Chapter 1

Introduction

The Giant Freshwater prawn (Macrobrachium rosenbergii de Man) is widely distributed in freshwater and brackishwater environments of Southeast Asian region. It is also commercially cultured in many regions of the world, Thailand, Philippines, Hawaii, Malaysia, (Holthuis, 1980). The larvae can survive and develop only in brackishwater, but juveniles and adults typically inhabit in low salinity and freshwater area. After the larval phase, postlarvae generally remain in brackishwater for a few weeks and then begin migrating toward freshwater.

In Thailand, this species is economically important for aquaculture; but in recent years, the size of cultured prawns seemed to be smaller than the previous generations. This incident might be due to the inbreeding effects (Menasveta, 1986). This problem could be solved by genetic manipulation through selective breeding. Since Macrobrachium rosenbergii were widely distributed in many areas. The geographic isolation and community structures could caused genetic variation and genetic or racial divergences. The existence of racial stocks within this species is needed to be determined for fishery managements purpose. The knowledge of racial stocks could lead to selective breeding and/or hybridization between selected stocks for genetic improvement programs in aquaculture.

In the past, several techniques were employed for studying genetic variation in decapods. The techniques can be categoried into 3 groups, i.e. morphology and physiology variation, allozymic variation and mitochondria variation.

Morphology and Physiology Variation

Koshy (1971) indicated that the number of teeth on the upper and lower edges of Macrobrachium dayanum were variable in number. These charactors did not significantly differ between two sexes but showed homogeneity in the population from Ohakuria Lake which could serve as a reliable diagnostic character of this population to distinguish it from other populations in different geographical areas. Williamson (1972) showed that morphology variation in Macrobrachium niloticum from Lake Chad might be distinguished from the Nile and Lake Rudolf by difference in proportion of leg 1 and 2, and the number of rostral teeth might provide a more reliable distinguishing characters. Goswami et al. (1986) studied morphometric variation in Penaeus merguiensis for the selection of broodstock and showed that morphometric characters, such as partial carapace length, exopod of uropod length and posterior abdominal circumference, either individually or in combination gave a more accurate estimate of tail weight than other variations.

Dobkin (1971) showed that Macrobrachium acanthurus from Florida could tolerate higher salinities than the Jamaica. Choudhury (1971) gave evidence of a possible difference in salinity tolerance between Macrobrachium carcinus from Barbados and Jamaica. In Macrobrachium rosenbergii, Lindenfelser (1980) collected samples of 11 wild populations and measured 16 morphometric variations that could separate this species into

Eastern groups and Western groups of the Indo-Malayan Archipelago and showed a few subspecific groups within each group. Dobkin et al. (1974) could not find any differences in pond performance between Thailand and Anuenue stocks of M. rosenbergii and no differences in various growth parameters between Anuenue and an ancestral Malaysian stocks (Malecha et al., 1980). Sarver et al. (1979) analyzed genetic stocks of M. rosenbergii from Thai, Australian, Sarawak and Anuenue stocks and found that physiological variation difference existed among these stocks. However, the further studies found high variable in mortality of offspring from three locations in Hawaii. That is difficult to estimate how much of the maternal variation is due to genetics and how much to the nutritional and other environmental factors affecting the female prawns or developing embryos (Sarver et al., 1981).

The identification of genetic variation with respect to morphometric or physiology variation was simple but not clearly understood. The phenotypic variations were possibly a affected by interaction between genotypes and environments. A same genetic stock could give different phenotype values in different environments, especially, the decapods.

Allozymic Variation

The usefulness of an enzyme system as a genetic marker in widely geographically separated population is determined by enzymes variability because all enzymes directly relative with genes. Proctor et al. (1974) studied Phosphoglucomutase (PGM) polymorphism in Penaeus aztecus and showed that there were no significant differences in PGM phenotype distribution between sexes or among samples of shrimp taken from Galveston Bay, Texas,

Vermilion and Barataria Bay, Louisiana. Tracey et al. (1975) surveyed enzyme electrophoretic variation in American lobsters (Homarus americanus) on eight geographically different populations and revealed little genetic variation but this species can be separated to inshore and offshore group. Hedgecock et al. (1976) compared the enzyme electrophoretic variation in American lobsters and European lobsters (Homarus gammarus) and found that American lobsters were electrophoretically similar to European lobsters. Trudeau (1978) used horizontal starch gel electrophoresis to examine 9 enzymes variation in 6 populations of Macrobrachium ohione. The result showed that they were genetically similar. Nelson and Hedgecock (1980) in an exhaustive review surveyed 44 species of decapod crustaceas and correlated enzymes electrophoretic variability levels with habitat and niche descriptors and found that in crustaceans electrophoretic genetic variability is low both in terms of the degree of polymorphism and average heterozygosity among loci. Lester et al. (1979) used enzyme electrophoresis to survey the economically important penaeid shrimps in the Gulf of Mexico i.e. Penaeus aztecus, P. seriferus and P. duorarum and found little subpopulation differentiation within each species. Fuller and Lester (1980) exhibited small genetic variability in grass shrimp, Palaemonetes pugio which caught from 9 different geographic areas and this variable was less in land locked ponds than in lagoons and bays. The 40 enzymes electrophoresis analyzed in 6 Metapenaeus species and 7 Penaeus species of fishery and aquaculture importance in Australia. The low levels of genetic variation was measured both in term of average heterozygosity and percentage of polymorphic loci in geographic differentiation area within species or between genus and species (Mulley and Latter, 1980; Mulley and Latter, 1981). Lester

(1983) investigated genetic differentiation among wild stocks of 5 Penaeus species in Southern United States using the 24 enzymes for electrophoresis technique. The results indicated low level of genetic variation within species or between species. For Macrobrachium rosenbergii, the intraspecific variation in allozymic have been extensively surveyed in 211 wild specimens from 9 different locations and showed relatively little allozymic variation in either within or among difference geographical areas (Hedgecock et al.,1979; Lindenfelser, 1980).

