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# Population structure of loggerhead turtle (*Caretta caretta*) nesting colonies in the Atlantic and Mediterranean as inferred from mitochondrial DNA control region sequences

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**Abstract** Mitochondrial (mt) DNA control region sequences were analyzed for 249 Atlantic and Mediterranean loggerhead turtles (*Caretta caretta* Linnaeus, 1758) to elucidate nesting population structure and phylogeographic patterns. Ten haplotypes were resolved among individuals sampled between 1987 and 1993, from ten major loggerhead nesting areas in the region. Two distinct phylogenetic lineages were distinguished, separated by an average of 5.1% sequence divergence. Haplotype frequency comparisons between pairs of populations showed significant differentiation between most regional nesting aggregates and revealed six demographically independent groups, corresponding to nesting beaches from: (1) North Carolina, South Carolina, Georgia and northeast Florida, USA; (2) southern Florida, USA; (3) northwest Florida, USA; (4) Quintana Roo, Mexico; (5) Bahia, Brazil; and (6) Peloponnesus Island, Greece. The distribution of mtDNA haplotypes is consistent with a natal homing scenario, in which nesting colonies separated by a few hundred kilometers represent isolated reproductive aggregates. However, a strong exception to this pattern was observed in the first group defined by mtDNA data (North Carolina to northeast Florida), which included samples from four nesting locations spread across thousands of kilometers of coastline. These locations were characterized by a single haplotype in 104 out of 105 samples, providing inadequate resolution of population divisions. In view of the subdivisions observed elsewhere, we attribute the lack of differentiation between North Carolina and northeast Florida to recent colonization of these warm temperate coastlines (after the Wisconsin glaciation) not to ongoing gene flow among spatially distinct nesting locations. The relationships among observed haplotypes suggest a biogeographic scenario defined by climate, natal homing, and rare dispersal events. The redefined relationships among nesting aggregations in the western Atlantic region (southeastern USA and adjacent Mexico) prompt a reconsideration of management strategies for nesting populations and corresponding habitats in this region.

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## Introduction

The loggerhead turtle (*Caretta caretta*) is distributed globally along tropical and subtropical latitudes but has a more temperate distribution than other cheloniid sea turtles (Pritchard and Trebbau 1984). Within the Atlantic region, the southeastern USA hosts the largest loggerhead nesting concentrations, comprising approxi-

mately 90% of the reproductive effort in this ocean basin (estimated from numbers provided by Murphy and Hopkins 1984; Meylan et al. 1995). Nesting beaches in Quintana Roo, Mexico and Bahia, Brazil support additional nesting habitat, with about 300 and 400 nesting females per year, respectively (Zurita et al. 1993; M. Marcovaldi, personal communication), and Kiparissia Bay, Greece, represents one of the largest rookeries in the Mediterranean, with about 300 nesting females per year (Margaritoulis 1988).

As with other species of sea turtles, the loggerhead life cycle consists of developmental stages which are segregated spatially and temporally. Juveniles are believed to spend their first few years drifting passively in ocean current systems or in floating sargassum rafts (Carr 1986; Bolten et al. 1998). Advanced juveniles subsequently shift to coastal feeding habitats (Carr 1987). After reaching sexual maturity (some 20 to 30 years later; Frazer and Ehrhart 1985; Klinger and Musick 1995), adult females undertake reproductive migrations spanning hundreds or thousands of kilometers (Meylan 1982). Tagging data indicate that most females migrate to the same nesting beaches in successive breeding seasons (Bjorndal et al. 1983), prompting several researchers to suggest that loggerhead turtles return to nest on their natal beach (Bowen et al. 1993a). Mark and recapture studies cannot, however, distinguish whether their site fidelity is the result of natal homing behavior or social imprinting (see Owens et al. 1982). Under the natal homing scenario, loggerhead hatchlings imprint on their natal beaches, and the adult females subsequently return to these beaches to lay eggs. Under the social facilitation scenario, first-time breeders follow experienced females to nesting beaches and imprint on that beach for successive nesting efforts. Although both hypotheses explain the strong site fidelity indicated by the tagging data, natal homing predicts genetic partitions between nesting loggerhead aggregations, while the social facilitation model would produce a homogeneous gene pool among regional nesting populations.

