THE GENETIC STRUCTURE OF A LOCAL LOGGERHEAD SEA TURTLE POPULATION BASED ON MITOCHONDRIAL DNA ANALYSIS

by

Connie Jo Sears

A thesis submitted to the Faculty of
the University of Charleston Graduate Program in Marine Biology
in partial fulfillment of the requirements for the Degree of Master of Science.

UNIVERSITY OF CHARLESTON, SOUTH CAROLINA

November 1993

APPROVED BY:

Sylvia A. Gallowa MAJOR ADVISOR	Nov 29, 1993 DATE
Charles K. Bu COMMITTEE	Nov. 29, 1993 DATE
Julian R. Harrison COMMITTEE	Nov 29, 1993 DATE
Sally R. Murphy COMMITTEE	Nov. 29, 1993 DATE
DEAN OF GRADUATE STUDIES	DATE

ACKNOWLEDGMENTS

I would like to thank the efforts of many people associated with this project. First and foremost is my major advisor, Dr. Sylvia Galloway, and my committee members: Dr. Charles Biernbaum, Dr. Julian Harrison, and Mrs. Sally Murphy. For technical expertise and support, I thank Dr. Cheryl Woodley. For the unselfish sharing of information, and for his encouragement, I thank Dr. Brian Bowen.

For support in data collection, data analysis, and tremendous volumes of library assistance I would like to thank the following people: Dr. Lee Chao, Dr. Robert Chapman, Ms. Ann Colbert, Dr. Greg Doucette, Mrs. Charlotte Hope, Mrs. Ann Horan, Mrs. Helen Ivy, Mr. Dan Johnson, Dr. John Keinath, Mr. Carl Kinerd, Mrs. Joan Logothetis, Mrs. Sylvia McCorkle, Mr. Phil Maier, Mrs. Katy Metzner-Roop, Ms. Kathy Moore, Mr. Doug Oakley, Capt. Carl Piggot, Dr. Jim Richardson, Mr. Tim Roop, Mrs. Gloria Seaborn, Capt. Mike Schwarz, Dr. Alan Shanks, Mrs. Jeanette Smith, Mr. Mark Thompson, Mr. Glen Ulrich, Dr. Robert Van Dolah, Mrs. Laura Webster, and Mrs. Lois Winemiller.

This research was supported in part by NOAA/NMFS Grant #NA90AA-H-MI225.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The purpose of this study was to identify the natal origins of juvenile loggerhead sea turtles found in the Charleston Harbor Entrance Channel (CHEC; Charleston, SC) feeding ground. Restriction fragment length polymorphisms (RFLP) were generated from mitochondrial DNA (mtDNA) purified from blood samples taken from thirty-three juvenile loggerhead turtles. These patterns (genotypes) were compared to haplotypes (groups of genotypes) from previously defined rookeries of the Southeastern United States (Florida, Georgia, and South Carolina) and Greece. It was determined that the population structure of the juveniles in the CHEC was not genetically identical to any one rookery but was a mix of the four rookery haplotypes. Although this supports the hypothesis of feeding area integration for juvenile loggerhead sea turtles relative to their natal rookeries, the higher-than-expected numbers of juveniles from the Georgia and South Carolina complexes indicate some degree of fidelity to feeding areas adjacent to natal rookeries.

INTRODUCTION

Loggerhead Natural History

The loggerhead sea turtle, *Caretta caretta* (Linnaeus 1758), is classified as a threatened species in United States waters under the Endangered Species Act of 1973. Loggerheads spend the majority of their lives at sea, only contacting land as mature nesting females or hatchlings. Upon leaving their nests, hatchlings become one of many nektonic organisms (able to move independently of ocean currents) found along border currents in the open ocean (Neshyba 1987). A twenty hour initial swimming frenzy takes the 20g hatchlings to *Sargassum* rafts along border currents of the Gulf Stream. Here they climb onto the mats and are at the mercy of the current direction. *Sargassum* mats provide protection to the hatchlings and allow foraging on various prey items such as coelenterates, molluscs, tunicates, crustaceans, worms, and small fish.

When there are no *Sargassum* mats, hatchlings aggregate at fronts. Floating debris at these fronts attract planktonic organisms that colonize and make survival of hatchlings feasible. Loggerheads are presumed to remain in the open ocean for approximately four or five years before they are seen again along coastal feeding grounds (Carr 1986, NMFS and USFWS 1991). Once leaving the *Sargassum* mats of the open ocean, Southeastern loggerhead turtles inhabit the continental shelf and are known to migrate as far north as Newfoundland (NMFS and USFWS 1991).

al. 1991) and are regularly found in estuarine environments including the Chesapeake Bay, harbors and sounds in North and South Carolina and Georgia, as well as the Indian River in Florida. Migrations of juvenile turtles may take them through many potential feeding areas. Butler et al. (1987) reported that there can be as many as three different groups of sea turtles occupying a feeding area at one time (adult males, adult females, and juveniles). In addition, it is proposed that several genetic populations may also be in a given feeding area (Smith et al. 1978).

In the Southeastern United States, nesting areas (rookeries) for loggerhead turtles are located from North Carolina (1.0% total US nests), through South Carolina (6.5% total US nests) and Georgia (1.5% total US nests), into Florida (91% total US nests). These nesting beaches total approximately 2,240km and produce 33% of the world's population of loggerheads. Eighty percent of the nesting in the United States occurs along the Atlantic coast of Florida, from Brevard to Broward Counties. Other significant nesting areas are along the coasts of Georgia, South Carolina, North Carolina, and the Gulf coast of Florida (NMFS and USFWS 1991). Rookeries may be defined by a single stretch of mainland beach or they may be comprised of many barrier islands. Low-density nesting areas appear to coincide with major beach development (Figure 1) and include the area between New Smyrna Beach and Jacksonville Beach, FL, the Grand Strand region on the north coast of South Carolina, and the coast along the Outer Banks of North Carolina (Hopkins and Richardson 1984).

Lines of Evidence for Natal Homing

There are two hypotheses concerning the nesting behaviors of female sea turtles, the natal homing hypothesis and the social facilitation hypothesis. The natal homing hypothesis states that females return to their natal rookery with high fidelity when time comes for them to nest. These return migrations cover hundreds or even thousands of kilometers between the nesting beach and the feeding grounds (Bustard 1979, Meylan et al. 1990). On the other hand, the social facilitation model suggests that inexperienced first-time breeding females will follow mature females to their nesting beaches. If that nesting incidence is a favorable experience for the new female, she will then return to that site to lay egg clutches in the future (Meylan et al. 1990, Bowen et al. 1991). In preliminary analyses of green turtle mitochondrial DNA (mtDNA) (Bowen et al. 1991), data supported the natal homing hypothesis.

Limpus et al. (1992) cautioned that there is no test that will define a turtle's natal beach within its rookery. Adult female loggerheads from Georgia and South Carolina do not necessarily exhibit nesting fidelity to one particular barrier island, but may have a range of up to fifteen kilometers (Hopkins and Murphy 1981). Limpus (1985) reported that 98.3% of Australian loggerhead recaptures occurred at the original tagging locality. The major difference in nesting between these two regions is that Australian loggerhead nesting beaches are either small cays on the Great Barrier Reef or small, isolated pocket beaches located on the mainland. Beaches along the Southeastern United States are either barrier islands of various sizes in North Carolina, South Carolina, and Georgia, or a nearly continuous mainland shore in Florida.

