Chapter I

Introduction

1.1 General introduction

The term prawn is more commonly used by the Americans to refer to species in the cultured Penaeidae, while marine prawn (or prawn) is more common in Asia-Pacific (Anderson, 1993). Prawns are the most valuable fisheries, particularly in terms of value of foreign exchange earning. This is because of a strong market demand, with the highest prices coming in the international markets. The United State of America and Japan are the two major consumer markets that have caused the rapid growth of the shrimp industry.

There are at least 60 species of commercially important penaeid prawns. In 2000, the world cultured prawn production account for 56% of *P. monodon*, 17% of *P. merguiensis*, 16% of *P. vannamei* and 11% of the others. In 1985, world production of farm-raised prawn (whole weight) hovered around 200,000 metric tons, about 10% of total world supplies of around 2 million metric tons. In 1995, commercial fishing produced around 2 million metric tons a year, so the 700,000 tons contributed by prawn farmers represents about 25% of world shrimp supplies. Thus, from 1985 to 1995, the production of farmed prawn increased 250%. Since 1995, viral and bacterial disease have slowed the growth of shrimp farming in the eastern and western hemispheres. Nonetheless, production set a new record in 1999. With lots of

technology in place, with new stains of prawn on the market, with case histories on many diseases, the industry appears poised for another spurt in production. If the production of farmed prawn were to increase by 200% in the 1995-2005 decade, production would be 2.1 million metric tons in 2005. Estimation of the commercial prawn fishery would be 1.8 million tons in 2005. That would place world prawn production at 3.9 million tons, with farmed prawn representing 54% of the total (Source: http://members.aol.com/brosenberr/History.html)

Thailand and Ecuador have the highest reliance on farmed prawn supply for export. In Thailand approximately 60% of the total harvest prawns was from culture. Prawn farms and hatcheries are scattered along the coastal areas of Thailand. Southern provinces (Nakorn Sri Thammarat and Surat Thani) account for the majority while those in the East (Chanthaburi) and Central regions (Samut Sakhon and Samut Songkhran) comprise the minority in terms of number. The intensive farming system (85%) had been used for *P. monodon* farming activity resulting in the consistent increase in the outcome production (Source: Department of fisheries).

Thailand is the world's largest exporter of prawn with a total export of 249,632 metric tons worth 107,890 million baht in the year 2000 (Table 1.1). Other major exporting countries are such as Ecuador, Mexico, India, China and Indonesia. The USA is the major importer of Thai prawn, accounting to approximately 49.33% of Thailand's total export in term of value in the year 2000 followed by Japan (22.19%) and Singapore (6.38%) (Source: Custom Department). Thai prawn farmers benefited from significant improvements in the black tiger prawn farming. However, due to the

unexpected outbreak of various diseases, production decreased by 8% and 7% in 1996 and 1997, respectively. Nevertheless, Thailand is still the largest *P. monodon* producer (Table 1.2).

The farming activity of *P. monodon* in Thailand has rapidly increased reflecting large annual production. The reasons for this are supported by several factors including the appropriate farming areas without the serious disturbing from typhoons or cyclone, small variable of sea water during seasons, and ideal soils and terrain for pond construction. Culture of *P. monodon* causes increasing national revenue, therefore this penaeid prawn species is economically important species in Thailand.

Table 1.1 Cultured prawn production in Thailand in the year 1998-2001.

Year	Culture Area (rai*)	Production (metric tons)	Quantity for export (metric tons)	Value for export (billion baht)
1998	495,000	220,000	156,176	58,343
1999	500,000	250,000	240,529	87,579
2000	560,000	290,000	249,632	107,890
2001	600,000	320,000	275,000	120,000

*6.25 rai = 1 hectare

Source : Shrimp culture newsletter (2001).

Table 1.2 Statistics illustrating the United State of America's import of prawn in the year 2000-2001.

Unit: metric tons

Country	2000	2001	% Change
Thailand	126,462.85	136,079.10	7.60
Vietnam	15,694.46	33,248.66	111.85
India	28,395.17	32,885.78	15.81
Mexico	29,075.57	30,028.12	3.28
China	18,189.24	28,032.29	54.11
Ecuador	19,096.43	26,762.22	40.14
Indonesia	16,737.73	15,830.53	-5.42
Venezuela	14,877.98	9,525.53	-35.98
Bangladesh	10,205.93	8,709.06	-14.67
Canada	8,845.14	6,713.00	-24.11
Honduras	8,618.34	9,661.61	22.41
Guyana	7,892.59	11,702.80	35.79
Brazil	5,896.79	9,797.69	66.15
Panama	5,851.40	6,894.67	17.83
others	29,257.03	34,473.67	17.83
Totals	345,096.62	400,344.73	16.01

Source : Shrimp culture newsletter (2001).

