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"Jabatan Perikanan Malaysia"

SEA TURTLE INFORMATION KIT

Volume 4

CONSERVATION GENETICS OF SEA TURTLE

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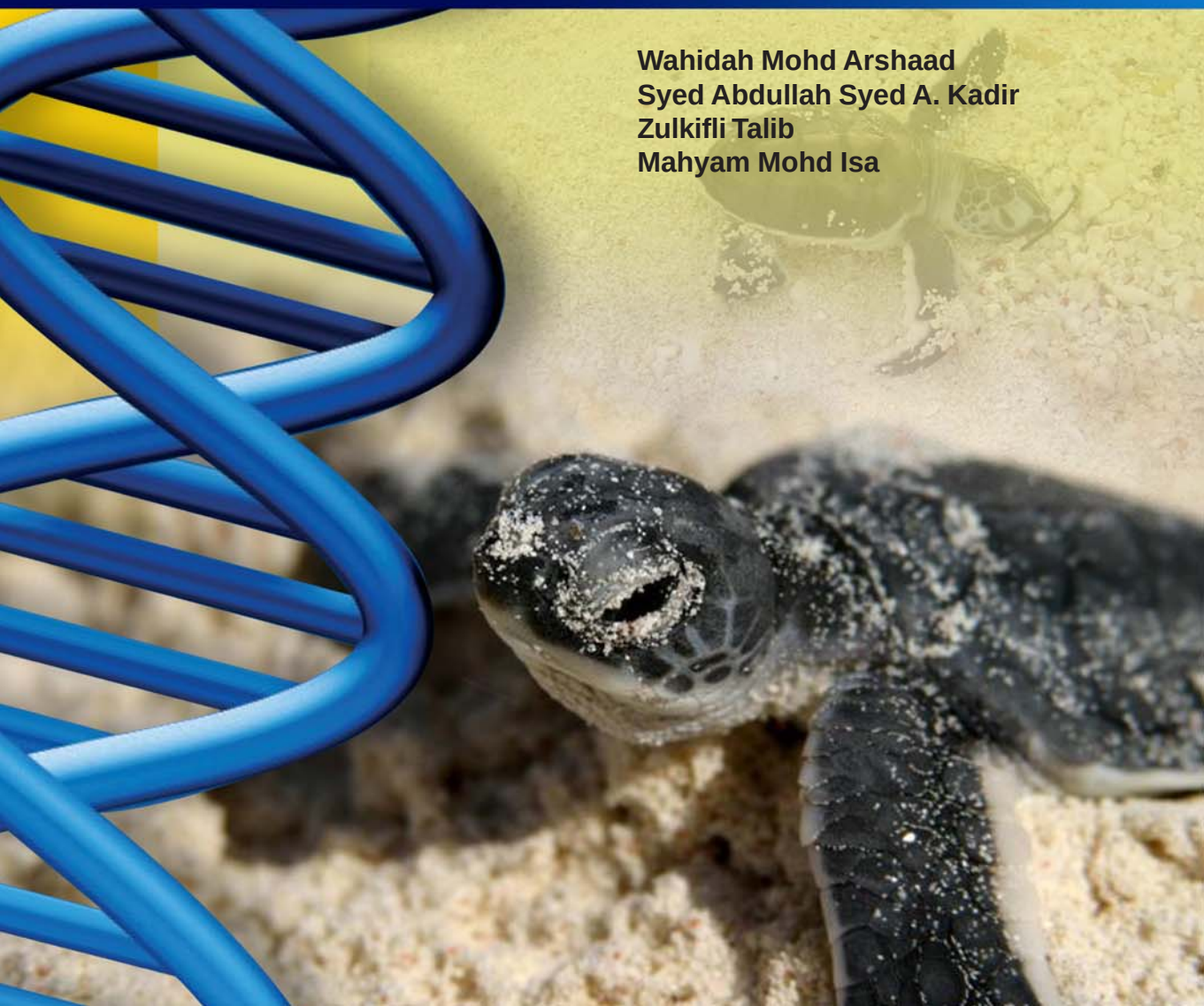