Nesting Female Migrations

Meylan (1981) and Meylan et al. (1983) showed that mature, Florida-nesting loggerheads have a tendency to migrate to the Bahamas and the Caribbean. In contrast, Georgia-nesting loggerheads are only found along the Eastern coast of the United States (Bell and Richardson 1978, Richardson unpublished data). These tagged females were recaptured in waters ranging from Jacksonville, FL, to the southern coast of New Jersey. A migratory path was proposed for Georgia's nesting females that took them from the local nesting beaches, north to Cape Hatteras (paralleling the Gulf Stream), and east into the warm mid-Atlantic water of the Sargasso Sea.

Environmental markers such as heavy metal concentrations in egg yolks were tested from four nesting sites along the mid-Atlantic coast of the United States (Canaveral National Seashore, FL; Cumberland Island National Seashore, GA; Cape Lookout National Seashore, NC; and Cape Hatteras National Seashore, NC). The heavy metals (e.g., aluminum, cadmium, cobalt, copper, iron, lead, mercury, nickel, zinc, etc.) were transferred into the blood system of the turtles after they consumed benthic invertebrates that had accumulated these compounds. Heavy metals were then incorporated into the yolks of eggs laid by nesting females. The concentration variations found between the four nesting areas suggest that feeding grounds utilized by adult female loggerheads were isolated from one another (Stoneburner et al. 1980).

Epibiota has been studied on carapaces of nesting females along a latitudinal gradient from Pritchard's Island, SC, to Hutchinson Island, FL. Of the forty-eight species (six phyla) of epibionts, there were two distinct groups: a northern one and a

southern one. The division between these two populations occurs between Cape Canaveral and Daytona Beach, FL. This separation of "carapace communities" serves as an indication that there are two distinct nesting populations of loggerhead turtles along the United States Eastern seaboard (Caine 1986). These four independent data sets (Florida and Georgia tag returns, heavy metals, and carapace epibionts) tend to support the concept of feeding area segregation for nesting females.

Endangered Species Act of 1973, Management Procedures, and Concerns

The Endangered Species Act of 1973 (ESA) was designed to identify and protect threatened and/or endangered species and develop plans for their recovery. There is a provision for the creation of conservation programs to protect these species by all Federal departments (USA 1975, 16 U.S.C. §1531 subsections [b] and [c]).

Responsibility for the conservation of all sea turtle species is under direct supervision of the US Fish and Wildlife Service (United States Department of the Interior) when sea turtles are on the beach, and the National Marine Fisheries Service (United States Department of Commerce) while the turtles are in the water. Coastal states in which sea turtles are found also have a keen interest and have developed programs to address limiting factors on both the nesting beaches and coastal waters. The ESA encourages individual states to create and enforce stricter and more comprehensive conservation measures. To do this, scientific information concerning specific population status is collected. Great interest in the stock structure of Southeastern loggerheads has encouraged genetic research. To delineate stocks of sea

turtles, significant genetic variation between identified stocks must be demonstrated.

Adequately describing a natural population's genetic structure is a major objective of population genetics (Avise et al. 1986). As a natural population, the long-lived sea turtle is not amenable to standard breeding-type studies to obtain genetic information. Conventional isozyme studies of nesting females did not yield sufficient detail to determine differences in stock structure for Southeastern loggerheads (Braddon-Galloway and Inabnett 1987). Therefore a different, more informative approach was needed.

Comprehensive information about specific species' genetics is needed to help strengthen the basis for management of threatened and endangered species (Laerm 1982). The conservation of these populations present a "challenging problem for developing a responsive and functional wildlife management program" (Smith et al. 1978). Population management is an important feature of conservation programs. Management of sea turtle populations requires the determination of the geographic stocks of turtles. In instances involving illegal possession, in cases of high mortality, or collection of life history data, it is important to determine the natal origin of juvenile and adult turtles. If nesting females or juveniles are depleted or reduced at a particular locale, the dispersal rate from other regions may not be sufficiently rapid to restock the original population over a timescale that can be meaningful to wildlife management agencies (Bowen et al. 1993).

Mitochondrial DNA provides a natural tag to address the issue without relying on metal or plastic tags or the duration of artificially applied markings. This natural

has even been suggested that since mtDNA has a faster rate of evolution, the genetic differentiation may be seen with smaller sample sizes (Graves et al. 1984, Wilson et al. 1985, Avise 1986b, Avise et al. 1987). Gorman and Renzi (1979) point out, however, that the routine use of small sample sizes is not advised due to the loss of information.

Usefulness of mtDNA in Sea Turtle Rookery Studies

Bowen et al. (1993) suggested that by analyzing the maternally inherited mtDNA and determining genotype differences, it is possible to look at migratory behavior and define population structures of female loggerheads. Genotypes of hatchlings from three Southeastern United States geographic rookeries (Florida, Georgia, and South Carolina) and the rookery in Greece were defined by Bowen et al. (1993) based on mtDNA restriction fragment length polymorphism (RFLP) profiles (Table 1). This procedure utilizes mtDNA molecules that have been digested into fragments with a particular restriction endonuclease. These fragments are separated on an agarose gel according to their molecular weight which, in turn, relates to the number of nucleotide base pairs present in the fragment. This process forms banding patterns, a genotype, that may be used as a type of genetic differentiation between sea turtle rookeries. For each restriction endonuclease, there may be multiple genotypes (Table 1) depending on the composition and size of the mtDNA found in loggerheads from each geographic rookery. When the genotypes of all the restrictions endonucleases have been determined, a haplotype is assigned (Table 2). These

Research Hypothesis

The research described herein was conducted based upon the following hypothesis:

The population of juvenile loggerhead sea turtles found in the Charleston Harbor Entrance Channel will be comprised of individuals from mixed geographic rookeries due to feeding ground integration.

MATERIALS AND METHODS

Sample Collection

Juvenile loggerheads were captured in the Charleston Harbor Entrance Channel using 18m mongoose-style trawl nets (10cm mesh) pulled by a 22.9m, double-rigged, St. Augustine shrimp trawler (the R/V Lady Lisa) with bottom towing times of fifteen to twenty minutes per tow. Turtles were measured, tagged (inconel series AAC, QQH, and PPV), and blood was taken. The thirty-three turtles used in this study ranged in size from 52.0cm to 88.5cm (straight line carapace measurements) with an average length of 67.8cm.

A "turtle board" (Figure 3) was fashioned which kept the turtle at an elevated angle and restricted its movement while blood was being drawn (after Owens and Ruiz 1980). Using heavy-duty screws and 90° angle metal supports, two 2" x 4" boards (approximately 8" long) were attached (approximately one foot from the end) perpendicularly to either side of a 5' long, 2" x 12" plank. Turtles were placed on the board with their head between the two supports. The opposite end of the board was propped up at approximately 45° to ensure that the front flippers could not touch the deck whereby the turtle might gain leverage and propel off the board. This also resulted in increased blood flow to the dorsal cervical sinus. The neck was kept as fully extended as possible by gently pressing the skull against the board and the dorsal

surface of the neck was swabbed with rubbing alcohol.

The needle (16 gauge, 1½ inch sterile hypodermic) was locked onto a 60cc syringe and carefully inserted into the neck at the location of the dorsal cervical sinus (Figure 4). The sinus was located about 1cm on either side of the dorsal midline of the neck between the posterior scutes of the skull and the nuchal scute on the carapace. The needle was inserted laterally to avoid striking the vertebral column and the angle of entry was perpendicular to the dorsal surface of the neck. If the dorsal sinus was located, there was no resistance in retracting the plunger. If the sinus was missed, there was a strong vacuum and the plunger would not move. If the blood did not flow into the tube, the depth of the needle (1cm to 3cm) was adjusted while making sure the needle was kept perpendicular to the dorsal surface of the neck. If the sinus was not located, the needle was removed and the procedure repeated in a more lateral, slightly medial, position possibly on the opposite side of the neck. After the blood was collected and the needle removed, the puncture site was swabbed with rubbing alcohol.

