Population Structure of Loggerhead Turtles (*Caretta caretta*) in the Northwestern Atlantic Ocean and Mediterranean Sea

BRIAN BOWEN*
JOHN C. AVISE
Department of Genetics
University of Georgia
Athens, GA 30602, U.S.A.

JAMES I. RICHARDSON
Institute of Ecology
University of Georgia
Athens, GA 30602, U.S.A.

ANNE B. MEYLAN
Florida Marine Research Institute
100 Eighth Avenue, S.E.
St. Petersburg, FL 33701, U.S.A.

DIMITRIS MARGARITOULIS
Sea Turtle Protection Society of Greece
Solomou 35, GR-106 82
Athens, Greece

SALLY R. HOPKINS-MURPHY
South Carolina Wildlife and Marine Resources Department
P.O. Box 12559
Charleston, SC 29422, U.S.A.

Abstract: *To assess population genetic structure and evolutionary relationships among nesting populations of loggerhead turtles (*Caretta caretta*), we analyzed mitochondrial (mt) DNA variation in 113 samples from four nesting beaches in the northwestern Atlantic Ocean and from one*

* *Current address: BEECS Genetic Analysis Core, P. O. Box 110699, University of Florida, Gainesville, FL 32611, U.S.A.*
Paper submitted November 5, 1992; revised manuscript accepted July 7, 1993.

Conservation Biology
Volume 7, No. 4, December 1993

Estructura poblacional de la tortuga caguama (*Caretta caretta*) en el océano Atlántico noroeste y el Mar Mediterraneo

Resumen: *Con el propósito de evaluar la estructura genética de las poblaciones y las relaciones evolutivas entre poblaciones anidadoras de la tortuga caguama (*Caretta caretta*), analizamos la variación en el ADN mitocondrial (ADNmt) de 113 muestras provenientes de cuatro playas de anidación en el Océano Atlántico noroccidental y de una en el Mar Mediterráneo. Las diferencias significativas en la frecuencia de haplotipos entre poblaciones anidadoras en Flo-*

nesting beach in the Mediterranean Sea. Significant differences in haplotype frequency between nesting populations in Florida and in Georgia/South Carolina, and between both of these assemblages and the Mediterranean nesting colony, indicate substantial restrictions on contemporary gene flow between regional populations, and therefore a strong tendency for natal homing by females. Nonetheless, this regional genetic structure appears shallow, indicating recent evolutionary connections among rookeries. Data from tag returns and mtDNA, as well as geological considerations, suggest that over short evolutionary time scales (perhaps a few thousand years), dispersal by female loggerheads is sufficient to allow colonization of appropriate habitat in proximity to established rookeries but is too low to significantly affect the population dynamics of rookeries on a contemporary time scale. These data indicate that nesting populations of the loggerhead turtle must be managed as demographically independent units. The population subdivisions based on mtDNA analyses are concordant with previously reported distinctions between Florida and Georgia/South Carolina nesting populations based on environmental markers, tag recaptures, and morphology.

ruda y en Georgia/Carolina del Sur, y entre estos dos grupos y la colonia anidadora en el Mediterráneo, indican limitaciones substanciales en el flujo genético contemporáneo entre poblaciones regionales y, por lo tanto, una tendencia de las hembras a anidar en sus playas de nacimiento ("natal homing"). A pesar de lo anterior, dado que las distancias genéticas son pequeñas se deducen conecciones evolutivas recientes entre colonias anidadoras. Los resultados de marcaje y del ADNmt, además de consideraciones geológicas, sugieren que a lo largo de escalas evolutivas pequeñas (quizá unos cuantos miles de años), la dispersión de caguamas hembras es suficiente para permitir la colonización de hábitats apropiados en la proximidad de playas de anidación establecidas pero es demasiado leve para afectar significativamente la dinámica de poblaciones de las colonias anidadoras en una escala de tiempo contemporánea. Nuestra información indica que las poblaciones anidadoras de la tortuga caguama deben ser manejadas como unidades demográficas independientes. La subdivisión poblacional basada en los análisis del ADNmt concuerdan con las distinciones ya reportadas entre las poblaciones anidadoras en Florida y Georgia/Carolina del Sur basadas en marcadores ambientales, recapturas de tortugas marcadas y morfología.

Introduction

The loggerhead turtle, *Caretta caretta*, is distributed widely in warm temperate and subtropical oceans. At intervals averaging two to three years, adult loggerheads depart from the foraging grounds on reproductive migrations that range in distance from a few to thousands of kilometers (Meylan 1982; Margaritoulis 1988*a*). Tagging data indicate that most females return faithfully to the same nesting beach, and both sexes return to the same foraging areas between reproductive migrations (Limpus et al. 1992). Loggerhead hatchlings leave the nesting beach to occupy oceanic current systems (such as the North Atlantic gyre), where they drift passively for five years or more before recruiting to coastal neritic zones (Carr 1986). Subadults may occupy coastal feeding grounds for a decade or more before their first reproductive migration (Carr 1987). Estimates of the age at maturity for western Atlantic loggerheads range from 12 to 30 years (Frazer & Ehrhart 1985; Zug et al. 1986; Klinger & Musick 1992).

