benzie, 2000) to 600 kms ((Klinbunga *et al.*, 1998).

The above analyses of reports of intraspecies allozyme and DNA polymorphism, levels of genetic variability, the significant differences in the genetic distances of populations or genetic homogeneity among populations in spite of their wide ranging geographic distributions may have valuable 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 basic aspects of the significance of these newer informations are that the smallest natural units of the species to be exploited for commercial purpose or to be conserved for genetic reasons are now known for the first time. Once such units of fisheries management are established, selection of genetically distinct breeders becomes possible for selective breeding programmes or for experimental purposes. Besides, the knowledge of the levels of genetic variability in the wild populations is very helpful to keep monitoring its changes in the founder populations stocked for commercial breeding programmes and to detect undesirable inbreeding in the wild populations, which may indicate over exploitation of stocks of the species. And finally, all these finer details on the population genetic variations and the undesirable changes induced by over exploitation should help to take proper steps to conserve the valuable genetic resources of the species investigated.

The above review on the concept of unit stock in fisheries management, the different methods of detection of such units and the results of application of these methods by different investigations worldwide reaffirm the comparative efficiency of these techniques. Finally, in this context, the decision to investigate the genetics of *Penaeus monodon* of India by morphometric, biochemical genetic and molecular genetic methods was appropriate and valuable.

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 frozen and transported to the laboratory in wet ice and then stored at -20° C until used for experiments. A total of 627 specimens of *P. monodon*, with a total length range of 100-300 mm were collected during 1996-2002.

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. Specimens in each sample were not grouped into size classes because of the following reasons. First, the length variables measured involved only distances between solid exoskeletal parts covering the head (carapace) and abdomen as shown and explained in the figure 2. Second, the total length measurement was used only for finding correlation with tail weight and it was not used for genetic stock differentiation (Table 4 (a, b) & 5 (a, b). Third, the truss network landmarks made for measuring the body shape of the specimens in each sample start and end between the hard exoskeletal components like, epigastric tooth to base of antennal flagellum, dorsal edge of carapace to ventral corner of carapace etc.

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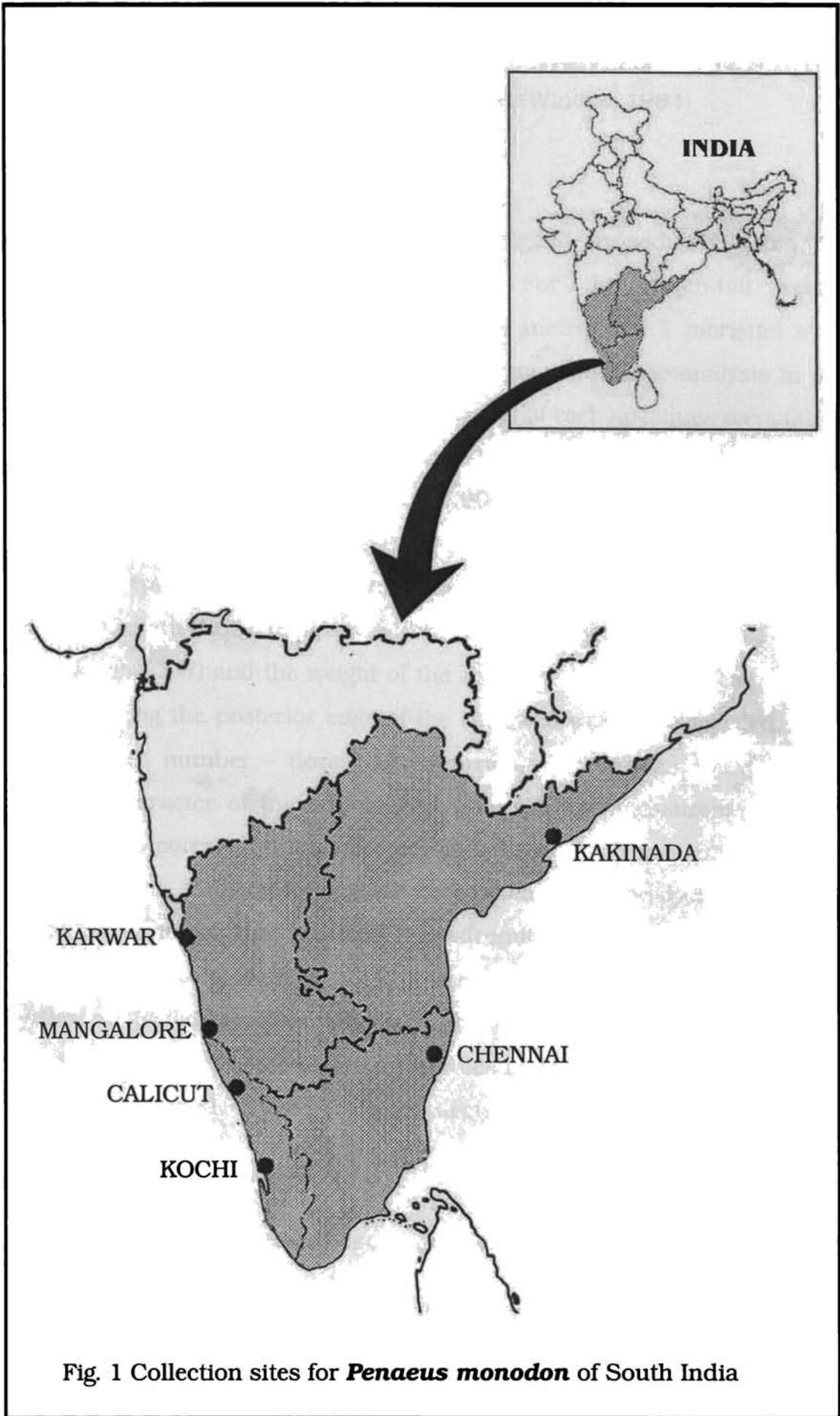
Fig. 1 Collection sites for *Penaeus monodon* of South India

(Fig. 3). Thus, irrespective of the size range of the specimens used, uniformity of morphometric measurements was assured and thus the resulting error in the measurement was least (Winans, 1984).

3.2.1a Length measurements (Fig. 2)

First, the thawed samples were weighed individually using the electronic weighing balance (Sartorius). For the length-tail weight correlation studies, a set of 10 variables (9 metric and 1 meristic) were measured but only 7 were selected for the morphometric analysis in the present study (Fig. 2). Length measurements of each specimen were taken linearly using a scale and dial caliper (0.05 mm accuracy) (Lester, 1983; Goswami et al., 1986; Lester, et al., 1990; Lester and Pante, 1992). The variables measured were – total length (TL), body length (BL), sixth segment depth (SSD), second abdominal segment depth (SAD), partial carapace length (PCL), carapace width (CW) and rostral length (RL). Total body weight (TW) and the weight of the abdomen-tail weight (TLW), which severed along the posterior edge of the carapace was also recorded. The rostral teeth number – dorsal and ventral – (RTN) was counted as a meristic character of the populations. The selected measurements were subjected to correlation matrix analysis between each measurement and the tail weight. Only four variables were taken for the analysis finally and the other variables that involved the extremities were excluded, as they were prone to easy damage. The correlation matrix thus obtained was further subjected to path-coefficient (direct effect) analysis to remove the indirect effect, of the inter related variables on the tail weight through the variable concerned. Thus the direct effects of each variable on tail weight (TLW) were obtained.

3.2.1b Truss network measurements (Fig. 3)

The body shape of each sample specimen was measured by truss network method (Lester et al., 1990; Lester and Pante, 1992). The

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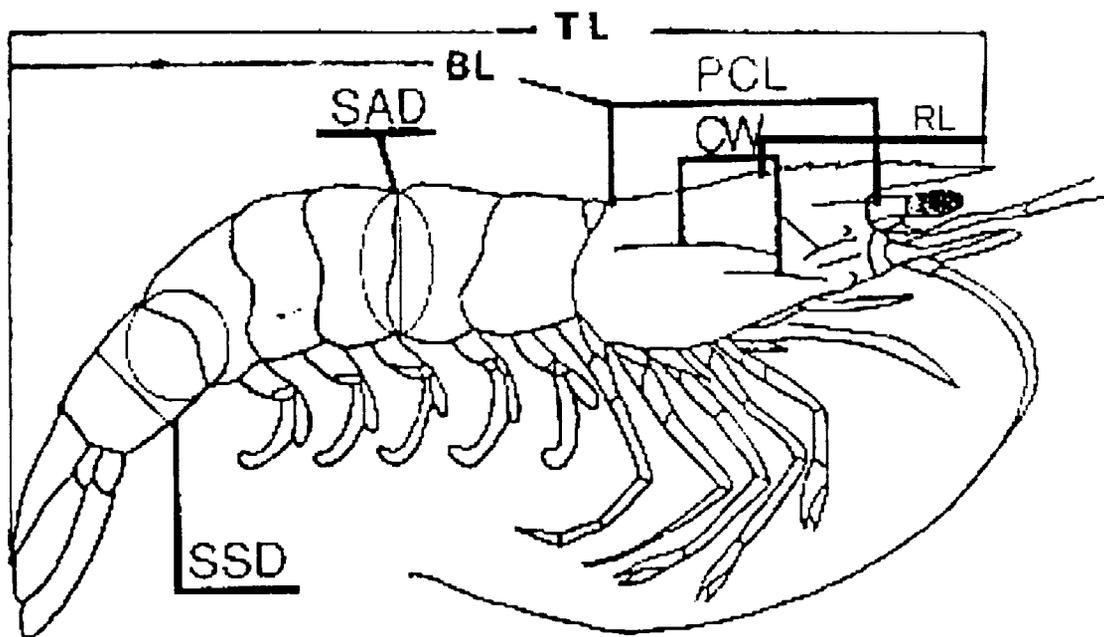
Variables

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 6th segment.
4.	Second abdominal segment depth (SAD)	:	Depth at the mid-point of the 2nd and 3rd 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*.

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 body 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 (picture template also used) (Fig. 3). Details 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.

The 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
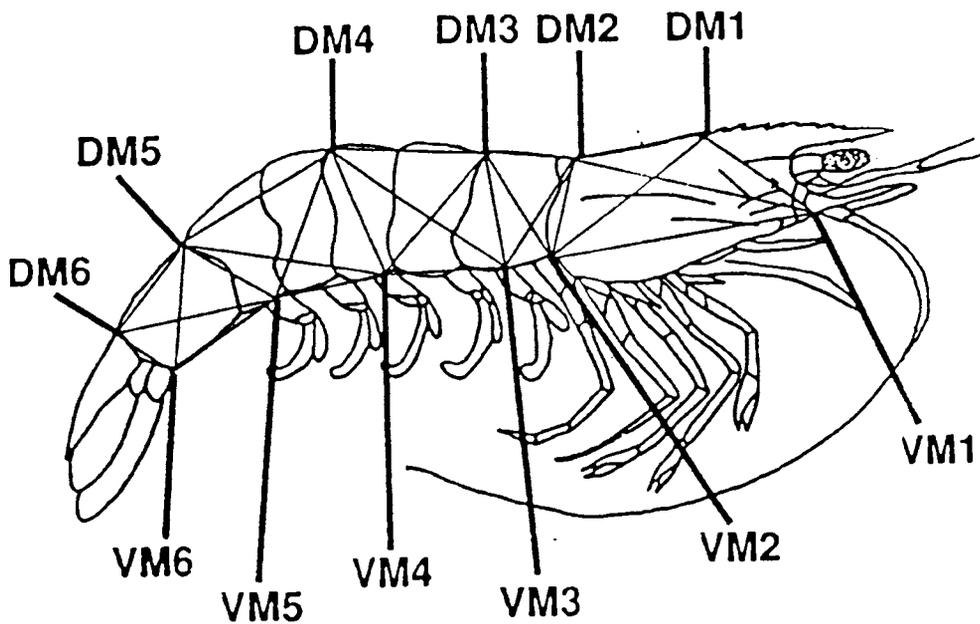
1.	**DM 1**	:	Epigastric tooth (anterior)
2.	**VM 1**	:	Base of the antennal flagellum
3.	**DM 2**	:	Posterior dorsal median edge of carapace
4.	**VM 2**	:	Posterior ventral corner of the carapace
5.	**DM 3**	:	Posterior dorsal edge of tergum of the 1st abdominal segment
6.	**VM 3**	:	Mid ventral point 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*.

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 (10,000 Xg) for 50 minutes. The supernatants were stored at ⁻20°C, until 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 1). 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 of each specimen (1g/1ml) was homogenized in ice-cold double distilled water (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 the horizontal slab gel apparatus (Multiphore II - Pharmacia, LKB). During standardisation procedures,

Table 1. 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

continuous and discontinuous buffer systems were used first. Electrophoresis was performed using gel buffers of different compositions (Table 2). Banding patterns were scored by designating loci 1, 2 etc. in the order of descending anodal mobility of a zone of allozyme bands. Nearly sixteen specific enzyme staining procedures were used (Table 3) and from these only the following nine enzyme systems (Table 7) 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 (αGPDH-8%), (6) Glucose phosphate isomerase (GPI-7.5%), (7) Octanol dehydrogenase (ODH-10%), (8) Malate dehydrogenase (sMDH-10%), (9) Phosphoglucomutase (PGM-7.5%) and (10) General protein (PROT-10%) – [Table 1, 7].

Buffer systems with different pH and molarity were tried for the present study (Table 2). 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 taken, while for the discontinuous system needed a stacking gel solution of 25 ml and separating gel solution of 50 ml. The amounts of gel buffer, ammonium persulphate (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.