Approximately 60cc of blood was withdrawn from twenty-nine of the netted juvenile loggerheads. Blood was divided into six 15ml plastic centrifuge tubes (10ml/tube) and spun in a Damon IEC Spinette table top centrifuge for five minutes. The serum was transferred into a second centrifuge tube and the two tubes were immediately frozen. Care was taken to leave a few millimeters of serum on top of the nucleated red blood cells (RBCs) to protect the thin layer of mitochondria-containing white blood cells (buffy coat) that formed at the cell/serum interface.

Blood was also collected from four juveniles in the CHEC by researchers from the Virginia Institute of Marine Science (VIMS). These samples were place in ACD solution B (an anticoagulent: 0.48% citric acid, 1.32% sodium citrate, 1.47% dextrose; White and Densmore 1992) in a 5:1 ratio (blood:ACD, respectively) and stored at 4°C.

Genomic DNA Isolation and Purification

The plug of frozen serum was removed from the top of the RBCs and approximately 1ml (±100µl) of sample was removed from the underside of the serum plug and from the top of the RBCs. This assured the greatest probability of obtaining a majority of the buffy coat but also substantially increased the nuclear DNA contamination. The cell/serum interfaces that formed as a result of settling in the four VIMS samples were also included in the data set.

Genomic DNA was isolated according to the procedure of White and Densmore (1992). Specifically, the samples were placed into individual 30ml Corex round-bottomed centrifuge tubes and 8ml SDS-urea, pH 6.8 (1% w/v SDS, 8M urea, 240mM Na_2HPO_4, 1mM EDTA) was added. The samples were vortexed until a homogeneous, viscous liquid was obtained and allowed to incubate thirty minutes at room temperature with light, occasional vortexing. An equal volume of freshly buffered PCI[1] (phenol:chloroform:isoamyl alcohol, 25:24:1) was added to each sample and

[1]Phenol was buffered in three separate steps using equal volumes of 1M Tris (pH 8), 0.1M Tris (pH 8), and chloroform:isoamyl alcohol (24:1) to the original volume of phenol. At each step, the mixture was centrifuged (five minutes at approximately 1100 x g) and the aqueous phase was removed after each step leaving the organic phase.

vortexed again until homogeneous. It was necessary to vigorously vortex each sample after the addition of the PCI to obtain a uniform mixture. The samples were incubated for ten minutes at room temperature with occasional light vortexing.

Each sample was weighed and balanced with SDS-urea, placed in an SA-600 rotor, and spun at 11,000 rpm (17,510 x g) at 0°C for thirty minutes. The aqueous phases were transferred into individual dialysis bags and dialyzed against TE (10mM Tris, 1mM EDTA, pH 8) for twenty-four hours (or until phenol smell disappeared). The resulting genomic DNA (5ml to 10ml) was removed from the bags and stored at 4°C.

Restriction Endonuclease Digestions

Twenty microliters of each sample were digested for three hours in a 30µl reaction of each of the following restriction endonucleases obtained from New England Biolabs (Beverly, MA): Ava II, BstN I, EcoR V, Hind III, and Stu I. After digestion, the samples were placed in a Speed-Vac (model AS290, Farmingdale, NY) and concentrated to 10µl using 3,000µ vacuum for ten minutes. Three microliters of loading dye (5X[2] TBE [450mM Tris borate, 10mM EDTA, pH 8], 0.25% bromphenol blue, 0.25% xylene cyanol, 15% ficoll) were added to each sample. Samples were then separated on a 0.8% agarose gel (SeaKem ME; FMC BioProducts, Rockland, ME) using 1X TBE (90mM Tris borate, 2mM EDTA, pH 8) running buffer. Four microliters of ethidium bromide (EtBr, 10mg/ml) were added to the gel for

[2]Use of the X implies that this solution is (X) times more concentrated than the base solution.

visualization purposes. A 1 kilobase (kb) ladder (Gibco BRL, Gaithersburg, MD) was run on the gel for standard base pair measurements. Gels were run at approximately seventy volts for sixteen hours (average 2.16 volts per cm). The bromphenol blue front migrated ahead of the xylene cyanol front at approximately 517 base pairs; therefore, the location of the bromphenol blue at the edge of the gel was used to determine the termination point for the run.

Gel Observation and Record

Each gel was rinsed in distilled water and observed under ultraviolet light (Foto/Prep I DNA UV Transilluminator; Fotodyne, New Berlin, WI) to verify that the samples had been digested. The EtBr fluorescence of the digested samples created a smear across each lane and this was used as an indicator to proceed with the Southern blot. The gel was photographed with a Polaroid MP-4 Land camera using Polaroid 667 black and white, low-light, instant photography film (f-stop 11, 5 or 6 second exposure, 3,000 ISO).

Southern Blot Procedure

Capillary action was used to transfer both nuclear and mitochondrial DNA from the gel onto Zeta-Probe GT nylon membrane (BioRad Laboratories, Melville, NY) through Southern blotting (BioRad 1991) (Figure 5). Large DNA fragments were depurinated by soaking the gel in 0.25N HCl for fifteen minutes. The HCl removes purine bases (adenines and guanines) which breaks the DNA into smaller fragments

and facilitates transfer. The gel was then denatured in a solution of 0.5M NaOH, 1M NaCl for thirty minutes to separate the double stranded DNA framents into two single strands. Finally, the gel was neutralized in 0.5M Tris-HCl, 3M NaCl (pH 7.4) for an additional thirty minutes.

Transfer buffer (10X SSC: 1.5M NaCl, 150mM sodium citrate) was placed in a large baking dish and a glass support plate was placed on top. A strip of 3MM Whatman chromatography paper (20cm x 43cm) was placed across the glass plate with the ends submerged in the buffer as a wick. Two pieces of chromatography paper (20cm x 25cm) were soaked in the buffer and laid on top of the wick. The gel was placed upside down on the paper squares (DNA is closer to the bottom of the gel and this allows an easier transfer of the DNA onto the nylon membrane) and a piece of membrane (20cm x 25cm), which had been soaked in distilled water, was placed on top of the gel. Two more pieces of chromatography paper squares were soaked and laid on top of the gel/nylon membrane setup. Care was taken at every step to remove all air bubbles by using a glass test tube to roll the surface.

The blot was covered with clear, plastic food wrap to reduce any evaporation of the buffer and the center (20cm x 25cm) over the gel was cut out. Paper towels (cut to the dimensions of the gel) were placed on top of the chromatography paper squares. It was important that the paper towels did not touch the gel to avoid a short circuit in the capillary action set up in the blot, resulting in incomplete transfer of DNA onto the membrane. All Southern blots were allowed to process overnight.

After the blot process was complete, the gel lanes and characterizing

before use, the probes were denatured at 99°C for seven minutes.

Hybridization of Southern Blot Membranes

Prehybridization of the membranes was carried out with 50ml hybridization solution (1mM EDTA; 0.25M Na_2HPO_4, pH 7.2; 7% SDS) at 65°C for five minutes in a hybridization oven (Hybridizer 700; Stratagene Cloning Systems, La Jolla, CA). Hybridization was accomplished using 50ml of the same EDTA/Na_2HPO_4/SDS solution plus the addition of approximately 20µl mtDNA probe (average $2.2x10^7$ counts per minute/µl) per membrane. This step was performed in the oven 65°C and allowed to continue overnight.

