POPULATION GENETICS OF SEA TURTLE IN SOUTHEAST ASIA

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INTRODUCTION

Population questions such as - is there population subdivisions or are populations genetically distinct from one another? is there gene flow among populations and how is genetic variation distributed among populations?- are fundamental to conservation. Questions that are more specific to sea turtle life history such as natal homing vs. social facilitation, male-mediated gene flows and multiple paternity also contribute to the same end. Molecular techniques have much to offer and when integrated with ecological and behavioral studies, can make a major impact on the understanding of processes and dynamics of biodiversity and its natural preservation (Karp and Edwards, 1996).

A comprehensive molecular study to assess the global population genetic structure of sea turtle was reported in the early 90's (Meylan *et al.*, 1990; Karl *et. al.*, 1992,). Subsequently, studies on populations nesting in specific localities around the world i.e. Indo-Pacific (Norman *et. al.*, 1994), Greater Carribean (Lahanas *et. al.*, 1994) and Australia (Fitz Simmons *et. al.*, 1997) were made. Some of their findings can be summarized as follows; (i) natal homing of females to distinct geographical regions was confirmed on both global and regional scales (ii) at least on smaller geographic range, breeding males, like females, are philopatric to courtship areas within their natal region and, (iii) resolving power of population discrimination is technique-dependent.

Given the geographic specificity of the published reports thus far, studies of sea turtles in other regions including Asean are necessary for a better understanding of the turtle world for the management and conservation of the species. This paper provides a brief description of the molecular approaches and a short report on the progress of the genetics study by MFRDMD/UKM.

Review of Molecular Markers

The choice of molecular markers for population studies are many. The PCR-based RAPD technique is an attractive proposition since no prior knowlwdge of the genome is required and has been applied in numerous population studies of many marine and terresterial species. Studies in sea turtles however have concentrated on mitochondria DNA and specific segments of nuclear DNA.

Mitochondrial DNA

mtDNA exhibits considerable variation among individuals both within and between populations thus it has proved to be an effective marker of population structure and patterns of geographic variation. Information about mtDNA variation in natural populations has come principally from comparisons of restriction enzyme fragment patterns of whole mtDNA genome or specific regions of mtDNA (Meylan *et. al.*, 1990; Bowen *et. al.*, 1992). In many of marine species, various regions of mtDNA (e.g. cyto b, ND-I, 5/6, D-loop) have been examined. Studies in sea turtle have largely confined to the control region. In addition direct sequencing of the mtDNA control region has been used for evaluating population variation (Lahanas *et. al.*, 1994, Encalada *et. al.*, 1996) and, ND4-leucine tRNA region and cytochrome b gene (Dutton *et. al.*, 1996; Bowen *et. al.*, 1993) for phylogeny studies. Restriction site variation of whole or specific regions offeres a limited glimpse of the amount of genetic variation that exists between groups. Direct nucleotide sequencing provides greater resolution than restriction site data for assessing mtDNA variation. Another point worth considering is that mtDNA is maternally inherited and may not yield a complete picture and can prompt misleading interpretation (Fitz Simmons *et. al.*, 1999).

Nuclear DNA

Population studies of nuclear DNA frequently comprises segments of DNA that are non-coding. Two nuclear DNA markers frequently used in sea turtle studies are anonymous single copy nuclear DNA (ascnDNA) and microsattelites, ascnDNA loci in sea turtle were first introduced by Karl *et. al.* (1992) and Karl and Avise, (1993). Seven ascnDNA polymorphic loci were characterized and used in various studies. The detection of variation in ascnDNA involves the development of specific primers flanking the ascnDNA locus and the use of these primers for amplification by PCR. The type of genetic variation most easily revealed by this method is restriction endonuclease enzyme cut site differences. The alleles at each locus are defined by the presence or absence of specific sites. Haplotype or genotype data at several loci for each individual are useful in determining levels of heterozygosity and elucidating population subdivision.

Microsatellites consists of short repeats (1-6 bp). Microsatellites are attractive markers because they are codominant and can be highly polymorphic. The major problem with these markers is that they may not already be available for the species under study and that they often showed limited cross-transferability to other general and even to other species within genus. Fitz Simmons *et. al.* (1995) successfully isolated and characterized nine $(CA)_n$ microsatellites in sea turtles and demonstrated a conservation of flanking sequences that permit the amplification of the loci across three general of Chelonids. The complete sequences of the loci can be obtained from the genebank and primers designed to amplify these loci are given by Fitz Simmons *et. al.* (1995). Their usefulness have been shown in studies related to population structure, male-biased gene flow and paternity assessment (Fitz Simmons *et. al.*, 1997; Fitz Simmons, 1998). These loci have been proved to be highly variable with 10-40 alleles per locus.

Sea turtle genetics in Southeast Asia

Reseach on the genetics of sea tutles in Southeast Asia is almost non-existent. There are a few ongoing studies but these are very much limited to small geographic areas such as the Sabah rookeries and Pulau Redang in Malaysia.

In the effort to develop expertise in sea turtle genetics, UKM was approached by MFRDMD in late 1997 with a single objective to assess the population of sea turtles, particularly the green turtle, from various nesting beaches on the coast of Malaysia. Precise scientific information on the methodologies with regards to sampling protocols i.e. tissue collection and sampling design, was lacking at the time. Given the limitations, we initially approached the problem using the RAPD technique. This technique is robust, simple and has been utilized extensively in our laboratory for fish genetics study. Four populations from nesting beaches of Pahang, Terengganu and Perak were studied. Twenty-one decamer primers (Genosys Biotechnologies) have been screened to date to generate RAPD profiles. The study is ongoing and therefore no attempt is made to present the results in great details. Rather, a brief qualitative description of the results is presented. From the 21 primers screened, eleven primers consistently produced scorable amplification products based on the basis of pattern clarity and the amount of detectable polymorphisms. A total of 59 scorable bands ranging in size from 300-1600 bp were produced of which 29 bands were polymorphic, i.e. present in some individuals and absent in others (Table 1). Research using a greater number of primers to build a complete and credible results of population variation is in progress.

Dr. Fitz Simmons (University of Queensland) has kindly provided the information necessary to enable us to pursue this study further by looking at the microsatellites and ascnDNA. Studies on these markers have begun and are targetted for completion by middle of year 2000.

Conclusion and Future outlook

Substantial genetic information exists for a few sea turtle populations throughout the world, but there is a paucity of even basic genetic information for the rest, including Southeast Asia. Genetic research on

these species has not been considered a high priority, has not been funded adequately and often is difficult to execute. The general lack of understanding of the genetics limits the effectiveness of current management and conservation policies. For a region placing importance on sea turtle conservation, Southeast Asia cannot affort to neglect the application of genetics in the conservation effort. More comprehensive genetic studies are required.

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Table 1: RAPD primers, number of scorable bands and approximate size of bands.

Primer	number of scorable bands (number of polymorphic bands)	approx size range (bp)
GEN1-50-08	7 (3)	430-1300
GEN1-50-09	7 (1)	500-1500
GEN1-60-07	6 (2)	320-1450
GEN1-60-09	9 (6)	300-1200
GEN2-50-11	8 (2)	350-1600
GEN2-50-16	8 (5)	370-1550
GEN2-50-18	5 (3)	830-1400
GEN3-50-22	5 (4)	300-1400
GEN3-50-26	4 (3)	650-1500
Total	59 (29)	

Figure 1: RAPD profiles generated using the GEN 2-50-16 (A) and GEN 2-50-18 (B) primers. Lanes 1-8 (Chendor), lane 9 (100 bp marker), Lanes 10-17 (Perak).