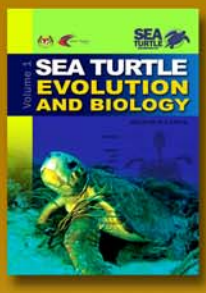

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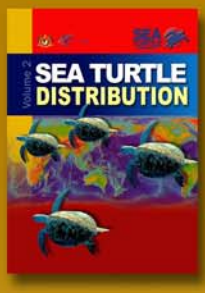

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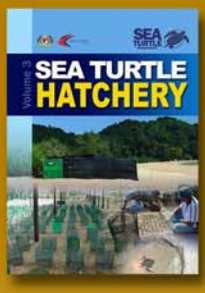

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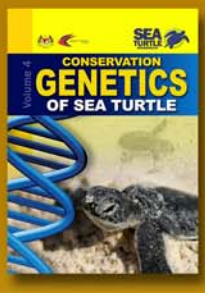

Volume 4

Volume 5

Sea Turtle Information Kit

Sea turtles are valued by people around the world. They are symbol of longevity, fertility, strength and protection from harm. However, sea turtles have also been exploited for their meat, eggs, shells and oil for years. This negatives impacts have accelerated the decline of the sea turtles population worldwide. The Sea Turtle Information Kit is specifically aimed at enhancing awareness, knowledge and understanding of the sea turtles among the public. It is hoped that the Sea Turtle Information Kit will help spread awareness among the public to protect and conserve the sea turtles and also the marine environment as a whole.

Message from the Honourable Dato' Junaidi bin Che Ayub, the Director-General of Fisheries Malaysia

The Southeast Asia holds a strong appeal to a myriad variety of sea creatures and one of these is the sea turtles. The region produces six of the seven living sea turtle species found worldwide and four of them are found nesting in Malaysia: green turtle (*Chelonia mydas*), leatherback (*Dermochelys coriacea*), hawksbill (*Eretmochelys imbricata*), and olive ridley (*Lepidochelys olivacea*).

Malaysia has, as early as in 1961, initiated and implemented conservation and management programs for the four species of sea turtles that occur in her waters. All the species which constitute a unique heritage in Malaysia have been accorded special attention through various conservation strategies to ensure their adequate conservation and protection.

In a world of diminishing natural heritage caused, in some cases by man-made pollution and overexploitation for commercial reasons, any effort to conserve the sea turtle from total annihilation is a virtuous idea that should be supported by all parties.

The Sea Turtle Information Kit is published to develop awareness, knowledge and understanding of sea turtles among the people. It is not easy to make people understand the serious and complex problems facing the sea turtles. However, with the publication of the Sea Turtle Information Kit it is hoped that it will drive home the message concerning the importance of sea turtles conservation.

I wish to congratulate the team for coming up with the Sea Turtle Information Kit. It is timely that such publication is produced to highlight the plight of the sea turtle. The sea turtles have been around since the dinosaurs' era. Let us protect these remarkable creatures and the habitats that they need to survive.

Dato' Junaidi bin Che Ayub
Putrajaya

1 December 2006

Foreword
Chief of
SEAFDEC-MFRDMD

The sea turtles have roamed Earth's oceans and sea for million of years. They were on Earth 150 millions years ago, and they have outlived almost all of the prehistoric animals with which they once shared the planet. Sea turtle survived the extinction of the dinosaurs and are still present in the world's ocean today.

Sea turtles once were found by the millions, but the demand for turtle meat, eggs, shell, leather and oil has greatly reduced their numbers. Their populations continue to decline because of the trade in sea turtle product and the loss of essential habitats.

Conservation is about reducing and removing the threat. But in reality, the work of conservation does not lie principally with the animals, plants and ecosystem but actually lies in dealing with humans. Although conservation programs are in existence, results in general have not been encouraging. The Sea Turtle Information Kit is produced with the intention of spreading awareness, knowledge and understanding to make people realize the importance of sea turtles conservation. It is our duty to make sure that the sea turtles still exist for our future generation to see.

I would like to take this opportunity to congratulate the team members headed by Ms Hjh. Mahyam bte Mohd Isa who have worked tirelessly to come up with this Sea Turtle Information Kit. Without their initiatives and sincere commitments, the Sea Turtle Information Kit would not have been realized.

Finally, I would like to express our thanks and gratitude to the Honorable Dato' Junaidi bin Che Ayub, the Director-General of Fisheries Malaysia, for the continuous support and confidence in the team members.