A single rinse and four washes were used after hybridization. The rinse was 2X SSC and the washes (in order) were 2X SSC/0.1% SDS, 0.5X SSC/0.1% SDS, 0.1X SSC/0.1% SDS, and 0.05X SSC/0.1% SDS. The rinse and the first three washes were carried out in the oven at 65°C for fifteen minutes each. The final wash was continued in a large plastic storage container until a Geiger counter (Series 900 mini-monitor; Research Products International Core, Mount Prospect, IL) reading of the membranes was 2 to 5 counts per second.

When washing was completed, the wet membranes were placed in individual plastic food storage bags, all air bubbles were removed, and the membranes were exposed to Kodak X-Omat AR film in Kodak X-Omatic cassettes with regular intensifying screens. Exposure times for these autoradiograms varied from 4 to 26 hours prior to film development. The membranes were then rehybridized using the

that profile and resulting genotype. Bands from individual test samples that did not match known genotype bands, and exhibited highly variable density, were considered to be ghost bands suspected to be nuclear contamination of the mtDNA preparation.

There are often several genotypes shared among a few organisms in mtDNA studies. Roff and Bentzen (1989) wrote: "Significance levels obtained from a X^2 contingency test are suspect when sample sizes are small. Traditionally this has meant that data must be combined. However, such an approach may obscure heterogeneity and hence potentially reduce the power of the statistical test." A test was developed where the data did not require pooling, therefore "the accuracy of the estimate of α...depends only on the number of randomizations of the original data set." The Roff-Bentzen test was used to determine the similarity between the CHEC loggerhead population haplotypes and the haplotypes of the Florida, Georgia, South Carolina, and Greek rookeries described by Bowen et al. (1993). This computer test generated 1,000 randomizations of the data set (in matrices) against a null hypothesis of homogeneity to obtain the expected X^2 distribution and provide a probability of heterogeneity (Roff and Bentzen 1989).

RESULTS

Blood from thirty-three juvenile loggerhead sea turtles captured in the Charleston Harbor Entrance Channel was used to examine the local juvenile population structure. The DNA was purified and RFLP patterns were obtained for each turtle using five restriction endonucleases shown to identify differences in turtles from Southeastern United States rookeries (Figures 6-10). Genotypes were determined for the turtles based on these patterns and haplotypes were identified using the same composite codes as Bowen et al. (1993) (Table 2).

Twelve individuals showed a strict Floridian/Grecian haplotype, eighteen turtles demonstrated a haplotype shared between the Florida, Georgia, and South Carolina rookeries, and three exhibited a haplotype exclusive to Georgia. Although the CHEC turtles were found to have haplotypes from all four rookeries (Greece, Florida, Georgia, and South Carolina), all thirty-three turtles shared the Atlantic B genotype when their DNA was digested with Hind III (Table 1 and Figure 9). While none of the thirty-three turtles from the CHEC showed the Atlantic A genotype, the Bowen et al. (1993) data set yielded two of 113 (1.7%) turtles with the Hind III/Atlantic A genotype. In comparison, the current data set of thirty-three would be predicted to have less than one turtle with this pattern.

The rare Georgia profile of EcoR V (Table 1 and Figure 8) was seen in three

of the thirty-three turtles. All three turtles, however, expressed a genotype that was very similar to turtles from the Florida rookeries based on the restriction endonuclease digests of Ava II, BstN I, Hind III, and Stu I profiles (Figures 6, 7, 9, and 10, respectively and Table 2).

No turtles were caught in the CHEC during the winter months when the water temperature was below 16°C. Eight of the fifty-one juvenile loggerheads that were tagged in this study were recaptured at a later date. Four of these (7.8% of the total number of juvenile turtles netted) were recaptured during the spring/summer of 1991 after being initially caught in the fall of 1990 (Van Dolah and Maier 1993).

A map was constructed (originally found in Bowen et al. 1993) that located the three Southeastern United States natal rookery haplotypes, the haplotypes of the rookery in Greece, as well as the haplotypes of the Charleston juveniles (Figure 11). Using these haplotypes in the Roff-Bentzen test for heterogenity, it was determined that the probability of the four natal rookeries (South Carolina, Georgia, Florida, and Greece) being genetically identical to the CHEC juveniles ranged from 0% to 1.5% (Table 3). In other words, there was at least a 98.5% chance that the five populations were different.

DISCUSSION

In this study of thirty-three juvenile loggerhead sea turtles, all showed the Hind III/Atlantic B genotype while no Atlantic A genotypes were found. In the work by Bowen et al. (1993), two turtles out of 113 (1.7% of the total) demonstrated the Atlantic A genotype of the Hind III digestion (including Greece). When compared with the turtles in this study, it is not surprising that there were no Atlantic A genotypes expressed since 1.7% of thirty-three equals six-tenths of a turtle.

The rare Georgia profile of the EcoR V digestion (genotype B, Table 1) was seen only in the three CHEC turtles that demonstrated the E haplotype (Table 2). In the Bowen et al. (1993) study, this genotype was seen in only one turtle from a Georgia nest and was given the notation of alpha, which indicates that the nesting female was a first-time arrival or even a stray from another rookery. In comparing haplotype E with haplotypes D (found only in Florida) and B (found mostly in Georgia and South Carolina), it can be seen that E and D share four out of five genotypes whereas E and B share only one out of five (Table 2). Based on this, loggerhead turtles expressing the E haplotype may be indicative of an ancestral Florida female and not one from the Georgia and/or South Carolina rookery system. One can speculate that the alpha female originated in Florida, her offspring hatched from a Georgia beach, and, after developing into juveniles, migrated north to feed in the

CHEC.

The four juvenile turtles that were recaptured after a known interval of at least three months absence (January, February, and March 1991), support the idea that some juvenile loggerheads exhibit fidelity to a particular feeding ground. There is currently no way to determine if 7.8% (four recaptures out of fifty-one turtles) is a typical percentage of returning juveniles but it does indicate that there are some juveniles that seem to prefer feeding in the same locality.

Florida has the only rookeries in the Southeastern United States in which the D haplotype is found, Georgia has the only recorded turtle with an E haplotype, and the B haplotype is shared between the rookeries from all three states (Figure 11). In this study, D constitutes over a third (36.4%) of the CHEC juvenile turtles (twelve of thirty-three), 9.1% are E (three of thirty-three), and the remaining 54.5% are comprised of the B haplotype (eighteen of thirty-three). Although there is evidence of mixing of Florida, Georgia, and South Carolina turtles in the CHEC, it appears that the mix is disproportionately composed of Georgia and South Carolina individuals as compared to the total percentage of nesting individuals associated with these rookeries.

When analyzing the ratios of haplotypes B, D, and E to the entire Southeastern United States rookery system (ignoring Greece) (Figure 11), it is estimated that 13% of haplotype B comes from Florida while 87% is from Georgia/South Carolina. One-hundred percent of haplotypes D and E originate in Florida and Georgia, respectively. Therefore, it can be estimated from these percentages that in the CHEC, for haplotype B, Florida contributes two turtles and Georgia/South Carolina contributes sixteen; for

adulthood. The preservation of South Carolina as a rookery depends upon the native females returning to deposit egg clutches. Conversely, if the CHEC loggerhead population can be protected against harm, which would lead to a greater number of juveniles surviving into adulthood, the South Carolina rookery has the opportunity to become stronger through increased female nesting.