In recent reports, mtDNA analyses have proven useful for defining demographic and evolutionary units among the marine turtles *Chelonia mydas* (green turtle) and *Lepidochelys* spp. (ridley turtles) (Bowen et al. 1991, 1992). The maternal inheritance of mtDNA lends this approach a special significance in evaluating aspects of marine turtle life history, including the possibility of natal homing by females (Meylan et al. 1990). Tag-recapture experiments indicate that female loggerheads typically return to the same nesting area (Bjorndal et al. 1983), but it is unclear whether this site fidelity is a product of natal homing behavior. In principle, a variety of mechanisms could explain female nest-site fidelity, including imprinting of hatchlings, genetic programming, or social interaction (see Owens et al. 1982). Notably, social interaction could account for nest-site fidelity without invoking natal homing; first-time nesters may follow experienced females from feeding grounds to suitable nesting locations, and then focus on that location for subsequent nesting efforts. Under this "social facilitation" scenario, recruitment to non-natal rookeries would provide an avenue of female-mediated gene flow between rookery populations that have overlapping feeding grounds. Alternatively, under a "natal homing" scenario, female-mediated gene flow between rookeries would be restricted or absent. Thus, the contrasting models of "social facilitation" versus "natal homing" generate distinct predictions about the distribution of mtDNA lineages among nesting assemblages.

Based on significant differences in the concentrations of heavy metals in eggs (Stoneburner et al. 1980) and in the composition of epibiota on adult females (Caine 1986), loggerhead nesting populations in the southeastern U.S. may be divided into at least two demographic units corresponding to Florida and Georgia/South Carolina. However, metal concentrations and epibiota are acquired through prolonged contact with a particular environment, and therefore are a product of the habitats utilized by females during non-nesting intervals. Thus, these "acquired" markers reflect differences in feeding areas or migration pathways but leave open the question of whether Georgia/South Carolina and Florida rookeries are genetically distinct. Here we seek to add an evolutionary perspective to the understanding of loggerhead population structure in the southeastern U.S. through analyses of innate, genetically inherited markers.

A second concern addressed in this study is the population structure of Mediterranean loggerhead turtles. It has been suggested that feeding grounds in the western Mediterranean contain more juveniles than could be generated by Mediterranean rookeries alone (Argano & Baldari 1983; Laurent 1990*a*; 1990*b*). Several researchers have speculated that North Atlantic turtles may enter the Mediterranean system via currents of the North Atlantic gyre (Carr 1987; Laurent 1990*b*), become trapped in the Mediterranean Basin by strong currents at the Straits of Gibraltar (Carr 1987), and eventually recruit to Mediterranean nesting colonies (Groombridge 1988). If Atlantic loggerheads recruit at high frequency to Mediterranean rookeries, this fact should be reflected in the genetic composition of western Atlantic and Mediterranean nesting populations.

Thus, this study was designed to assess the genetic and demographic integrity of nesting populations both within one region (the northwestern Atlantic), and among rookeries on a geographic scale consistent with known loggerhead migrations (the North Atlantic Ocean and Mediterranean Sea). Given the magnitude of loggerhead movements, both micro- and macrogeographic scales are relevant to loggerhead conservation. As noted by Limpus et al. (1992), "no one country controls the fate of a given turtle population."

Materials and Methods

Between 1987 and 1991, 113 nests were sampled from: (1) Kiparissia Bay, western Peloponnesus, Greece (n = 21); (2) Cape Romain, South Carolina, U.S.A. (n = 19); (3) Cumberland Island and Little Cumberland Island, Georgia, U.S.A. (n = 44); (4) Broward County and St. Lucie County, eastern Florida, U.S.A. (n = 15); and (5) Key Island (Collier County), western Florida, U.S.A. (n = 14).

Nests were sampled for two eggs or for one hatchling during one typical internesting interval (12–14 days) to assure that the same female was not resampled. Two eggs were taken to offset mortality during transportation, because loggerhead eggs are very sensitive to motion during the first few weeks of development (Limpus et al. 1979). Eggs were incubated for six to eight weeks prior to processing, and hatchlings were sacrificed at appropriate lab facilities. Because siblings are expected to be identical with respect to mtDNA genotype, sample sizes refer to the number of nests sampled.

Closed-circular mtDNA was isolated from soft tissues (hatchlings) or whole embryos (eggs) by CsCl-ethidium bromide density gradient centrifugation (Lansman et al. 1981). Purified mtDNAs were digested with the 17 informative four-, five- and six-base cutting restriction enzymes listed in Table 1. In addition, representative samples were digested with *Bam*HI, *Cla*I, *Eco*RI, *Kpn*I, *Nsi*I, *Sac*I, *Sal*I, and *Sma*I, but these enzymes proved to be phylogenetically uninformative, producing either one or no cuts in loggerhead samples. Digestion fragments were end-labelled with ^{35}S nucleotides and separated on 1.0–1.7% agarose gels. Restriction fragments were visualized by autoradiography and assigned molecular weights on the basis of comparison to a 1-kb ladder standard.

Estimates of nucleotide sequence divergence (p values) were calculated by the "site" approach of Nei and Li (1979); haplotype and nucleotide diversities were estimated by the methods described by Nei and Tajima (1981) and Nei and Li (1979), respectively. Restriction fragment profiles were described using composite letter codes and were joined into a parsimony network that interrelates observed restriction fragment patterns.