Raja Mohammad Noordin bin Raja Omar
Kuala Terengganu

1 December 2006

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INTRODUCTION

Animals, plants, and other species become endangered when their normal habitats are lost or altered. Effective management of ecosystems can help to preserve threatened species.

The **Science of Conservation Biology** studies individuals and populations that have been affected by habitat loss, exploitation, and/or environmental change. That information is used to make informed decisions to ensure the survival of organisms in the future.

The **Science of Genetics** is the study of inheritance and the investigation of the genes responsible for inherited traits.

Conservation Biology + Genetics = Conservation Genetics

Conservation genetics as a distinct discipline became prominent in the early 1980s. Conservation in the past has been addressed from a mathematical, evolutionary, or taxonomic point of view. Genetic studies supply conservation scientists and ecological managers with new insights into the extent of diversity among the individuals in a population. Without using genetics, we may conserve the wrong population or waste valuable resources on a population that is not endangered. Conservation genetics has become an important tool in studies of animal and plant populations.

Genetic methods are currently being used to;

- uncover taxonomic relationship,
- define distinct population segments and evolutionary significant units,
- evaluate population viability,
- determine migration and gene flow patterns,
- detect hybridization,

- better understand behavior and mating systems,
- estimate population sizes and
- identify presence or absence of endangered species.

As a result, genetic data plays an important role in conservation and management decisions.

WHEN DO SCIENTISTS USE CONSERVATION GENETICS?

1) When habitat destruction or other factors put a population at risk, scientists and conservation managers target that population for investigation.

2) When total species becomes small; surveillance of that species becomes critical. When the population of a species is small to begin with, further reduction of their remaining numbers can sharply reduce genetic diversity.

WHAT GENETIC TECHNIQUES CAN DO?

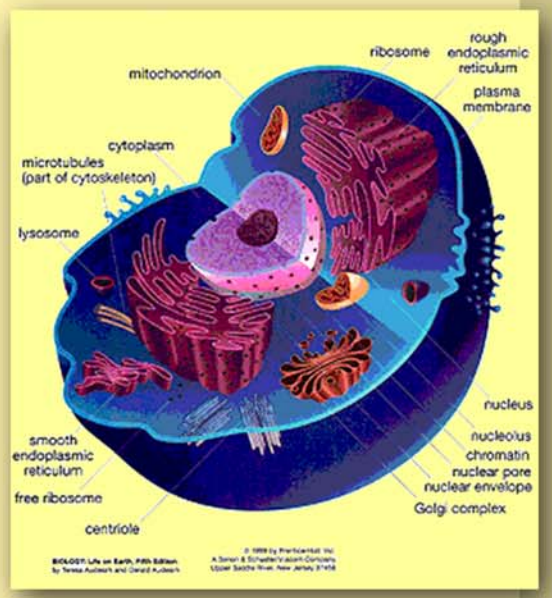
In the last decade genetic techniques have illuminated several aspects of sea turtle life history:

- Do female turtles return to nest on their natal beach?
- How many male contribute to a clutch?
- Do female provide an avenue for gene flow between nesting colonies?
- Can DNA fingerprints be used to trace sea turtle migration?
- What are evolutionary relationships among sea turtle species?

Thousands of sea turtle around the world have been tagged to collect information about their growth rates, reproductive cycles and migration routes. After decades of studying sea turtle, much has been learned. However, many mysteries still remain.

Resolution of population in sea turtles is confounded by extensive migrations made by most species as juveniles and breeding adults. These migrations highlight the need to identify the geographic range of feeding habitats that support a specific breeding population and, conversely, to assess proportions of different breeding population present in particular feeding ground or harvest. The molecular data are most informative when integrate with field studies, especially tag-recapture studies.

analysis. For this reason, the control region is recognized as the mtDNA segment of choice for nesting beach survey.



DNA AND GENETIC MARKERS

DNA or deoxyribonucleic acid is the chemical inside the nucleus of all cells that carries genetic instructions for making living organisms.