1.2 Taxonomy of *P.monodon*

The taxonomic definition of the black tiger prawn is as follows (Bailey-Brook and Moss, 1992):

Phylum Arthropoda

Class Crustacea

Subclass Malacostraca

Suborder Natantia

Infraorder Penaeid

Superfamily Penaeoidae

Family Penaeidae Rafinesque,1815

Genus *Penaeus* Fabricius, 1798

Subgenus *Penaeus*

Species *monodon*

Scientific name: *Penaeus monodon* Fabricius, 1798.

English common name: black tiger prawn or

giant tiger shrimp

1.3 Morphology

Externally the prawn can be divided basically into the thorax and abdomen (Figure1.1). The thorax (or head) is covered by a single, immobile carapace which protects internal organs and support muscle origin. The rostrum, extending beyond the tip of the antennular peduncle, is sigmoidal in shape, and has 6-8 dorsal and 2-4 ventral teeth, mostly 7 and 3, respectively. The carapace is carinate with the adrostral

carina, almost reaching to or not as far as the epigastric or first tooth. The gastro-orbital carina occupies the posterior one-third to one half distance between the post-orbital marine of the carapace and the hepatic spine. The hepatic carina is prominent and slightly curved, extending behind the antennal spine. The antennular flagellum is sub-equal or slightly longer than the peduncle. The walking legs or pereiopods are the thoracic appendages. Exopods are present on the 1^{st} and 4^{th} but absent on the 5^{th} pereiopods. Gills are formed from sac-like out growths of the base of the walking legs and sit in branchial chambers on either side of the thorax. The carapace extends laterally to cover the gills completely. The abdomen has the obvious segmentation of invertebrates. A pair of swimming legs or pleopods arise appendages of each of the six abdominal segments. A tail fan comprisee of a telson, which bears the anus, and two uropods attaches to the last (6^{th}) abdominal segment. The telson has deep median groove, without dorso-lateral spines. A rapid ventral flexion of the abdomen with the tail fan produces the quick backward dart characteristic of prawn (Anderson, 1993).

1.4 Life cycle

Development of penaeid prawn is complex. It begins with a larvae hatching from the fertilized egg to the first stage, nauplius, followed by protozoea, mysis and post larval stages (Figure 1.2). These require the development times about 1-5 days, 5 days, 4-5 days and 6-15 days, respectively (Solis, 1988). Prawn larvae are naturally planktonic in behaviour. Swimming is possible using antennae in nauplii, antennae and thoracic appendages in protozoa and thoracic appendages in mysis larvae. The normal adult slow swimming using the pleopods (abdominal appendages) is seen in the

postlarvae. Nauplii are about 0.3 mm long at hatching and are characterized by being totally planktonic and positively phototaxic; they exist entirely on their own egg yolk. The larvae being to feed as protozoea. They are filter feeders and consume particles of the correct size. They are approximately 1 mm in length, with a narrow elongated thorax and abdomen, and a loose-fitting carapace. Paired eyes, a rostrum and feeding appendages are present for the first time. The second metamorphic change is seen when the third protozoea stage moults into the first mysis stage. Mysids have five pairs of functioning pereiopods (thoracic appendages). The carapace now covers all the thoracic segments. The mysids swim in a more adult manner and actively seek out photoplankton and zooplankton to feed on. The final metamorphosis is to the post-larvae stage, where a full complement of functioning appendages are present.

Post-larvae continue molting as they grow. They migrate shoreward and settle in nursery areas close to shore or in estuaries, where they grow quickly to juvenile and subsequently to sub-adult, they highly tolerate variable physico-chemical factor in their living environment. Sub-adults migrate back to the sea where they are finally mature and able to mate and spawn. The life span of penaeid prawns are rarely longer than two years (Anderson, 1993).