Table 2 Composition of the buffer systems selected for electrophoresis of enzymes in *P. monodon* of South India

Buffers	Electrode buffer		pH	Gel buffer				
				Stacking		pH	*Separating*		pH
1.Tris- Citric- EDTA (Ayala et al., 1972)	0.135 M Tris 0.045 M Citric acid 0.00125 M EDTA	16.35g/l 9.45g/l 0.47g/l	7.0	0.009 M Tris 0.003 M Citric acid 0.00125 M EDTA	1.09g/l 0.63g/l 0.47g/l	7.0	0.009 M Tris 0.003Citric acid 0.00125 M EDTA	1.09g/l 0.63g/l 0.47g/l	7.0
2.Tris- Citric acid - Lithium Hydroxide - Boric acid	**A** 0.0472 M Tris 0.023 M Citric acid **B** 0.03 M Lithium Hydroxide 0.19 M Boric acid	8.7g/l 4.8g/l 1.2g/l 11.8g/l	7.25	A B DDH$_2$0	90ml 42ml 168ml	6.1	A B DDH$_2$0	90ml 42ml 168ml	8.2
3.Tris- Citrate Lithium Hydroxide - Boric acid	0.03 M Lithium Hydroxide 0.19 M Boric acid	1.2g/l 11.8g/l	8.1	0.072 M Tris 0.023 M Citric acid	8.7g/l 4.8g/l	6.8	0.072 M Tris 0.023 M Citric acid	8.7g/l 4.8g/l	8.8
*4.Tris- Glycine Tris- HCl	2 M Tris C140.2 M Glycine	25ml 15.04g/l	8.3	1.8 M Tris 21.81g/100ml 1N HCl to adjust the pH		8.9	0.05 M Tris 6.06g/ 100ml 1N HCl to adjust the pH		6.8

* Buffer system selected

The staining recipe used for the isozyme detection were modified from that of Shaw and Prasad (1970) and Shaklee *et al.* (1990). The stock solutions used were also of the same concentration mentioned in the original recipe.   The details of the staining recipe including the modifications for these sixteen enzymes studies are given below.

Table 3. 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 phosphate Isomerase	5.3.1.9	GPI
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
Isocitratedehydrogenase	1.1.1.42	mIDHP
L – Iditoldehydrogenase	1.1.1.14	sIDDH
Malate dehydrogenase	1.1.1.37	sMDH
Octanol dehydrogenase	1.1.1.73	ODH
Phosphoglucomutase	5.4.2.2	PGM

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

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$_2$HPO$_4$	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
NaCl	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	50 mg
When ready to stain, added:	
Black K salt	20 mg
Incubated at 37°C.	

15. Glucose phosphate Isomerase (GPI)

0.2 M Tris-HCl (pH 8.0)	25 ml
0.1 M $MgCl_2$	7.5 ml
18 mM Fructose-6-Phosphate	1.75 ml
G-6-PDH	10 ml
1 % NADP	0.625 ml
When ready to stain, added:	
1 % PMS	0.5 ml
1 % MTT	1 ml
Agar overlay, Incubated at 37°C	

16. Phosphoglucomutase (PGM)

0.2 M Tris-HCl (pH 8.0)	5 ml
0.1 M $MgCl_2$	5 ml
Glucose-1-Phosphate	5 ml
G-1, 6, diphosphate	2.5 ml
G-6-PDH	4 ml
1 % NADP	0.5 ml
Double distilled water	25ml
When ready to stain, added:	
1 % PMS	0.5 ml
1 % MTT	1 ml
Agar overlay, Incubated at 37°C	

17. General Protein (PROT)

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

Destainer

Acetic acid	500 ml
Methanol (95 %)	1500 ml
Make up to 5 % with DDW, mix well.	

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

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 synthesized by the DNA molecule. The molecular technique – random amplified polymorphic DNA (RAPD), 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 *et al.*, 1982; Garcia and Benzie, 1995).

3.2.3.1 Random Amplified Polymorphic DNA (RAPD)

3.2.3.1a 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 Operon 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 (1 µl 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 Operon 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 1X TEB 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 ten OPA primers amplified the isolated DNA samples producing sharp and repeatable bands. However only OPA-2 and OPA-4 revealed DNA variations between samples from geographically isolated locations; hence these two primers alone were used for further studies.

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. TEB 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

3.3.1a Morphometric Analysis

The length measurement on four variables of 428 specimens 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 four different variables on the tail weight were assessed.

3.3.1b Truss Network Analysis

The truss data on body shape (X and Y co-ordinate values for each landmark) specimens from six locations 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, 9, 10)

The zymograms were obtained after following the electrophoretic and histochemical staining procedures described on pages (22 - 27) of the thesis. The zymogram patterns were drawn or photographed immediately after histochemical staining. The enzyme activity obtained on the gel was differentiated into specific zones of enzyme loci. The fastest moving zone was marked as locus 1 and the slower one as locus 2. The zone having bands with different electrophoretic mobilities was counted as polymorphic and the one without as monomorphic loci. Since the differences in the electrophoretic mobilities of bands in a polymorphic locus were negligible, their mobilities of each band was actually measured to distinguish the multiple form of the alleles at the locus. As a general practice, the commonest band was given the electrophoretic mobility value 100. All the other bands were given actually measured additional or lesser values than the 100 base value. Thus there were bands with values, 77, 100, 124, 166, 183 (Fig. 7). In descriptive genetic terms, there were slow (100), slower (77), fast (124), faster (166), single bands or multiple bands (alleles) at a locus. Since, protein/allozyme bands are co-dominant allelic products (genotypes), a single banded genotype was counted as a homozygote formed of homozygous alleles where as a two banded

genotype was as heterozygote, formed of two heterozygous alleles at the locus. When genotypes were formed of more than two different alleles already considered, then the locus was counted as multiple allelic locus as in ODH enzyme with alleles 81, 91, 100, 109 (Fig. 9 & Plate 4, 5). More over, when an isozyme genotype had only two bands, the enzyme structure was described as monomeric and when it was formed of three bands, it is considered as a product of dimeric enzyme structure as in the case of ODH (Fig. 9). The number of different genotypes observed at each locus was counted in each sample. These counts of the genotype formed the basic genetic data on the population tested or compared. The data on genotype counts were used to estimate the frequencies of alleles, observed and expected frequencies of genotype, heterozygosities, proportion of polymorphic loci, mean effective and average number of alleles, the degree of allelic frequency differences between populations and the genetic distance between populations. The goodness of fit between the expected and the observed genotype frequencies (Hardy-Weinberg equilibrium) 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 (the genetic variability) was measured by the parameters like - heterozygosities, proportion of polymorphic loci, mean effective number of alleles, average number of alleles, genetic distance and F_{ST}.

The following appropriate standard statistical formulae were applied to estimate the significance of biochemical genetic variability between the sample populations. The computer programme which was also used to detect all the parameters of genetic variation for the present investigation was Genepop version 3.1 (Raymond and Rousset, 1998).

3.3.2.1 F-analyses

A co-ancestry assessment of the individuals between the populations was done by applying the methodology suggested by Weir and 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, $I = I_{XY}/ \Sigma I_X I_Y$

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

Genetic distance was taken as $-\ln I$.

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 (Table 8). The DNA marker applied along with RAPD samples helped to determine the molecular sizes of the DNA fractions of the prawn samples.

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

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 (Table 16).

RESULTS

4. RESULTS

4.1 Morphometrics

4.1.1 Correlation with tail weight

The correlation and path-coefficient analysis of the variables (SSD, SAD, PCL, CW) on the tail weight (TLW) of both males (0.9605 & 0.3097) and females (0.9639 & 0.4881) of *P. monodon* from the six locations were given in Table 4 & 5. From the path-coefficient (direct effects) analysis, it was found that partial carapace length (PCL) was the variable having the highest correlation with the tail weight (TLW) of the species irrespective of their sex.

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 1996-2002 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 6) 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 from that of other stations though there is mixing up of samples. Further analysis was attempted by shearing the principal components of all the

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

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

Table 5(a) 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 5(b) Path-Coefficient (Direct Effects) on TLW (Female)

Variables	SSD	SAD	PCL	CW
Effects	-0.0214	0.3087	**0.4881**	0.2022
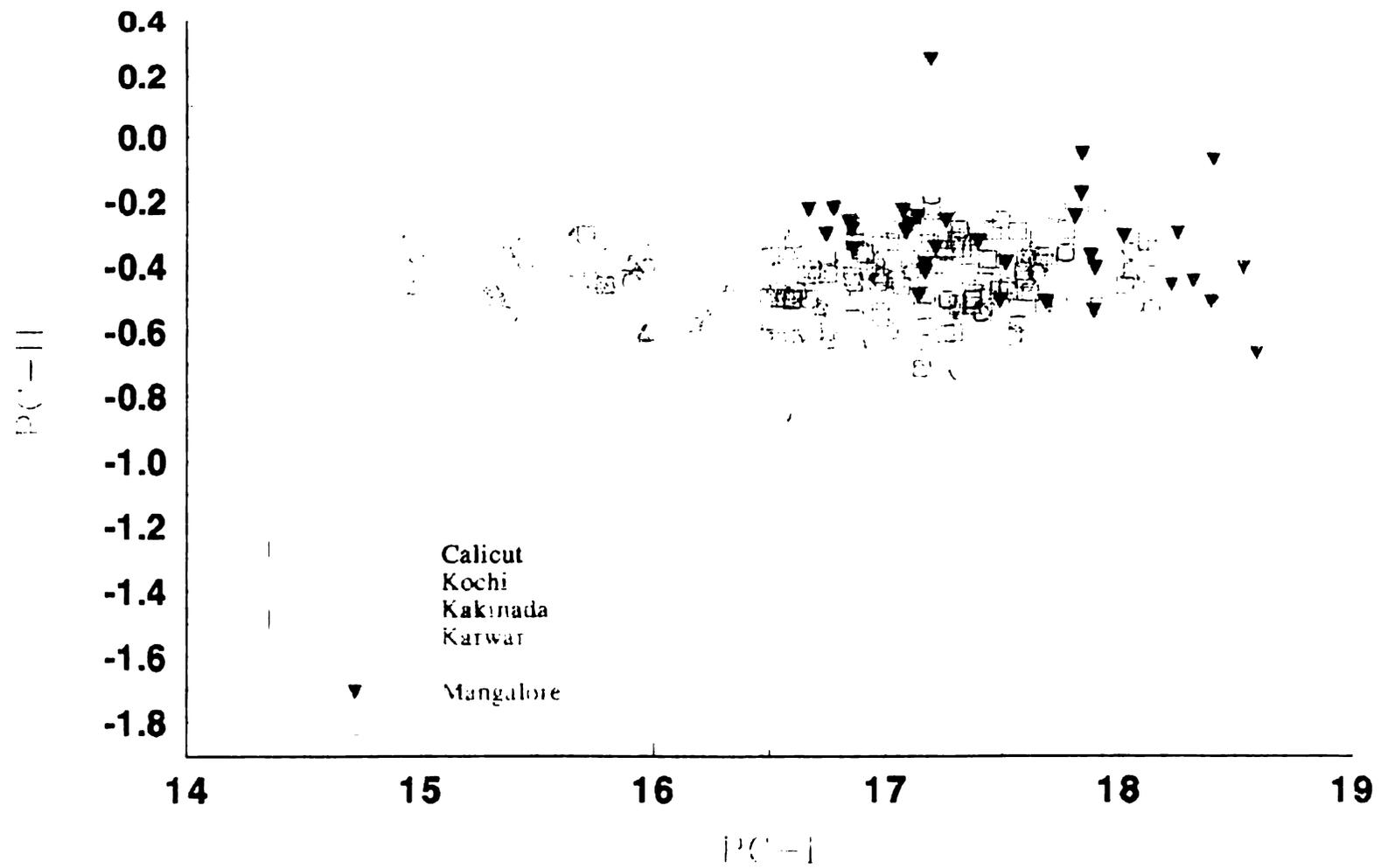
Fig.4 Morphometric profiles (truss measurements) of six
populations of *P. monodon* of South India

Table 6. 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
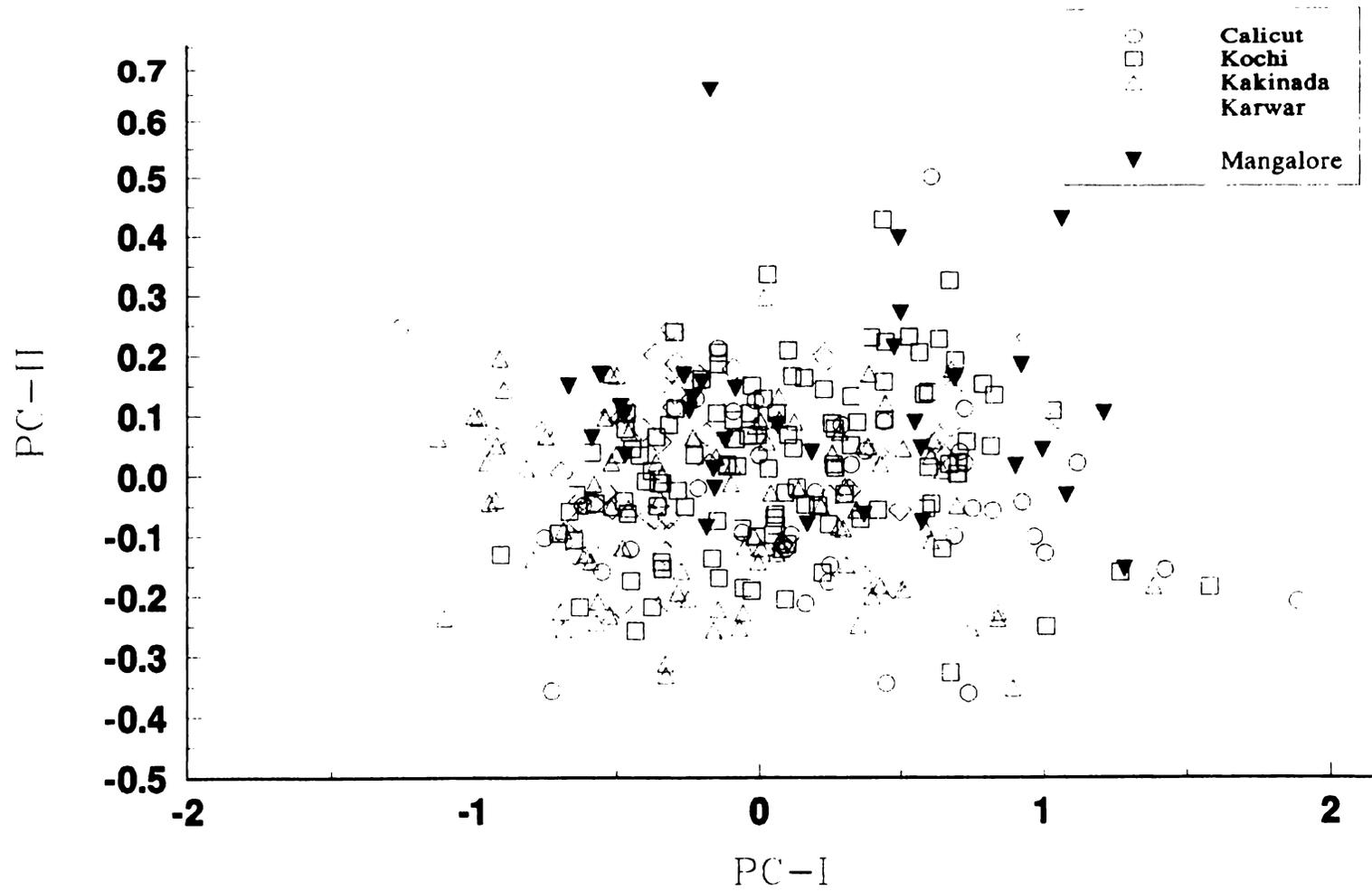
Fig.5 Morphometric profiles (sheared PC scores of truss measurements) of six
populations of *P. monodon* of South India

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 (Fig. 5).