Because we are interested in genetic relationships among turtles from particular pairs of nesting beaches (such as those that may occupy the same feeding grounds), some of the analyses described below include pairwise rookery comparisons (although results of multiple pairwise comparisons are not statistically independent). Pairs of rookeries were tested for significant differences in haplotype frequency by the G test, with Yates's correction for small sample size (Sokal & Rohlf 1981). Pairwise estimates of inter-rookery gene flow (Nm) were also calculated from G_{ST} values ($Nm = 1/2a(1/G_{ST} - 1)$), where $a = (L/L - 1)^2$ and L is the number

Table 1. Description and distribution of mtDNA genotypes observed in loggerhead turtles.*

Composite Code	*mtDNA Genotype*	*Rookery Location*	*Number of Nests*
A	*DCCCCCCCCACCCCCBC*	Georgia, U.S.A.	2
B	*DCCCCCCCCBCCCCCBC*	South Carolina, U.S.A.	19
		Georgia, U.S.A.	41
		East Florida, U.S.A.	4
		West Florida, U.S.A.	5
C	*DCCCCBCCCBCCCCCBC*	East Florida, U.S.A.	1
D	*ACCCCDCCCBBCCCCCC*	East Florida, U.S.A.	10
		West Florida, U.S.A.	9
E	*ACCCCDCBCBBCCCCCC*	Kiparissia, Greece	21
		Georgia, U.S.A.	1

** Italicized letters refer to mtDNA restriction-fragment profiles produced by (from left to right): AvaII, BclI, BglI, BglII, BstEII, BstNI, DraII, EcoRV, HindIII, HindIII, MspI, NdeI, PvuII, SpeI, SstII, StuI, and XbaI. For each enzyme, adjacent letters in the alphabet indicate that fragment profiles differ by a single restriction-site gain or loss; nonadjacent letters differ by at least two sites.*

of demes (Takahata & Palumbi 1985; Nei 1987). Calculations were conducted under assumptions of large I_i (hence $a \approx 1$) rather than small I_i (hence $a \approx 4$), providing a more conservative evaluation of population structure in the sense that the resulting Nm values are four-fold higher. Finally, an estimate of mean migration rate (Nm) among rookeries was calculated by the private-allele method (Slatkin 1985), using the equation in Slatkin and Barton (1989).

Results

Five genotypes were observed among the 113 loggerhead nests sampled (Table 1), with a mean of 82 restriction sites scored per individual. Digestion profiles are available from the senior author upon request. All restriction site changes could be explained by specifiable gains or losses of particular restriction sites.

Turtle species tend to exhibit relatively low levels of genetic variation and differentiation (Avise et al. 1992; Karl et al. 1992; but see Scribner et al. 1986), and previous studies indicate that loggerhead turtles have comparatively low genetic diversity at protein electrophoretic loci (Smith et al. 1978; Gyuris & Limpus 1988). The current study extends this qualitative conclusion to loggerhead mtDNA: overall haplotypic and nucleotide diversities in surveyed loggerheads were 0.505 and 0.0018, respectively (Table 2). These estimates, and the levels of sequence divergence among all observed haplotypes (Table 3), are at the low end of the spectrum of such values reported for comparisons of conspecific vertebrates (Avise et al. 1987, 1989).

The size of the loggerhead mtDNA molecule was estimated to be 16.6 kilobases (kb). Variation in mtDNA genome size, however, was revealed by many of the restriction enzyme fragment profiles. Discrete size classes differed by roughly 80–100 bases and spanned a total range of about 0.4 kb in a single region of the mtDNA molecule. Because the evolutionary dynamics of these size polymorphisms are uncertain, and because size polymorphisms were distributed more or less continuously across our assayed locations, these variants were excluded from population analyses. All subsequent discussion will concern restriction-site changes only.

Table 2. Loggerhead turtle haplotype and nucleotide diversities (Nei & Tajima 1981; Nei & Li 1979).

	Haplotype Diversity (h)	*Nucleotide Diversity (π)*
Greece	0.000	0.0000
South Carolina	0.000	0.0000
Georgia	0.132	0.0002
East Florida	0.514	0.0018
West Florida	0.495	0.0018
Overall	0.505	0.0018

Table 3. Estimates of sequence divergence between the mtDNA clones described in Table 1, based on the site approach of Nei and Li (1979).

mtDNA clone	A	B	C	D	E
A	——	0.0010	0.0023	0.0089	0.0100
B		——	0.0012	0.0078	0.0089
C			——	0.0092	0.0103
D				——	0.0010
E					——

Two distinct groups of haplotypes were observed in the mtDNA phylogeny (*A, B, C* versus *D, E* haplotypes in Figure 1), and these groups differ at a mean level of sequence divergence $p = 0.008$. This magnitude of divergence is similar to the deepest fork observed in a global phylogeny for green turtle mtDNA ($p = 0.007$), which partitioned Atlantic-Mediterranean from Indian-Pacific phylads (Bowen et al. 1992). In contrast, both major groupings in the loggerhead mtDNA phylogeny are observed within the Atlantic Ocean and co-occur in the Florida and Georgia nesting populations.

To some extent, the low level of genetic variation observed in this study impairs our ability to resolve regional population issues. Rookery samples are dominated by two mtDNA genotypes (*B* and *D* in Table 1 and Figure 2). Nevertheless, the distribution of these genotypes has a strong geographic component. Genotype *D*,

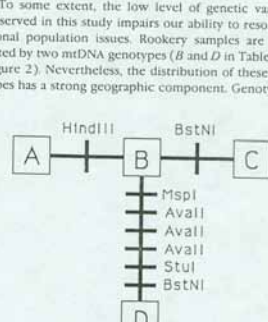

Figure 1. Parsimony network summarizing the interrelationships among mtDNA haplotypes observed in the survey of northwestern Atlantic and Mediterranean loggerhead rookeries. Letters refer to the composite genotypes described in Table 1. Each dash corresponds to a single restriction-site gain or loss, with the corresponding enzyme indicated above or to the left of the dash.

observed in 67% of eastern Florida samples (n = 15) and 64% of western Florida samples (n = 14), is completely absent from the Georgia (n = 44) and South Carolina (n = 19) collections (Fig. 2). Genotype frequencies differed significantly in eight of ten pairwise rookery comparisons (Table 4). Notably, the cases where genotype frequencies were not significantly different involved proximal nesting beaches: (1) eastern Florida and western Florida; and (2) Georgia and South Carolina. We conclude that loggerhead rookeries of the southeastern U.S. comprise at least two genetic populations, between which contemporary gene flow is low (Table 4).