Previous sea turtle genetic surveys have successfully employed mitochondrial (mt) DNA polymorphisms to assess population partitions and the possibility of female natal homing among several marine turtles, including green turtles, *Chelonia mydas* (Bowen et al. 1992; Allard et al. 1994; Lahanas et al. 1994; Norman et al. 1994; Encalada et al. 1996), hawksbill turtles, *Eretmochelys imbricata* (Broderick et al. 1994; Bass et al. 1996), leatherback turtles, *Dermochelys coriacea* (Dutton 1995), and loggerhead turtles, *Caretta caretta* (Bowen et al. 1993a, 1994). These studies found varied levels of population subdivision among species, and similarly diverse degrees of intraspecific natal homing behavior. In the case of the loggerhead turtle in the southeastern USA, previous molecular studies (Bowen et al. 1993a) confirmed distinctions, based on morphology and epibiota (Stoneburner et al. 1980; Caine 1986), between nesting populations in Florida and Georgia/South Carolina. A Mediterranean (Greek) colony was also found to be demographically distinct from West Atlantic nesting colonies (Bowen et al. 1993a). This earlier survey did not include the entire range of loggerhead nesting in the Northwest Atlantic, and was hampered to some extent by the low haplotype diversity observed with restriction fragment length polymorphisms (RFLPs).

In the present study we assess the population genetic composition of Atlantic and Mediterranean loggerhead turtle nesting colonies through the analysis of sequences from the control region of the mtDNA, a region which yielded an approximate sixfold greater resolution than RFLPs in a green turtle survey (Encalada et al. 1996). The increased resolution, and more thorough sampling across the loggerhead geographic range, provide a more detailed understanding of population structure of Atlantic and Mediterranean nesting aggregates. A related goal is to determine whether the relationships among mtDNA haplotypes provide a phylogeographic scenario that can account for the colonization of loggerhead maternal lineages in the region.

This research can provide two substantial advances in sea turtle conservation. First, earlier work established that widely separated nesting colonies were genetically distinct, but could not resolve the geographic limits of individual nesting populations (Schroth et al. 1996). The more detailed survey conducted here is intended to define geographic partitions among management units, especially for the cluster of nesting beaches in the

**Table 1** *Caretta caretta*. Polymorphic sites in mtDNA control region sequences of Atlantic loggerhead turtles. Dashes denote instances of insertion/deletion events

| Halotype | Sites: 32 | 35 | 37 | 51 | 53 | 63 | 96 | 104 | 161 | 162 | 188 | 210 | 230 | 244 | 246 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | T | G | T | T | T | A | A | A | C | G | G | C | C | G | C |
| B | C | A | C | C | – | G | A | G | T | A | G | T | T | A | T |
| C | C | A | C | C | – | G | A | G | T | A | A | T | T | A | T |
| D | T | G | T | T | T | A | A | A | C | A | G | C | C | A | C |
| E | C | A | C | C | – | G | A | G | T | A | G | T | T | A | T |
| F | C | A | C | C | – | G | A | G | T | A | G | T | T | A | T |
| G | C | A | C | C | – | G | A | G | T | A | G | T | T | A | T |
| H | C | A | C | C | – | G | A | G | C | A | G | T | T | A | T |
| I | C | A | C | T | – | G | G | G | T | A | G | T | T | A | T |
| J | C | A | C | C | – | G | A | G | T | A | G | T | T | A | T |

southeastern USA. Second, rookery-specific mtDNA markers can be used to determine the origin of sea turtles in distant feeding locations (Bowen et al. 1995; Bolten et al. 1998). In cases where oceanic or coastal feeding aggregates are susceptible to fishery mortality, the value of this information is readily apparent. Wildlife managers need to know which nesting populations are reduced by commercial fisheries, and a relatively complete rookery survey is the essential foundation for genetic studies of feeding-ground composition.

