Chapter I

Introduction

The term "shrimp" is commonly used by FAO to refer to species in the marine Penaeidae, while prawn is more commonly used to call freshwater Palaemonidae. Shrimps 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. United State of America and Japan are the two major consuming markets that have caused a rapid growth of this industry. In 1982 about 4.84 % of the total world harvest shrimps was from culture whereas the other 95.16% was from normal fisheries. In 1993, cultured shrimp production was increased to 22.50% of the total world shrimp production (Table 1.1) (Shrimp culture newsletter group, 1995a). With all of the tropical shrimp fisheries are overexploited, any increases in supply to the market place will be from increased of the cultured production. There are at least 60 species of commercially important penaeid shrimps. In 1994 the world cultured shrimp production accounted for 61% of *P. monodon*, 15% of *P.vannamei* and 24% of the others. In Thailand, cultured shrimp production (mainly, *P.monodon*) accounted for 1.34% of total production in 1983 and dramatically increased to 67% of the total production in 1991. The intensive

farming system has been widely used for *P.monodon* farming actively resulting in the consistent increase in the outcome production. Among all members of Penaeidae, *P.monodon* is the most economically cultured species in the Southeast Asia (Anderson 1993; Shrimp culture newsletter group, 1995a).

The total world cultured shrimp production had increased rapidly from 298,573 MT in 1986 to 762,000 MT in 1995. Thailand is the leader in cultured shrimp for five consecutive years, followed by Ecuador, Indonesia and India. (Table 1.2) (Anderson, 1993; Asian shrimp culture council, 1995). Thailand exports a huge quantity of cultured shrimps which provide a substantial income to the country. (Table 1.3) (Shrimp culture newsletter group, 1995b). For example in 1993, approximately 50% of the value of marine animals exported came from cultured shrimp production. As a result, cultured shrimp production has economically important value of Thai marine animal production and the most economically important cultured species is the black tiger prawn (*Penaeus monodon*, Fabricius) (Shrimp culture newsletter group, 1994).

Table 1.1 World total shrimp production: 1982-1993.

Production (MT)			
Year	Cultured shrimp production	Captured shrimp production	Total production
1982	84,000	1,652,000	1,736,000
1983	143,000	1,683,000	1,826,000
1984	174,000	1,733,000	1,907,000
1985	213,000	1,908,000	2,121,000
1986	309,000	1,909,000	2,218,000
1987	551,000	1,733,000	2,284,000
1988	604,000	1,914,000	2,518,000
1989	611,000	1,832,000	2,443,000
1990	633,000	1,968,000	2,601,000
1991	690,000	2,118,000	2,808,000
1992	721,000	2,191,000	2,912,000
1993	610,000	2,100,000	2,710,000

Source : Shrimp culture newsletter 1995.

Table 1.2 World cultured shrimp production: 1993-1995.

Country	Head-on Production (MT)			Variance' 94/95	
	1993	1994	1995	MT	%
Thailand	209,000	250,000*	270,000*	+20,000	+8
Ecuador	76,000	100,000	100,000	-	-
Indonesia	100,000	100,000	80,000	-20,000	-20
China	30,000	35,000	70,000	+35,000	+100
India	55,000	70,000	60,000	-10,000	-14
Vietnam	40,000	50,000	50,000	-	-
Bangladesh	30,000	35,000	30,000	-5,000	-14
Taiwan	20,000	25,000	30,000	+5,000	+20
Philippines	25,000	30,000	20,000	-10,000	-33
Mexico	9,000	12,000	12,000	-	-
other	45,000	51,000	40,000	-11,000	-17
Total	639,000	758,000	762,000	+4,000	+1

Source: Asian shrimp news, 1995.

Table 1.3 Exports of Thailand shrimp: 1986-1995.