The tagging data of Georgia and Florida nesting females, supported by the carapace epibiont and egg heavy metal concentration studies, suggest migratory patterns for adult females but also raises important questions regarding juvenile loggerheads. Do juvenile turtles from the Eastern seaboard rookeries return to these locations once they leave their pelagic habitats? Do they migrate into the Caribbean to feed? Do juvenile turtles from Floridian rookeries stay in the more tropical regions or do they travel northward to feed along the Georgia, South Carolina, North Carolina, and Virginia coasts? Information gathered from this study indicates that the population of juvenile turtles feeding in the CHEC is composed of individuals from all Southeastern rookeries (South Carolina, Georgia, and Florida). This information, however, is based only upon thirty-three juveniles from one locality. Migratory patterns of Southeastern juvenile loggerheads remains basically unknown. Once the genotypes and haplotypes of juveniles from feeding grounds throughout the Southeast (i.e., Virginia, Georgia, and Florida) are determined, then it will be possible to distinguish individual populations of sea turtles and their possible migratory routes.

Implications from studies such as this have important impacts on the management of sea turtle populations occurring along coastal states. If long term

investigations can demonstrate that juvenile sea turtles undergo repetitive migrations to a particular feeding area, then care must be taken to protect that local population. When populations become depleted (either naturally or through man-made situations), turtles will be unable to reproduce themselves and continue their genetic lineage.

The population structure for loggerhead rookeries has been described by Bowen et al. (1993) as shallow with a low female gene flow. This means that if a population of loggerhead sea turtles is impacted in a way that is harmful to that population, then it is unlikely that the population will recover and become revitalized through regional dispersal and recruitment. It has been demonstrated that negative changes are not compensated by natural recruitment from nearby rookery areas (Parsons 1962). Dispersal of juvenile turtles over numerous coastal feeding areas may reduce the impact of adverse circumstances on the survival of the population.

CONCLUSIONS

It can be concluded from this study that there is definite loggerhead sea turtle feeding ground integration at the Charleston Harbor Entrance Channel. The results reported indicate that the population of juvenile sea turtles in the CHEC does not exhibit a ratio of haplotypes similar to the haplotype ratios found in the individual rookeries of the Southeastern United States or Greece (Figure 11). There is a very low probability that these five populations are the same (p: 0% and 1.5%) (Table 3). This indicates that the Charleston population is not representative of any single rookery but is an assembly of juveniles recruited from natal beaches throughout the Southeast. It should be pointed out, though, that there is a disproportionate representation from the Georgia/South Carolina rookeries. The mechanism(s) of juvenile population recruitment is unknown and has not been predicted. This is due to the fact that comprehensive population models fail since they are hypothetical and have no predictive capacity (Hopkins and Richardson 1984).

It is suspected that similar results could be found in other feeding areas off the Southeastern United States due to extensive migratory patterns of the juvenile turtles and abundant feeding opportunities along the Southeastern Atlantic coast. One may predict that the feeding areas from the Chesapeake Bay through Georgia would exhibit a higher percentage of juveniles from the Georgia and South Carolina rookeries while

areas located off the Florida coast might experience a higher percentage of Florida individuals. This prediction is based on the assumption from this research that there is some degree of juvenile fidelity to feeding areas associated with natal rookeries.

This research is in accord with task number 2214 of the recovery plan for loggerhead sea turtles prepared by the US Fish and Wildlife Service and the National Marine Fisheries Service (NMFS and USFWS 1991). This task expresses the need to determine breeding population origins for juvenile turtles in United States waters. This study provides direct evidence of the natal origins for one group of juvenile loggerhead sea turtles. Application of this approach to other localized juvenile sea turtle populations will provide the opportunity to obtain a broader picture of one phase of the life history of these wide ranging animals.

LITERATURE CITED

Avise, J. C. 1986a. Mitochondrial DNA and the evolutionary genetics of higher animals. Phil. Trans. R. Soc. Lond. B312:325-342.

----. 1986b. Molecular systematics of birds. Pt. Reyes Bird Observ. 74:4-7.

----, J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: The bridge between population genetics and systematics. Ann. Rev. Ecol. Syst. 18:489-522.

Avise, J. C., G. S. Helfman, N. C. Saunders, and L. S. Hales. 1986. Mitochondrial DNA differentiation in North Atlantic eels: Population genetic consequences of an unusual life history pattern. Proc. Natl. Acad. Sci. USA. 83:4350-4354.

Avise, J. C. and W. S. Nelson. 1989. Molecular genetic relationships of the extinct dusky seaside sparrow. Sci. 243:646-648.

Ayala, F. J. 1982. Population and evolutionary genetics: A primer. Benjamin/Cummings Pub. Co., Menlo Park, CA.

Bell, R. and J. I. Richardson. 1978. An analysis of tag recoveries from loggerhead sea turtles (*Caretta caretta*) nesting on Little Cumberland Island, Georgia. Fl. Mar. Res. Pub. 33:1-66.

BioRad. 1991. Zeta-probe GT (genomic tested) blotting membranes: Instruction manual.

Birky, C. W., Jr., T. Maruyama, and P. Fuerst. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103:513-527.

Bowen, B. W., A. B. Meylan, and J. C. Avise. 1989. An odyssey of the green sea turtle: Ascension Island revisited. Proc. Natl. Acad. Sci. USA 86:573-576.

Bowen, B. W., A. B. Meylan, J. P. Ross, C. J. Limpus, G. H. Balazs, and J. C. Avise. 1991. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46:865-881.

Bowen, B. W., J. I. Richardson, A. B. Meylan, D. Margaritoulis, S. R. Hopkins-Murphy, and J. C. Avise. 1993. Population structure of loggerhead turtles (*Caretta caretta*) in the northwestern Atlantic Ocean and Mediterranean Sea. Conserv. Biol. In press.

Braddon-Galloway, S. and T. Inabnett. 1987. Analysis of genetic variance of sea turtles common in the Southeast U.S. and the Caribbean, using PAG-IEF coupled with specific enzyme staining. Pages 180-181 in Kumpf, H. E. et al. (eds.), Proceedings of the stock identification workshop, 5-7 Nov 1985, Panama City Beach, Florida. NOAA Tech. Memo. NMFS-SEFC-199.

Bustard, H. R. 1979. Population dynamics of sea turtles. Pages 523-540 in Harless, M. and H. M. Morlock (eds.), Turtles: Perspectives and research. Wiley Interscience, New York.

Butler, R. W., W. A. Nelson, and T. A. Henwood. 1987. A trawl survey method for estimating loggerhead turtle, *Caretta caretta*, abundance in five Eastern Florida channels and inlets. Fish. Bull. 85(3):447-453.

Caine, E. A. 1986. Carapace epibionts of nesting loggerhead sea turtles: Atlantic coast of U.S.A. J. Exp. Mar. Biol. Ecol. 95:15-26.

Cann, R. L., M. Stoneking, and A. C. Wilson. 1987. Mitochondrial DNA and human evolution. Nature 325:31-36.

Carr, A. 1986. Rips, FADS, and little loggerheads. Biosci. 36(2):92-100.

Gorman, G. C. and J. Renzi, Jr. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: Effects of sample size. Copeia 1979(2):242-249.

Graves, J. E., S. D. Ferris, and A. E. Dizon. 1984. Close genetic similarity of Atlantic and Pacific skipjack tuna (*Katsuwonus pelamis*) demonstrated with restriction endonuclease analysis of mitochondrial DNA. Mar. Biol. 79:315-319.

Hopkins, S. R. and T. M. Murphy. 1981. Reproductive ecology of *Caretta caretta* in South Carolina. Study completion report. October 1, 1976 to September 30, 1980. E-1, Study No. VI-A-1. South Carolina Wildlife and Marine Resources Department.

Hopkins, S. R. and J. I. Richardson (eds.). 1984. Recovery plan for marine turtles: Prepared by the marine turtle recovery team. U.S. Government Printing Office, Washington, D.C.

