Chapter I

Introduction

Zoogeographically, turtles first appearance in the fossil record was approximately 200 million years ago but marine turtles was not known to exist until 50 million years later (Pritchard, 1979 cited in Bowen *et al.*, 1993). Although all turtle species are classified into the order Testudines, their taxonomic status beyond suborder level are still questionable. Only 8 species are taxonomically recorded in marine turtles. They are composed of leatherback turtle, *Dermochelys coriacea*, green turtle, *Chelonia mydas*, black turtle, *C. agassizi*, flatback turtle, *Natator depressus*, loggerhead turtle, *Caretta caretta*, olive ridley turtle, *Lepidochelys olivacea*, Kemp's ridley turtle, *L. kempi*, and hawksbill turtle, *Eretmochelys imbricata*. All of these are formally announced by the International Union of the Conversation of Nature and Natural Resources to be threatened or endangered species.

Generally, marine turtles are distributed over vast geographic areas covering both tropical and subtropical regions where water temperature is over 19°C all year round. For instance, *C. mydas* and *E. imbricata* are found along the coasts in the Atlantic Ocean from New England to Argentina, Gulf of Mexico, Caribbean Waters and the India Ocean. While *C. caretta* and *D. coriacea* are not only distributed throughout the Atlantic and the Indian Oceans but also in the Mediterranean Sea, *L. kempi* is only found in the Gulf of

Mexico and along the east coast of the United States. For *L. olivacea*, the greatest voyager among all marine turtle species exhibits a very long distance feeding and nesting grounds covering the Eastern Pacific and Indian Oceans. Geographic distribution and habitat of *N. depressus* was not clear as there have been no publications on such aspects in this species (Hirth, 1971and Rene, 1990)

A recent taxonomic status for marine turtles can be described as follows (Bowen *et al.*, 1993):

Order Testudines

Family Dermochelyidae - *D. coriacea*

Family Cheloniidae

Tribe Chelonini

Genus *Chelonia* - *C. mydas*

- *C. agassizi*

Genus *Natator* - *N. depressus*

Tribe Carettini

Genus *Caretta* - *C. caretta*

Genus *Lepidochelys* - *L. olivacea*

- *L. kempi*

Genus *Eretmochelys* - *E. imbricata*

In Thailand, only 4 native marine turtles are reported: They are *L. olivacea* (the Thai common name: Tau Ya) and *D. coriacea*

(Tau Mapheung) only found in the Andaman Sea and *C. mydas* (Tau Tanu) as well as *E. Imbricata* (Tau Kra) which are found in both the Andaman Sea and the Gulf of Thailand (Boonlert Phasuk, 1995). Geographic distribution of *C. mydas* is illustrated in Fig. 1.1 and its morphological characters are shown in Fig. 1.2 Marine turtles are poikilothermic reptile species spending their whole lives in the sea except during the reproductive season when females migrate over a vast geographic area to lay their fertilised eggs (about 80-200 fertilised eggs a clutch) on the clam sandy beach (Vinai Gromin, Sea Turtle Conservation Center of the Royal Thai Navy, unpublished data). On the basis of tagging studies, it was additionally shown that marine turtles travel over a large geographic distance for their feeding habitats (sometimes thousands of kilometres). Female marine turtles migrate to the nesting beaches, which are generally the same places that they were born, to lay fertilised eggs during the breeding season. The females show, precisely but not perfectly, nest site fidelity (repeatedly return to the nesting beach) throughout their lives (Carr, 1986; Carr, Carr and Meylan, 1978 and Meyland, 1982 cited in Bowen *et al.*, 1993). Like other reptiles, development of sexes in newly hatching marine turtles is depended on environmental temperature during the incubation period. At higher temperatures (at least 31°C) almost all of the newly hatching are subsequently developed to be females. Nonetheless, at a lower temperatures