Year	Quantity		Value	
	MT	Difference	Billion baths	Difference
1986	28,063	-	4,391	-
1987	33,909	+21	5,749	+31
1988	49,829	+47	9,701	+69
1989	74,294	+49	16,057	+66
1990	84,724	+14	20,454	+27
1991	121,240	+43	26,681	+30
1992	140,432	+16	31,696	+19
1993	148,889	+6	37,843	+19
1994	190,650	+28	48,109	+27
1995	202,000	-	50,600	-

Source: Shrimp culture newsletter, 1995.

Taxonomy

The taxonomic definition of black tiger prawn is as follow:

Phylum Arthropoda

Class Crustacea

Subclass Malacostraca

Order Decapoda

Suborder Natantia

Infraorder Penaeid

Family Penaeidae Rafinesque,1815

Genus *Penaeus* Fabricius, 1798

Subgenus *Penaeus*

Species *monodon*

Scientific name: *Penaeus (Penaeus) monodon* Fabricius, 1798.

Common name: giant tiger prawn (or shrimp) or black tiger prawn (or shrimp)

FAO name : giant tiger shrimp (or prawn)

Morphology

Externally the shrimp can be basically divided into the thorax and abdomen (Fig.1.1). The thorax (or head) is covered by a single, immobile carapace which protects the internal organs and supports muscle origin. The rostrum, extending beyond the tip of the antennular peduncle, is sigmoidal in
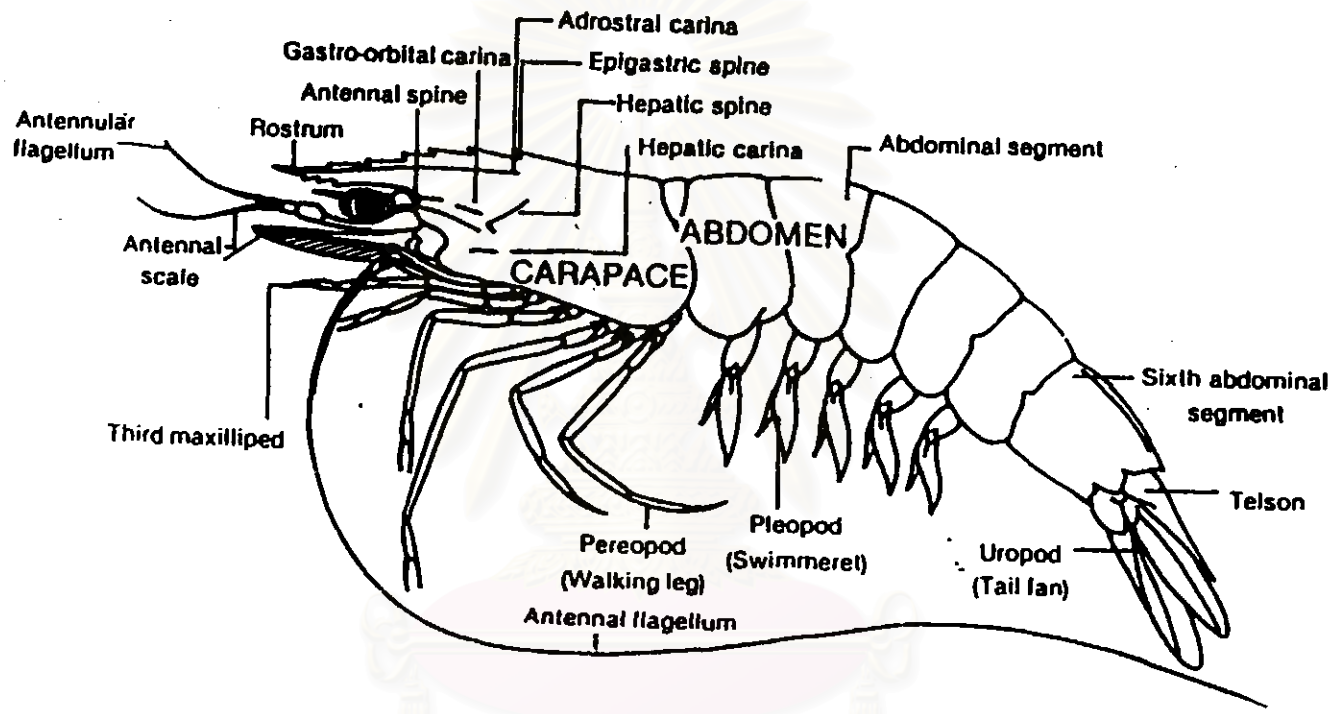
Figure 1.1 Lateral view of *P.monodon* showing important parts.