Genetic marker is a segment of DNA with an identifiable physical location on a chromosome whose inheritance can be followed. A marker can be a gene, or it can be some section of DNA with no known function. Because DNA segments that lie near each other on a chromosome tend to be inherited together, markers are often used as indirect ways of tracking the inheritance pattern of genes that have not yet been identified, but whose approximate locations are known. Different genetic markers have different scopes and different advantages and disadvantages.

Mitochondrial DNA (mtDNA) has proved particularly effective for detecting population structure in the sea turtle. The resolving power of mtDNA assays is technique-dependant and several studies have reported enhanced population discrimination using rapidly evolving control region rather than whole genome restriction fragment (RFLP)

Population studies of nuclear DNA typically use segments of the genome that do not code for specific protein products. These non-coding regions accumulate mutation more rapidly than protein coding regions, and thereby provide greater sensitivity. The nuclear DNA segments that are appropriate for sea turtle population studies include anonymous single copy nuclear DNA (ascnDNA), minisatellites and microsatellites. Minisatellite and microsatellite techniques, popularly known as DNA fingerprinting, have also been used to assess pedigrees and the possibility of multiple paternity in the sea turtle nests.

Microsatellite DNA is extremely useful for population studies because single loci with as many as 30-50 alleles can be examined. It also called “simple sequence repeat (SSRs)” and are composed of tandem repeat 2-, 4-, or 6- base repeat units (such as CA, CAAC, or GGACC). Microsatellite loci are analyzed by amplifying the target region using PCR, followed by electrophoresis through a high resolution of gel such as acrylamide gel to allow resolution of alleles that may differ in size by as few as two base pairs.

DNA sequences are the most detailed analysis of DNA differentiation that can be obtained by sequencing the region of interest from different individuals. Using PCR, specific region can be targeted for amplification if the sequence of the conserve regions flanking the region of interest is available. The amplified fragment can then be sequenced.

SYNERGY BETWEEN GENETIC SURVEYS AND TAGGING STUDIES

Genetic data and information from tag returns can interact in three ways:

1) Tagging studies will generate hypotheses about migration patterns that are testable with genetic data. In several sea turtle species, hypotheses about the reproduction of migrations of sea turtles, formulated on the basis of tag-recapture studies, have been evaluated with genetic surveys.

2) Tagging data can be used to test weather nesting populations that appear to be united by extensive gene flow (base on genetic data) also show frequent exchange of nesting female turtles among adjacent nesting habitats that are genetically homogeneous.

3) Molecular data can provide novel perspectives that can be tested subsequently through tagging programs. For example, genetic data may indicate that a breeding population extends beyond the borders of intensive tagging studies – this inference can be tested by extending mark-recapture across a broader geographic scale. Finally, genetic data may demonstrate rare range long-distance colonization events which are difficult to document by tagging alone (FitzSimmon *et al*. 1997).

DNA SAMPLING METHOD

DNA is found in nearly all cells of all organisms and it can be recovered from both living and dead tissues. Only **nanograms** of DNA are needed for analysis (when amplified by PCR – Polymerase Chain Reaction). The molecule is so stable that recognizable sequence can remain intact for hundreds of millions of years. For most application, fresh or recently preserved DNA is needed for analysis. All materials and solution that use during conducting the sample DNA need to be sterilized to avoid any contamination. Blood and tissue muscle can be used as DNA sample.

Blood as DNA sample.

Tissue muscle collected by using biopsy punch.

DNA sampling: Tissue muscle collection

The area of sampling was sterilized using ethanol and the equipment used need to be sterilized also.

Only 1 cm^2 tissue muscle was collected.

Tissue sample was stored in vial containing 90% ethanol.

Vial containing tissue sample with label was stored in storage box.

MOLECULAR LABORATORY FACILITIES

Marine Fishery Resources Development and Management Department (MFRDMD) had established a molecular laboratory. Most of the equipment was funded by Japanese Government through Japanese International Cooperation Agency (JICA) and Trust Fund II Research Program from SEAFDEC.