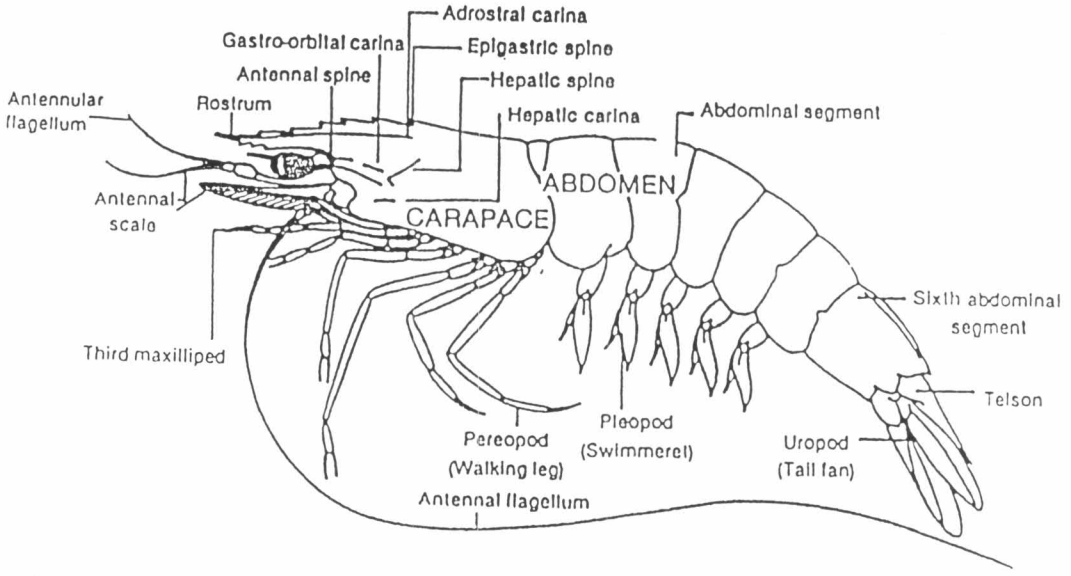

Figure 1.1 Lateral view of *P. monodon* showing important parts (Anderson, 1993).

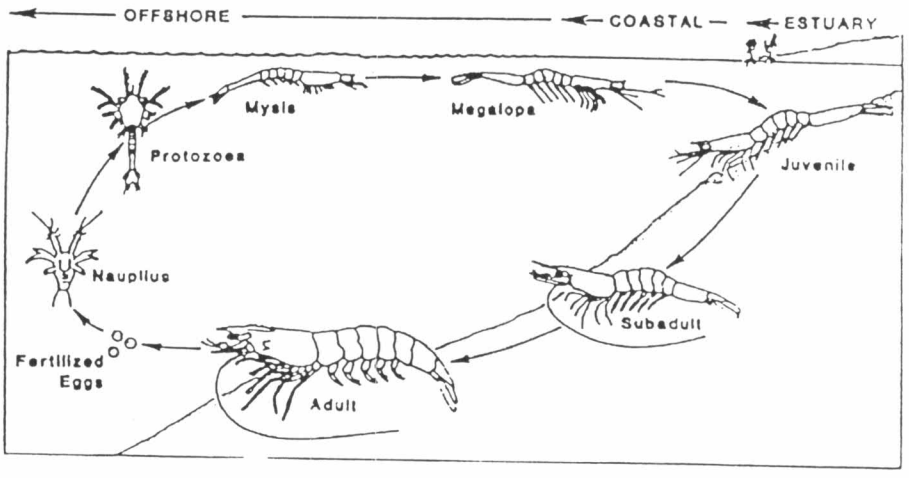

Figure 1.2 The life cycle of the black tiger prawn, *P. monodon*, with stages in different Habitats (Baily-Brook and Moss, 1992).

1.5 Distribution

The black tiger prawn (*P. monodon*) is naturally distributed in the major part of Indo-West Pacific region. It is commonly found in the East and Southeast Africa, through the Red Sea and Arabian Gulf, around the Indian subcontinent, and throughout the Malasian Archipelago to Northern Australia and Japan (Figure 1.3). It is a marine species inhabits in mud or sand bottoms at all depths from shallows to 110 meters (360 feet), so it can be caught from offshore or inshore as well as from tidal zones. (Dore and Frimodt, 1987).