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 1st and 4th but absent on the 5th 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 comprise of a telson, which bears the anus and two uropods, attached to the last (6th) 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 shrimp (Anderson,1993).

Distribution

P.monodon is the largest penaeid shrimp. It can tolerate a wide range of fluctuating environments (e.g., water temperature, salinity) in tropical areas and performing well in ponds at high density. Most of the adults inhabit deeper

water down to 162 m. The fry, juvenile and adolescent stages occupy shore areas and mangrove estuaries (Panya Suwansamut, 1991; Anderson, 1993). *P.monodon* is naturally distributed in the Indo-West Pacific region; South Africa, Tanzania, Kenya, Somalia, Madagascar, Saudi Arabia, Oman, Pakistan, India, Bangladesh, Sri Lanka, Indonesia, Thailand, Malaysia, Singapore, Philippines, Hong kong, Taiwan, Korea, Japan, Australia and Papua New Guinea (Motoh, 1985). In the Gulf of Thailand, this species is widely distributed. The abundance of it is found at the depth of 26-30 m., closed to the coastal areas, the mouths of the rivers and the islands (Atchara Vibhasiri, 1989).

The farming areas of penaeid are found throughout the world (Table 1.4). However, South-East Asia is the center of the most farming areas of *P.monodon* in the world (Anderson,1993).

Life cycle

During the spawning season, adult shrimps undertake spawning migration to various depths on the continental where copulation occurs. The number of spawned eggs depend on the size of spawners. The fecundity of spawners at 70-150 g. in size is approximately 1,000,000 -1,200,000 eggs. In *P.monodon* the thelycum modified for internal storage of sperm ("close thelycum"), mating occurs just after the female moult. At this time the male inserts the spermatophore (encapsulated spermatozoa) through the soft cuticle of

Table 1.4 Some of the prawns and shrimps farmed throughout the world.

Scientific name*	Common name	Natural distribution	Cultured in
P.monodon	Black (black) tiger prawn	Indo-West Pacific	Taiwan, SE Asia+, China, India, Vietnam
P.chinensis (*P. orientalis*)	Chinese white shrimp or Oriental shrimp	West Pacific	China, Korea
P. vannamei	Western white shrimp	Eastern Pacific	Ecuador, Central and South America
P.merguiensis	Banana prawn	Indo-West Pacific	SE-Asia, India, China
P.indicus	Indian white prawn	Indo-West Pacific	SE-Asia, India
M.ensis	Greasy back shrimp	Indo-West Pacific	Taiwan
P. stylirostris	Blue shrimp	Eastern Pacific	Central and South America
P. japonicus	Kuruma prawn	Indo-West Pacific	Japan, Korea, Taiwan, Italy
P. aztecus	Northern brown shrimp	Western Atlantic	USA
P. duorarum	Northern pink shrimp	Western Atlantic	USA
P.setiferus	White shrimp	Western Atlantic	USA
P. penicillatus	Red-tailed prawn	Indo-West Pacific	China, Taiwan
P.semisulcatus	Green tiger prawn	Indo-West Pacific and Eastern Mediterranean	Taiwan
P.esculentus	Brown tiger prawn	Indo-West Pacific	Australia
P. schmitti	Southern White shrimp	Western Atlantic	Central and South America
P. californienis	Yellow-leg shrimp	Eastern Pacific	USA
M.dobsoni	Kadal shrimp	Indo-West Pacific	India

*P = *Penaeus*, M = *Metapenaeus*.