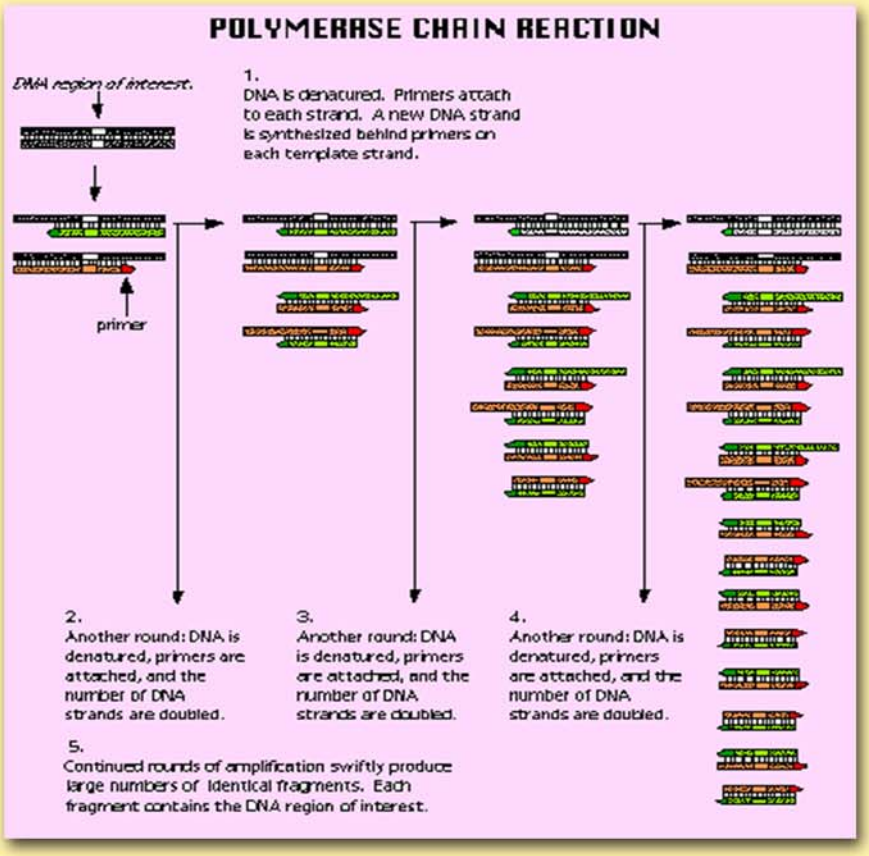
POLYMERASE CHAIN REACTION

PCR is a process xeroxing a strand of DNA interest based on a specialized polymerase enzyme. The mixture is heated to separate the strands of double-stranded DNA containing the target sequence and then cooled to allow :

(1) The primers to find and bind to their complementary sequences on separated strands.
(2) The polymerase to extend the primers into new complementary strands.

Repeated heating and cooling cycles multiply the target DNA exponentially, since each new double strand separates to become two templates for further synthesis. In about 1 hour, 20 PCR cycles can amplify the target by a million fold.

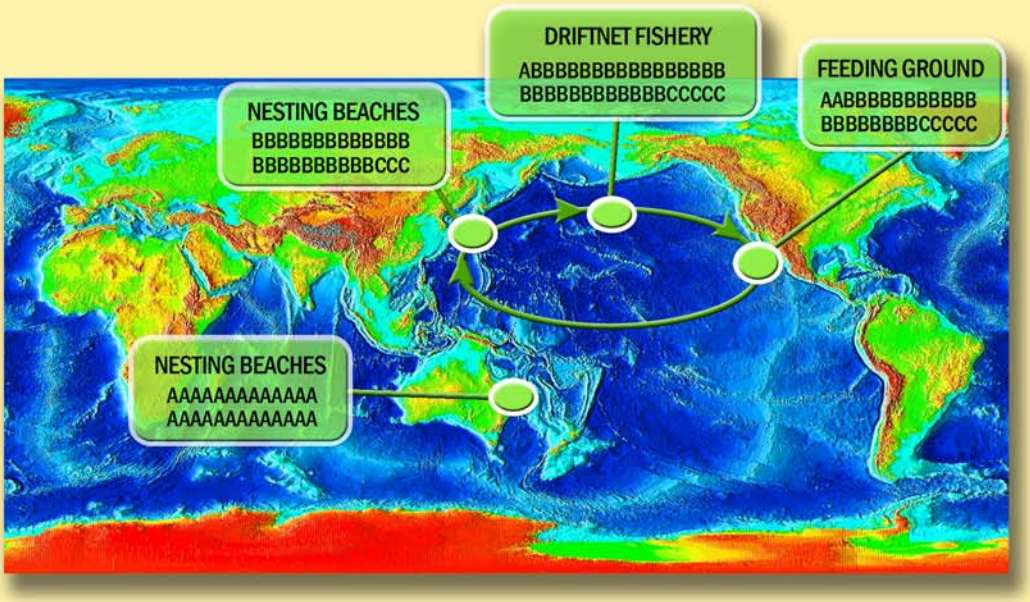
The only protection PCRs have is the technique of the analyst, use of control samples to monitor contaminants and careful interpretation. Most forensic laboratories perform negative controls, blank samples that will often detect contaminants in the laboratories.