1.6 Exploitation

Due mainly to the strong demand and the high price of *P. monodon* in international markets, *P. monodon* industry in Thailand has rapidly expanded. This causes the increasing use of wild female broodstock, because farming of *P. monodon* relies on wild female for the seed production. However, broodstock availability declines, the cost of gravid females will be increased imposing and increasing burden on the financial viability of hatchery operations. Reliance on wild seed and/or broodstock can limit availability of postlarvae either seasonally or geographically in area without abundant indigenous stocks. Demand for seed has also encouraged transfer of live prawns between geographically distinct areas resulting in the spreading of certain pathogens. Distribution of seed stock heavily infected with significant pathogens can have important negative effects for growers. For all these reasons, domestication is a better alternative which allows convenient disease prevention and

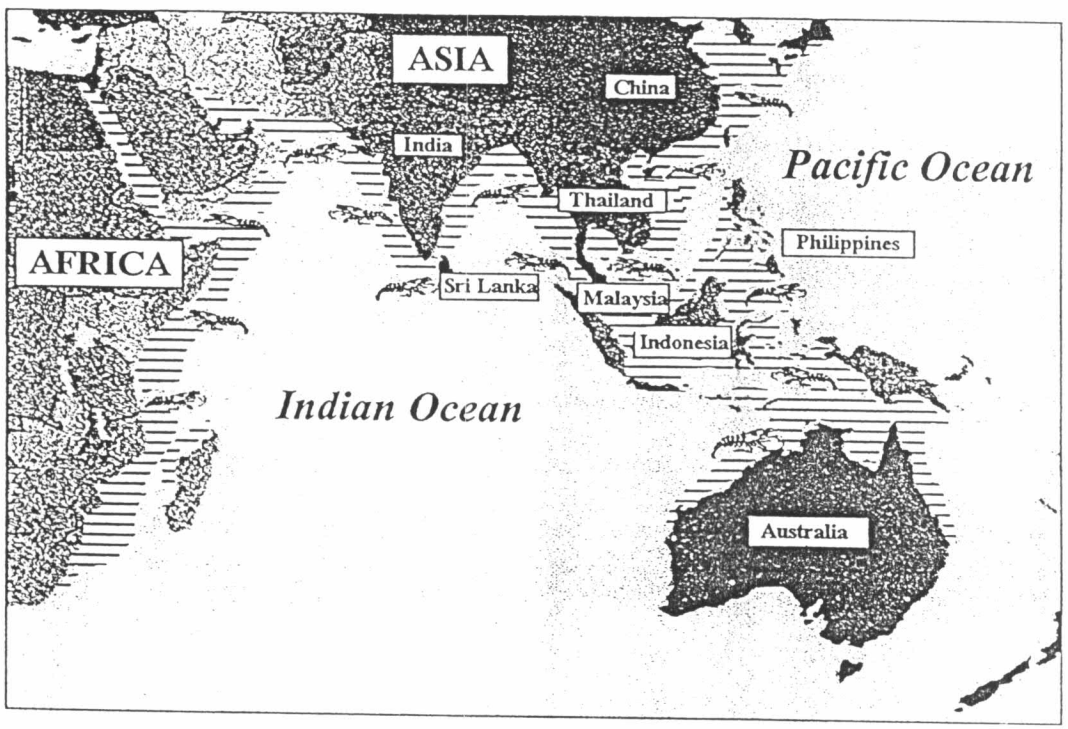

Figure 1.3 Geographic distributions of *P. monodon* in the Indo-West Pacific regions (Grey et al., 1983)

control. The genetic selection may also be achieved though this process. (Browdy, 1996).

The primary value of genetic markers is to assist in the domestication process and to improve the domestication prediction. The markers produce information that can be used for domestication. They can provide answers to paternity testing and evaluation of polymorphism within groups under going selection to minimize effects of inbreeding.

Most of genetic researcher today is devoted to developing genetic maps; some of genes, some of chromosomes, and some of the entire genome. A genetic map is much like an atlas which shows the locations of genes or DNA fragments that have been identified, some of which expresses themselves as desirable traits for the farmer. When these markers are identified and characterized, each can be used as a signpost to help locate genes that expresses the desired trait (faster growing animal, disease resistance, nutrition efficiency).

Genetic improvement through domestication provides the capability of culturing a better quality animals in less time, with greater survival and at less cost than animals removed from the wild (Carvoalho and Pitcher, 1995; Fukuoka and Okuno, 2001).

1.7 Genetic markers

The development of molecular genetic markers has led to intense investigation and characterization at the genetic level. The advantage provided by molecular

markers over classical genetic marker systems is the ability to tap and utilize information on naturally occurring polymorphism within populations. Most natural populations have relatively high levels of polymorphism due to small changes in DNA, sequence such as point mutation, base substitutions, insertions, deletions and translocations, most of which are phenotypically neutral. New technologies allow the detection of these polymorphisms and have proven extremely efficient in the discrimination of individuals. Such polymorphisms and the molecular markers associated with them are inherited in Mendelian fashion. They are extremely abundant and found throughout the genome. In addition, developmental, tissue specific and environmental factors do not influence the detection of these polymorphisms, making them excellent genetic markers (Kaga et al., 1996; Weising et al., 1995).