4.2 Biochemical Genetics

4.2.1 Standardisation of Methodology (Tables 1 & 2)

The discontinuous buffer system composed of tris-glycine as electrode buffer (0.2M, pH 8.3), tris-HCl as separating gel buffer (1.8M, pH 8.9) and tris-HCl as stacking gel buffer (0.5M, pH 6.8); 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. Ten percent gel for ODH, EST, sMDH and general protein, 8% for AO, FBALD, αGPDH and 7.5% for AK, GPI and PGM were selected for electrophoresis.

Though 16 enzyme systems were tested (Table 3), only 9 (AO, FBALD, αGPDH, AK, EST, GPI, sMDH, ODH and PGM) could be detected with good resolution by specific enzyme activity in *Penaeus monodon*.

The rest showed poor (GDH), or no activity (ACP) or inconsistent activity (mIDHP) or poor resolution (G6PDH). Since sIDDH, ADH, HK and AK showed same zymogram patterns, so only AK was selected for genetic analysis. The enzymes FBALD, EST, GPI, ODH and PGM were single locus enzymes. The AO, AK, αGPDH and sMDH had two loci each.

The enzyme loci, AO-1, AO-2, FBALD, NSDH αGPDH, AK-1, AK-2, EST, GPI, sMDH, ODH and PGM were polymorphic in all the tested samples. The details of the results under each category are described below (Table 7).

4.2.2 Polymorphic Enzymes

1. 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 genotypes were observed at this first zone. The alleles AO-1*100 and AO-1*110 accounted for the heterozygous genotype (100/110) and the single banded homozygous genotype (100/100) at the first locus. The alleles AO-2*50 and AO-2*100 accounted for the heterozygous genotype (50/100). The allele AO-2*100 produced the homozygous phenotype (100/100) at the second locus. The third zone with inconsistent banding patterns were not scored. The first zone also showed minor bands in some individuals. The enzyme AO has a monomeric structure in P. monodon (Fig. 6 and Plate 1a).

2. FBALD (Fructose-biphosphate aldolase)

The enzyme showed three zones of enzyme activity - the fastest moving zone (bluish red) alone showed consistent banding patterns. The

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

Sl.No.	Loci	Enzyme Number	Polymorphism	No. of alleles
1	FBALD	4.1.2.13	Polymorphic	2
2	AO-1	1.2.3.1	Polymorphic	2
3	AO-2	1.2.3.1	Polymorphic	2
4	NSDH	:	Polymorphic	2
5	αGPDH	1.1.1.8	Polymorphic	2
6	AK-1	2.7.4.3	Polymorphic	2
7	AK-2	2.7.4.3	Polymorphic	2
8	EST	3.1.1.-	Polymorphic	2
9	GPI	5.3.1.9	Polymorphic	2
10	sMDH	1.1.1.37	Polymorphic	2
11	ODH	1.1.1.73	Polymorphic	4
12	PGM	5.4.2.2	Polymorphic	2
13	PROTEIN			
	1-5 & 7		Monomorphic	6
	6		Polymorphic	2
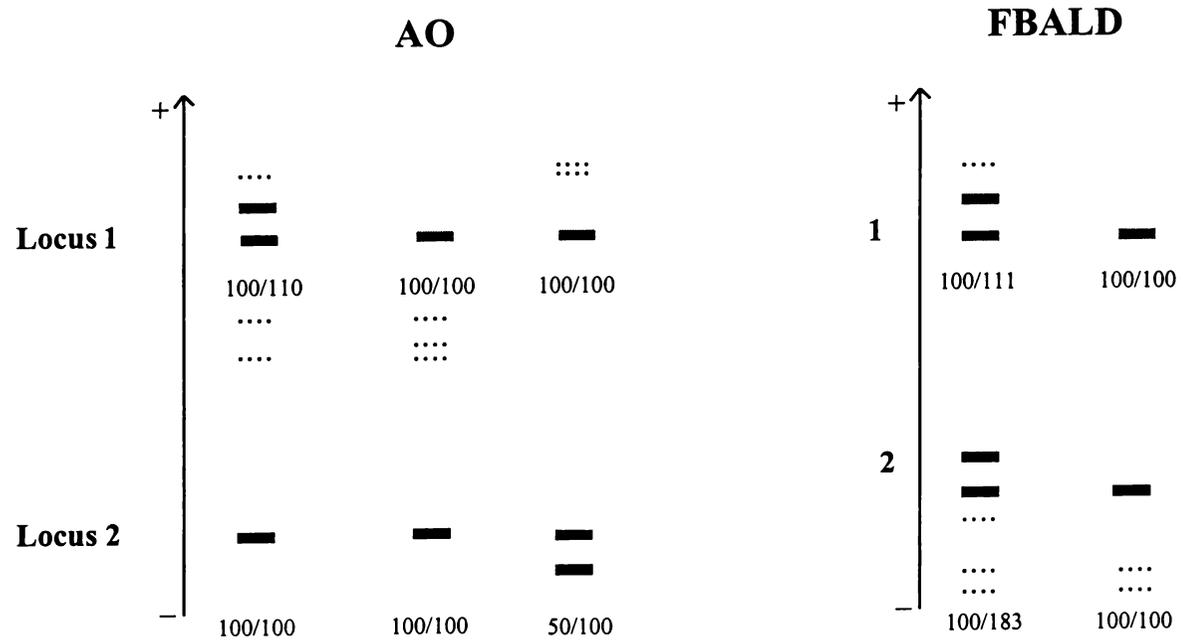
Fig. 6 Zymogram patterns of AO and FBALD in muscle tissue of *P. monodon* of South India

PLATE – 1

(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   * 100/100:   *Homozygote*

FBALD   * 100/111:   *Heterozygote*

PLATE 1
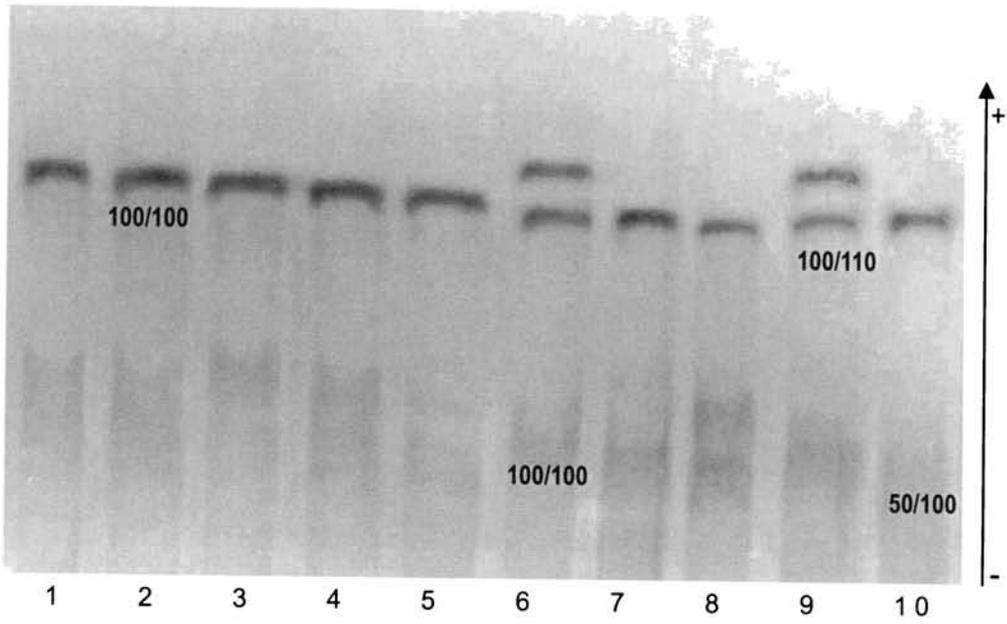
(a) **Aldehyde oxidase**
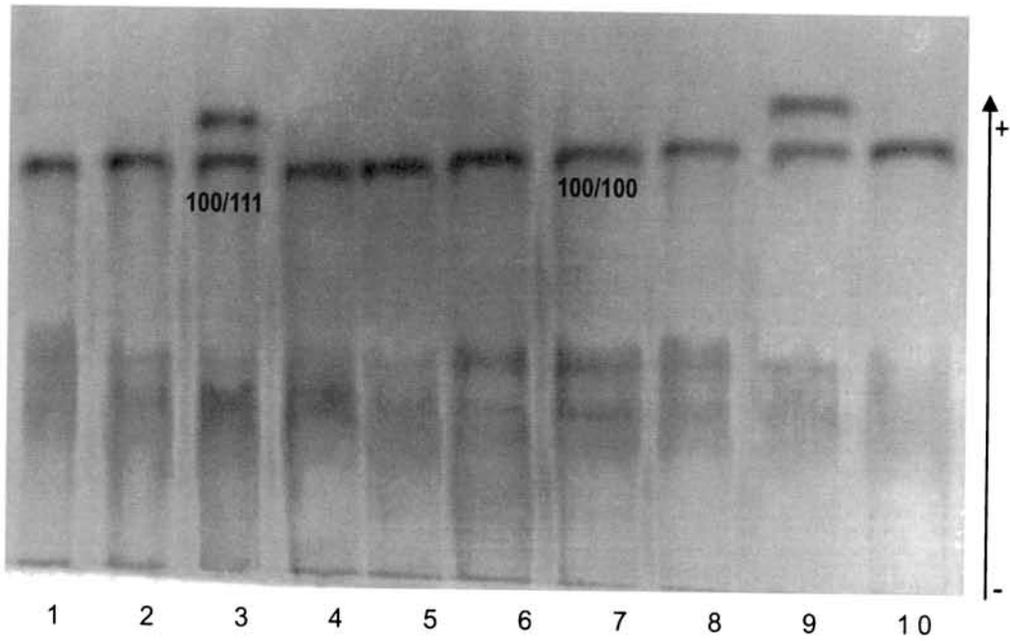
(b) **Fructose Biphosphate Aldolose**

other two zones were inconsistent and hence were discarded from further analyses. The first zone (locus) produced two and single banded polymorphic genotypes. The alleles, *FBALD*100* and *FBALD*111* accounted for the two banded heterozygous genotype (100/111) and allele *FBALD*100* for the single banded homozygous genotype (100/100). FBALD has a monomeric structure in *P. monodon* (Fig. 6 and Plate 1b).

3. αGPDH (Glycerol-3-phosphate dehydrogenase)

αGPDH enzyme also showed three zones of enzyme activity. The fastest moving bluish red zone produced single and double banded genotypes. The double banded heterozygote may be a product of monomeric enzyme structure. On the contrary, the second zone produced double banded homozygotes and triple banded heterozygotes. The enzyme showed a typical dimeric structure here. The alleles that produced two and three banded genotypes were *αGPDH*100* and *αGPDH*166* (Fig. 7, Plate 2a). The second band of the two banded homozygous genotype (100/100) had migration rate equal to that of the middle band of the three banded genotype. The reason for the occurrence of monomeric structural band pattern at the first zone needs some explanation, since it is not compatible with that of dimeric structure of αGPDH enzyme for which it was stained with the substrate αDL-glycerophosphate. The two banded heterozygote at the first zone then should be an expression of an unknown monomeric enzyme. This may be comparable with that of achromatic zone of (nothing dehydrogenase?) tetrazolium oxidase (Brewer, 1967) that can appear along with lactate dehydrogenase test using DL-lactate as substrate. Tetrazolium oxidase was formed by its enzymatic reaction with the pigment NBT in the presence of NAD. It does not require the αDL-glycerophosphate as substrate. The single and two banded enzyme pattern observed here in the present investigation therefore could be due to some other non-specific enzyme reaction with non-specific substrate contained in the enzyme
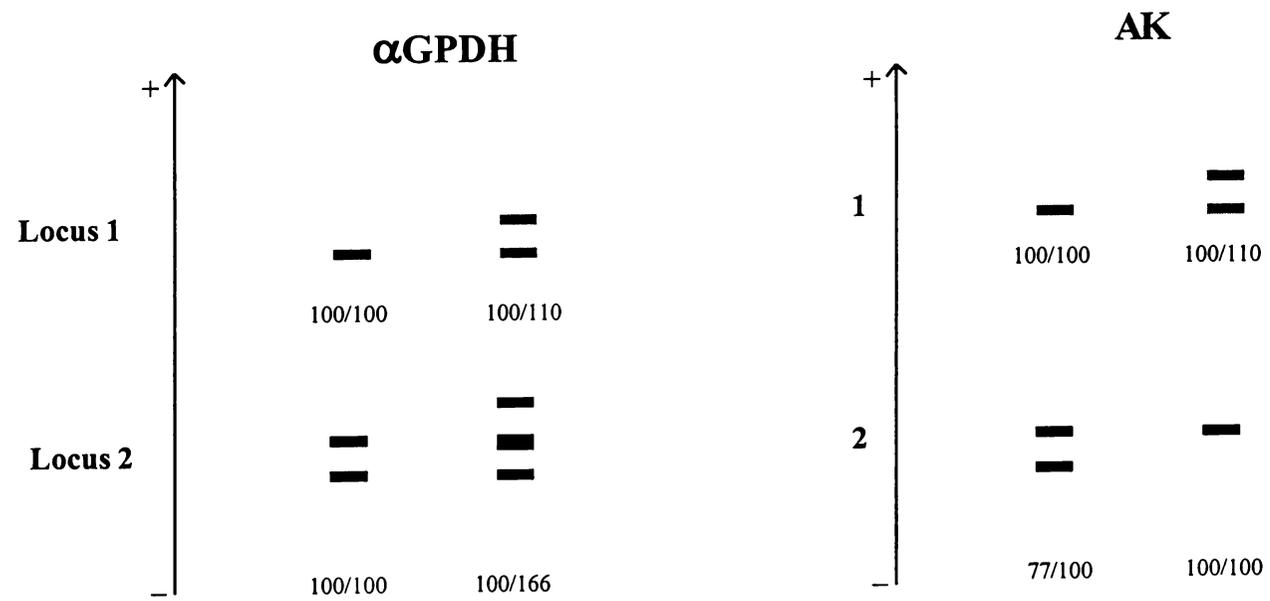
Fig. 7 Zymogram patterns of αGPDH and AK in muscle tissue of *P. monodon* of South India