DNA POPULATION STUDY

DNA markers based on mitochondrial (mtDNA) control region sequences are widely used in the turtle population study. Some findings that had been published are:

1) Recent studies of mtDNA variation among the turtle population are consistent with the hypothesis that females return to beaches in their natal region to nest as adults.
2) Breeding males, like female, are philopatric to courtship areas within their natal region (FitzSimmons *et al*. 1997).
3) Trans-Pacific migrations of loggerhead turtle (*Caretta caretta*). Japan is the primary source (95%) of turtles in the North Pacific Current and around Baja California. Australian nesting colonies may contribute the remaining 5% of these feeding aggregates (Bowen *et al*. 1995).
4) Nucleotide sequences from the cytochrome b-gene of mtDNA were used to resolve phylogenetic controversies and to assess molecular evolutionary rates in marine turtles (Cheloniodea) (Bowen *et al*. 1993). The research group found special relevance to conservation biology include discovery of a distant relationship between Natator and other chiloniid species, the paraphyly of *Chelonia mydas* with respect to *Chelonia agassizi*, and genetic distinctiveness of *Lepidochelys kempi* from *Lepidochelys olivacea*. Sequence divergences at intergeneric and interfamilial levels, when assessed against fossil-based separation times, support previous suggestions (from micro-evolutionary comparisons) that mitochondrial DNA in sea turtles evolves much more slowly than under "conventional" vertebrate clock.

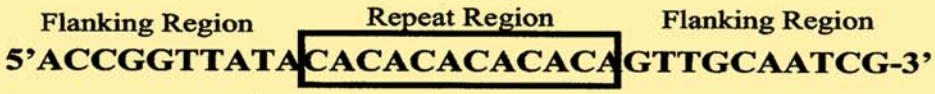


Collecting locations and mtDNA haplotype distribution on Pacific nesting beaches and pelagic developmental habitats (Bowen *et al*. 1995).

DNA PATERNITY ANALYSIS

One of the most commonly used forensic DNA marker is Variable Number Tandem Repeats (VNTR). A VNTR is a region of the DNA where there is a small sequence of DNA repeated over and over again. They are found in non-coding regions of the genome (sometimes referred to as "junk DNA"). The VNTRs are commonly divided into two categories: minisatellites and microsatellites.

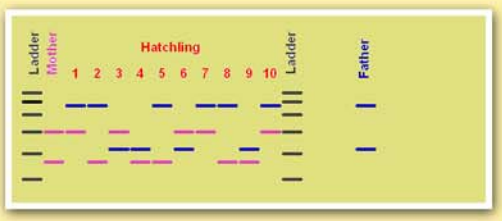
Minisatellites are typically made of short sequences of DNA about 10-30 base pair long, repeated in tandem multiple times. Microsatellites are very small repeats, typically 2 to 4 bases in length. For example, one of the most common microsatellites used is the CA repeat. In this case, variation among different alleles would be due to the differences in the number of CA repeat units. Diagram of the complimentary strand for the CA microsatellite allele is as follows:



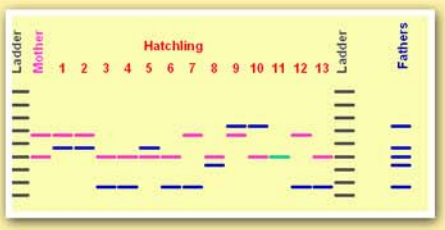
Repeat regions have high rate of mutation due to alterations in the number of the repeats. So it is often likely that the two alleles in one individual will have different number of repeats. For example, individual #1 may have CACACA at a particular locus while individual #2 may have CACACACACACA.