The Mediterranean sample was fixed for one mtDNA genotype (haplotype *D* in Table 1) that was observed at 64–67% frequency in Florida samples and absent in

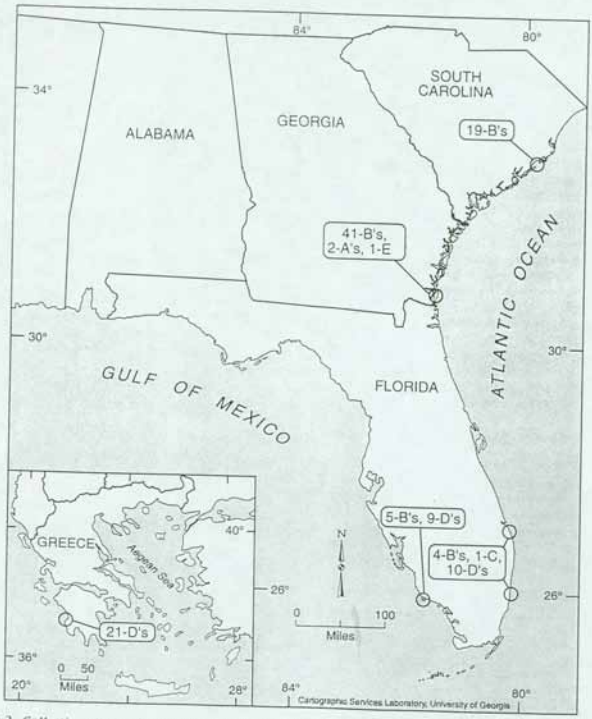

Figure 2. Collection sites and genotypes observed at each of five Atlantic and Mediterranean rookeries of the loggerhead turtle. Each letter refers to the composite mtDNA genotype of an individual turtle (Table 1).

Table 4. Pairwise comparisons of haplotype frequencies among nesting beaches with a G-test of independence* (above diagonal) and Nm values based on the G_{st} estimates (below diagonal).**

	I	II	III	IV	V
I. South Carolina	—	0.3	19.9	13.5	60.0
		N.S.	$P < 0.001$	$P < 0.001$	$P < 0.001$
II. Georgia	1.1	—	13.0	25.8	7.6
			$P < 0.001$	$P < 0.001$	$P < 0.001$
III. East Florida	0.8	3.3	—	0.1	6.0
				N.S.	$P < 0.02$
IV. West Florida	0.8	3.3	high	—	6.4
					$P < 0.02$
V. Greece	0.0	0.3	1.3	1.2	—

* Using Yates's correction (Sokal & Rohlf 1981).

** Takahata & Palumbi 1985.

Georgia and South Carolina. Pairwise comparisons of the Mediterranean versus West Atlantic rookeries yielded significant G values and low estimates of inter-rookery migration (ranging from $Nm = 0.0$ to $Nm = 1.3$ migrants per generation [Table 4]). Thus, the Mediterranean rookery differs significantly in genetic composition from nesting beaches in the northwestern Atlantic.

Based on the private allele method (Slatkin 1985), a mean value of $Nm = 2.0$ is estimated for the assayed loggerhead nesting populations. This value is qualitatively similar to values derived from the G_{st} approach (Table 4). In general, values of Nm greater than about one to four indicate that gene flow is sufficient to maintain a relatively homogeneous gene pool, whereas lower values indicate that gene flow is not sufficient to prevent divergence of isolated gene pools by genetic drift (Birky et al. 1983; Slatkin 1987). Several caveats concerning Nm estimates merit consideration, however. First, the estimates generated here are from a single gene genealogy. More accurate estimates of gene flow would be expected from an analysis of multiple independent loci (although they would not then apply strictly to female lineages that are of special interest here). Second, some of the rookeries surveyed may be the product of recent colonization events (see below), such that assumptions of population equilibrium are not met (although Slatkin and Barton [1989] suggest that the approaches used here to estimate Nm are relatively insensitive to this type of bias). Finally, the theoretical basis for these estimates is still under development, and empirical calibrations are currently unavailable. For these reasons, Nm estimates should be interpreted as *general* indicators of the magnitude of genetic exchange.

Discussion

The nesting beaches of the southeastern United States, taken together, comprise the second largest reproductive aggregate of loggerhead turtles in the world, with approximately 45,000 nesting females (Murphy & Hopkins 1984). Within this region, however, nesting habitat is not continuous. The nesting beaches in Florida and Georgia/South Carolina are separated by several hundred kilometers of beach in which loggerhead nesting density is low (Murphy & Hopkins-Murphy 1989). In the Mediterranean, prominent nesting aggregates are reported from Zakynthos and Peloponnesus in Greece and along the adjacent coastline of Turkey (Groombridge 1988). Notably, the loggerheads that nest at these Mediterranean locations are significantly smaller than those that nest in the western Atlantic (Margaritoulis 1982, 1988*b*).