1.7.1 Isozymes

Many proteins can be analyzed simply and rapidly and have proven to be useful and reliable genetic markers. Both enzymes and nonenzyme proteins have been used as genetic markers. Non-enzyme proteins are usually analyzed by one or two dimensional polyacrymide gel electrophoresis (PAGE) where normally several bands or spots relating to different molecular forms of the protein are observed. In the case of enzymes, staining based on their specific activity is usually exploited for their detection and analysis, therefore non-denaturing starch or polyacrylamide gels are used. Differences or polymorphisms due to different molecular forms of the enzyme but with conserved activity are detected by differential migration within the gels. Differences may be due either to changes at the DNA level which cause amino-acid

substitutions and changes in the charge of the protein or post-translation modifications such as glycosylation which lead to changes in molecular weight (Avis, 1994; Carvalho and Pitcher, 1995; Weising, 1995).

Enzymes have been developed as molecular markers since the 1950's and Markert and Moller (1959) introduced the term of enzyme to describe the different molecular forms of an enzyme. However, different forms of an enzyme may be expressed either by different loci or by different alleles at the same locus. The term allozyme has therefore been accepted to describe forms associated with different alleles at the same locus and isozyme to describe different forms from distinct loci.

Isozymes has been used as "tags" to follow agronomically important simple traits such as nematode resistance in tomato (Rick and Fobes, 1974). A gene is "tagged" when a molecular marker is found to be very closely linked to the trait of interest, presence of the marker is a very strong indication that the gene associated with the trait of interest is also present. Isozymes have also been used in the analysis of complex or "quantitative" trait loci (QTLs) in which several genes are responsible for the phenotype (Misevie, Gerie and Tadie, 1990).

Protein based markers have the advantages that they are cheap and need no sophisticated equipment. They are codominant making them appropriate for heterozygosity studies. However, the resolution of protein electrophoresis is not always adequate for detecting differences between populations or individuals (Carvoalho and Pitcher, 1995). There are certain limitations to the allozyme studies. A new allele will only be detected if nucleotide substitution results in an amino acid

substitution that affects the electrophoretic mobility of the protein. Since sixteen out of 20 common amino acids are electrostatically neutral, mutations do not usually alter the total charge of the protein. Only 30% of all nucleotide substitutions result in polymorphic fragment patterns. Therefore, allozyme analysis underestimates the genetic variability (Selander and Whittam, 1983; Weising et al., 1995).

1.7.2 Restriction Fragment Length Polymorphism (RFLPs)

The first DNA based molecular markers were developed to detect polymorphisms produced by changes in the distance between two restriction enzyme sites. These polymorphisms are caused by mutations in or around the restriction sites. The size of a given fragment can also change as a result of an internal deletion or an insertion in the fragment of interest (Botstein et al., 1980).

Obviously a simple restriction digest of DNA would produce a smear of bands in which changes in individual fragments would be possible to detect, therefore the Southern blot technique is used to detect specific restriction fragments. Single copy genomic DNA clones or cDNA clones are used as probes for specific regions of the genome (Hallerman and Beckman, 1988).

RFLP markers like isozymes, are co-dominant, detecting both alleles at a given locus, results are consistent when the same probes are used in different laboratories and in the case of cDNAs and many genomic clones the probes are directly associated with coding sequences (Chase, Ortega and Vallejos, 1991).

1.7.3 mt-RFLPs

Mitochondrion is the cell organelle found in cytoplasm of eukaryotes. Each mitochondrion contains 5 - 10 copies of double-stranded circular DNA. The animal mitochondrial genome is approximately 16,000-20,000 bp in length coding for 13 protein coding genes (NADH dehydrogenase, ND, subunits 1, 2, 3, 4, 4L, 5 and 6 ; cytochrome b, three subunits of cytochrome oxidase, COI, II and III, and two subunits of ATP synthetase (ATPase 6 and 8), 2 genes coding for ribosomal RNAs (16S and 12S rRNA), 22 transfer RNA coding genes) (King and Stansfield, 1985; Park and Moran, 1994). Generally, mitochondria are inherited martiarchally except in some species (e.g. *Mytilus edulis*, *M. galloprovencialis*) whose contribution of paternal mitochondria is observed (Margoulas and Zouros, 1993). The mutation rate of mtDNA is much more rapidly than that of single-copy nuclear genes reflecting its potential to be used for determination of intraspecific genetic variation among geographically different populations (Brown et al., 1979; Lynch and Jarrell, 1993).