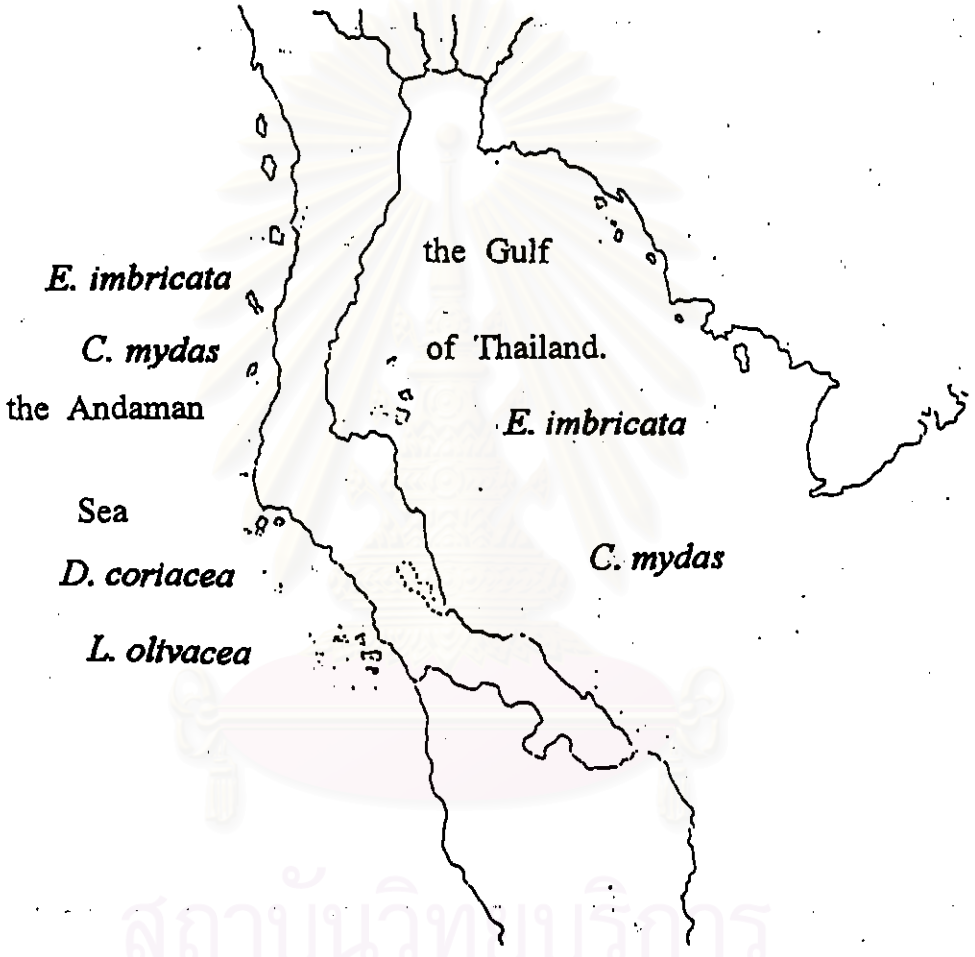

Figure 1.1 Distribution of *C. mydas* in Thailand.

This species can be naturally found in both the Andaman Sea and the Gulf of Thailand.

(between 22° and 28°C), almost all of the hatching juveniles are developed to males (Bull and Vogt, 1979; Bull, Vogt and Bulmer, 1982). Therefore, all marine turtles are gender-biased. Sex differentiation of marine turtles cannot be externally identified until they reach the mature stage (about 7-15 years old). Male turtle has a longer and sharper tail.

Figure 1.2 The external morphology of *C. mydas*.

Decreasing of marine turtles in Thailand and its neighbouring country

As mentioned previously, 4 native marine turtle species are found in the Thai waters. However, their number has decreased in Thailand and its neighbours since 1970. Of all the native species, the opportunities of *C. mydas* to maintain its census population sizes through the reproductive success seem to be severely disturbed by human. The reason for this is resulted from highly demands for consuming of *C. mydas* eggs. This and other factors including

destruction of its nesting areas have effectively decreased the number of natural *C. mydas* in Thailand.