PLATE – 2

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

*NSDH*100/100 : *Homozygote*

*NSDH*100/110 : *Heterozygote*

*αGPDH * 100/100* : *Homozygote*

αGPDH 100/166* : *Heterozygote*

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

*sMDH * 100/100:* *Homozygote*

*sMDH * 100/124 :* *Heterozygote*

PLATE 2
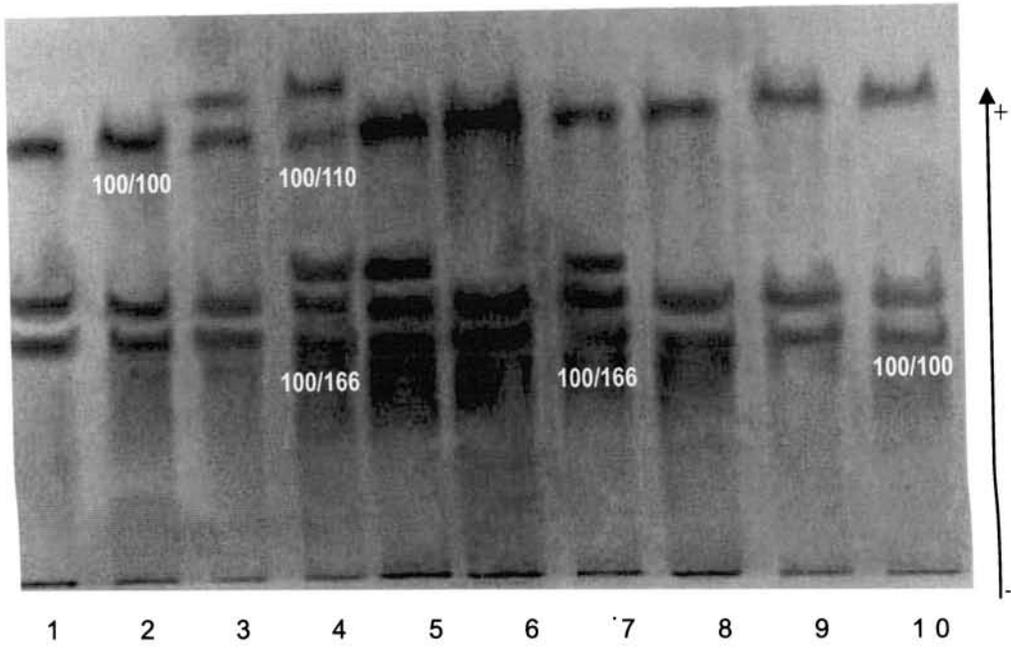
(a) Glycerol-3-phosphate dehydrogenase (αGPDH)
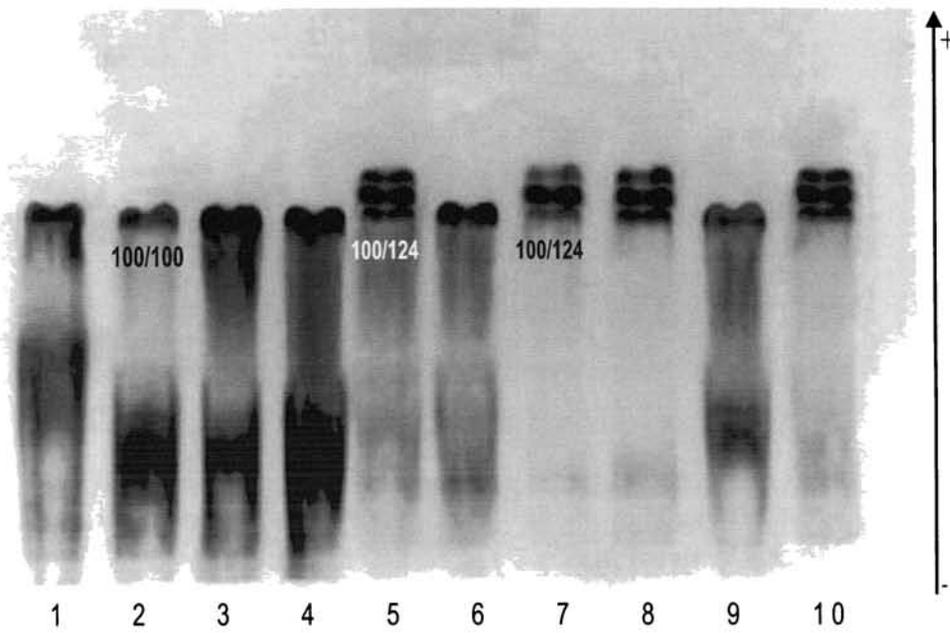
(b) Malate dehydrogenase

recipe used here for detection of αGPDH. A study of the functional dynamics of this non-specific enzyme detected here alone may reveal the reasons for the present observation (Koehn, 1970; Shaw and Prasad, 1970). For the purpose of interpretation of the data from this non-specific dehydrogenase enzyme, its locus has been designated as NSDH(non-specific dehydrogenase).

4.   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 genotypes. Single banded genotype (100/100) at the first locus were formed by the allele *AK-1*100* and two banded (100/110) by the alleles *AK-1*100* and *AK-1*110*. The second locus also having single banded and double banded genotypes which were homozygous and heterozygous respectively. The alleles of locus-2 were *AK-2*100* and *AK-2*77* accounted for heterozygous (77/100) and *AK-2*100* for homozygous (100/100) genotypes. The enzyme was monomeric in the species (Fig. 7).

5.   EST (Esterase)

The enzyme esterase also expressed three zones of activity (Fig. 8 and Plate 3b). The fastest and slowest moving zones formed of inconsistent bands were not scored. The banding patterns 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*100* and *EST*117* produced heterozygous genotype (100/117). The homozygous genotype (100/100) was formed of *EST*100* allele. The esterase in *P. monodon* is monomeric in structure.

GPI

Locus 1

100/100

100/116   116/116

EST

Locus 1

100/117   100/100
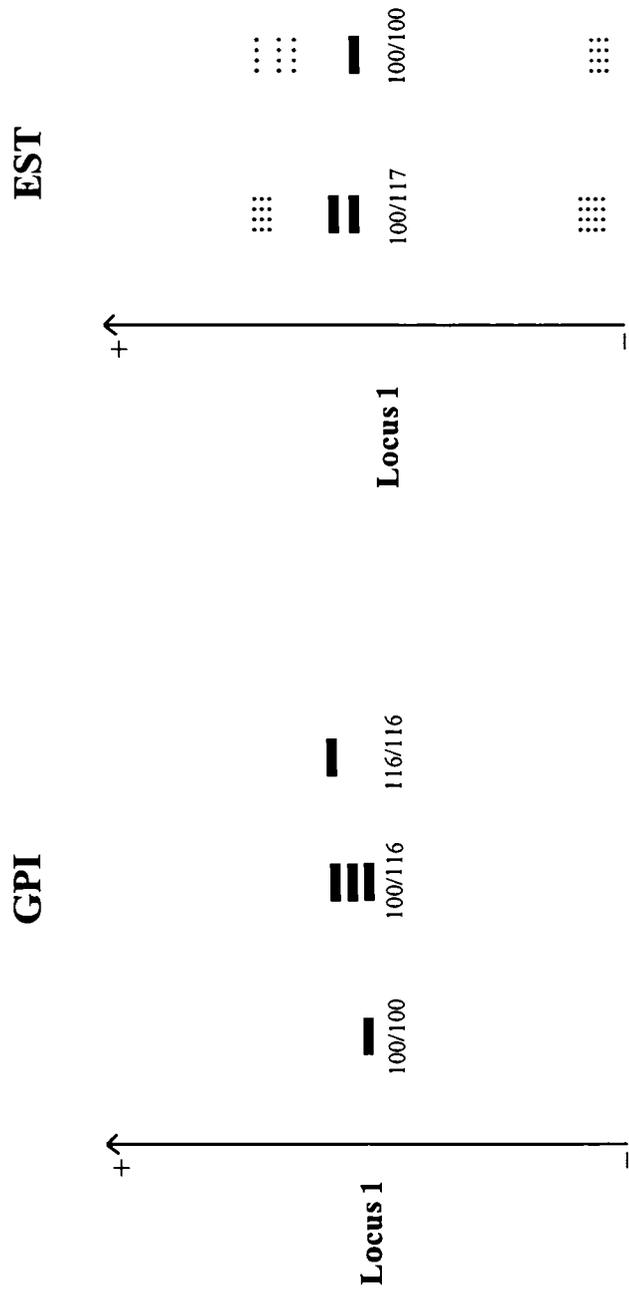
Fig. 8   Zymogram patterns of GPI and EST in muscle tissue of *P. monodon* of South India

PLATE – 3

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

GPI* 100/100 : Homozygote

GPI* 100/116 : Heterozygote

GPI* 116/116 : Homozygote

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

EST * 100/100 : Homozygote

EST * 100/117 : Heterozygote

PLATE 3
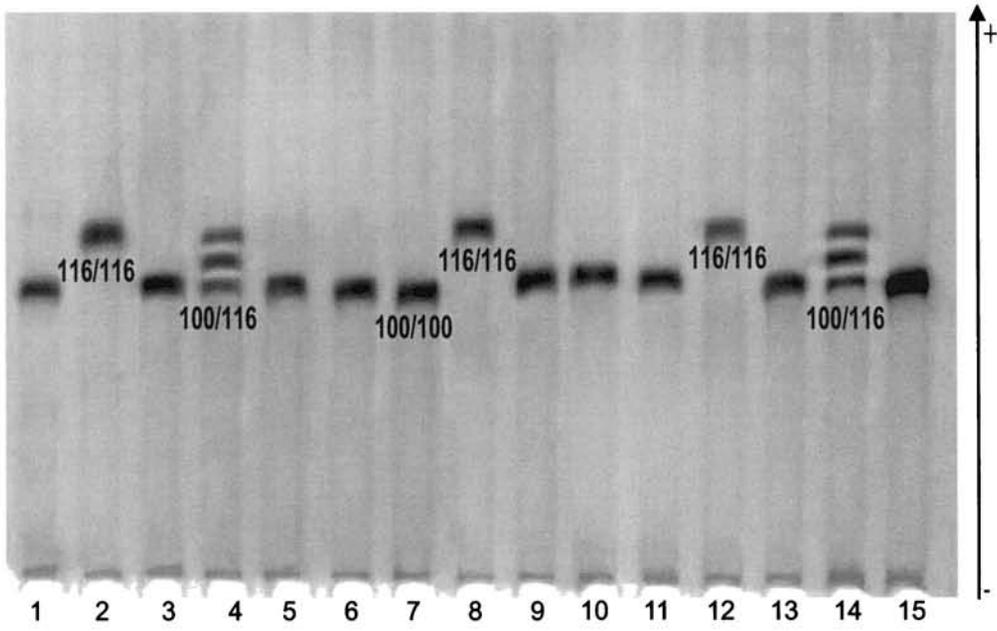
(a) Glucose phosphate isomerase
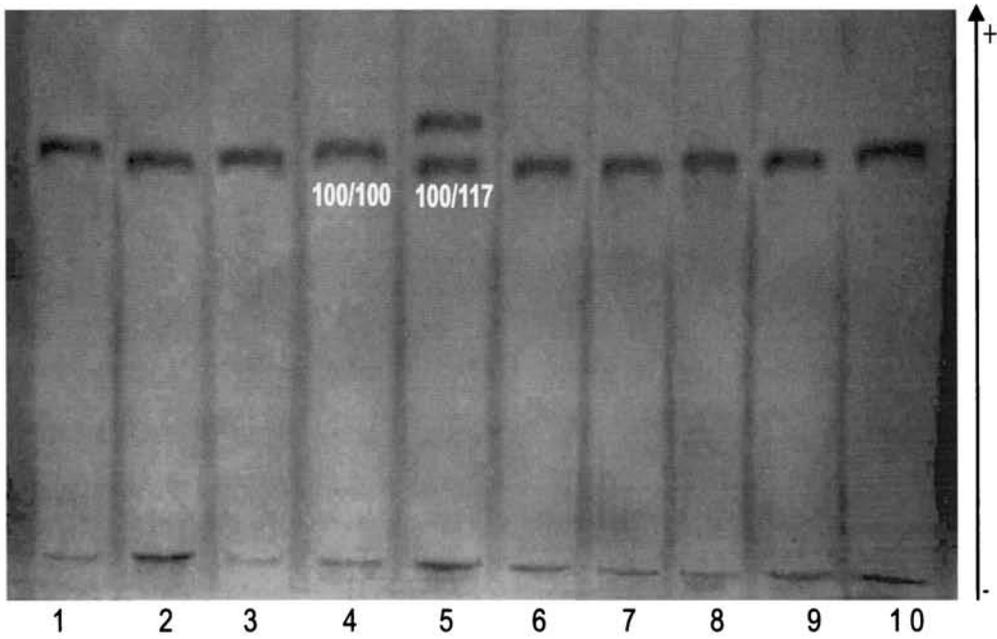
(b) Esterase

6.   GPI (Glucose phosphate isomerase)

The GPI enzyme showed only one major zone of activity in *P. monodon*. Three different genotypes were expressed at its locus (Fig. 8 and Plate 3a). There were two homozygotes [(100/100), (116/116)] and heterozygote (100/116). The alleles *GPI*100* and *GPI*116* accounted for the observed genotypes. The three banded nature of the heterozygote indicates that GPI enzyme has a dimeric structure in the species.