Restriction site of fragment analyses of mtDNA and direct sequencing of specific regions of the mitochondrial genome following amplification by the polymerase chain reaction (PCR) are currently the methods of choice for the majority of population level studies (White et al., 1992).

1.7.4 Randomly Amplified Polymorphic DNA (RAPD) Markers

RAPD markers were developed using the polymerase chain reaction (PCR) methodology. In a normal PCR reaction two distinct oligonucleotide primers of known

sequence, flanking a region of interest are used to synthesize the DNA fragment between the two primers. Repeated synthesis of the fragment leads to an amplified number of copies of the specific fragment bounded by the oligonucleotides (Mullis, 1990). In a RAPD reaction, a single 10-nucleotide sequence chosen at random is used as a primer. Genomic regions flanked by inverted repeats of the oligonucleotide will be synthesized from the single primer. Amplified PCR fragments are visualized under UV light following separation on an agarose gel and staining with ethidium bromide. In the case of RAPDs, the polymorphisms detected in different individuals are due to mutations or changes in DNA sequence either at the regions homologous to the oligonucleotide primer or in the surrounding sequences (Welsh and McClelland, 1990; Williams et al., 1990). These changes may inhibit oligonucleotide hybridization causing the PCR reaction to fail and no amplified fragment to be produced or may cause the size of the fragment to change. Often, however, loss or movement of the region of homology to the oligonucleotide primer presents the situation that distances between primers are greater than the limits of a standard PCR technique (greater than 2 Kb) leading to failure in the amplification reaction and loss of band.

Polymorphisms are detected as presence or absence of bands and although in theory heterozygotes should give lower intensity bands, very small changes in reaction conditions or DNA concentration can mask this effect, therefore it is usually not possible to distinguish heterozygotes. For this reason the RAPD marker system unlike isozymes and RFLPs is a dominant/recessive system.

RAPD markers have become widely used in many organisms since they are relatively cheap and simple to use, the only sophisticated equipment necessary being a thermocycler. No radioactivity is needed and commercially available oligonucleotide primers can be used directly to screen for polymorphism. The main criticisms of random amplification methods is the low reproducibility between different laboratories since the reactions are extremely susceptible to changes in buffer composition, DNA concentrations and even different models of thermocyclers (Weising et al., 1995).

1.7.5 Amplified Fragment Length Polymorphisms (AFLPs)

The AFLP technique is also a PCR based technique, however unlike RAPDs, specific restriction fragments are targeted for amplification. Genomic DNA is digested with two different restriction enzymes, normally a hexacutter and a tetracutter (recognizing a four base pair sequence). Specific adapter molecules are then ligated to the ends of the restriction fragments. The oligonucleotide primers for the PCR reactions correspond to these adapter molecules. A greater specificity is achieved by adding an extra nucleotide to the PCR primer corresponding to the internal nucleotide following the restriction site. A preliminary amplification reaction is carried out using this +1 primer allowing the amplification of only a subset of the population of digested molecules. This initial amplification is allowed by a second amplification using an oligonucleotide primer with three extra bases, therefore only a fraction of the originally amplified fragments is subsequently amplified i.e. those processing precisely the combination of internal bases determined by the primers. Different combinations of three base sequences at each restriction site lead to analysis of different fraction of the

genome. In order to detect the amplified fragments, one of the +3 primers has radioactive or fluorescent label attached, the amplification products are then run out on a polyacrylamide sequencing gel and visualized by autoradiography or by scanning in a special apparatus designed to detect fluorescent compounds. The general idea behind the AFLP technique is to obtain information simultaneously for many restriction fragment polymorphisms but in an easily manageable fashion, therefore although initial digestion, ligation and amplification reactions involve the whole genome, various steps within the protocol lead us to analyze only a small proportion of genome (Lin and Kuo, 1995; Vos et al., 1995).