In Malaysia, the amount of *D. coriacea* has been drastically decreased for approximately 98 % in the last three decades. This seriously indicates that *D. coriacea* are required to be conserved otherwise it will be extinct. Accordingly, a policy which are well followed in practice need to be developed in Malaysia as soon as possible. (Athikom Jaturawan, Sea Turtle Conservation Center of the Royal Thai Navy, unpublished data)

Previously, *C. mydas* was found in several provinces of Thailand including Samut Sakorn, Prachuap Khiri Khan, Aung Thong Island at Suratthani, Pa Dang Island at Satun, Kuraburi beach at Phangnga but it is extremely difficult to find any marine turtles at those places at present (Phasuk, 1995). Chantrapornsyl (1992 cited in Phasuk, 1995) reported that the total nests of *L. olivacea* at the Prathong Island (Phangnga) were decreased about 10% during 1979-1990. Incredibly, approximately 70% of *L. olivacea* nests at Nai Yang beach (Phuket) were dramatically decreased during 1978-1983. In the future, if the number of nests and marine turtles *per se* are decreased at this rate, it is possible that they will finally be extinct. From their biological requirement, they need the private breeding grounds. Nevertheless, almost beaches in Thailand have been changed to be tourist places resulting in destruction of their natural breeding areas.

After hatching, newly hatching juveniles need a luminous stimulation from a moon and stars to lead them correctly into the sea. Unfortunately, this

basic behaviour can be interfered by other light sources causing them confused of their way from the artificial stimulation. In the sea, juvenile marine turtles were further hunted by their biological predators (Limpus, 1993). Because of many biological and non-biological factors during their development, it was estimated that the survival rate of marine turtles, in general, from newly hatching until a reproductive stage was only 0.001-0.002% (Hughes *et al.*, 1974 cited in Somchart *et al.*, 1994).

It should be emphasised, however, that human is the most important predator for marine turtles. The reason for this is that human kills marine turtles at every stages of their development with sensible (e.g. for food) and non-sensible (e.g. hunting) reasons. The turtle's shell can be used to produce an ornament while body fat has been widely used in cosmetic businesses. Rubbish e.g. plastic bag left by tourists, are also dangerous to marine turtles as they eat such plastic bag with a misunderstanding to be their natural food (Sea Turtle Conservation Center of the Royal Thai Navy, unpublished data). Due to a few numbers of their population sizes, the Conversation of International Trade in Endangered Species (CITES) classified marine turtles to be a group of species to be conserved since 1975 (Phasuk, 1995). Nevertheless, the number of marine turtles in several countries has still decreased. Therefore, many countries including Thailand have set up the conservation programmes to save them in several aspects but in reality, the output of such purposes are not quite successful.

Conversation of C. mydas and other native marine turtles in Thailand.

There are several different ways to conserve natural marine turtles. Many countries have set up the policy that killing, hunting or even culturing of turtles for commercial purposes are prohibited. Moreover, normal fisheries using trawlers should not be allowed as marine turtles can be trapped from such capture activity. Therefore, the most popular conservation approach used by several countries such as Australia, Malaysia, Mozambiquean and Thailand is the "Head Starting Programme" (Hendrickson, 1958; Mrosovsky *et. al.*, 1981; Bustard, 1972 cited in Phasuk, 1995). The main objective of this programme is to collect eggs from the nests and subsequently to hatch them in the conservation stations. Generally, the incubation period until hatching is about 30-60 days. Juveniles are then fed with chopped fish until they are 5 months old before being released to the sea for restocking. There are 2 major (following the Head Starting Programme) stations nursed marine turtle in Thailand; at the Sattahip Naval Base in the inner Gulf of Thailand and at the Third Squadron Fleet in the Andaman Sea. However, several problems have arisen during the conservation programme because of a lack of basic biological disciplines composing of systematic, population genetics and dynamics of marine turtle species. In terms of molecular genetics, there have been several publications concerning population genetic parameters in marine turtles such as sex-determination and intraspecific population structure (Bull, Vogt and Bulmer, 1982 and Bowen *et al.*, 1992). The latter allows accurate estimation of various parameters in investigated populations such as subgroups of

population , gene flow or evolutionary history (Engel *et. at.*, 1996). Unfortunately, publications on such topics have never been reported from all native marine turtles in Thailand. Therefore, management of these endangered species has been carried out without any sensible assumption. Theoretically, suitable management programmes can be initiated using various basic knowledge including population genetics and dynamics of marine turtle species. The objectives of such management are able to be separated into either a short or a long term.