+SE Asia = Indonesia, Philippines, Thailand, Brunei, Singapore and Malaysia

the thelycum, a special seminal receptacle for the storage of spermatozoa. During eggs are extruded from the paired genital pore located at the base of 3rd pereiopods. Meanwhile , spermatozoa are also extruded from the thelycum for fertilization. The time required for each spawning is approximately 3-5 min. Fertilized eggs gradually sink to the bottom. Hatching of the eggs, occurs about 12 hours later. The larval stages consist of three to six nauplius, three protozoea and three mysis substages. This larval development period is between 10-14 days depended on temperature and feeding level. Mysis III larvae metamorphose into post-larvae (PL) which have all the appendages and organs as in adult shrimps.

Postlarvae continue moulting 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 spawns are rarely longer than two years (Panya Suwansamut, 1991; Anderson, 1993).

<u>Why employ molecular genetic markers in *Penaeus monodon*?</u>

Black tiger prawn, *Penaeus monodon* is an economically important species. The culture of this species has rapidly developed in the last decade.

However, the culture of this species depends on wild caught spawners that can finally result in a serious bottleneck effect. At present this exploitation has resulted in increasing pressure on wild populations. As the industry grow, some of the most heavily harvest stocks are showing signs of over-exploitation. If, however, broodstock availability declines, the cost of gravid females will be increased imposing and increasing burden on the financial viability of hatchery operations. Some efforts have been made to enhance wild populations but genetic effects of the release of large numbers of hatchery reared seed with unknown origin may harmful to local population. 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 shrimps 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, the breeding program is required to manage this species. The used of captive broodstocks will offer many advantages including disease control, maintenance of genetic diversity and selection to improve commercially important traits (Browdy, 1996).

Basic knowledge on population genetics is essential for improving a selective breeding program. These data can provide information on rare alleles, decrease heterozygosities, an increased level of inbreeding within a breeding

program. Population genetic analysis provided genetic markers which may be utilized for making hatchery broodstock in order to maintain line purity, and for constructing sophisticated breeding efforts. Using molecular data, scientists can developed breeding programs aimed at specific needs (Garcia et al., 1994.).

Molecular markers for population genetic analysis

Traditional methods such as comparative anatomy, morphology and physiology had been used to evaluated genetic variability in the past but these methods are not adequate for studying intraspecific genetic variation of several species. During the past decade, traditional methods have been increasingly complemented by molecular techniques, the development of so-called "molecular markers" which are based on polymorphisms found in protein or DNA molecules.

Morphological characters have long been used for identification and classification of organisms at different levels (genera, families and species). Levels of variability can be estimated from morphological characters and their responses to selection and their genetic background can be determined. Morphological characters are, often, influenced by the environment. Molecular methods, on the other hand, are not effected by the environment and, provide almost unlimited numbers of potential markers. Theoretically, the general properties of the desirable molecular marker are (1) highly polymorphic

behavior (2) codominant inheritance (allows to discriminate homo- and heterozygotic states in diploid organisms) (3) frequent occurrence in the genome (4) everly distribution throughout the genome (5) selectively neutral behavior (i.e., no pleiotropic effects) (6) easy access (7) easy and fast assay (e.g. by proceduces amenable to automation and highly reproducible). The data from the analysis of these markers are acceptable among laboratories.

No molecular markers that fulfill all of these criteria are available yet. However, according to the systems of study, a suitable molecular marker can already be chosen from a variety of marker systems, each of which combines at least some of the above-mentioned properties (Weising et al., 1995).

A. Protein markers

For a long period of time, the first molecular approach employed widely in population genetic studies was protein electrophoresis of various enzymes (allozyme and isozyme systems). Generally, only allozyme data is used in either systematic study or phylogenetic analysis. Allozyme methods were introduced in the mid-1960s had been dominated in molecular systematics until the late 1980s where other DNA approaches have increasingly used instead. The use of allozyme and other DNA techniques opened a new approach to understand population and the evolution of organisms.