Laerm, J. 1982. Genetic determination of the status of an endangered species of pocket gopher in Georgia. J. Wildl. Manage. 46(2):513-518.

Limpus, C. J. 1985. A study of the loggerhead sea turtle, *Caretta caretta*, in Eastern Australia. Ph.D. dissertation, Univ. of Queensland, St. Lucia, Australia.

----, J. D. Miller, C. J. Parmenter, D. Reimer, N. McLachlan, and R. Webb. 1992. Migration of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles to and from Eastern Australian rookeries. Wildl. Res. 19:347-358.

Linnaeus, C. 1758. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Ed. 10, Tomus 1. L. Salvii, Stockholm, Sweden, 823 pp.

Meylan, A. B. 1981. Sea turtle migration - evidence from tag returns. Pages 91-100 in Bjorndal, K. A. (ed.), Biology and conservation of sea turtles. Smithsonian Inst. Press, Washington D.C.

----, K. A. Bjorndal, and B. J. Turner. 1983. Sea turtle nesting at Melbourne Beach, Florida II. Post-nesting movements of *Caretta caretta*. Biol. Cons. 26:79-90.

Meylan, A. B., B. W. Bowen, and J. C. Avise. 1990. A genetic test of the natal homing versus social facilitation models for green turtle migration. Sci. 248:724-730.

National Marine Fisheries Service and U.S. Fish and Wildlife Service. 1991. Recovery plan for U.S. population of loggerhead turtle. National Marine Fisheries Service, Washington D.C.

Neshyba, S. 1987. Oceanography: Perspectives on a fluid earth. John Wiley and Sons, Inc., New York.

Owens, D. W. and G. J. Ruiz. 1980. New methods of obtaining blood and cerebrospinal fluid from marine turtles. Herpetologica 36(1):17-20.

Parsons, J. 1962. The green turtle and man. Univ. of Florida Press, Gainsville.

Roff, D. A. and P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: X^2 and the problem of small samples. Mol. Biol. Evol. 6(5):539-545.

Slatkin, M. and W. P. Maddison. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. Genetics 123:603-613.

Smith, M. H., H. O. Hillestad, M. N. Manlove, D. O. Straney, and J. M. Dean. 1978. Management implications of genetic variability in loggerhead and green sea turtles. 13th Congress of Game Biologists, p. 302-312.

Stoneburner, D. L., M. N. Nicora, and E. R. Blood. 1980. Heavy metals in loggerhead sea turtle eggs (*Caretta caretta*): Evidence to support the hypothesis that demes exist in the western Atlantic population. J. Herp. 14(2):171-175.

Stryer, L. 1988. Biochemistry, 3rd edition. W. H. Freeman and Co., New York.

United States of America. 1975. A compilation of Federal Laws relating to conservation and development of our Nation's fish and wildlife resources, environmental quality, and oceanography. U.S. Government Printing Office, Washington, D.C.

Van Dolah, R. F. and P. P. Maier. 1993. The distribution of loggerhead turtles (*Caretta caretta*) in the entrance channel of Charleston Harbor, South Carolina, U.S.A. J. Coastal Res. In press.

Wallace, D. C. 1986. Mitochondrial genes and disease. Hosp. Pract. 21(10):77-92.

Watson, J. D., N. H. Hopkins, J. W. Roberts, J. A. Steitz, and A. M. Weiner. 1987. Molecular biology of the gene, 4th edition. Benjamin/Cummings Pub. Co., Inc., Menlo Park, CA.

White, P. S. and L. D. Densmore III. 1992. Mitochondrial DNA isolation. Pages 29-58 in Hoelzel, A. R. (ed.), Molecular genetic analysis of populations: A practical approach. IRL Press, Oxford.

Wilson, A. C., R. L. Cann, S. M. Carr, M. George, U. B. Gyllensten, K. M. Helm-Bychowski, R. G. Higuchi, S. R. Palumbi, E. M. Prager, R. D. Sage, and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. 26:375-400.

Ava II		BstN I			EcoR V		Hind III		Stu I	
A	*D*	*B*	*C*	*D*	*B*	*C*	*A*	*B*	*B*	*C*
Florida, Greece	Georgia	Oman, Brazil	Georgia, Florida	Florida, Greece	Georgia (rare)	global	Atlantic	Atlantic, Greece	Georgia	Florida, Greece
7205		7003	7003	7003	10386		7003		3593[1]	3593[1]
6249		4875				7003		5286[1]	3191	3191
	5433		3118[1]			5743[1]	3867	3867	2294	2294
	4747			2070	3772	3772	2978	2978	2201	
	1874		1670	1670			1639	1639	1550	1550
1609	1609	1390	1390	1390			1579	1579	1522	1522
1390		1342	1342	1342				1550		1188
858	858	1168	1168	1168						959
832	832			914					943	943
	795-716[1]	637	637	637					610	610
	675									
	628									

[1] This is a size variable region.

Table 1. Restriction fragment length polymorphism base pair values and resulting genotypes of restriction endonucleases used (after Bowen et al. 1993).

GENOTYPE[1]	HAPLOTYPE[2]	FLORIDA[2]	GEORGIA[2]	SOUTH CAROLINA[2]	GREECE[2]	CHEC
DCCAB	A	0	2	0	0	0
DCCBB	B	9	41	19	0	18
DBCBB	C	1	0	0	0	0
ADCBC	D	19	0	0	21	12
ADBBC	E	0	1	0	0	3

[1] Genotype letters refer to RFLP profile designations (Table 1) for the following restriction endonucleases (in order): Ava II, BstN I, EcoR V, Hind III, and Stu I.

[2] Composite haplotypes and rookery information from Bowen et al. (1993).

Table 2. Mitochondrial DNA genotypes and composite haplotypes found in turtles from the rookeries of the Southeastern United States and Greece. Juvenile turtles from the CHEC are included for comparison. Numbers correspond to individuals per haplotype per locality.

	Greece	Florida	Georgia	South Carolina	CHEC
Greece	X	0.002	0.0	0.0	0.0
Florida		X	0.0	0.0	0.015
Georgia			X	0.367	0.0
South Carolina				X	0.0
CHEC					X

Table 3. Roff-Bentzen test for heterogeneity. Values are the probability that the populations in comparison are the same. Numbers of individuals from localities in Table 2 were used in value determinations.

Figure 1.

Nesting areas in the Southeastern United States (Hopkins-Murphy pers. comm.).

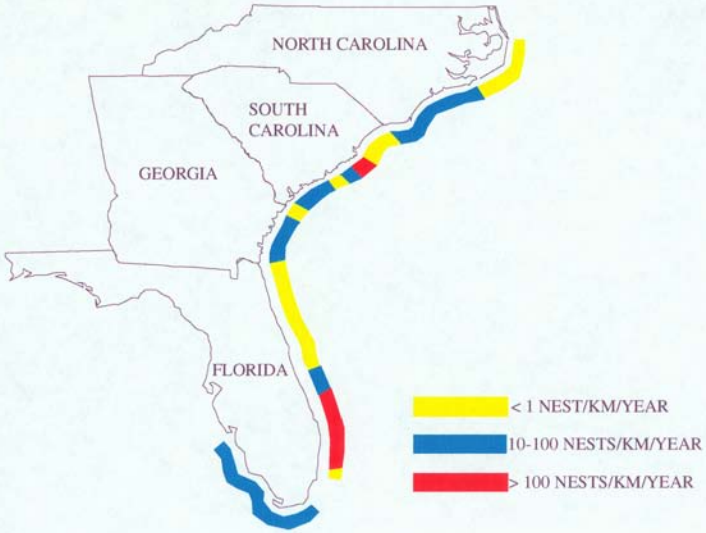

NORTH CAROLINA
SOUTH CAROLINA
GEORGIA
FLORIDA
< 1 NEST/KM/YEAR
10-100 NESTS/KM/YEAR
> 100 NESTS/KM/YEAR

Figure 2.