7.   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 genotypes were scored. The alleles *sMDH*100* and *sMDH*124* produced the homozygote (100/100) and the heterozygote (100/124) genotypes (Fig. 9 and Plate 2b).

8.   ODH (Octanol dehydrogenase)

The ODH enzyme showed only one major zone of activity in *P. monodon*. Seven different genotypes were expressed at its locus (Fig. 9 and Plates 4 & 5). However only four of these (81/100, 81/109, 91/100, 100/109) were three banded and others were single banded. The alleles responsible for the seven genotypes were *ODH*81*, *ODH*91*, *ODH*100* and *ODH*109*. The allele 100 accounted for homozygous genotype (100/100). Thus ODH showed a dimeric structure in *P. monodon*. The slowest moving minor bands also formed inconsistent phenotypes.
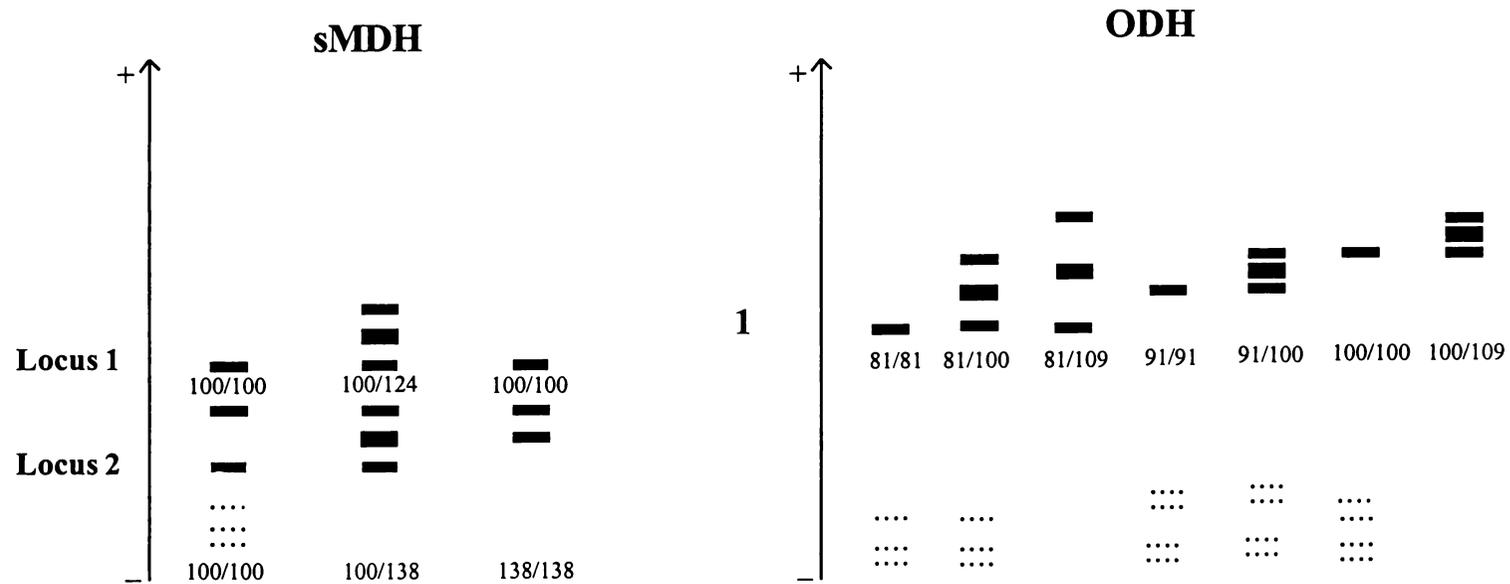
Fig. 9   Zymogram patterns of sMDH and ODH in muscle tissue of *P. monodon* of South India

PLATE – 4

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

ODH *91/91   :   Homozygote

ODH *100/100 :   Homozygote

ODH *100/109 :   Heterozygote

ODH *81/100   :   Heterozygote

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

ODH *100/100 :   Homozygote

ODH *91/100   :   Heterozygote

ODH *81/109   :   Heterozygote

PLATE 4
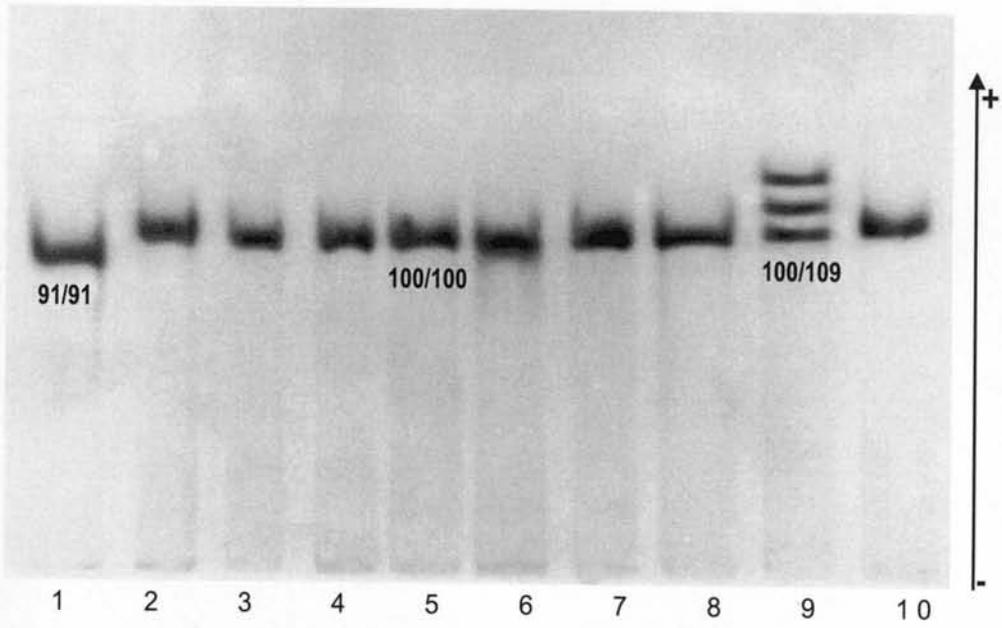
(a) Octanol dehydrogenase
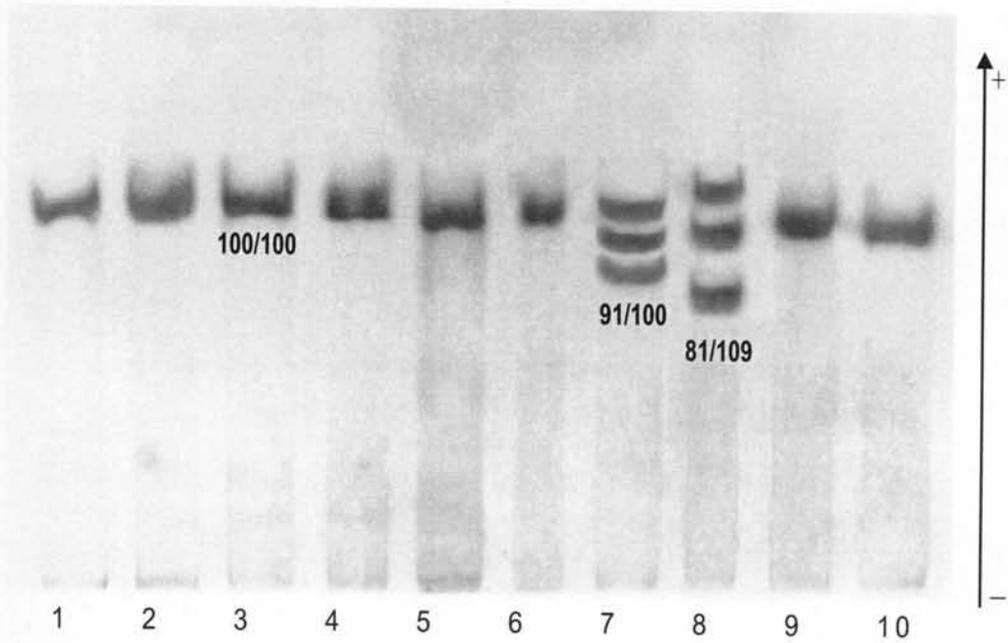
(b) Octanol dehydrogenase

PLATE – 5

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

ODH * 81/81   :   Homozygote

ODH * 100/100 :   Homozygote

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

ODH * 81/100   :   Heterozygote

ODH * 100/100 :   Homozygote

PLATE 5

81/81

100/100

1   2   3   4   5   6

(a) Octanol dehydrogenase

100/100

81/100

1   2   3   4   5   6   7   8
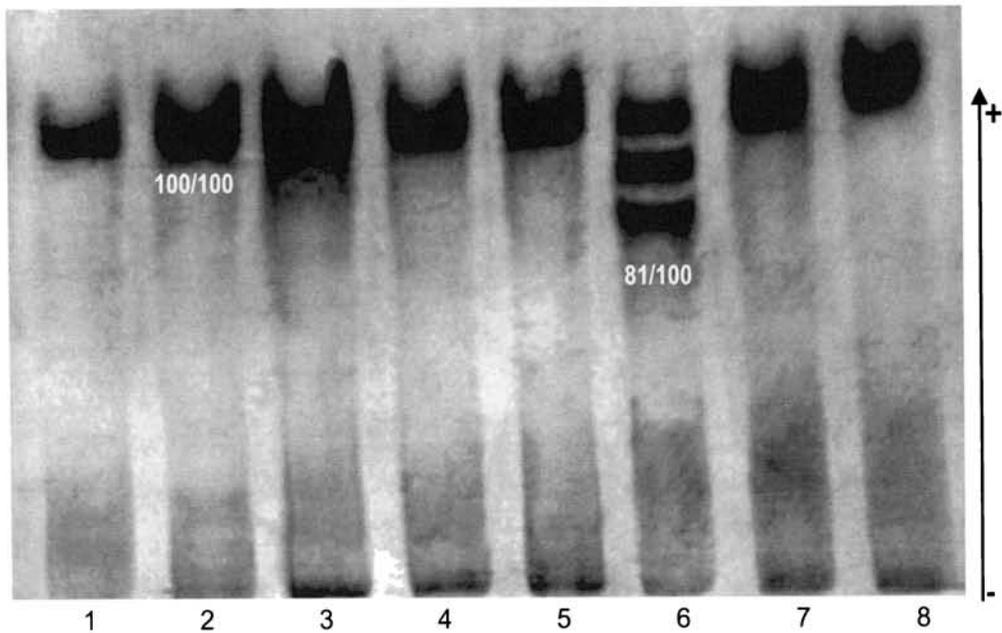
(b) Octanol dehydrogenase

9. PGM (Phosphoglucomutase)

The PGM enzyme showed only one major zone of activity in *P. monodon*. Three different genotypes (100/100, 100/108, 108/108) were expressed by the enzyme (Fig. 10 and Plate 6b). The two alleles responsible for the three phenotypes were *PGM*100* and *PGM*108*. PGM showed monomeric pattern in *P. monodon*.

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.

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.

4.2.3 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. 10 and Plate 6a). All tissues showed about ten

42
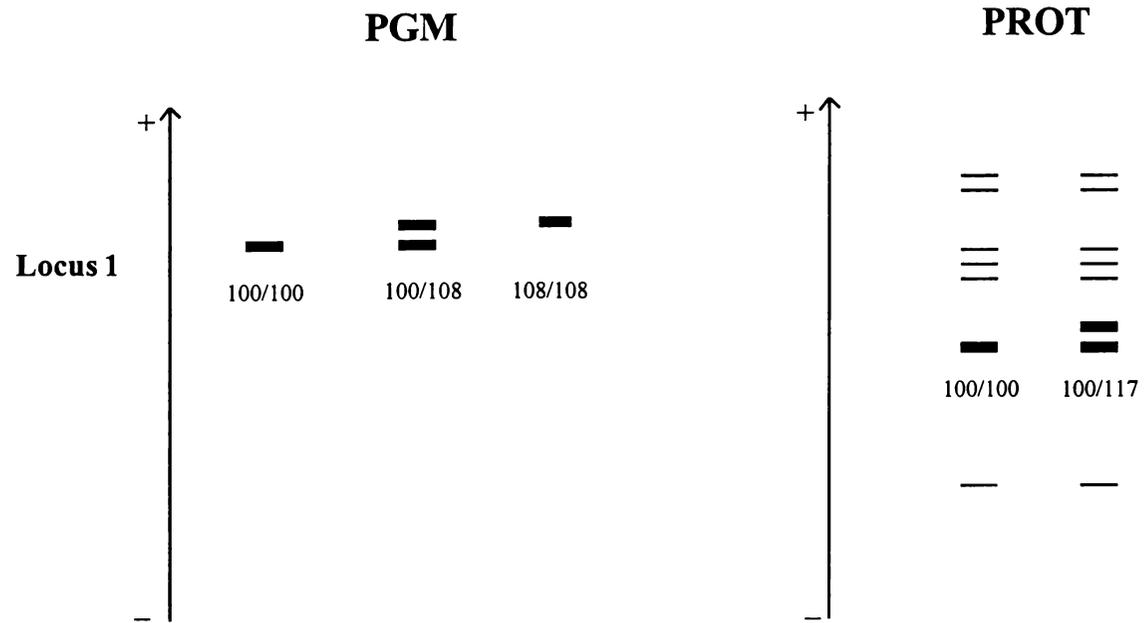
Fig.10 Zymogram patterns of PGM and PROT in muscle tissue of *P. monodon* of South India

PLATE – 6

(a) 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

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

PGM * 100/100	:	Homozygote
PGM * 100/108	:	Heterozygote
PGM * 108/108	:	Homozygote