AFLPs have been shown to detect high levels of polymorphism in many different organisms. The polymorphism in this case has the same basis as for RFLPs, namely mutations in or around the restriction sites of the enzymes used in the initial digest. In spite of the complexity of the protocol for carrying out AFLP analysis and the preparation of sequencing gels, many reports of mapping and tagging using the AFLP technique have been published recently. The AFLP technique, like RAPDs, could be easily automated for high sample throughput (Cervera et al., 1996; Hill et al., 1996).

1.7.6 Variable Number of Tandem Repeats (VNTRS)

VNTR markers are characterized by a core sequence which consists of a number of identical repeated sequences. They can be divided into three categories; satellite, minisatellite and microsatellite, based on the repeat length (O'Reilly and wright, 1995)

1.7.6.1 Satellites

Satellite DNA is a repetitive DNA that contains tandemly repeated short nucleotide sequences. The repeat unit may be from one to a few hundred nucleotides long. In some mammals, certain satellite DNA may occur as millions of copies per genome (Alberts et al., 1983). However, they are not as variable in size within populations as the other members of highly repetitive DNA family.

1.7.6.2 Minisatellites

Minisatellites is a repeating DNA sequence ranging between 15-70 bp per unit and 0.5-30 kb in size (Koreth et al., 1996). Minisatellites are found within noncoding regions of genomic DNA. The variation between alleles or polymorphism produced by minisatellites is due to different numbers of repeats at the same locus on homologous chromosome. These differences in copy number on homologous chromosomes may be due to for example to unequal crossing over during recombination or perhaps to errors during replicative processes (Nagylaki, 1988). In order to utilize minisatellite markers, it is necessary to develop a method to examine minisatellites at a specific locus. If a Southern blot is carried out using minisatellite sequences, normally a complex pattern of bands (often called a fingerprint) is observed. This is due to the fact that a single minisatellite sequence may be found at different locations in the genome and all these regions are simultaneously detected by hybridization. These complex patterns are extremely useful in the discrimination between individuals or fingerprinting analysis, where the probability of two individuals having exactly the same banding pattern is extremely low (Rogstad, Patton and Schaal, 1988).

1.7.6.3 Microsatellites

Microsatellite are short tandemly repeated sequence motifs consisting of repeat units of 1-6 bp in length. They are highly abundant in eukaryotic genome, but also occur in prokaryotes at low frequencies. It was estimated that one microsatellite locus may be found every 10 kb in eukaryotic genomes (Tautz, 1989). They seldom include more than about 70 repeat units and are interspersed thought the genome (Schlotterer, 1998). Like minisatellite, the variability in microsatellite regions arise from changing in the number of repeated sequences which is proposed to be from slipped-strand misparing or slippage during DNA replication (Schlotter and Taulz, 1992). Microsatellite alleles exhibit codominant inheritance fashion (Queller, Strassman and Hughes, 1993).

The number of repeats varies in different individuals, and so the different repeats can be regarded as "polymorphic" alleles at that "locus". To reveal polymorphic microsatellite sequences, it is necessary to sequence the conserved flanking DNA, and to design PCR primers that will amplify the repeat sequences. Sizing of the PCR products on high resolution gels allows the determination of the number of repeats and any variation in repeat number between the different alleles (O'Reilly and Wright, 1995).

Microsatellite combine several features of the ultimate marker: (1) they are typically codominant and multiallelic; (2) they are highly polymorphic allowing precise discrimination even closely related individuals; (3) they are abundant and disperse in genome; (4) they can be efficiently analysed by a rapid and simple PCR

assay; (5) microsatellite marker genotyping can be semi-automated in multiplex assays; (6) marker information based on primer sequences, may be easily shared between laboratories improving cooperative efforts in research and development.

Microsatellites are so widespread throughout eukaryotic genome that they are being used as genetic markers for cows (Beckmann and Soller, 1990; Fries, 1993), sheep (Crawford et al., 1991), horses (Marklund et al., 1994) and human (Weber, 1990). They also are being used as genetic markers for sea bass (Bahri-Sfar et al., 2000), abalone (Selvamani, Degnan and Degnan, 2001), European flat oyster (Naciri et al., 1995), crab (Desvignes et al., 2001) and other species.

Microsatellite can be used in a wide variety of applications including pedigree analysis, identification of individual animals, determining parentage, and selective breeding to minimize effects of inbreeding. They can also be used as markers to indicate the presence of genes that are otherwise difficult to detect, and as markers for quantitative traits such as disease resistance or growth rate. Computerized digital comparisons of DNA typing from a sample population against a reference species library (of markers) reveals the variations involved, allowing selection of only those broodstock animals with desired traits. Such technology will revolutionize prawn aquaculture and promote overall industry growth through domestication, greater production efficiency, and increased profits (Prasad et al., 2000; Fukuoka and Okuno, 2001).