In terms of the distribution of mtDNA lineages, the various Atlantic and Mediterranean nesting populations assayed in this study are significantly different, a conclusion consistent with results of a protein electrophoretic analysis of loggerhead rookeries in Queensland, Australia (Gyuris & Limpus 1988). But we observed more sharing of mtDNA genotypes among loggerhead rookeries than was observed in green turtles, a fact reflected in somewhat higher estimates of mean migration rate based on Slatkin's (1985) private allele method: $Nm \approx 2.0$ for Atlantic loggerheads, compared with $Nm \approx 0.3$ for Atlantic green turtles (Bowen et al. 1992). It is possible that these migration estimates reflect a higher level of dispersal in female loggerhead turtles relative to green turtles; movement between spatially distinct green turtle rookeries is known to be extremely rare (Meylan 1982). On the other hand, the higher migration estimate for loggerheads may be an artifact of sampling design, because four of the five sampled nesting beaches are in one geographic province (the southeastern United States). Additional data will be required to determine whether nesting loggerhead turtles are less site-specific than are nesting green turtles.

Evolutionary History of the Western Atlantic and Mediterranean Rookeries

Climatic processes have undoubtedly influenced the contemporary distribution and population structure of loggerhead nesting assemblages. Loggerhead eggs require a minimum of 60 days above 25°C to incubate successfully, such that cold temperate conditions in the Mediterranean (Buckley et al. 1982; McCoy 1980) may have precluded nesting here during the most recent glacial period (18,000 to 12,000 years before the present). In the western Atlantic, loggerheads possibly nested in southern Florida during glacial intervals, but almost certainly not at present-day rookery locations in Georgia, South Carolina, and North Carolina (see Hedgpeth 1954). Thus, the contemporary distribution of nesting beaches in the southeastern United States may be the product of colonization events over the last

12,000 years, as loggerhead females extended the northern boundary of the nesting range. One consequence of this colonization process could be a progressive loss of mtDNA diversity in more northerly rookeries, as maternal lineages are filtered through a series of colonization bottlenecks. Estimates of haplotypic and genotypic diversity (Table 2) are consistent with this scenario.

In the southeastern U.S., this colonization process has apparently been sufficient to extend the northern limits of nesting by 1000 km within the last 12,000 years (fewer than 600 loggerhead generations). In the Mediterranean, habitats that were too cold to support nesting and feeding 12,000 years ago are now utilized extensively by loggerhead turtles. Furthermore, the presence of the *D* genotype at 100% frequency in samples from Greece indicates that this colony shared a recent common ancestor with western Atlantic populations. Thus, conclusions drawn from climatic history, mtDNA data, and tagging studies are concordant in indicating that loggerheads are active colonizers that can occupy newly opened habitat over relatively short evolutionary time scales. In other words, contact between loggerhead rookeries in the West Atlantic and Mediterranean has been sufficient to prevent pronounced evolutionary divergence, despite a restriction of contemporary gene flow between nesting assemblages (Table 4).

Regional Population Structure—Southeastern United States

MtDNA data indicate that nesting loggerhead turtles in the southeastern United States are divided into Georgia/South Carolina and Florida cohorts, a conclusion also supported by subtle differences in morphology (Stoneburner 1980). Many coastal marine organisms of the southeastern United States show a phylogeographic discontinuity in this area (Bowen & Avise 1990; Avise 1992), including an estuarine terrapin (Lamb & Avise 1992). But the apparent separation in loggerheads is difficult to reconcile with some aspects of tag-recapture data. While most nesting females return to the same beach in successive nesting seasons, a small fraction of tagged turtles has been observed nesting at alternative sites (Dodd 1988). Bjorndal et al. (1983) reviewed 25 cases of nesting-beach relocations in the southeast region, and LeBuff (1974) reported that a female tagged on a western Florida nesting beach was observed nesting on the east coast of Florida (550 km distant) four years later. More directly relevant to this discussion, 11 tagged loggerheads were observed to nest at both Georgia and eastern Florida sample locations during the period of 1978–1985 (J. I. Richardson, unpublished data).

The significant difference in observed mtDNA haplotype frequencies between Florida and Georgia/South Carolina rookeries appears to be inconsistent with this propensity for nesting relocations, because the exchange of even one migrant per generation is sufficient in theory to maintain alleles in similar frequencies in populations at equilibrium (Slatkin 1987; but see Allendorf 1983). One possible explanation is that Georgia/South Carolina and Florida nesting populations have not reached an equilibrium condition. Support for this possibility stems (1) from climatic data: the Georgia and South Carolina rookeries were probably unsuitable for nesting 12,000 years (or about 600 loggerhead generations) before the present, and (2) from contemporary demographic data: nesting populations have been reduced in the last several decades by mortality associated with incidental capture in the shrimp fishery (Henwood & Stuntz 1987; National Research Council 1990). Either of these factors could be sufficient to abrogate assumptions of population equilibrium. A related hypothesis is that these nesting relocations are a relatively recent phenomenon, perhaps induced by human encroachment on nesting grounds and adjacent internesting habitat. Inferential support for this scenario is provided by field observations: turtles disturbed during nesting are more likely than undisturbed turtles to relocate to an adjacent beach, and they may use the new beach for subsequent nesting efforts (T. M. Murphy, personal communication).