## Materials and methods

Samples from 249 *Caretta caretta* (Linnaeus, 1758) individuals were collected between 1987 and 1993 from ten major loggerhead nesting aggregates throughout the Atlantic Ocean and Mediterranean Sea. Locations sampled were Quintana Roo, Mexico ($n = 20$); Bahia, Brazil ($n = 11$); Kiparissia Bay, Peloponnesus Island, Greece ($n = 21$); and seven nesting areas in the southeastern USA. The latter samples were collected from North Carolina: Bald Head Island ($n = 8$), Cape Lookout ($n = 9$), Topsail Beach ($n = 8$), Camp Lejuene ($n = 2$), and Caswell Beach ($n = 1$); South Carolina: Cape Romain ($n = 20$); Georgia: Cumberland Island and Little Cumberland Island ($n = 43$); northeast Florida (NEFL): Amelia Island ($n = 12$), Jacksonville Beach ($n = 1$) and Guana River area ($n = 1$); southeast Florida (SEFL): Melbourne Beach ($n = 6$), Hutchinson Island ($n = 9$) and Port Everglades ($n = 10$); southwest Florida (SWFL): Key Island ($n = 15$) and Sarasota County ($n = 10$); and northwest Florida (NWFL): Eglin Air Force Base ($n = 21$), Panama City ($n = 1$), Apalachicola ($n = 1$), Tyndall Beach ($n = 7$), and areas adjacent to St. George and St. Joseph ($n = 12$). All samples from Greece, South Carolina, Georgia, and some of the samples from SE and SW Florida are the same as those used by Bowen et al. (1993a) in RFLP analyses.

Most samples consisted of two eggs or one hatchling from each nest (procedures described by Bowen et al. 1993a). Mexican samples consisted of blood obtained from the dorsal cervical sinus of nesting females or hatchlings (following the procedure of Owens and Ruiz 1980). Whole genomic DNA was isolated from tissue or blood samples by a series of phenol/chloroform extractions following a protocol modified from Hillis et al. (1996). Genomic DNA was subsequently resuspended in 1× TE buffer. A mtDNA region including 391 nucleotide sites from the control region was PCR-amplified (Innis et al. 1990) using biotinylated primers CR-1 (5′-TTG TAC ATC TAC TTA TTT ACC AC-3′) and CR-2 (5′-GTA CGT ACA AGT AAA ACT ACC GTA TGC C-3′) (Norman et al. 1994). These oligonucleotides included a universal M13 primer extension to facilitate sequencing via a dye-primer procedure (see below). Amplified double-stranded PCR products were purified using Dynal streptavidin-coated magnetic particles (Dynal, Sweden) and denatured with 0.2 *N* NaOH. Throughout the PCR amplification procedures, negative control (template-free) reactions were conducted to detect and guard against contamination. Single-stranded products were sequenced at the DNA Sequencing Core Laboratory of the University of Florida in a robotic workstation (Applied Biosystems Model 800) using fluorescently labelled universal M13 primers. The strand complementary to the biotinylated strand was sequenced. Dideoxy-terminated reaction products were separated and analyzed in an automated sequencer (Applied Biosystems Model 373A), and sequences were scored directly from the chromatogram output. All samples were sequenced using forward primers. Reverse sequences were obtained for representative samples to confirm each haplotype designation.

Sequences were aligned by eye and/or using the SeqEd editor provided with the automated sequencer 373A (Applied Biosystems). Haplotypes were assigned letter codes and were joined by hand into a parsimony network. Estimates of nucleotide sequence divergence ($p$-values) were calculated using the Jukes–Cantor method (Jukes and Cantor 1969); haplotype ($h$) and nucleotide ($\pi$) diversities were estimated by the method of Nei (1987, Eqs. 8.4 and 10.5, respectively). Relationships between the observed mtDNA genotypes were assessed by the neighbor-joining algorithm (Saitou and Nei 1987) available in the program MEGA (Kumar et al. 1993), with bootstrapping (100 replicates).