The protein markers are based on protein polymorphisms. This method

involves the separation of native proteins under electric field. The proteins are identified by histochemical staining to reveal the enzymes. The majority of protein markers are represent by allozymes.

Allozyme electrophoresis has been successfully applied to many organisms from bacteria to numerous animal and plant species. The studies have encompassed various fields (e.g., physiology, biochemistry, genetic breeding) and purposes (e.g., population structure analysis, mating system planning) (Avise, 1994).

Allozyme analysis has been used as the standard molecular technique in population genetic research. This technique has been enormously successful for three primary reasons. First, it is inexpensive particularly in comparison to other molecular genetic methods. Second the method allows for quick processing time. A laboratory can assay hundreds of samples per day for many different loci and this translates to low labor costs as well. Finally, allozyme markers are codominant therefore both alleles in a diploid organism are usually clearly identifiable. Accordingly heterozygotes can be discriminated from homozygotes, which is a prerequisite for estimation of allele frequencies in population genetic 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 resultes 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).

B. DNA markers

Although traditional allozyme analysis serves as the basis for many studies, the limitation of polymorphic markers in the investigated populations have frequently limited the power of analysis. The DNA of techniques have been developed. They uncover a great number of DNA markers. Because each individual DNA sequence is unique, this sequence information can be exploited for any studies as genetic markers. (Hallerman and Beckman, 1988).

Molecular markers at the DNA level as opposed to morphological characters or allozymes have several advantages. First, since the genotype of organism is examined directly, environmental and developmental influences on the phenotype are not a concern. Second, since different regions of DNA evolve at different rates, appropriate regions may be chosen for a given study. Third, DNA markers are not restricted to coding regions. Therefore, an almost

unlimited number of detectable polymorphisms exist. Finally a variety of techniques have been developed; each of which has the potential to provide suitable markers for a particular problem (Vernon, Jones, and Noble 1995; Weising et al., 1995).

1. DNA-level variation

Variation at the DNA level can be generalized into two categories, namely, base substitution and insertion/deletion. The simplest form of variation is a single nucleotide substitution or point mutation. Insertion or deletion of one or more nucleotides can occur within a DNA sequence. The actual insertion/deletion can be single nucleotide or hundreds to thousands of nucleotides long. A common type of insertion/deletion mutation is copy number variation. The variation results from a difference in the number of copies of a basic unit, or core sequence. The sequence on either side of the variant region is the same in different individuals. Another type of insertion variation is found in some multi-copy elements, such as Alu sequence. Some of these elements display a high degree of polymorphism at specific insertion sites.

The proportion of DNA sites that are polymorphic will depends on certain species-specific population parameters, population size and mutation rate in particular. In general, the level of polymorphism is expected to be inversely correlated with the functional constraints of the DNA. Coding regions of the genes, for example, tend to have little polymorphism compared to introns

and noncoding regions. By this account of variation, it is widely thought that the highest level of polymorphism will be found in regions of DNA having no function. The most likely place to find polymorphism, therefore, is in noncoding regions of DNA. Introns, for example, are generally relatively polymorphic (Kreitman, 1990; Carvalho and Pitcher, 1995).

2. DNA techniques used in population genetic

2.1 Restriction fragment length polymorphisms

Restriction fragment length polymorphisms (RFLPs) are based on differences in fragment lengths obtained by digesting the DNA samples with restriction endonucleases (Yu, Deynze and Pauls, 1993). Digestion of a particular DNA fragment with enzymes result in a reproducible set of fragments of well defined lengths. Point mutations as well as insertions or deletions within the restriction enzyme recognition sequences result in an altered pattern of restriction fragments (Weising et al., 1995).