In a short term, genetic diversity within a particular population of *C. mydas* is importance to evaluate the status of such population whether it needs to be conserved. In the long term, the most important goal for conservation of *C. mydas* is to protect its local gene pools to ensure sustainable availability of its genetic diversity. Heterozygosity is one of the most important population genetic parameters generally used to estimate levels of genetic diversity in this species. Moreover, conservation biologists require the information on the numbers and sizes of *C. mydas* populations in Thailand. Based on this knowledge, an appropriate breeding programme can then be set up. Hatchery propagated *C. mydas* juveniles may be released into their original habitats without destroying the native gene pools.

At present, the numbers of *C. mydas* are severely decreased therefore several government organisations within the country have established the conservation programmes without a basic knowledge on population structure of this species. Thus, unintentional mixing of natural *C. mydas* may occurred

from artificially propagated stocks originated from different *C. mydas* populations.

Based on the fact that behaviour and biological characters of individual *C. mydas* populations may differ in different environment and ecology (climatic variants), hybridisation between different *C. mydas* stocks may result in irretrievable loss of environmental adaptability of the local populations. Thus, a study on determination of genetic variation level in this species is essential in both conservation programme and management as well as further basic researches in this taxon (Ferguson *et al.*, 1995).

Molecular techniques commonly used in studies of genetic variation of animal Taxa

Several different biological methods such as morphology, life history and behaviour have been utilised for classical taxonomy of various species. It is well accepted that such classical techniques have several limitations. Accordingly, molecular genetic markers including both protein and DNA markers are increasingly employed in population genetic and systematic studies (Park and Moran, 1994 and Ferguson et al., 1995 Weising *et al.*, 1995). In contrast to studies at the protein level, which synonymous mutations cannot able to be detected, DNA approaches provide direct genetic data which are much more sensitive than that from analysis of protein polymorphisms. As a result, analysis of DNA polymorphisms is widely used at present. Furthermore, DNA analysis requires only small amount of starting tissue especially in PCR

amplification. Specimens used for DNA analysis e.g. blood or small tissue pieces can be non-lethal collected from experimental animals and simply kept in ethanol whereas samples for allozyme analysis need several organs to be used depending on investigated enzyme loci so that killing of investigated individuals cannot be avoid. The other reason in favour of genetic variation analysis at the DNA level is that DNA is much more stable than proteins. This results in much simpler and more convenient sampling strategies (Ferguson *et al.*, 1995).

The main disadvantages in using DNA markers for population genetic studies is that relatively long experimental period and high cost of chemicals are required. In comparison to allozyme analysis, DNA based techniques are much more complicated and time consuming. Theoretically, perfect markers that can be applied to all applications can not be obtained from only one approach. Base on the fact that several molecular techniques can be used for evaluation of the same population genetic parameters, selection of a molecular marker is largely depended on facilities available in the laboratory *per se* or a particular question needed to be investigated (Ferguson *et al.*, 1995 and Weising *et al.*, 1995). Commonly used molecular techniques are composed of protein eletrophoresis (allozymes), Restriction fragment length polymorphisims (RFLP), Randomly amplified polymorphic DNA (RAPD) and microsatellite loci.