To compare haplotype frequencies between pairs of populations, a chi-squared test of independence (Sokal and Rohlf 1981) was used with the Monte Carlo randomization method (Roff and Bentzen 1989) in the program CHIRXC (Zaykin and Pudovkin 1993). The program AMOVA (analysis of molecular variance; Excoffier et al. 1992) was used to assess the proportion of genetic variation within and among nesting colonies.

Estimates of gene flow between pairs of colonies ($Nm$) were calculated from the $G_{st}$ analog in AMOVA ($\Phi_{st}$), using the equation $Nm = 0.5\ (1/G_{st} - 1)$ (Takahata and Palumbi 1985; Nei 1987). Mean migration rate across all rookeries was calculated by the private allele method (Slatkin 1985; Barton and Slatkin 1986), using the equation of Slatkin and Barton (1989).

## Results

The mtDNA control region sequences were aligned for 380 bp. Twenty-six polymorphic sites were detected, consisting of 21 transitions, no transversions, and five deletions/insertions (indels; Table 1). Four of the five indels involved single sites and one involved a 6-bp segment (sites 355–360). Site 358 contained both a transition (A → G) and an indel. The indels were treated as single mutation events throughout this analysis. Only one instance of suspected homoplasy was observed. In this

| 259 | 294 | 312 | 314 | 315 | 317 | 321 | 327 | 355 | 356 | 357 | 358 | 359 | 360 | 363 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | – | A | A | T | A | A | C | – | – | – | – | – | – | A |
| G | G | A | G | C | G | A | T | G | C | A | A | G | T | A |
| G | G | A | G | C | G | A | T | G | C | A | A | G | T | A |
| A | – | A | A | T | A | A | C | – | – | – | – | – | – | A |
| G | G | A | G | C | G | A | T | G | C | A | A | G | T | – |
| G | G | A | G | C | G | – | T | G | C | A | A | G | T | A |
| G | G | G | G | C | G | A | T | G | C | A | A | G | T | A |
| G | G | A | G | C | G | A | T | G | C | A | A | G | T | A |
| G | G | A | G | C | G | A | T | G | C | A | A | G | T | A |
| G | G | A | G | C | G | A | T | G | C | A | G | G | T | A |

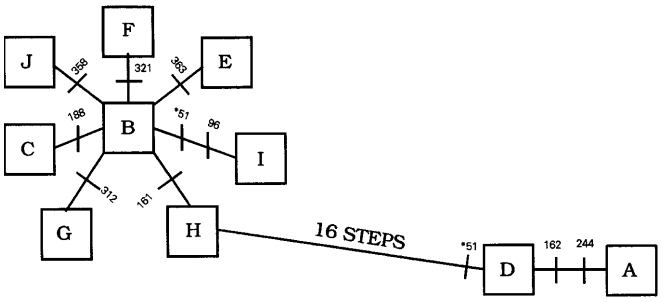


**Fig. 1** *Caretta caretta.* Unrooted parsimony network of ten Atlantic and Mediterranean haplotypes. Letters correspond to haplotypes presented in Tables 1 and 2. Mutation steps distinguishing haplotypes are represented by line divisions along branches. Haplotypes H and D are separated by 17 mutations at sites 32, 35, 37, 51, 53, 63, 104, 210, 230, 246, 259, 294, 314, 315, 317, 327, and the 6-bp indel (sites 355–360). The asterisk beside site 51 designates an instance of assumed homoplasy

case, haplotypes separated by 20 to 22 polymorphisms (haplotypes D and A versus haplotype I in Fig. 1) contained a C → T transition at site 51 (see Table 1).