Double stranded, circular mitochondrial DNA, indicating genes coding for specific proteins or nucleic acids (Wallace 1986, Watson et al. 1987, Stryer 1988).

NADH dehydrogenase - These genes code for various subunits of nicotinamide adenine dinucleotide. This coenzyme is important in the cellular regulation of free energy.

Cytochrome B - This codes for the cytochrome b subunit of cytochrome reductase. This protein acts as a bridge between cytochrome c (see below) and ferrous sulfate molecules in the proton pump of the inner mitochondrial membrane.

ATP synthase - This complex is responsible for the genes that code for the transformation of ADP into ATP.

Ribosomal RNA - This area carries the genes needed to create the 16S and 12S ribosomal subunits.

Transfer RNA - These multiple gene sites carry the DNA used to make tRNA molecules.

Cytochrome C - The water-soluble proteins (cytochrome c) of cytochrome reductase are coded here. This functions in pumping protons across the inner mitochondrial membrane.

Non-coding regions - These areas do not code for cellular products. Whenever a gene is located on either the heavy or light strand, one of these non-coding spaces occur on the corresponding area of the other strand.

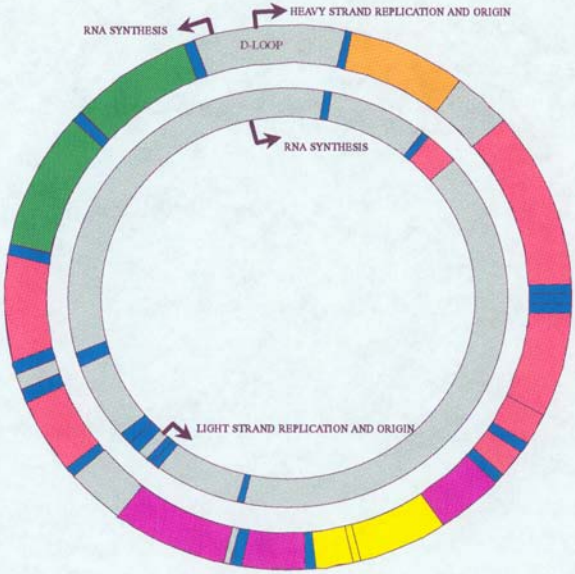

RNA SYNTHESIS
HEAVY STRAND REPLICATION AND ORIGIN
D-LOOP
RNA SYNTHESIS
LIGHT STRAND REPLICATION AND ORIGIN

Figure 3.

Turtle board in propped position showing shoulder supports. This is in relative size to an average juvenile loggerhead.

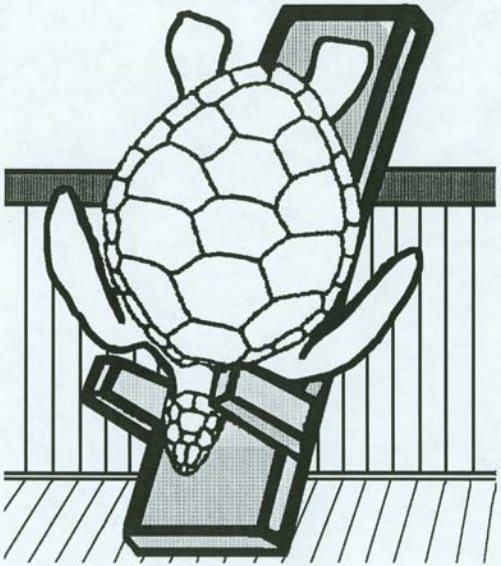


Figure 4.

Location diagram of the dorsal cervical sinus of a sea turtle, the dorsal midline, and point of needle insertion.

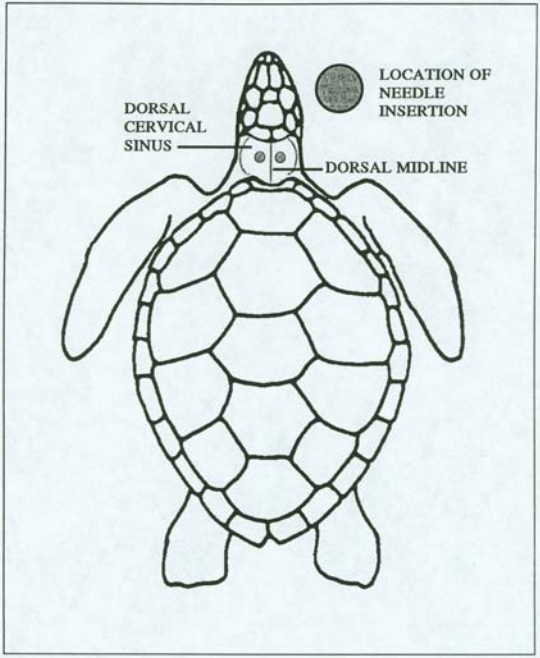
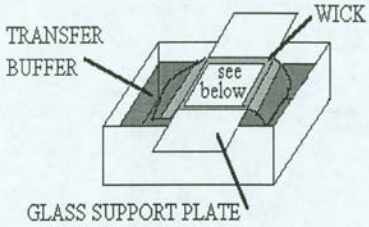

LOCATION OF
NEEDLE
INSERTION
DORSAL
CERVICAL
SINUS
DORSAL MIDLINE

Figure 5.

The arrangement of a Southern blot.


WICK
TRANSFER
BUFFER
see
below
GLASS SUPPORT PLATE

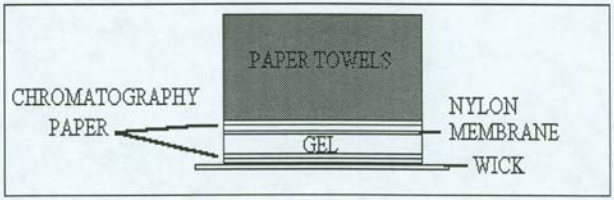

PAPER TOWELS
CHROMATOGRAPHY
PAPER
NYLON
MEMBRANE
GEL
WICK

Figure 6.

Ava II RFLP indicating patterns and designated genotypes determined by Bowen et al. (1993). Three bands in genotype D are not resolved in this autoradiogram and are not shown in the profile diagram (approximately 755, 675 and 628 basepairs - Table 1). The right hand column denotes molecular base pair fragment standards by kilobases. Gel lanes, in order from the left to right, are as follows:

#1 - QQR 143/144	#13 - QQH 521	#25 - QQH 581
#2 - AAC 299	#14 - QQH 522	#26 - QQH 583
#3 - QQH 509	#15 - QQH 557	#27 - QQH 586
#4 - QQH 510	#16 - QQH 559	#28 - PPV 895
#5 - QQH 511	#17 - QQH 565	#29 - PPV 897
#6 - QQH 512	#18 - QQH 566	#30 - PPV 898
#7 - QQH 513	#19 - QQH 567	#31 - standards
#8 - QQH 516	#20 - QQH 570	#32 - QQZ 317/318
#9 - QQH 517	#21 - QQH 571	#33 - QQZ 321/322
#10 - QQH 518	#22 - QQH 573	#34 - QQZ 325/326
#11 - QQH 519	#23 - QQH 574	#35 - QQZ 327/328
#12 - QQH 520	#24 - QQH 575	#36 - QQZ 329/330

Individuals with tags AAC, PPV, QQH, and QQR were obtained from the VanDolah and Maier (1993) study. Tags identified by QQZ were from individuals captured by VIMS researchers. QQH 516 and QQZ 321/322 were adult turtles and not used in this study.