PLATE 6

100/100

100/117

1 2 3 4 5 6 7 8 9 10

(b) General Protein

108/108

100/108

100/100

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
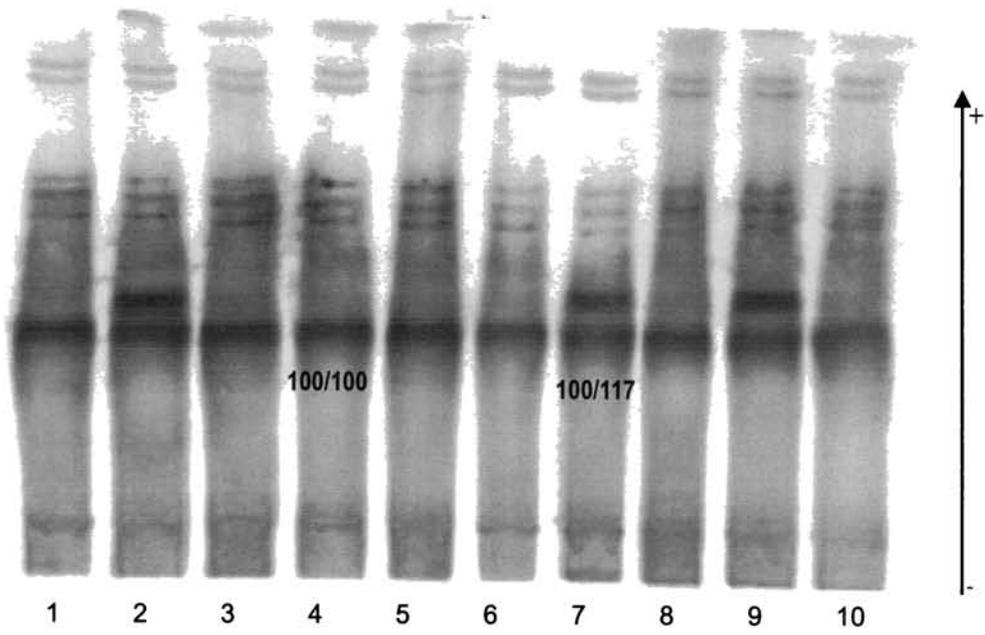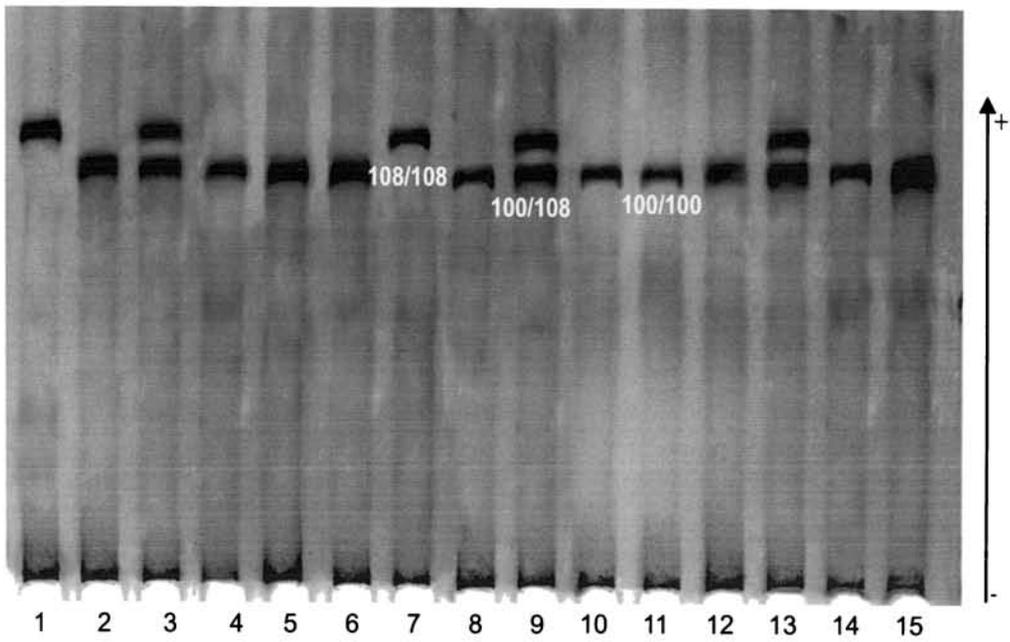
(b) Phosphoglucomutase

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 (nDNA)

4.3.1a Polymerase Chain Reaction

The Operon 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, the samples were analysed using only these two primers. The RAPD profiles revealed by these two primers are described below.

OPA 2

Twenty specimens, ten each from Chennai and Kochi were screened with OPA 2 (Fig. 11 & Plate 7a, b). It produced a total of 16 bands in the species, *P. monodon* (Table 8 The kilobases of the OPA-2 fragments varied from 0.1 to 1.9 kb (0.1, 0.125, 1.3, 0.2, 0.56, 0.85, 0.87, 0.95, 1.0, 1.1, 1.15, 1.2, 1.4, 1.5, 1.6, 1.9 kb). The significant aspects of the results were that the number of fragments present in the two samples of the species was significantly different. Six RAPD fragments (0.1, 0.2, 0.85, 0.87, 0.95, 1.9 Kb) were unique to Kochi population. The other ten fragments were common to both the samples (Table 8 A).

OPA 4

Twenty specimens, 10 each from Kochi and Chennai were also screened with OPA 4. Fifteen fragments or the bands were produced by the OPA 4 in the species (Plate 8a, b). The kb sizes of the fragments were

Table 8. 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	0.85	0.87	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 & 16 are unique OPA-2 fractions to Kochi.

Similarly, fractions 1 & 11 are unique OPA-4 fractions to Chennai whereas the fractions 9 & 15 are unique OPA-4 fractions to Kochi.
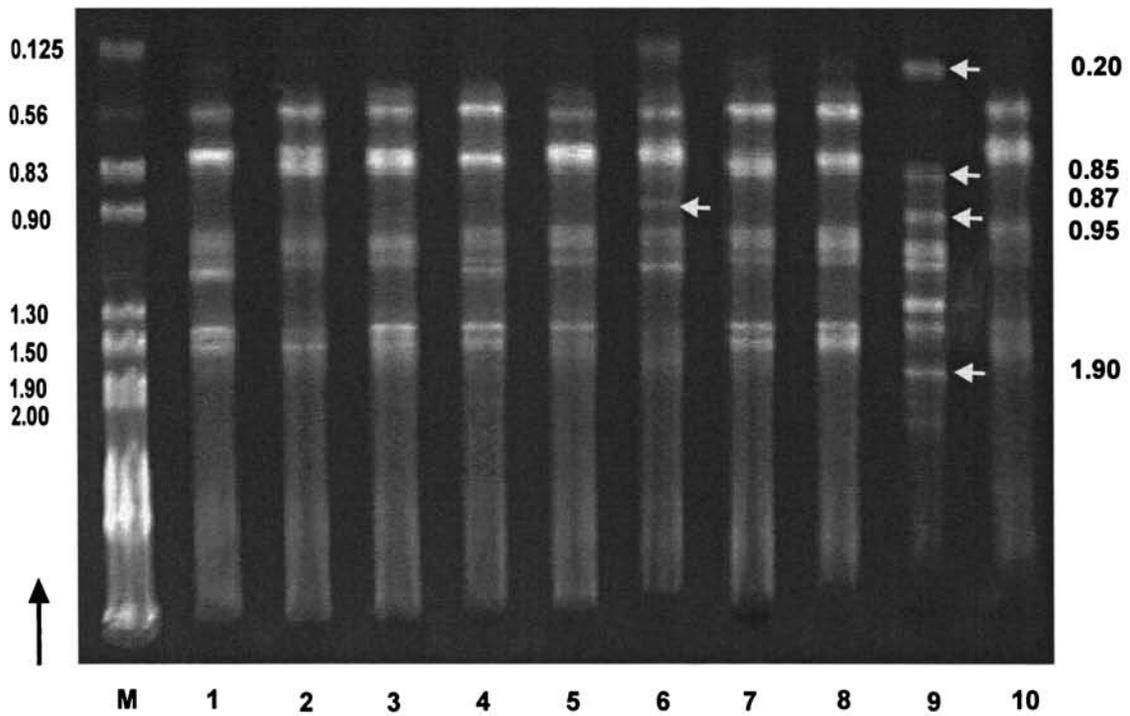
Fig.11 RAPD (OPA-2) banding pattern of *P. monodon* of South India

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.**

PLATE – 7

(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 7
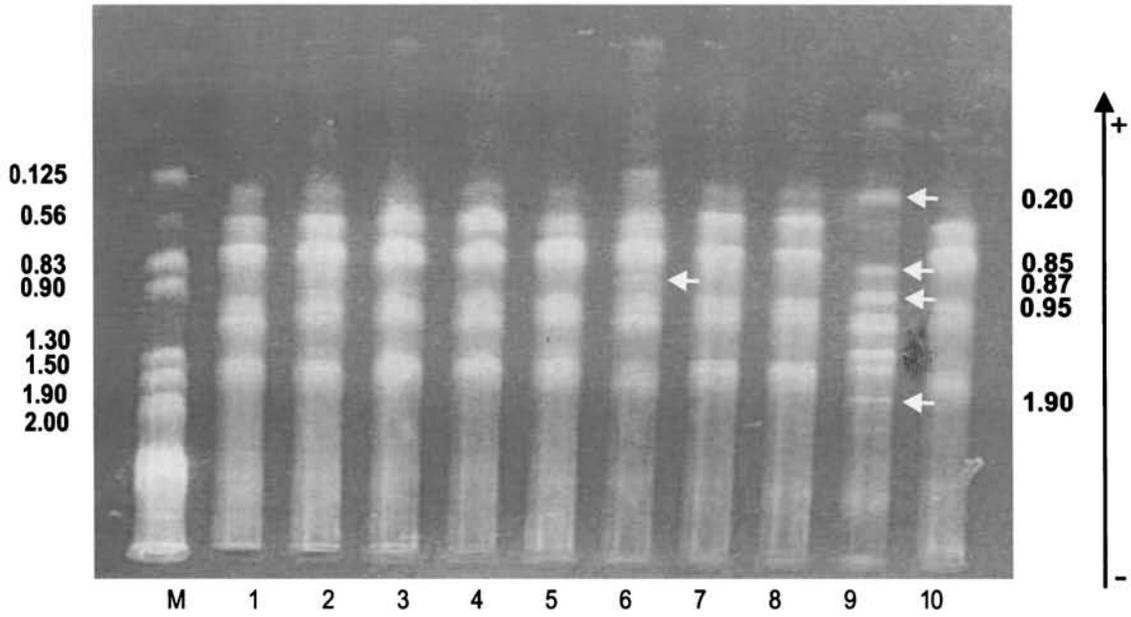
(a) RAPD (OPA-2) Banding pattern of
P. monodon of Chennai and Kochi
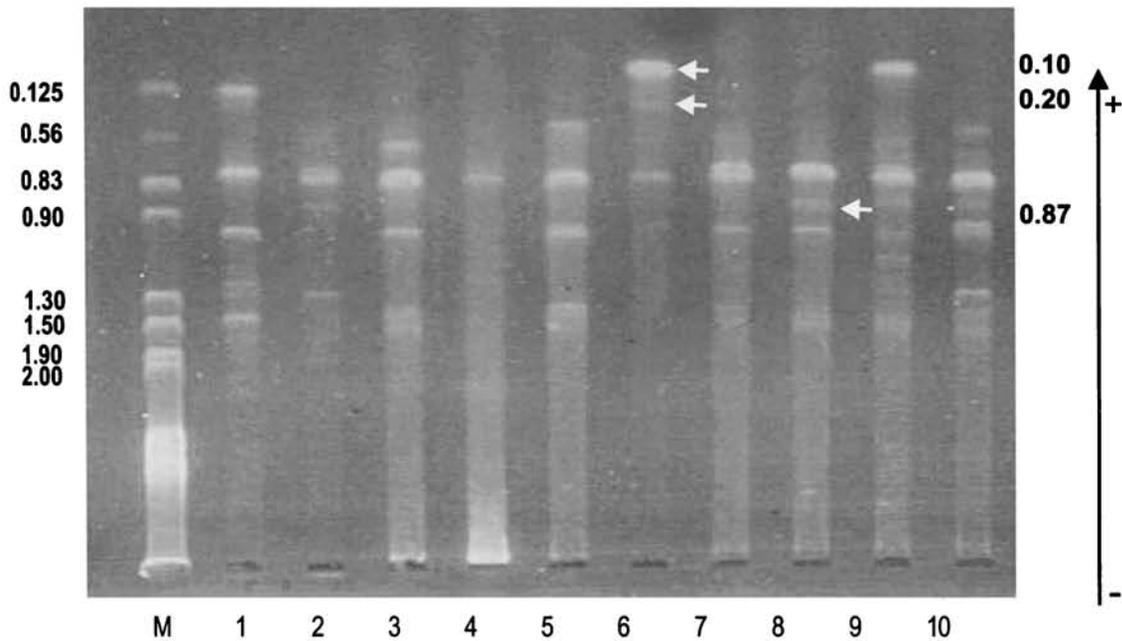
(b) RAPD (OPA-2) Banding pattern of
P. monodon of Chennai and Kochi