Ten mtDNA haplotypes were distinguished among the 249 samples (Table 2). Sequences are archived in GenBank (Accession Numbers AJ001074–AJ001083). When arranged into an unrooted parsimony network, the observed haplotypes group into two distinct clusters: (1) B, C, E, F, G, H, I, J versus (2) A, D (Fig. 1). The two groups were separated by 17 mutation steps, and haplotypes within each group differed by a maximum of three and two mutations, respectively. This arrangement was confirmed by neighbor-joining analysis, with bootstrap support distinguishing these two groups at the 100% level. The mean sequence divergence between the two clusters, $p = 0.05$, is comparable to the deepest known separation of green turtles (distinguishing Atlantic versus Pacific lineages: $p = 0.04$; Encalada 1995), and is about seven times higher than the sequence divergence observed for green turtles within the Atlantic/Mediterranean basin: $p = 0.008$ (Encalada et al. 1996). The sequence divergence observed with this data set (control region sequences) is approximately six times higher than that produced by the RFLP survey over the entire mitochondrial genome (Bowen et al. 1993a). This ratio is almost identical to that found in a comparison of control region sequences and RFLP data in Atlantic green turtles (Encalada et al. 1996), and is similar to that found in control region sequence versus RFLP comparisons for Pacific green turtles (eightfold increase; Norman et al. 1994).

Two haplotypes, A and B, accounted for 88% of the individuals (Table 2; Fig. 2), but the distribution of haplotypes still showed a strong geographic component. Within the southeastern USA (inset in Fig. 2), haplotype B was observed at 48% frequency in southern Florida (SEFL and SWFL), 9.5% in NW Florida, and 1% in NE Florida, Georgia, North and South Carolina. On the other hand, haplotype A was observed at 100% frequency in North and South Carolina, 98% in Georgia, 100% in NE Florida, 81% in NW Florida, but at only 44% in southern Florida (SE and SW combined). Haplotype frequencies between pairs of locales in the southeastern USA showed significant differences in 10 of 21 comparisons (Table 3). The instances where genotype frequencies were not significantly different involved adjacent areas: (1) NE Florida, Georgia, South Carolina, North Carolina (hereafter referred to as the NEFL/NC samples); and (2) SE and SW Florida (hereafter referred to as southern FL samples). Since the sample locations within each one of these two groups were indistinguishable, these were combined in subsequent analyses. Pair-wise comparisons between NW Florida and the NEFL/NC colonies showed nonsignificant or borderline significant *P*-values for two of the four comparisons ($P = 0.051$ and $P = 0.091$; Table 3), but a comparison of the combined NEFL/NC samples and NW Florida, was highly significant ($\chi^2 = 17.53$, $P \sim 0.000$). This difference, and the significant genetic distinction of NW Florida from southern Florida (its nearest neighbor), as well as NW Florida's geographic separation from the northeastern nesting locations, prompt consideration of NW Florida as an independent population unit. We conclude that loggerhead colonies within the southeastern USA are comprised of at least three genetic units corresponding to (1) northeastern Florida to North Carolina (NEFL/NC); (2) southern Florida (SW and SE Florida); and (3) NW Florida (Panhandle region),

**Table 2** *Caretta caretta.* Distribution of mtDNA haplotypes for Atlantic and Mediterranean loggerhead turtles

| Haplotype | NW Florida | SW Florida | SE Florida | NE Florida | Georgia | South Carolina | North Carolina | Mexico | Greece | Brazil |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 34 | 10 | 12 | 14 | 42 | 20 | 28 | | | |
| B | 4 | 12 | 12 | | 1 | | | 11 | 19 | |
| C | 2 | 2 | | | | | | 2 | | |
| D | | | | | | | | | | 11 |
| E | | | 1 | | | | | | | |
| F | | | | | | | | | 2 | |
| G | 2 | 1 | | | | | | | | |
| H | | | | | | | | 1 | | |
| I | | | | | | | | 1 | | |
| J | | | | | | | | 5 | | |
| Total | 42 | 25 | 25 | 14 | 43 | 20 | 28 | 20 | 21 | 11 |