1. Allozyme electrophoresis

Allozyme analysis is the first molecular tool used in population genetic studies. Charges in protein molecules are resulted from side chain groups making its isoelectric point (pI) different from one protein to others. The net charged in the protein molecules can be positive or negative depending on their environmental pH. The different charges of the same proteins result in their distinguishable migration on supporting medium (e.g. polyacrylamide or starch gel etc.) after electrophoresis. Besides that, sizes of proteins also affect their migration (Hoelzel and Dover, 1991). Almost all of the protein markers used in population genetic studies are allozymes because specifically histological staining can be combined with suitable electrophoretic conditions. When an electrophoretically size-fractionated enzyme react to its specific substrate in the presence of a co-factor, a catalyst and oxidised MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenltetrazolium bromide) or NBT (Nitroblue tetrazolium), the result can be directly visualized as protein bands (in blue) in the gel. For fluorescent staining, the resulting products can be seen under ultraviolet light (Hunter and Markert, 1957 cited in Park and Moran, 1993). An allozyme approach is a reasonably powerful technique causing a large number of individuals to be determined in a limited period of time. However, it also has some limitations. For instance, synonymous mutation is not able to be detected. Moreover, substitutions causing changes of one non-polar amino acid to another do not alter the electrophoretic mobility of the protein. Accordingly, allozymes underestimate levels of genetic variation. Due to its low ability to

detect genetic polymorphism, allozymes may not be an appropriate technique for evaluation of genetic variability in endangered species like *C. mydas* because an assumption on single large reproductive population of *C. mydas* can be wrongly accepted (Hoelzel and Dover, 1991; Park and Moran, 1994 and Ward and Grewe, 1994).

2. Restriction fragment length polymorphisms (RFLPs) of animal mitochondrial DNA (mtDNA)

Animal mtDNA is a extrachromosomal DNA found in mitochondria. It is circular double stranded DNA ranging from 15-20 kb in sizes (Hoelzel and Dover, 1991). MtDNA inherits maternally in nearly all animal species. Although deletions and insertions can be occurred, single-base substitutions are predominated. This coupling with extremely rare rearrangement of mtDNA allow the mitochondrial genome to be salient to trace precisely matriarchal relationship at the higher orders of taxa in which other markers may not accurately provide such basic data for phylogenetic reconstruction. Generally, substitution rate in the mitochondrial genome is higher than that of single copy genes in the nuclear genome for approximately 5-10 times. The mitochondrial genome is regarded as a single locus even though 13 protein coding genes, 22 tRNA genes, 12s and 16s rRNA genes are found. The reason for this is that all genes in the animnal mtDNA are tightly linked together. PCR has been recently used to amplify part of mtDNA before subjected to digestion of amplifies DNA fragment with restriction endonucleases. With this approach, different part of mtDNA can be chosen for classification of animal at different level e.g. PCR-

RFLP of 12s and 16s are rRNA probably suitable, unless tested, at interspecific level while various protein coding genes may be intraspecifically useful. Considering its mode of transmission and segregation, mtDNA is haploid reflecting its higher sensitive ability than that of single copy genes for detection of genetic variation level in animal taxa. The effective population size estimated from mtDNA analysis are one-fourth of that from the nuclear loci. This indicates that mtDNA markers are much more sensitive to random genetic drift and bottleneck events than the conventional approach based on allozyme and single copy nuclear DNA (Park and Moran, 1994; and Hoelzel and Dover, 1991).

On the other point of views, species experienced prolonged bottleneck effects (a dramatically decreased in effective population sizes) e.g. in several endangered species may result in an inability to detect intrapopulation genetic diversity. In this case, additional nuclear DNA markers which are at least equally in mutation rate (e.g. microsatellite loci) as that of mtDNA should be further used (Hoelzel and Dover, 1991).