In eukaryote, the genome is so large that digestion with a typical restriction enzyme produces hundreds of thousands of fragments. The DNA appears as a continuous smear on the gel upon EtBr staining. It is therefore necessary to visualize only particular fragments. The specific fragments are made visible by hybridization with specific labeled probes (Hallerman and Beckman, 1988; Avise, 1994; Carvolho and Pitcher, 1995). RFLPs have been used   extensively to develop genomic maps, establish linkages to traits and

develop phylogenetic trees. (Yu et al., 1993).

2.2 Hybridization-based fingerprinting

In 1985 Jeffreys et al. introduced multilocus DNA finger-printing techniques which reveals enormous variability in a wide range of organisms. Multilocus DNA fingerprinting detects genetic variation at hypervariable regions (HVR). The HVR consists of tandem repeats of short sequence. Hypervariability at these minisatellites or microsatellites result from change in the number of repeats, presumably driven either by unequal recombination or by slippage at replication forks leading to the gain or loss of repeat units. The resulting length variability can be high, and the frequency of heterozygotes can sometimes approach 100% (Jeffreys, 1987; Jeffreys and Pena, 1993). The technique of classical DNA fingerprinting (hybridization-based fingerprinting) is methodologically derived from RFLP analysis.

Two categories of multilocus probes are mainly used. The first category comprises of cloned DNA fragment called " minisattellite". Its tandem repeat is about 10 to 60 bp long. The second category is examplified by "simple sequences" or "microsatellites" whose tandem repeats is 1 to 5 bp long (Ali, Muller, and Epplen, 1986; Weising et al., 1995).

The resulting DNA fingerprints allow the identification of individuals in populations. Thus frequently permit assignments of paternity and thereby

promote analysis systems on a fine scale. They provide a potential source of markers for population studies where genetic variation is extremely limited. They should be especially valuable in systems where other markers are lacking (Turner, Elder and Laughlin, 1991). RFLPs and hybridization-based fingerprinting markers have advantages that they are phenotypically neutral, codominantly inherited, non specific to growth stages. However, RFLPs and hybridization-based fingerprinting require fairly large amounts of DNA and are time-consuming and expensive.

2.3 PCR based fingerprinting, randomly amplified polymorphic DNA analysis

Randomly amplified polymorphic DNA analysis (RAPD) has been as a method of detecting polymorphisms since 1990 (Williams et al., 1990). This technique is based on the polymerase chain reaction (PCR). With standard PCR, it is first necessary to determine the sequence of the DNA to be analyzed. Then two specific primers complementary to sequences flanking the target segment are synthesized and used to prime the DNA amplification reaction. The RAPD method, however uses a single arbitrary primer and lower annealing temperature than the average PCR reaction. The arbitrary primers used for the procedure are usually at 10 bp in size. They have GC content of 50 to 80% and do not contain palindromic sequences. The number and size of fragment(s) that are amplified are dependent on the primer and the genomic

DNA used. For a shorter primer, the probability that two priming sites occur in the genome close to each other in an inverted orientation increases. A subset of elongation products in the first cycle can serve as templates for the following cycles because it may have primer annealing sites within the templates suitable for amplification. Priming sites are randomly distributed throughout the genome. The various of DNA fragments of different lengths amplified in this fashion are inherited as classical Mendelian traits and, thus, can be used for genetic analysis (Paran, Kessili, and Michelmore, 1991; Yu, et al., 1993). A single nucleotide substitution in a primer can result in a complete change in the RAPD pattern, this is an indication of the sensitivity of the system. The RAPD products are detected as DNA fragment length polymorphism in multiple loci by the presence or absence of bands at various positions in agarose gel after electrophoresis (Mullis, Ferre, and Gibbs, 1994). DNA polymorphism revealed by this method results from either chromosome rearrangements within the amplified sequence, deletion of priming sites, insertion or deletion of the sequence between of the priming sites and base substitution in priming sites (Michelmore, Paran, and Kessili, 1991; Paran et al., 1991). Insertion of a large piece of DNA between the two annealing sites may render the original fragment too large to be amplified, resulting in its absence in the gel. The deletion of a DNA fragment carrying one of the two primer annealing sites also results in loss of a fragment. A nucleotide substitution may affect the annealing of one of the two primers at a given site. Insertion or deletion of a small pieces of DNA can

lead to change in size of amplified fragment.