PLATE – 8

(a) 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

(b) 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

PLATE 8
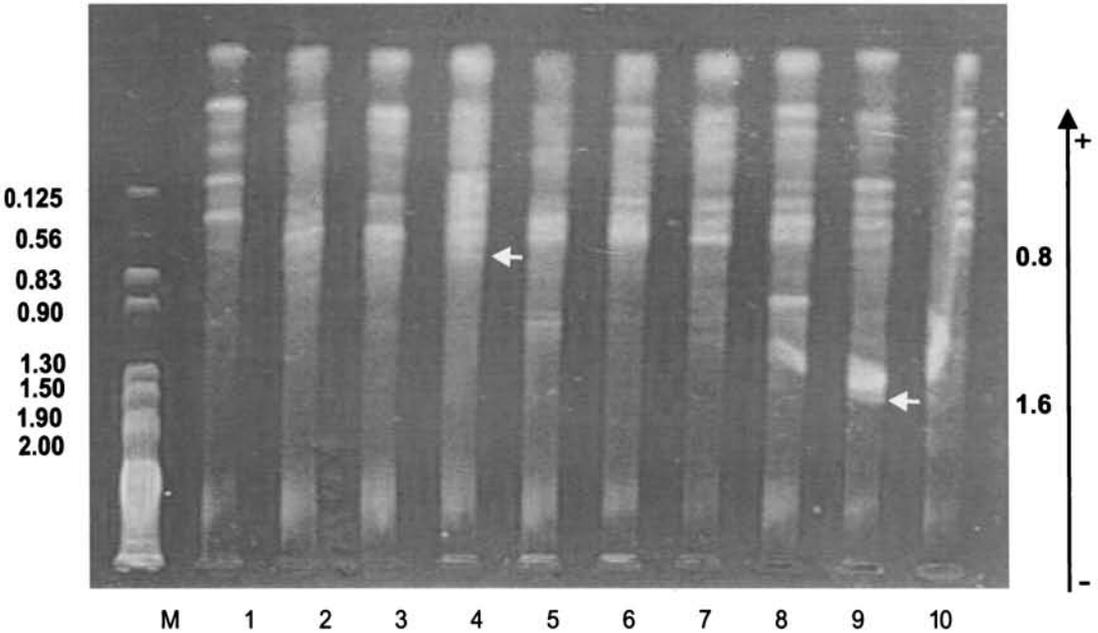
(a) RAPD (OPA-4) Banding pattern of
***P. monodon* of Kochi**
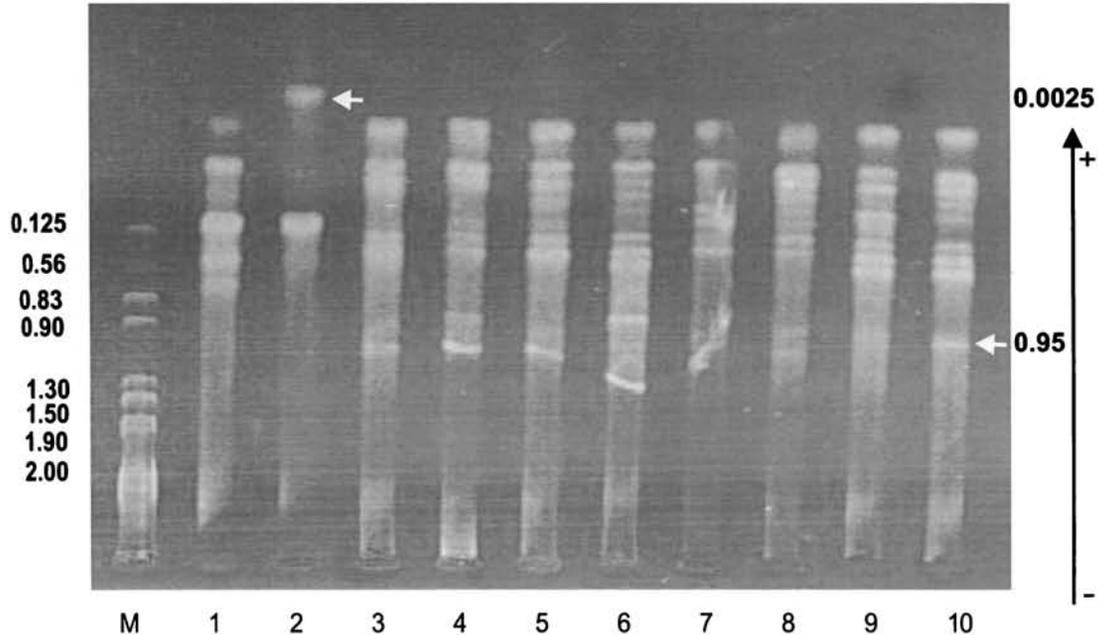
(b) RAPD (OPA-4) Banding pattern of
***P. monodon* of Chennai**

0.0025, 0.003, 0.005, 0.009, 0.1, 0.125, 0.3, 0.56, 0.8, 0.9, 0.95, 1.0, 1.1 1.4 and 1.6.   However, Kochi and Chennai populations could be differentiated by their own two unique fragments. The unique fragments of the Kochi were 0.80 and 1.6 Kb (Table 8B & Plate 8a) whereas, that of Chennai were 0025 and 0.95 Kb (Table 8B & Plate 8b).

4.4   Analyses of the Data

4.4.1 Morphometrics

4.4.1a   Correlation with tail weight

Nine metric and only one meristic variables were measured to detect variation among the random samples from different locations (Fig. 2). 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) only were considered for the analyses. Among these four variables, PCL showed the highest correlation with the tail weight (TLW) in both males (0.9605) and females (0.9639); path-coefficient (direct effects) in males (0.3097) and in 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 the highest correlation with the tail weight irrespective of sex (Table 4a, b & 5a, b).

4.4.1b Truss network analysis

Truss data of P. monodon from six centres (Karwar, Mangalore, Calicut, Kochi, Chennai and Kakinada) collected during 1996-2002 were used for the analysis. The 26 truss measurements (Fig. 3) 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 6) of the total

variations in the truss data. Thus two principal components accounted for 89.15% of the variations in truss measurements data. 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 that of the other localities though there was some overlapping of samples. Further analysis was done 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. Then, 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 thirteen polymorphic loci (AO-1, AO-2, FBALD, NSD, αGPDH, AK-1, AK-2, EST, GPI, sMDH, ODH, PGM and PROT-6) in all the samples tested. The general protein showed one polymorphic and six monomorphic loci. A total of thirty four alleles were detected in the species, *P. monodon.* (Table 9 & Fig. 6, 7, 8, 9, 10).

4.4.2.1 Allele Frequency

The frequencies of twenty six alleles at twelve 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 9). The AK enzyme was not tested in other four populations. The enzymes that could not be tested in some 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 two centres

Table 9. Allele frequencies of isozymes in *P.monodon* of South India

Locus	Allele	Kochi	Calicut	Mangalore	Karwar	Chennai	Kakinada
(N) FBALD		(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
(N) AO - 1		(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
(N) AO - 2		(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
(N) NSDH		(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
(N) αGPDH		(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	
(N) AK - 1		(10)	(0)	(0)	(0)	(20)	(0)
	100	1.00	NT	NT	NT	0.95	NT
	110					0.05	
(N) AK - 2		(10)	(0)	(0)	(0)	(20)	(0)
	77					0.33	
	100	1.00	NT	NT	NT	0.67	NT
(N) EST		(10)	(20)	(10)	(0)	(40)	(0)
	100	1.00	0.90	1.00	NT	0.95	NT
	117		0.10			0.05	
(N) GPI		(23)	(0)	(0)	(0)	(22)	(0)
	100	0.83	NT	NT	NT	0.84	NT
	116	0.17				0.16	
(N) sMDH		(30)	(20)	(10)	(19)	(50)	(30)
	100	1.00	1.00	1.00	1.00	0.99	1.00
	124					0.01	
(N) ODH		(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	0.82	0.97	0.90
	109	0.00			0.07	0.00	0.03
(N) PGM		(23)	(0)	(0)	(0)	(22)	(0)
	100	0.83	NT	NT	NT	0.84	NT
	111	0.17				0.16	
(N) PROTEIN 1 - 5 & 7 6		(60)	(20)	(20)	(20)	(60)	(30)
	100	1.00	1.00	1.00	1.00	1.00	1.00
	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

(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 frequencies of the observed and the expected genotypes were determined. 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 fifty one comparisons made (out of 58 values). Significant deviations (P< 0.05) were obtained only at four out of 24 loci, ie., AK-2 in Chennai, GPI in Kochi and Chennai, ODH in Chennai and Karwar, PGM locus in Kochi and Chennai (Table 10). The observed and the expected genotype frequencies were in Hardy-Weinberg equilibrium condition at all other loci in the six populations compared and hence, the observed variant banding patterns were considered as genetic variations controlled by the respective loci detected. The observed genetic variations were 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 alleles 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

The average values of twenty six alleles detected at twelve enzyme loci and seven general protein loci in six sample populations are shown in table 13. The average number of alleles varied from 1.16 to 2.00. The overall averages of the alleles between east and west coast populations were 1.59 and 1.93 respectively.

Table 10. Observed and expected (within parenthesis) 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) FBALD		**40**		**20**		**10**		**19**		**60**		**30**	
	100/100	34(34.6)	0.33	16(16.2)	0.247	8(8.1)	0.123	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) AO - 1		**40**		**20**		**10**		**19**		**60**		**30**	
	100/100	36(36.1)	0.111	16(16.2)	0.247	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)	0.061	0(0.05)	0.055					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) NSDH		**20**		**20**		**0**		**19**		**10**		**30**	
	100/100	16(16.2)	0.247	16(16.2)	0.247	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	100/100	20(20)	0	19(19.2)	0.074	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) AK - 1		**10**		**0**		**0**		**0**		**20**		**0**	
	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	—
(N) EST		**10**		**20**		**10**		**0**		**40**		**0**	
	100/100	10(10)	0	16(16.2)	0.247	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) GPI		**23**		**0**		**0**		**0**		**22**		**0**	
	100/100	18(15.84)	11.70*	NT	—	NT	—	NT	—	17 (15.52)	13.36*	NT	—
	100/116	2 (6.49)								2 (5.91)			
	116/116	3 (0.66)								3 (0.56)			
(N) sMDH		**30**		**20**		**10**		**19**		**50**		**30**	
	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) ODH		**40**		**20**		**10**		**19**		**58**		**30**	
	81/81									1(0.05)			
	81/100									1(3.38)			
	81/109							1(0.93)					
	91/91	0(0.04)						1(0.12)				0(0.15)	
	91/100	3(2.33)						1(2.49)				4(3.78)	
	100/100	37(37.64)	0.204	20(20)	0	10(10)	0	15(12.78)	8.4646*	56(54.57)	19.7633*	24(24.3)	0.2856
	100/109							0(2.18)				2(1.62)	
	109/109							0(0.09)				0(0.03)	
(N) PGM		**23**		**0**		**0**		**0**		**22**		**0**	
	100/100	18(15.84)	11.70*	NT	—	NT	—	NT	—	17 (15.52)	13.36*	NT	—
	100/111	2 (6.49)								2 (5.91)			
	111/111	3 (0.66)								3 (0.56)			
PROTEIN **(N)** 1 -5 & 7		**60**		**20**		**20**		**20**		**60**		**30**	
	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)	0.055	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 are shown in the table 13. It varied from 1.04 (Mangalore) to 1.22 (Karwar) in the west coast populations and 1.14 (Chennai) to 1.19 (Kakinada) in the east coast populations.

4.4.2.3c Heterozygosity

The observed and expected Heterozygosity values of *P. monodon* are shown in table 13. The observed heterozygosity values varied from 0.05 (Mangalore) to 0.21 (Karwar) in the species. The highest value of heterozygosity (0.21) was shown by Karwar (west coast) and the next (0.17) in Chennai (east coast). The expected values varied from 0.05 to 0.18. It ranged from 0.05 (Mangalore) to 0.15 (Karwar) in west coast samples (Kochi, Calicut Mangalore and Karwar). In the east coast, it varied from 0.09 (Kakinada) to 0.18 (Chennai). The mean expected heterozygosity value per individual was 0.115 and observed heterozygosity was 0.122.

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) genetic similarity analyses for paired populations. The results of these analyses are shown below.

4.4.2.3d F_{ST} Analysis

The F_{ST} values showed the levels of genetic differentiation among the populations compared (Table 11). Fifteen pairwise comparisons produced 85 F_{ST} values. Of these, 82 were negative, the three positive

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Table 11. F_{ST} values for the pairwise comparisons of isozyme genetic variabilities in *P. monodon of South India*

Table 11. F_{ST} values for the pairwise comparisons of isozyme genetic variabilities in *P. monodon of South India*

	FBALD	AO-1	AO-2	*NSDH*	αGPDH	ODH	sMDH	AK-1	AK-2	GPI	PGM
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]	0.03608[l]	0.03608[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 12. 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	0.9839	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	0.0161	0.0029	0.0077	0.0134	----	0.9915
Kakinada	0.0039	0.0023	0.0054	0.0065	0.0085	----

Table 13. Summary of Genetic Variation data in six populations of *P. monodon*

		Kochi	Calicut	Mangalore	Karwar	Chennai	Kakinada
				Locations			
Sample size		83	20	10	20	82	30
Number of loci (enzyme & protein)		19	19	19	19	19	19
Number of loci (enzyme)		12	12	12	12	12	12
Proportion of polymorphic loci (protein & enzyme)		63%	63%	63%	63%	63%	63%
Proportion of polymorphic loci (enzyme)		90%	90%	90%	90%	90%	90%
Average heterozygosity per locus	He	0.14	0.08	0.05	0.15	0.18	0.09
	Ho	0.10	0.09	0.05	0.21	0.17	0.11
Effective number of alleles		1.14	1.13	1.04	1.22	1.14	1.19
Average number of alleles/locus		1.58	1.75	1.16	1.86	2.00	1.86

F_{ST} values (0 - 0.05) produced by AK-2, GPI and PGM loci were shown only between Kochi and Chennai. This may indicate some inherent biochemical genetic heterogeneity between east and west populations of the species. The F_{ST} value 0.031 was quoted as significant in *P. monodon* of Australia (Benzie *et al.* 1992) with P<0.001.

4.4.2.3e   Genetic Identity

Nei's (1972) values of genetic similarity between paired populations obtained are presented in table 12. The similarity values ranged from 0.9839 to 0.9977 in the six populations from east and west coast. The lowest similarity (S) value (0.9839) recorded was between Kochi (west coast) and Chennai (east coast). The corresponding value of genetic distance (D) was 0.0161. The lowest distance value recorded was between Kakinada and Calicut (D = 0.0023) and the highest between Kochi and Chennai (D = 0.0161). The highest value (0.0161) produced between Kochi in the west coast and Chennai in the east coast also suggests that the genetic structure of these two populations may be heterogeneous. The reason for comparable lowest genetic distance value between Kakinada and Calicut samples may be that the enzyme AK that showed maximum allele frequency difference between Kochi and Chennai was not tested in Kakinada.