In turtle study, DNA microsatellite marker can be used to determine whether all hatchlings in a nest have the same father or different fathers. If different fathers contribute to a single nest of hatchlings, this is called multiple paternities. Hatchlings that have the same mother but different fathers are called half-sibs.

Paternity analysis can be done by looking at the pattern of gel electrophoresis band (one band = one allele). **Single father will present one to two paternal alleles, while clutches with three to four paternal alleles represented offspring from a mating between one female and two male turtles**. If more than four paternal alleles were present, the clutch was assumed to has a minimum of three fathers (Moore *et al*. 2002).



a) DNA microsatellite PCR products from a nesting mother and their hatchlings with single father.



b) DNA microsatellite PCR product from a nesting mother and their hatchling with multiple paternity (>3).

Multiple fathers have been provisionally detected in a study of green turtle clutches at Tortuguero, Costa Rica (Parker *et al*. 1996). Croix and FitzSimmons (1998) found very low incidence of multiple paternity in green turtle (*Chelonia mydas*) nesting in the southern Great Barrier Reef. Kemp's ridley (*Lepidochelys kempii*) clutches at Rancho Nuevo, Mexico had high rate of multiple paternity (Kichler *et al*. 1999). Bollmer *et al*. (1999) found that one of three loggerhead clutches from Melbourne Beach, FL, had multiple fathers, with two fathers more likely than three.

Hoekert *et al*. (2000) had studied mating system of olive ridley sea turtle in the Galibi Nature Reserve in Suriname, Western Atlantic using 2 polymorphic microsatellite markers. In 8 clutches that have been studied, 2 clutches were found in which multiple paternities had occurred. Multiple paternities occur at least to some extent in the olive ridley sea turtle.

Generally, multiple paternities were common, with 31% of all nests possessing multiple fathers. Almost 10% of the multiple fathered nests had three or more fathers – more fathers than have previously been reported for sea turtle species. The advantages of multiple mating are many. If the adult sex ratio is skewed in this population, it may be that females choose to mate with the first male that they encounter to ensure fertilization, and then they can 'upgrade' if they happen with a better male later on (Birkhead *et al*. 1993; Evan & Magurran 2000). Female loggerheads probably mate at the beginning of the nesting season and store sperm to fertilize the clutches for that season (as female green turtles do), allowing ample opportunity for sperm mixing and competition (Miller 1997; FitzSimmons 1998). Avoidance of genetic incompatibility (Zeh & Zeh 1996; Newcomer *et al*. 1999; Vala *et al*. 2000) and selection of competitive sperm (Smith 1984; Watson 1991; Madsen *et al*. 1992; Bear & Schmid-Hempel 1999) are also plausible hypothesis for maintaining polyandry in sea turtle.

CLONING

Every year, number of turtle nest showed declining trend. These species disappear due to hunting, poaching and competition between humans and other animals for scare resources and most of all, loss habitat. With the advent of cloning, wildlife conservationists have a new tool in their efforts to protect endangered species from extinction. Cloning is the new last line of defense for this reptile, after habitat preservation, poaching control and hatchery.

Dolly: World's first cloned adult animal

Since the reported birth of a cloned lamb, Dolly, in 1997, conservation biologists have become aware of the possibility of using reproductive and developmental technologies to manage endangered species. Whether cloning technologies can be used in conservation biology remains controversial, while some scientist advocate that cloning can offer many benefits, some argue that to cloning endangered species is too expensive for a field that is always in need of more money.

A pair of new born World's first cloned calves (1998)

Copycat: World's first cloned kitten (2001)

Although other methods are more efficient than cloning but they don't always succeed. **In those instances, cloning may be the only way to prevent the loss of a species forever such as leatherback.** Cloning should be the last resort for less efficient method than habitat preservation, poaching control and hatchery.

Idaho Gem: World's first cloned mule (2003).

ANDi: First rhesus monkey cloned by embryo splitting

When a species perishes, we lose the unique survival strategies encoded in its genes over millions of years of evolution. We lose the opportunity to understand those strategies, which may hold essential clues to our own survival as a species. And we lose the singular beauty and spirit of that life form.

~Maryjo Koch

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