Although, the allozymic variation could be used for studying genetic variation in many organisms. But in decapods, this technique showed low level of allozymic variation both in term of the degree of polymorphism and average heterozygosity among loci. All in all there appears to be insufficient phenotypic variation in Macrobrachium rosenbergii to permit the possibility of a response to identify genetic or racial divergences.

Mitochondria DNA Variation

At present, the direct study on genetic materials has been developed. The comparision of the degree of nucleotide substitution in genetic materials should be a more direct genetic variation than comparision of amino acid substitution in proteins or phenotypic differences in chromosomes. The genetic materials which useful for studying genetic variation in chromosomal DNA and mitochondrial DNA. In multicellular animals, mitochondria DNA (mtDNA) was about 4.4 - 5.6 um. (table 1) and found in the matrix of mitochondria. The mtDNA contain only a small percentage of the genes necessary to produce the organelles'RNA and proteins, a few of the subunit of the respiratory enzymes

Table 1 showed size and structure of mtDNA in some organisms

source	structure	contour length (um)
Mammalia		
Man	circular	4.8-5.3
Monkey	circular	5.5
Ox	circular	5.1-5.3
Sheep	circular	5.4
Rat	circular	4.9-5.4
Mouse (Liver)	circular	5.0-5.1
Mouse (L-cells)	circular	4.7
Guinea pig	circular	5.6
Hamster	circular	5.1
Aves		
Chick	circular	5.1-5.4
Duck	circular	5.1
Amphibia		
Frog (*R. pipiens*)	circular	5.9
Toad (*X. laevis*)	circular	5.7
Axolotle (*S. mexicanum*)	circular	4.9
Mud puppy (*N. maculosus*)	circular	4.9
Osteichthyes		
Crap	circular	5.4
Echinoidea		
Sea urchin (*L. pictus*)	circular	4.6-4.9
Insecta		
Fly (*M. domestica*)	circular	5.2
Protozoa		
Tetrahymena pyriformis	linear	17.6
Fungi		
Saccharomyces	circular	25.0

Sagar (1972) refer to Borst (1970)

present in the inner membrane (Sheeler and Bianchi, 1987; Mckee and Poyton, 1984; Bingham and Nagley, 1983).

The mtDNA in animals were simple closed - circular DNA and strikingly uniform in size and structure in the organisms (Whittaker and Danks, 1978). There was evidence that mtDNA evolved much faster than most nuclear DNA (Brown et al., 1979). Thus, in general, conspecific populations and close related species showed greater differentiation of mtDNA than nuclear DNA. Besides mtDNA was inherited maternally and did not recombine. A pair of breeding individuals could transmit only one type of mtDNA and that was a tool for relating individuals to one another. The mtDNA was homogeneous for a given organ and the mtDNA molecules were identical from one organ to another within an individual (Potter et al., 1975).

Over the past several years, mtDNA had been used as a good marker for genetic variation within and among close-related species of animals for stocks identification. Hayashi et al. (1979) found the intraspecies divergence of mtDNA sequences in three type of black rats (Rattus rattus) were about 8 % (1200 bp) and four types of Norway rats (Rattus norvegicus) were about 1% (150 bp). Densmore et al. (1985) found change in mtDNA base substitution of lizards (Cnemidophorus tesselatus). Skibinski and Edwards (1986) revealed significant differences in frequencies of particular mtDNA genotype in two marine mussels, Mytilus galloprovincialis population and M. edulis population. Avise et al. (1984) showed genetic variation in mtDNA of bluegill sunfish, Lepomis macrochirus, and used to identify stocks which was better than morphometric or allozymic. Bermingham et al. (1986) indicated that size polymorphism in mtDNA of lower vertebrates could be separate into 3 genetic stocks for bowfin fish, Amia calva, and treefrog (Hyla sp.). Olivo et al. (1983)

showed four different mtDNA sequences which could be distinguish the Holstein cow stocks. Harrison et al. (1985) surveyed the wild crickets (Gryllus firmus) and could identify 5 subpopulations by the analysis of mtDNA restriction fragment patterns. Desalle et al. (1987) analyzed mtDNA in Drosophila mercatorum subgroup flies and indicated a high degree of genetic relatedness among the geographically separated strains but the analysis of many isozymes showed low levels of variability which was similar to D. melanogaster, D. simulans and D. virilis (Shah and Langley, 1979; Hudson, 1982).

The purpose of the present study is to characterize mtDNA of Macrobrachium rosenbergii among geographically separated populations with an emphasis on a genetic marker for identifying the racial stocks of the M. rosenbergii. Specifically, a diagnostic DNA fragment from the mtDNA will be sought and used for genetic stocks identification by the restriction fragment length polymorphism (RFLP) in the mtDNA. The study on RFLP of mtDNA could provide a direct approach to the study of genetic variability which is not the relative contribution of environmental influences to those morphological, physiological, ecological and behavioural differences. Besides this method can be used to study genetic variation in other commercially important decapods with on application genetic improvement in fishery and aquaculture programs.