Ava II

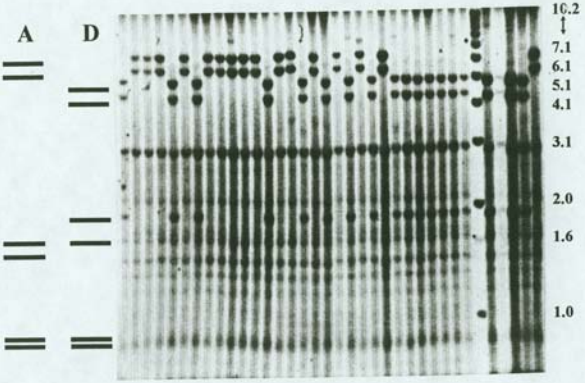


Figure 7.

BstN I RFLP with genotypes, molecular standards, and gel lanes as described in Figure 6. The 637 base pair band (Table 1) common to all three genotypes is not shown in this autoradiogram. The 3.1 kb band of genotype C is a size variable region due to differing lengths in the D-loop portion of mtDNA (Figure 2). Bands that have a dot are part of the RFLP pattern while the others are ghost bands (see text page 20). The location of molecular standards was ascertained by visual comparisons using gel photographs.

BstN I

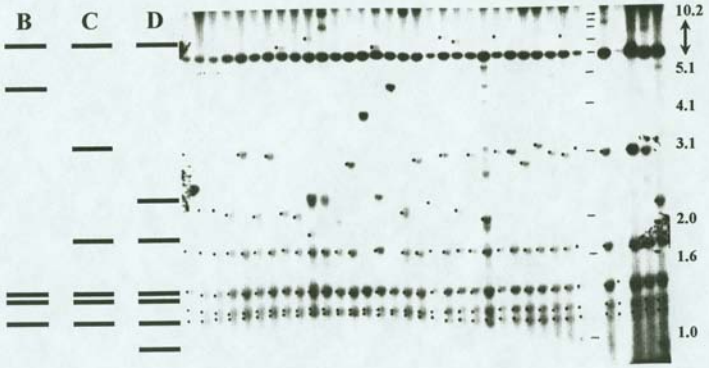


Figure 8.

EcoR V RFLP with genotypes, molecular standards, and gel lanes as described in Figure 6. The 5.7 kb band of genotype C is a size variable (see Figure 7).

ADDENDUM: Genotype D should read as genotype B (see Table 1).

EcoR V

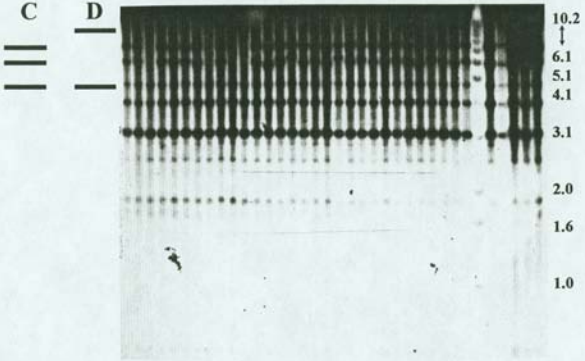


Figure 9.

Hind III RFLP with genotypes, molecular standards, and gel lanes as described in Figure 6. The bands occurring around 5.3 kb are size variables (see Figure 7). The asterisk denotes a band location in the genotype B profile that was not resolved in this autoradiogram.

Hind III

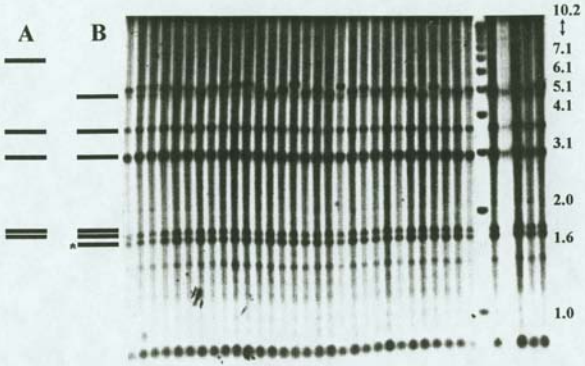


Figure 10.

Stu I RFLP with genotypes, molecular standards, and gel lanes as described in Figure 6. There is a size variable region around 3.6 kb (see Figure 7) in both genotypes.

Stu I

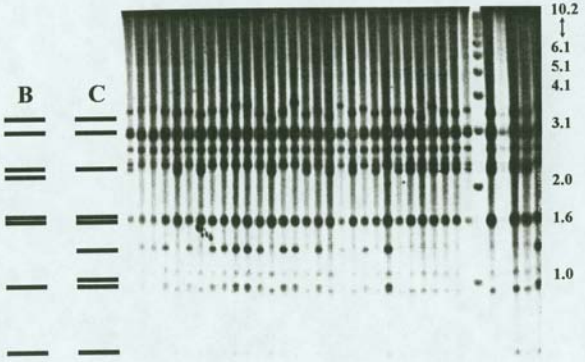
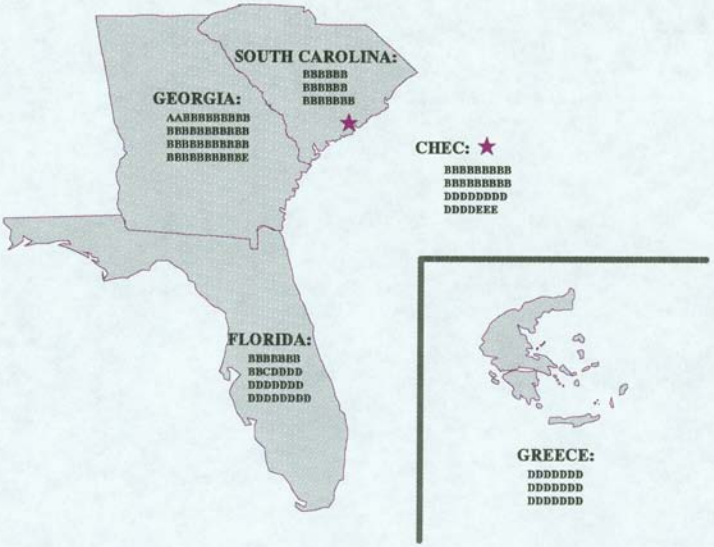


Figure 11.

Southeastern United States map showing rookery and CHEC locations with haplotypes of associated populations (Greece is shown for reference). South Carolina, Georgia, Florida, and Greece haplotypes are from Bowen et al. (1993). Haplotype ratios for each locality are as follows:

South Carolina:	0.00 A:	1.00 B:	0.00 C:	0.00 D:	0.00E
Georgia:	0.05 A:	0.93 B:	0.00 C:	0.00 D:	0.02 E
Florida:	0.00 A:	0.31 B:	0.03 C:	0.66 D:	0.00 E
Greece:	0.00 A:	0.00 B:	0.00 C:	1.00 D:	0.00 E
CHEC:	0.00 A:	0.55 B:	0.00 C:	0.36 D:	0.09 E


SOUTH CAROLINA:
BBBBBB
BBBBBB
BBBBBBB
GEORGIA:
AABBBBBBBBB
BBBBBBBBBBB
BBBBBBBBBBB
BBBBBBBBBBE
CHEC:
BBBBBBBBB
BBBBBBBBB
DDDDDDDD
DDDDEEE
FLORIDA:
BBBEBBB
BBCDDDD
DDDDDDD
DDDDDDDD
GREECE:
DDDDDDD
DDDDDDD
DDDDDDD