3. Random amplified polymorphic DNA (RAPD)

This approach allows multiple regions of genome to be amplified randomly though the polymerase chain reaction (PCR) using arbitrary primers, usually 10-12 nucleotides long. This is simple and rapid approach used in population genetic studies because a prior knowledge of investigated genome is not required. RAPD is different from the conventional PCR in the sense that a single primer, rather than a set of two primers is employed in the amplification

reaction. Theoretically, 10 nucleotides will anneal to target DNA approximately every million base pairs (Hoelzel and Green, 1992). The products obtained are usually smaller than 3-4 kb. When an appropriate primer is used, small numbers of amplified fragments are obtained. DNA polymorphisms in individuals and populations are caused by point mutation, deletion or insertion of DNA at the priming site (Hoelzel and Green, 1992). Unless investigated, RAPD bands are treated in a dominant manner and can be explained by Mendel's laws. Accordingly, the presence of amplified fragment may reflect either a homozygous (AA) or heterozygous (Aa) situations. Only the absence of the fragment reveals the aa genotype. This disadvantage of RAPD results in an inability to estimate allele frequencies in a population and/or a species because the homozygous (AA) can not be dissociated from heterozygous (Aa). Nonetheless, RAPD is still widely used in studies of population structure because unlimited number of markers can be theoretically created though unlimited number of random primers. Moreover, the technique *per se* is less tedious than any other DNA based techniques. This makes RAPD extremely useful for population studies particularly when dealing with a large numbers of specimens. The most important disadvantage of RAPD is mainly due to its reproducibility. Therefore, care must be taken when this approach is employed for determination of phylogenetic relationships, e.g. among marine turtle species, where their systematic allocation are still not clear (Ward and Grewe, 1994 and Ferguson, 1995).

4. Microsatellites DNA polymorphisms

Koreth *et al.* (1996) reported that microsatellites DNA are shortly repeated arrays distributed along in genome. They are roughly presented at 35,000 to 100,000 copies in the human genome. The sizes of repeat units is range between 1 to 6 bp of different sequences. For example, white-footed mouse, *Peromyscus leucopus*, has different microsatellite loci such as 75,000 $(GT)_n$ loci, 50,000 of $(CT)_n$ loci as well as 40 of $(TA)_n$ and $(CG)_n$ loci (Janecek *et al.*, 1993 cited in Engel *et al.*, 1996). At present dinucleotides repeated microsatellite are commonly employed as reported in publications. This is mainly due to its higher content in the genome than tri and tetranucleotides repeated microsatellites. It is believed that microsatellites lack of a functional constraint causing highly polymorphic nature of these DNA markers. The mutation rate of microsatellite is estimated to be approximately 10^{-2}-10^{-5} per generation (A chance to find one mutated offspring among 100-10,000 full-sib progeny).

Weising *et al.* (1995) described general properties required for useful molecular marker. Based on their suggestions, microsatellite loci seem to be ideal for various population genetic application in comparison to other markers. The reason of this is that microsatellite loci are highly polymorphic therefore level of genetic variation detected with these are theoretically higher than that from allozyme, single copy nuclear DNA (scnDNA), and mtDNA.

Like allozyme and scnDNA, microsatellites segregate in a co-dominant fashion allowing discrimination of homozygotes from heterozygotes which is

extremely important parameter for basic population genetic studies. Moreover, they are also frequently and (possibly) evenly distributed in the genome which is contradictory to all other widely used markers except RAPD. On the basic of their polymorphic nature, different microsatellite loci can be selected to suitable application. Highly polymorphic loci are able to be used for individuality and parentage analysis whereas lower polymorphic loci (approximately 10 alleles per locus) are extremely useful for stock structure analysis. Study of genetic polymorphisms in the Atlantic cod, *Gadus morhua*, based on microsatellite markers found several loci with about 90% heterozygosity. Each of these loci contain more than 25 alleles reflecting the ability to be employed these highly polymorphic markers in genome mapping of *G. morhua* (Ruzzante *et al.*, 1996). In contrast to RAPD, microsatellite markers are amplified from, generally, locus-specific primers so results are much more reproducible than those from a single random primer like RAPD approach. Disadvantages of microsatellites seems to be their complicated approach to obtained locus-specific primers. This step are tedious and time consuming (Angers *et al.*, 1995; FitzSimmons *et al.*, 1995 and Bowers *et al.*, 1996). As a result, homologous DNA primers from publication should be, if possible, firstly looking for prior to development of additional homologous primer in the laboratory through DNA cloning and sequencing.