The heavy metal concentration of loggerhead egg shells differs significantly between Florida and Georgia/South Carolina rookeries (Stoneburner et al. 1980), a distinction that is further supported by differences in the epibiota assemblages of nesting turtles (Caine 1986). Since both epibiota and heavy metals accumulate during non-nesting intervals, these environmental markers indicate some level of segregation on feeding grounds or migratory routes. Notably, tag recoveries support a similar partition: Florida nesting turtles are recovered along the southeastern U.S., in the Bahamas, and in the Caribbean (Meylan 1982; Meylan et al. 1983), while Georgia nesting turtles have been recovered almost exclusively on the east coast of the U.S. (Bell & Richardson 1978; Richardson 1982, unpublished data). Thus, both environmentally-acquired markers and human-applied tags indicate some segregation on feeding grounds.

Acquired markers (heavy metals, epibiota, and tags) and innate genetic markers (mtDNA) support a concordant partitioning of nesting populations into Florida versus Georgia/South Carolina units. But these two classes of markers elucidate very different aspects of loggerhead natural history. The genetic markers indicate that loggerhead females tend to nest in the vicinity of their natal rookery, whereas the acquired markers indicate that Florida and Georgia/South Carolina nesting populations tend to segregate on feeding grounds as well. Taken together, these distinct classes of information al-

low a more complete picture of loggerhead migratory behavior to emerge.

Mediterranean Recruitment and Natal Homing

The mtDNA data indicate that regional loggerhead nesting populations are genetically distinct, but a complete test of natal homing requires information on feeding ground composition as well. If nesting populations maintain separate feeding grounds, then genetic expectations under the natal homing and social facilitation models converge because turtles are not confronted with the option of following experienced breeders to a non-natal rookery. Therefore, the optimal genetic tests of these competing hypotheses involve nesting populations that share feeding areas.

The supposition that juvenile loggerheads from North Atlantic nesting beaches occur on Mediterranean feeding grounds is based on three lines of evidence. First, Carr (1987) noted that more juvenile loggerheads occupy Mediterranean feeding grounds than could be generated by the Mediterranean rookeries alone (see also Argano & Baldari 1983). Second, a loggerhead tagged in the Azores subsequently was recovered in the Mediterranean (Bolten et al. 1992). Third, the North Atlantic current system (believed to passively transport juvenile loggerheads) branches into the Mediterranean (Estrada et al. 1985). Groombridge (1988) speculated that surface currents and oceanic topology may trap pelagic-stage (juvenile) loggerheads in the Mediterranean Basin, and that some of these strays may remain there to breed.

If one accepts the premise that juveniles from North Atlantic nesting beaches occupy Mediterranean feeding grounds, then mtDNA data provide a critical test of natal homing for these turtles. Under a social facilitation model, considerable recruitment of North Atlantic juveniles onto Mediterranean nesting beaches is expected, resulting in a sharing of common mtDNA lineages. Contrary to these expectations, samples from one of the largest Mediterranean rookeries (Kiparissia Bay, Greece) contained only genotype *D* of the two genotypes (*B* and *D*) that dominated northwestern Atlantic samples. Thus, the mtDNA data indicate that female-mediated gene flow between the northwestern Atlantic and Mediterranean rookeries is limited (Table 4). These data are inconsistent with a major role for social facilitation. If juveniles are trapped in the Mediterranean, as Groombridge (1988) suggests, they apparently are not recruiting to Mediterranean rookeries at levels sufficient to homogenize haplotype frequencies or to affect contemporary demographics.

Conservation Concerns

Mitochondrial lineages are useful for addressing the female component of population structure, but they leave parallel questions about male dispersal unresolved. In terms of conservation genetics and management of marine turtles, the population structure of females is of special interest because nesting females ultimately govern the reproductive success of these species. However, other aspects of marine turtle conservation may benefit from an understanding of the male component of population genetic structure. For example, do males mate with females from other rookeries, facilitating nuclear gene flow between nesting populations that are isolated with respect to female lineages? If regional nesting populations are linked by male-mediated gene flow, then these assemblages may be buffered to some extent from the reduced genetic diversity associated with population bottlenecks. While a paternal analog to mtDNA is unavailable, many of these issues may be addressed with analysis of nuclear (n) DNA loci. In green turtles, restriction fragment analysis of nDNA loci demonstrate that nesting populations are less structured with respect to these biparentally-inherited markers than is the case for maternally-inherited lineages (Karl et al. 1992). The implications of these data are that male green turtles provide an avenue of gene flow between nesting populations. It remains to be seen whether the same conclusion applies to loggerhead turtles. Nonetheless, these complementary approaches (in this case, nDNA and mtDNA analyses) are necessary to resolve the complex genetic architecture of marine turtle populations.

The mtDNA data reported here indicate a significant population genetic structure for loggerhead turtle rookeries in the North Atlantic Ocean and Mediterranean Sea. Contemporary female-mediated gene flow between regions is negligible (Table 4), yet all loggerhead populations are related very closely in an evolutionary sense. What do these results imply for the management of threatened and endangered populations? Over short evolutionary time scales (perhaps a few thousand years), female dispersal apparently is sufficient to allow colonization of appropriate habitats in proximity to established rookeries. Both tag returns and mtDNA data indicate, however, that female gene flow is too low to have a significant impact on population dynamics on a contemporary scale. Therefore, if nesting females are depleted or extirpated at one rookery, regional dispersal will not be sufficient to replenish this resource over a time scale that is meaningful to wildlife management agencies. This conclusion holds regardless of the magnitude of inter-rookery gene flow mediated by males. Accordingly, nesting populations must be considered to be demographically independent.

Acknowledgments

For field assistance we gratefully recognize M. Camhi, P. Castaneda, C. Coogan, L. Ehrhart, G. Garris, L. Fisher, S. Karl, R. Klinger, L. Letson, R. Mezich, N. Richardson, B.