4.4.3   Molecular Genetics

4.4.3.1 Random Amplified Polymorphic DNA

The number of RAPD bands produced by each primer was counted from the photographs and the band sharing indices between the individuals were estimated using Nei's (1987) formula. These index values were averaged over the primers in order to find out the mean genetic similarities among the individuals within the east and west coast

Table 14. 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 15. 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

Table16. 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%)**

populations and between the east and west coast populations. The similarity values of each specimen in the total population ranged between 0.35 and 1.00 (Table 15). The mean percentage similarity among the individuals of Kochi population for OPA 2 and 4 was 74%, whereas that of Chennai population was only 69%. The mean percent similarity between the Kochi and Chennai populations for the primers was 78% (Table 16). In other words, 32% of the RAPD banding patterns between Kochi and Chennai are different.

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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 vital informations can be gained by standard 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 viz., multivariate analysis of body shape variations, electrophoretic analysis of the tissue isozymes and analysis of patterns of randomly amplified DNA. The significance of the results produced by these three independent methods applied on the species are discussed below separately.

Morphometrics

Twenty six truss measurements of body variations present in 428 specimens from six sample populations (Karwar, Mangalore, Calicut, Kochi - west coast and Chennai, Kakinada - east coast) of South India were used to compute the 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 body 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 the present morphometric study did not support the earlier

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hypothesis of stock differences based on stock assessment results (Rao *et al.*, 1993).

The first report of evidence of morphometric differentiation between sample populations in penaeid species is that of Horton (1982). He detected significantly different morphometric variations in samples of *P. stylirostris* and *P. vannamei*. However, he could not conclude whether these differences can form a basis for genetic stock differences (Lester and Pante, 1992). On the other hand, past reports of stock separations by multivariate analysis of morphometrics of fishes are many (Ihssen *et al.*, 1981; Winans, 1984).

However, the lack of significant morphological differences even between east and west coast samples of *P. monodon* of South India studies here need not always mean that these coastal populations are interbreeding and hence, genetically similar stocks. 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, the above hypothesis of morphometric homogeneity of these six populations of *P. monodon* was tested by an alternative, more efficient biochemical genetic method. The significance of the results of the biochemical genetic analysis of the six sample populations of *P. monodon* have been discussed below.

Biochemical Genetics

Surprisingly, the overall results of the biochemical genetic analysis also support the morphometric homogeneity of these six populations. The overall biochemical genetic variations shown between east and west coast samples were also not significantly different. The above second hypothesis of stock homogeneity presented here is based on the lack of

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significant differences in the allele frequencies estimated at a total of about twelve enzyme and seven protein loci (Table 9), F_{ST} values estimated for fifteen pairwise comparisons of genetic variabilities at twelve enzyme loci (Table 11) and fifteen values of genetic similarities made for six population samples (Table 12). Interestingly, comparable reports of insignificant biochemical genetic variations among geographical populations of other penaeid species with reference to the allozyme loci examined in many 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, there are also examples of reports of genetic stock differences shown by significantly different allozyme frequencies in the species of prawns and fishes. 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). Probably, the earliest examples of detection of reproductively isolated biochemical 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 reproductive isolation of stocks in the red fish (*Sebastes mentella*), anchovy (*Engraulis encarasicholus*), sockeye salmon (*Oncorhynchus nerka*) were detected even by repeated sampling for several years (Altukhov, 1981).

It appears that the chances of geographic isolation and differentiation of stocks due to evolutionary forces like migration, genetic drift and natural selection (Dobzhansky, 1960; Ayala and Keiger, 1980, 1984) are more than lack of it by 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 may be many and may suit to specific cases. A major reason for showing lack of differences may be 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.

The above reasoning is evident in the report of Benzie *et al.* (1992). Seventy five enzymes had to be screened to detect seven polymorphic enzymes. However, allele frequencies only at PGM, GPI and MPI loci alone helped to detect the 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 for the same species. Another important theoretical reasoning for the observation of closely similar allozyme frequencies even between two distant populations may be that a migration of about fifty individuals per generation (Allendorf and Phelps, 1981) can mask the inherent stock differences. The probable reason for the lack of significant allele frequency differences, especially between the east and west coast samples of the *P. monodon* of South India examined here may be that the number of potential polymorphic loci examined were insufficient. The number of polymorphic enzymes used for genetic stock differentiation of east and west cost populations of Australia by Benzie *et al.* (1992) was seven and in the present investigation was nine, out of which only three enzymes were not in common. The GPI and PGM enzymes that showed significantly different allele frequencies in the Australian populations of *P. monodon* did not show comparable stock differences in the Indian species of *P. monodon* examined here.

A general conclusion that emerges out of the above debate over the lack of significant allozyme frequencies or its significant differences between distant populations as reported by Benzie *et al.* (1992) is that such phenomenon may depend on the species or even the geographic areas examined even for the same species as in *P. monodon*. In other words, though PGM and GPI allozyme loci were strong genetic markers for the Australian populations of *P. monodon*, these two enzymes could not differentiate its populations of east and west cost of India.

Preliminary tagging experiments of prawns showed that mixing of prawn populations of east and west coast of South India may not be taking place (Annon, 1982; Vijayaraghavan *et al.*, 1982). The mixing of these two populations by migrations is probably prevented by the hydrographical barrier (Ramamirtham and Jayaraman, 1960). On the contrary, 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 the distribution of specific alleles at ODH and AK loci appears to support non-mixing hypothesis of east and west coast populations of the species. For example, the allele, *ODH*81* was absent in Kochi while its frequency was 0.03 in Chennai, though sample size was sufficiently large in both areas. Another allele *ODH*91* was absent in Chennai population but present in Kochi (0.03), Karwar (0.08) and Kakinada (0.07) frequency levels. 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 9); though allele frequencies at other eleven enzyme loci were not significantly different. Comparable rare allele phenomenon was also reported in *P. indicus* (Bindhu Paul, 2000). The presence or absence of such rare or private alleles in populations may imply their separate stock identity (Jamieson, 1974; Ayala and Keiger, 1980, 1984). If the rare alleles are indicative of stock isolation, the present observation of the absence of the allele *ODH*81* in Kochi and its presence in Karwar also

suggests that these two local populations in the west coast are also heterogeneous populations. On the same reasoning, presence of the allele *ODH*91* in Kakinada samples and its absence in Chennai samples should mean that these two east coast populations may be also isolated. If any of these two populations are proved to be really isolated, these rare alleles can be utilized as genetic markers for selection of breeders from a desired stock for selective breeding programmes (Lester, 1983; Goswami *et al.*, 1986; Lester and Pante, 1992).

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 average heterozygosity for *P. monodon* from South India was 0.122 (Table 13) which is comparatively much higher than that of other penaeids and the value shows that the species from South India has high genetic variability at the allozyme loci examined here. The genetic variability reported for the species from South east Asia is also at lower level (Sodsuk, 1996). The recent review (Benzie, 2000) of allozyme variability in twenty seven species of penaeus and metapenaeus showed that allozyme variability may vary from 0.008 as estimated in two populations of *P. monodon* to 0.089 in *P. aztecus*. The genetic variability reported here in *P. monodon* of South India (0.122) is much higher than thirty percent over the highest reported (0.089) in penaeids (Benzie, 2000) and (0.083) reported for *P. monodon* from South-east Indian Ocean (Forbes *et al.*, 1999; de la Rosa-Velez *et al.*, 2000). The logical reasons for such significant differences in the genetic variability reports by different investigators even on the same species may be due to differences in the allozymes examined and differences in the number of loci examined or differences in the techniques followed. Since the method and techniques applied by different investigators are bound to vary, even for the same species, the reported levels of genetic variability may not be the natural for the species. Application of closely comparable

experimental alone can reveal the real picture of the natural genetic variability present in the different geographical populations of one species. The most probable reason for the reported differences in the genetic variability of *P. monodon* of South India, Australia and that of South-east Indian ocean is that these geographically different populations are genetically heterogeneous.

The above critical analysis of the biochemical genetic results led us to conclude and make the following suggestions. The overall close genetic similarities at the twelve allozyme loci indicate that all the six populations of *P. monodon* of South India including its east and west coast populations have homogeneous stock structure. Since, the ODH rare alleles 81 and 91 are either present or absent in the Kochi and Chennai populations and since the frequency of the AK-2 allele 100 *(AK-2*100)* was 1.00 in Kochi while it had a frequency of only 0.67 in Chennai (Table 9), these two loci should be screened in larger samples in these two populations to confirm the present hypothesis that these rare alleles indicate the inherent genetic heterogeneity of its populations. The above suspected heterogeneity especially of Kochi and Chennai populations is also indicated by the highest F_{ST} (0.03708) (Table 11) and highest genetic distance (0.0161) values respectively obtained only for these two populations (Table 12). Now let us finally discuss the more interesting molecular genetic results obtained for Kochi and Chennai populations that strongly support the above stock heterogeneity hypothesis.

Molecular Genetics

Though the total numbers of the RAPD fractions produced by the Operon primer, OPA-2 and OPA-4 were sixteen and fifteen respectively. The number of fractions present in Kochi and Chennai were significantly very different.. For example, Chennai had only ten out of sixteen fractions presented in Kochi. In other words, six kilobased fractions (0.1, 0.2, 0.85,

0.87, 0.95, 1.9 Kb) of the primer OPA-2 were unique to Kochi sample (Table 8 A). Similar significant differences were shown by OPA-4. Each sample was short of two but fractions with different kilobases. For example, the fractions 0.0025 and 0.95 Kb were absent in Kochi. 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 8 B). Hence, the above significant differences caused by the unique RAPD 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 7a, b & 8a, b) between individuals of each sample also mean that level of DNA variability is also significantly different in Kochi and Chennai (Table 14 & 15). 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 a 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 the natural genetic diversities in penaeid prawns, especially in *P. monodon* which fact is more evident in the present report. Because the genetic stock structure differences present in the east and west cost populations of *P. monodon* of South India could be clearly detected only by RAPD method and not by the morphometric or allozyme methods.

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

to select separately the desired east or the 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 now be used to detect any possible mixing of these two stocks, especially, during selective breeding programmes or larval rearing period.

Nevertheless, a word of caution also to be mentioned here. The present conclusions are based on only a few specimens and a single RAPD 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* of South India. At the same time, the very possible aspect of the RAPD experimental conditions that favours the present conclusion is to be highlighted here. The significantly different RAPD profiles obtained here were produced on a single gel under uniform electrophoretic and staining conditions. Hence, the RAPD experimental error that might have affected the results and the conclusion was the least possible. This is further strengthened by the fact that the RAPD differences were shown only by OPA-2 and OPA-4 primers where as all the other eight OPA primers tried produced only non-polymorphic RAPD profiles.

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 by truss network method and allozyme and protein variations detected by electrophoresis were not significantly different in six populations (Karwar, Mangalore, Calicut, Kochi, Chennai, Kakinada) of *P. monodon* of South India. However, the presence or absence of ODH and AK rare alleles between east and west coast populations may indicate inherent stock differences in the species. The species has comparatively high biochemical genetic variability. The

unique RAPD fractions detected in Kochi and Chennai samples alone strongly suggest that these two populations may be distinct genetic stocks.

The significance of the present conclusions may be based on the original findings corroborated by detailed future studies.

CONCLUSION

6. CONCLUSION

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 and path-coefficient (direct effect) to the tail weight (TLW) in *P. monodon* (Table 4 a, b & 5 a, b).

3. The estimated average number of alleles per locus (1.71), percentage of polymorphic loci (90%) and the average heterozygosity (0.122) reveal that the species, *P. monodon* of South India has high genetic variability (Table 13).

4. The statistical comparisons of the biochemical genetic variations among 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. However, the presence of ODH and AK allozyme rare alleles detected in the Kochi and Chennai samples indicates that the east and west coast populations of the species may be genetically heterogeneous populations.

5. The results of molecular genetic method obtained here were strikingly different from that of the other two methods. The random amplified polymorphic DNA (RAPD) profiles (Plate 7a, 7b & 8a, 8b) discovered for the first time in the specimens from Kochi and Chennai were significantly different. Hence, it is concluded that the molecular genetic stock structure of these two populations is heterogeneous (Table 15 & 16).

SUGGESTIONS

7. SUGGESTIONS

1. A detailed biochemical genetic screening of all the populations of *Penaeus monodon* of India, using a wide range of allozyme genetic markers including glucose phosphate isomerase (GPI), phospho-glucomutase (PGM) mannose-6-phosphate isomerase (MPI) (Lester and Pante, 1992; Benzie *et al.*, 2000) along with Adenylate kinase (AK) which already showed significantly different allozyme frequencies between Kochi and Chennai populations tested.

2. The present 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 microsatellite techniques have produced the highest genetic variability in the *P. monodon* of Thailand (Supungul *et al.* 2000), the same technique is also to be applied in the future investigations of the Indian species.

SUMMARY

8. SUMMARY

The thesis contains the detailed aspects of an investigation entitled **"Genetic Studies of the marine penaeid prawn** *Penaeus monodon* **Fabricius, 1798"** from South India. 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 and path-coefficient (direct effect) to the tail weight (Table 4a, b & 5a, b).

The body shape variations were measured by truss network method (Fig. 3) and these were 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 that the measured morphometric variations are not significantly different in the six samples. 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 twelve 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 at AK-2 locus. The AK enzyme was not tested in other populations (Table 9).

The estimated values of average number of allozyme alleles per locus (1.71), heterozygosity (0.122) and percentage of polymorphic loci (90%) indicate that the species has comparatively high biochemical genetic variability (Table 13). The statistical significance of the 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 11). 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 polymerise 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, six were absent in the Chennai sample. Of the fifteen OPA-4 fractions, two different fractions were absent in Kochi (0.0025, 0.95 kb) and Chennai (0.80, 1.6 kb)samples. In other words, six 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 8 & Plate 7 & 8).

Since the RAPD profiles of Kochi and Chennai were significantly different (Plate 7, 8), the molecular genetic stock structure of Kochi and Chennai populations of the species is 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 also highlighted.

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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