Population genetic studies in C. mydas and other marine turtle species

Studies on molecular population genetics and systematics of *C. mydas* are rather limited therefore review of literature on such topics in this study will also be from other related species.

It has been reported that conventional allozyme analysis are not sensitive enough for determination of intraspecific population differentiation in several species (Ferguson *et al.*, 1995; Lanzaro *et al.*, 1995 and Ruzzante *et al.*, 1996). This is mainly resulted from limited number of polymorphic markers obtained through such analysis. Bonhomme *et al.* (1987 cited in Karl and Avise, 1993) surveyed level of genetic polymorphisms in *C. mydas* collected from several rookeries around the world. From 23 allozyme loci investigated, only two low-frequency polymorphic loci were reported. Based on this approach, it was directly indicated extremely low level of genetic diversity within *C. mydas*. It was then concluded that extensive gene flow among rookeries homogenised detectable population substructure in this taxon. As a result, all subsequent researches on population genetic and systematic studies in *C. mydas* have emphasised on DNA based approaches.

A phylogenetic relationships at molecular level among all marine turtles were recently reported on the basis of sequence divergence in cytochrome b regions. Generally, the topology of a phylogenetic tree constructed from the mtDNA divergence was comparable to that from a classical taxonomic method. Moreover, several important information for conservation biology was discovered. This included an ability to detect a distant relationship between

Natator and other cheloniidae species. Where the paraphyly of *C. mydas* with respect to *C. agassizi*, and clearly genetic distinctiveness between *L. kempi* and *L. olivacea* were observed (Bowen *et al.*, 1993)

More importantly, it was also found that the evolutionary rate of mtDNA in marine turtles was much more slowly than that reported in vertebrates. It was estimated to be approximately 0.4% sequence divergence per million years between pairs of lineage in marine turtles rather than a conventional evolutionary rate of 2% sequence divergence per million years (Brown, 1979 cited in Bowen *et al.*, 1993).

To investigated population structure and gene flow in *C. mydas*, Karl *et al.* (1992) developed anonymous nuclear DNA loci to be used for RFLP analysis. The genomic DNA library of *C. mydas* was then constructed. Seven anonymous positive clones after hybridisation with total DNA probe were chosen namely CM-01, CM-12, CM-14, CM-28, CM-39, CM-45 and CM-67. Five of which were single locus markers while the rest (CM-14 and CM-67) were occurred in a low copy number of the genome.

Seven pairs of locus-specific primers were developed and used to investigate a total of 256 *C. mydas* specimens collected from 15 nesting populations at macrogeographical areas with 40 restriction endonucleases. Sixteen of which cleaved at least one site resulting in a total of 166 restriction sites for overall loci. However, only 9 of these were polymorphic. It should be noted, however, that genetic diversity of *C. mydas* was not observed when CM-01 and CM-28 were employed. Geographic heterogeneity analyses based

on χ^2 and F_{ST} were statistically significant indicated that population structure is well existed in this species. Furthermore, the highest genetic distance among populations observed was approximately 0.26. This also supported the large differences in distribution of allele frequencies among populations. A positive relationship between genetic and geographic distances (isolation by distance model) was clearly observed.

Female *C. mydas* inhabits breeding grounds over vast geographic areas. During reproductive seasons, female migrate for a long distance from foraging areas to a particular nesting location. From capture and recapture of tagged adults, it is well known that *C. mydas* exhibits strong nest-site fidelity. Theoretically, mtDNA can be used to examine female natal homing based on the basic assumption that genetic distances among different rookeries should be clearly observed regarding to matriarchal-transmitted fashion of the mtDNA. This marker is believed to be one of the most suitable markers for population genetic and dynamic studies of *C. mydas* which gender-biased is occurred.