Schroeder, C. Sears, G. Smith, W. Weber, M. Zacks, and J. Zurita. For logistic support we are indebted to G. Acres, R. Carthy, R. Ferl, M. Harris, J. A. Huff, S. Jacobsen, J. Musick, W. Nelson, E. Possardt, T. Richardson, D. Walker, J. Woody, the Broward County Environmental Quality Control Board, the Caribbean Conservation Corporation, The Conservancy, the Florida Department of Natural Resources, the Georgia Department of Natural Resources, the National Marine Fisheries Service (U.S.), the Sea Turtle Protection Society of Greece, the South Carolina Wildlife and Marine Resources Department, and the U.S. Fish and Wildlife Service. K. Bjorndal, A. Bolten, L. Laurent, C. Limpus, and N. Mrosovsky provided insightful reviews of an earlier version of this report. We thank A. Abreu for translation and constructive comments. The map was drafted by J. Ingram at the Cartographic Lab, Geography Department, University of Georgia. This research was supported by the National Geographic Society, the National Science Foundation, the Interdisciplinary Center for Biotechnology Research (University of Florida), and by a Genetics Training grant to B. W. Bowen from the National Institutes of Health. Funding for the South Carolina rookery samples was provided by the National Oceanographic and Atmospheric Administration (NOAA). The views expressed herein are those of the authors and do not reflect the views of NOAA or any of its subagencies.

Literature Cited

Allendorf, F. W. 1983. Isolation, gene flow, and genetic differentiation among populations. Pages 51–65 in C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas, editors. Genetics and Conservation. Benjamin/Cummings, London, England.

Argano, R., and F. Baldari. 1983. Status of Western Mediterranean sea turtles. Rapports de Commission Internationale de la Mer Mediterranee **28**(5):233–235.

Avise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna: A case history with lessons for conservation biology. Oikos **63**:62–76.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics **18**:489–522.

Avise, J. C., B. W. Bowen, and T. Lamb. 1989. DNA fingerprints from hypervariable mitochondrial genotypes. Molecular Biology and Evolution **6**:258–269.

Avise, J. C., B. W. Bowen, T. Lamb, A. B. Meylan, and E. Bermingham. 1992. Mitochondrial DNA evolution at a turtle's pace: Evidence for low genetic variability and reduced microevolutionary rate in the Testudines. Molecular Biology and Evolution **9**:457–473.

Bell, R., and J. I. Richardson. 1978. An analysis of tag recoveries from loggerhead sea turtles (*Caretta caretta*) nesting on Little Cumberland Island, Georgia. Florida Marine Research Publications **33**:1–66.

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.

Bjorndal, K. A., A. B. Meylan, and B. J. Turner. 1983. Sea turtle nesting at Melbourne Beach, Florida, I. Size, growth, and reproductive biology. Biological Conservation **26**:65–77.

Bolten, A. B., H. R. Martins, K. A. Bjorndal, M. Cocco, and G. Gerosa. 1992. *Caretta caretta* (loggerhead) pelagic movement and growth. Herpetological Review **23**:116.

Bowen, B. W., and J. C. Avise. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: Influence of zoogeographic factors and life-history patterns. Marine Biology **107**:371–381.

Bowen, B. W., A. B. Meylan, and J. C. Avise. 1991. Evolutionary distinctiveness of the endangered Kemp's ridley sea turtle. Nature **352**:709–711.

Bowen, B. W., A. B. Meylan, J. P. Ross, C. J. Limpus, G. H. Balazs, and J. C. Avise. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution **46**(4):865–881.

Buckley, H. A., L. R. Johnson, N. J. Shackleton, and R. A. Blow. 1982. Late glacial to recent cores from the eastern Mediterranean. Deep Sea Research **29**:739–766.

Caine, E. A. 1986. Carapace epibionts of nesting loggerhead sea turtles: Atlantic coast of U.S.A. Journal of Experimental Marine Biology and Ecology **95**:15–26.

Carr, A. 1986. Rips, FADS, and little loggerheads. Bioscience **36**(2):92–100.

Carr, A. 1987. New perspectives on the pelagic stage of sea turtle development. Conservation Biology **1**:103–121.

Dodd, C. K., Jr. 1988. Synopsis of the biological data on the loggerhead sea turtle *Caretta caretta* (Linnaeus 1758). U.S. Fish and Wildlife Service Biological Report **88**(14):1–110.

Estrada, M., F. Vives, and M. Alcaraz. 1985. Life and the productivity of the open sea. Pages 148–197 in M. Margalef, editor. Western Mediterranean. Key Environments Series. Pergamon Press Ltd., London, England.

Frazer, N. B., and L. M. Ehrhart. 1985. Preliminary growth models for green, *Chelonia mydas*, and loggerhead, *Caretta caretta*, turtles in the wild. Copeia **1985**:73–79.

Groombridge, B. 1988. Marine turtles in the Mediterranean: Distribution, population status, conservation. Report T-PVS (88) 42. Council of Europe, Environment Conservation and Management Division, Strasbourg, France.

Gyuris, E., and C. J. Limpus. 1988. The loggerhead turtle *Caretta caretta* in Queensland: Population breeding structure. Australia Wildlife Research **15**:197–209.

Hedgpeth, J. W. 1954. An introduction to the zoogeography of the northern Gulf of Mexico with reference to the invertebrate fauna. Pages 111–211 in Publication 3, Institute of Marine Science, University of Texas, Port Aransas, Texas.