The amplification products can be separated by agarose gelelectropheresis or nondenaturing polyacrylamide gel electrophoresis. The choice of system depends on the size of the DNA fragments to be separated. Agarose gel electrophoresis is relatively simple to perform and separates DNA fragments from 0.5 to 25 kb in size. Polyacrylamide gel electrophoresis is the method of choice to separate DNA fragments less than 1 kb. The DNA in the gel can be visualized by staining with ethidium bromide or by silver staining which gives better resolution, especially of minimal length-difference fragments in polyacrylamide gel electrophoresis (Yu et al., 1993).

The advantages of using RAPD markers are the followings. First, RAPD analysis is simpler, faster, and demands less technical expertise. Second, RAPD-PCR requires small amount of DNA. Third, the same single short primers usually can be used for the analyses of widely different taxonomic group. Forth, prior sequence data of the target DNA is not necessary. Fifth, the availability of a large number of random primers makes RAPD-PCR a powerful tool for detecting numerous polymorphism, which are useful for the studies of population genetics. Finally, RAPD-PCR does not require probes, DNA libraries, and radioactive chemicals (Williams et al., 1993; Narang et al., 1994).

There are some disadvantages in using the RAPD-PCR approach for

population genetics, genetic mapping, and taxonomic studies. First, many fragments (especially those arising from mispairing of a primer with the genomic DNA) may not be reproducible among different laboratories because amplifications are sensitive to slightly change in temperature cycle.

Second, in contrast to RFLP (on autoradiographs), which are usually codominant, most of the polymorphisms detected by RAPD-PCR are inherited as dominant markers. Therefore, homozygotes and heterozygotes can not be differentiated. In RAPD-PCR, both genotypes will show one band because the size difference is more likely to appear as the present or absent of a band.

Third, RAPD bands of the same size may not actually have the same or similar sequence. Comigrating RAPD bands may not be allelic (Narang et al., 1994).

Genetic Studies in Crustaceans

Dating back to the earliest years of modern genetics, the crustacean genetic literature had never been summarized, except for more specific reviews such as those on sex determination, chromosomal evolution in copepods or the genetics of genera such as *Artemia* or *Daphnia*. In the 1940s, the synthetic or neo-Darwinian theory of evolution and the "new systematics" cast a spot light on polychromatisms as visible examples of polyallelic systems, though to be the medium of genetic change. Polychromatism is difference in colour pattern. The

first comprehensive genetic studies of visible polymorphisms in marine organisms began. There soon appeared treatments of polychromatisms of the copepod *Tisbe reticulata*, the flabelliferan isopod *Sphaeroma serratum*, the asellote isopod *Jaera albifrons* and a few decapods.

By 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 of crustacean declined. The enzyme polymorphisms posses significant advantages, including codominant expression and much greater frequency of occurrence (only a very small percentage of crustacean species exhibit well-marked polychromatism).

Protein polymorphism data from 97 crustacean species had been surveyed for variation at 12 to 43 loci ($\bar{x}$ =23). 30.5% of the loci in the average population are polymorphic and 7.3% of the loci in an average individual are heterozygous ($\bar{H}$, Mean heterozygosities = 0.073). Nevo, et al.(1984) had been reported mean heterozygosities of 0.082 for crustaceans (Hedgecock et al., 1982).