1.8 Genetic markers in penaeid prawns

Dating back to the mid-1960s, electrophoretic techniques for protein separation followed by histochemical localization of specific enzymes allowed geneticists, for the first time, to survey natural populations for the amount of variation in a random sample of primary gene products. As investigators turned to biochemical techniques. The interest in externally visible polymorphisms declined. The enzyme polymorphisms posses significant advantages, including codominant expression (Hedgecock et al., 1982).

The identification of the genetic diversity in penaeid prawn is through the examination of allozyme variability indicates relatively few allozyme polymorphisms. Low levels of genetic variation and little geographic differentiation within wild penaeid prawn species have been reported (Garcia et al., 1994). Using three penaeid prawns from the Gulf of Mexico, *Penaeus aztecus*, *P. setiferus* and *P. duorarum*, Lester (1979), could not demonstrate the significant differences among locality differentiation of allozyme frequencies. However, Benzie et al. (1992) found significant allozyme frequency differences among Australian populations of *P. monodon*. However, mtDNA analysis indicated higher levels of variation among the Australian populations of *P. monodon*, suggesting that DNA analysis would provide a better source of markers for penaeid prawns.

A randomly amplified polymorphic DNA (RAPD) analysis has been demonstrated as useful markers in penaeid prawn breeding programs (Garcia and Benzie, 1995). The percentage of RAPD markers generated that were polymorphic (6-

7%) was similar to that observed in other organisms. A RAPD marker specific to the population originated from Ecuador was reported by Garcia et al. 1994. Tassanakajon et al. (1997) reported that the primer UBC 428 generated a RAPD marker that was found only in *P. monodon* originating from Satun-Trang, located in the Andaman Sea. A RAPD marker generated by UBC 268 was also found specific to the population of *P. monodon* from the Gulf of Thailand (Klinbunga et al., 2001). These results suggested the potential use of these markers as population specific markers. The RAPD analysis of wild populations of Thai *P. monodon* suggested high level of polymorphism and the existence of population differentiation (Tassanakajon et al., 1998).

Mitochondrial DNA restriction fragment length polymorphism (mtDNA-RFLP) was successfully used for determination of genetic diversity in 3 wild populations of *P. monodon* (Klinbunga et al., 1999). Recently, PCR-RFLPs of 2 mtDNA genes (16s rDNA and COI-COII) of *P. monodon* samples from the 2 coastal areas of Thailand showed high average nucleotide diversity within samples of Thai *P. monodon* compared to several marine organisms (Klinbunga et al., 2001).

The use of microsatellite markers in penaeid prawns was first demonstrated by Wolfus et al. (1997). A microsatellite locus was employed to determine the allelic inheritance within 14 families of *P. vannamei* stocks. Tassanakajon et al. (1998) isolated and characterized 2 microsatellite loci from a *P. monodon* genomic library and reported difficulties of microsatellite development in this species. This is in concordance with that reported in *P. japonicus* (Moore et al., 1997). Long repeat arrays

of dinucleotide microsatellites were found in penaeid prawns making the design of useful primers difficult. This limited their application in genome mapping because only small number of microsatellites can be developed. Nevertheless, microsatellite markers were successfully used to illustrate geographic population differentiation of *P. monodon* in Thailand and Australia (Supangul et al., 2000, Brooker et al., 2000). Recently, Wilson et al. (2002) constructed an initial genetic linkage map of *P. monodon* from 3 reference families using AFLP markers. This linkage map has 20 linkage groups covering a total genetic distance of 1412 cM. A more defined *P. monodon* map require sufficient markers including microsatellites to create larger number of linkage groups covering a long genetic distance.

1.9 Objective of the thesis

To obtain sufficient useful microsatellite markers in *P. monodon* for application in prawn breeding program and genetic mapping, we isolated and characterized tri- and tetranucleotide microsatellite sequences from several types of prawn genomic library.

The aim of this thesis is to isolate and characterize tri- and tetranucleotide microsatellite sequences for application in DNA typing of *P. monodon*. Microsatellite loci developed in this study, were tested to determine the level of polymorphism and microsatellite allelic patterns. Multiplex analysis was developed to increase the efficiency of genotype detection. The microsatellite loci were also applied for linkage mapping.