Henwood, T. A., and W. E. Stuntz. 1987. Analysis of sea turtle captures and mortality during commercial shrimp trawling. Fisheries Bulletin 85:813–817.

Karl, S. A., B. W. Bowen, and J. C. Avise. 1992. Global population structure and male-mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analyses of anonymous nuclear DNA regions. Genetics 131:163–173.

Klinger, R. C., and J. A. Musick. 1992. Annular growth layers in juvenile loggerhead turtles (*Caretta caretta*). Bulletin of Marine Science 51:224–230.

Lamb, T., and J. C. Avise. 1992. Molecular and population genetic aspects of mitochondrial DNA variability in the diamondback terrapin, *Malaclemys terrapin*. Journal of Heredity 83:262–269.

Lansman, R. A., R. O. Shade, J. F. Shapira, and J. C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. Journal of Molecular Evolution 17:214–226.

Laurent, L. 1990*a*. Les tortues marines en Algerie et au Maroc (Mediterranee). Bulletin de la Societe Herpetologique de France 55:1–23.

Laurent, L. 1990*b*. L'origine des tortues caouannes, *Caretta caretta* (Linnaeus 1758) de Mediterranee Occidentale. Rapports de Commission Internationale de la Mer Mediterranee 32:240.

LeBuff, C. R., Jr. 1974. Unusual nesting relocation in the loggerhead turtle, *Caretta caretta*. Herpetologica 30:29–31.

Limpus, C. J., V. Baker, and J. D. Miller. 1979. Movement induced mortality of loggerhead eggs. Herpetologica 35:335–338.

Limpus, C. J., J. D. Miller, C. J. Parmenter, D. Reimer, N. McLachland, and R. Webb. 1992. Migration of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles to and from eastern Australian rookeries. Wildlife Research 19:347–358.

Margaritoulis, D. 1982. Observations on loggerhead sea turtle, *Caretta caretta*, activity during three nesting seasons (1977–1979) in Zakynthos, Greece. Biological Conservation 24:195–204.

Margaritoulis, D. 1988*a*. Post-nesting movements of loggerhead sea turtles tagged in Greece. Rapports de Commission Internationale de la Mer Mediterranee 31(2):284.

Margaritoulis, D. 1988*b*. Nesting of the loggerhead sea turtle, *Caretta caretta*, on the shores of Kiparissia Bay, Greece. Mesogee 48:59–65.

McCoy, F. W. 1980. Climate changes in the eastern Mediterranean area during the past 240,000 years. Pages 79–100 in C. Doumas, editor. Thera and the Aegean World II: Papers and Proceedings of the Second International Scientific Congress, Santorini, Greece, August 1978. Thera and the Aegean World, London, England.

Meylan, A. B. 1982. Sea turtle migration—evidence from tag returns. Pages 91–100 in K. A. Bjorndal, editor. Biology and conservation of sea turtles. Smithsonian Institution Press, Washington, D. C.

Meylan, A. B., K. A. Bjorndal, and B. J. Turner. 1983. Sea turtle nesting at Melbourne Beach, Florida, II. Post-nesting movement of *Caretta caretta*. Biological Conservation 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. Science 248:724–727.

Murphy, T. M., and S. R. Hopkins. 1984. Aerial and ground surveys of marine turtle nesting beaches in the southeast region, U.S. Report no. NA83-GA-C-00021. National Marine Fisheries Service, Southeast Fisheries Center Miami, Florida.

Murphy, T. M., and S. R. Hopkins-Murphy. 1989. Sea turtle and shrimp fishing interactions: A summary and critique of relevant information. Center for Marine Conservation, Washington, D.C.

National Research Council. 1990. Decline of the sea turtles: Causes and prevention. National Academy Press. Washington, D.C.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., and W.-H. Li. 1979. Mathematical models for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Science U.S.A. 76:5269–5273.

Nei, M., and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145–163.

Owens, D. W., M. A. Grassman, and J. R. Hendrickson. 1982. The imprinting hypothesis and sea turtle reproduction. Herpetologica 38:124–135.

Richardson, J. I. 1982. A population model for adult female loggerhead sea turtles (*Caretta caretta*) nesting in Georgia. Ph.D. Dissertation. University of Georgia, Athens, Georgia.

Scribner, K. T., J. E. Evans, S. J. Morreale, M. H. Smith, and J. W. Gibbons. 1986. Genetic divergence among populations of yellow-bellied slider turtle (*Pseudemys scripta*) separated by aquatic and terrestrial habitats. Copeia 1986(3):691–700.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evolution 39:53–65.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Slatkin, M., and N. H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349–1368.

Smith, M. H., H. O. Hillstad, M. N. Manlove, D. O. Straney, and J. M. Dean. 1978. Management implications of genetic variability in loggerhead and green sea turtles. Pages 302–312 in Pro-

ceedings of the 15th International Congress of Game Biologists. Wildlife Society, Washington, D.C.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. 2nd edition. W. H. Freeman, San Francisco, California.

Stonehurner, D. L. 1980. Body depth: An indicator of morphological variation among nesting groups of adult loggerhead sea turtles (*Caretta caretta*). Journal of Herpetology 14(2):205–206.

Stonehurner, 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. Journal of Herpetology 14(2):171–175.

Takahata, N., and S. R. Palumbi. 1985. Extranuclear differentiation and gene flow in the finite island model. Genetics 109:441–457.

Zug, G. R., A. H. Wynn, and C. Ruckdeschel. 1986. Age determination of loggerhead sea turtles, *Caretta caretta*, by incremental growth marks in the skeleton. Smithsonian Contributions to Zoology 427:1–34.