Bowen *et al.* (1993) studied mtDNA restriction site analysis of 226 *C. mydas* individuals collected from different rookeries around the world. It was found that phylogenetic tree constructed from nucleotide sequence divergence (0.67%) separated all specimens into either the Atlantic-Mediterranean or the Indo-Pacific Oceans clades. Genetic substructure was also seen within each ocean basin evidenced by fixed or nearly fixed differences in constituent haplotypes of each rookeries.

Based on such evidences, results from mtDNA restriction site polymorphisms was able to prove female natal homing of *C. mydas* without the need to use a tediously direct method such as repeated capture approach. Additional evidence on natal homing of *C. mydas* was supported by sequencing of PCR-amplified control region in the mitochondrial genome of the Costa Rica (n=15) and Florida samples (n=24). These are the same sample set reported in Bowen *et al.* (1992) allowing direct comparison of data on the same specimens on different analytical approaches. The results from this and previous studies on female natal homing were concordant (Allard *et al.*, 1994).

An extensive survey of phylogeography and population structure of *C. mydas* covering a larger geographic area (the Atlantic and Mediterranean populations) than that reported by Allard *et al.* (1994) was latterly published. Sequence variation analysis of control region (487 bp from 147 individuals) amplified through PCR provided a total of 20 polymorphic sites consisting of 17 transitions, 2 transversions and an insertion of a 10 bp repeat. The results of the experiment indicated significant genetic differences among rookeries and a strong evidence for philopatry natal homing of nesting females.

As described earlier, the control region of *C. mydas* mtDNA is the most popular gene portion used for population structure analysis. Additionally, Norman *et al.* (1994) also sequenced 384-385 bp fragments which were amplified from the control region of 15 individuals collected as representatives from 12 rookeries in the Indo-Pacific region. Pairwise sequence divergence estimates ranged from 0.00 to 6.98% (mean = 3.67%) which were extremely

higher than those obtained through analysis of the whole mtDNA of the same individuals using RFLP approach (mean = 0.46%, sequence divergence ranged between 0.00-1.02%).

Furthermore, PCR-RFLP analysis of 384-385 bp amplified product of control region from 256 individuals digested with *Eco*RI and *Mse*I gave 1 and 5 restriction patterns, respectively. Analysis of geographic heterogeneity revealed significance differences in allele frequencies of *C. mydas* from the Indo-Pacific region implying an occurrence of its intraspecific population differentiation.

FitzSimmons *et al.* (1995) developed microsatellite loci in 3 marine turtle species including *C. mydas*, *C. caretta* and *E. imbricata*. Six highly polymorphic loci consisting of Cm3, Cm58, Cm72 Cm84 from *C. mydas*; Cc117 from *C. caretta* and Ei8 from *E. imbricata* were selected to test their ability to be used for amplification of the same DNA loci from several other heterologous species. The species used in the experiment included the three species described above, *D. coriacea*, *L. olivacea* and *N. depressus* (freshwater turtle). While Cm3 and Cm58 revealed polymorphic patterns in all species except in *C. caretta* and *L. olivacea*, Cm72 gave monomorphic results in *D. coriacea* and *E. imbricata* but not in others. On the other hand, Ei8 locus was only monomorphic in *C. mydas*. The remaining two loci (Cm84 and Cc117) were variable across all marine turtles.

For population differentiation analysis, significance in distribution of allele frequencies between eastern (n = 20) and western Australian *C. mydas*

(n = 14) were found when analysed with Cm3 and Cm58. This essentially indicated substructuring in the *C. mydas* in Australia.

Prior to the present research, there have been no publications concerning population genetic studies of *C. mydas* in Thailand with any molecular markers. Based on the fact that microsatellite markers are more appropriate than others for determination of population architecture in endangered species like *C. mydas* because level of intraspecific genetic variability in this species may be rather limited. Hence, there is a need to explore the possibility of using this approach to determine genetic variation and population structure in *C. mydas*. The data obtained can serve as an initial evidence for further studies of molecular genetics of *C. mydas*. Moreover, the basic information from this study can be used as the rational behind a decision to set up the most effective and appropriate programme of *C. mydas* in Thailand.