Decapods are known to display relatively low level of enzyme

polymorphisms. Mean heterozygosities of 0.048 were reported for decapods. Geographic variation among conspecific decapod populations had been tested by allozyme variation. The grass shrimp, *Palaemonetes pugio* has a much more fragmented distribution than does the penaeid shrimp, being sometimes isolates in small ponds. These pond populations tend to have less allozyme variation compared with open bay populations, and Nei's statistics show significant genetic subdivision among samples from the Galveston, Texas, area (Fuller and Lester, 1980). Likewise pandalid shrimp, although, not subject to obvious gene flow barriers, exhibits some slight allozyme frequency variation, at least among wider spread localities (Hedgecock et al., 1982).

The identification of the genetic diversity in Penaeid Shrimp is through the examination of allozyme variability indicates relatively few allozyme polymorphisms. Low levels of genetic variation and little geographic differentiation within wild penaeid shrimp species have been reported (Garcia et al., 1994). Using three penaeid shrimps 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 using Nei's (1973) procedures for partitioning gene diversity. 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. RFLPs have been used to examine the degree of genetic diversity in the mitochondrial and nuclear DNA of *P.vannamei* (Garcia and Benzie, 1995). The large differences in mtDNA between species of *Penaeus* have been reported by direct mtDNA sequencing and RFLP analysis (Garcia et al., 1994). RAPD technique has also been developed for monitoring genetic diversity. The results from the RAPD technique are found to be consistent with those of other techniques for the identification of individuals at the population and subspecies levels and for the investigation of the genetic relatedness of population (Garcia et al., 1994).

By using RAPD technique, D'Amato and Corach (1996) reported a high level diversity detected in *Macrobrachium borellii* from Arroyo Pescodo stream and Canteras de Berisso, an artificial pond with a surface area of 4 ha. Polymorphic RAPD bands/individuals range from 33.33-50.00% in both populations. The average percentage difference (APD), as derived by Gilbert et al. (1990) and Yuhki and O'Brient (1990), was calculated for the two populations. The APD for Pescodo and Berisso (close population) are more similar than that of the Pescado (open population). The measure of genetic distance [Nei's genetic (D) = 0.001] indicates high genetic similarity among populations. Nevertheless, population-specific marker was found.

Garcia et al. (1994) had evaluated the genetic diversity of *P. vannamei*

by using 3 different techniques, namely, restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNA (RAPD) and allozyme variation. The RAPD technique revealed a high level of genetic variation which polymorphic bands/individuals range from 39-77%. A population and family-specific marker was found in this technique. For RFLPs technique, variability was found in cytochrome oxidase subunit I (CO I) of mtDNA, digested with *Hha* I or *Cfo*I enzymes, among the populations and families. Allozyme variation was examined on thirty loci. Seven loci were polymorphic. The percentages of polymorphic loci range from 3.33-16.67%. The levels of heterozygosity were low and consistent with reports by other researchers. Garcia et al.(1994) concluded that the low levels of allozyme variability in the populations were not high enough for the establishment of a breeding program based on genetic variation. Therefore, mtDNA polymorphisms can be used together with nuclear DNA polymorphisms to establish a breeding program (Garcia et al., 1994).

Garcia and Benzie (1995) had also investigated the RAPD patterns in six families of *P. monodon*. The percentage of polymorphic bands was 6.2%. The ratios of segregation for each family were consistent with Mendelian inheritance. They suggested that the levels of variation were similar to those observed in other taxa, and were likely to be adequate to obtain markers to assist selective breeding programs (Garcia and Benzie, 1995).

In conclusion, although RAPD technique has some disadvantages, it is superior to other methods in uncovering genetic variability for distinguishing of difficult taxa. Therefore, this research described herein possibility of using the RAPD technique to evaluate genetic variability in wide populations of Thai *P.monodon.*

Objectives

1. To find a suitable system (optimal conditions and primers) for identification of DNA-markers in wild populations of *P.monodon* by PCR-based fingerprinting method.

2. To assess the genetic variation among wild populations of *P.monodon* from the Andaman Sea and the Gulf of Thailand.

3. To evaluate the evolutionary relationship among wide populations of *P.monodon* based on the RAPD data.