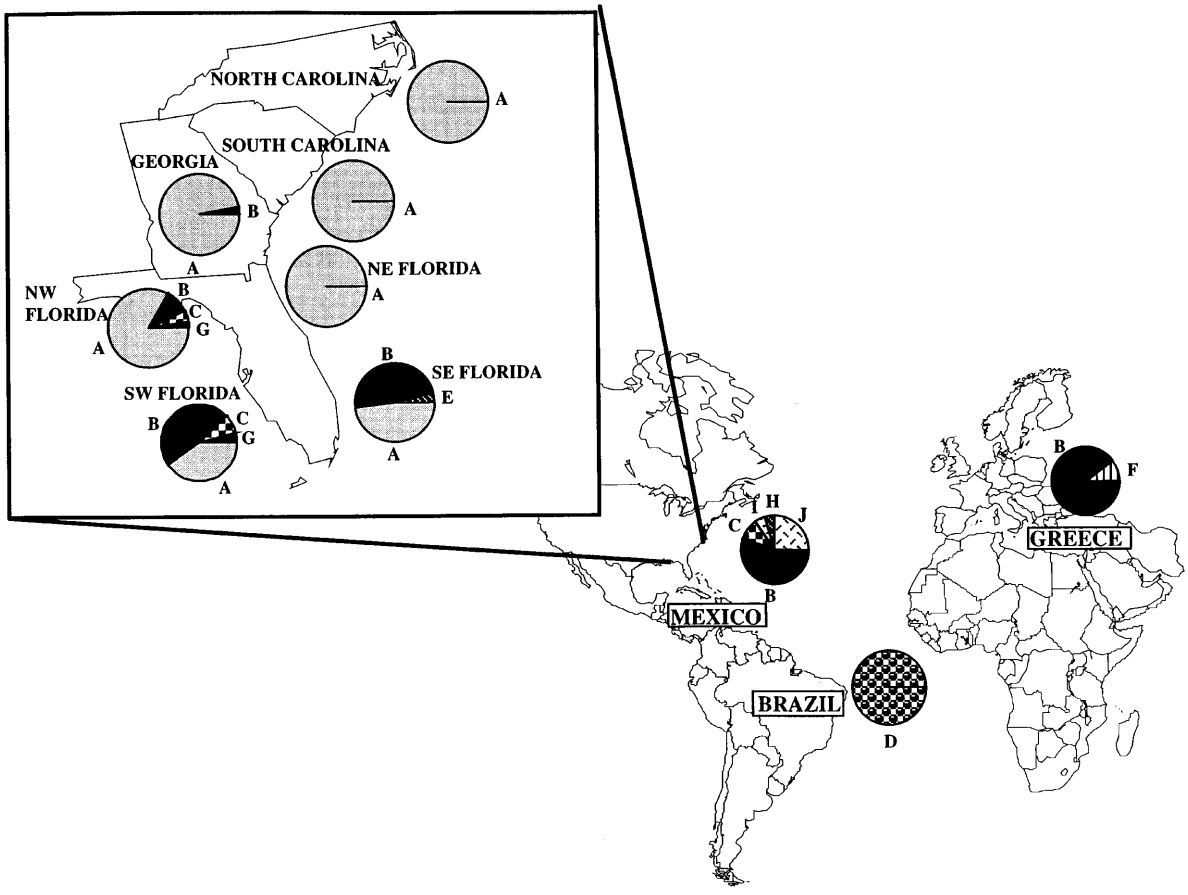


**Fig. 2** *Caretta caretta.* Sample locations of ten nesting areas. Pie charts designate the frequency of haplotypes in each location (see Table 2). *Inset*: sample locations in the southeastern USA

among which there is restricted female-mediated gene flow (Table 3).

Genotype frequency comparisons between Mexico and all other colonies show significant chi-square values, thus distinguishing the Mexican population as a separate genetic and demographic unit. The Brazilian sample was fixed for one mtDNA genotype (haplotype D), which was observed only in this region. Although the Mediterranean sample shared haplotype B with rookeries in the northwestern Atlantic, significant chi-squared values distinguished Greece from all other Atlantic colonies (Table 3). Distinction among these nesting areas was also reflected in the AMOVA, which attributed 64% of the total variation to differences among the six genetically defined nesting populations ($\Phi = 0.638$). Estimates of migration among these six population units were low: average $Nm \approx 0.3$ based on the distribution of private alleles ($Nm \approx 1.1$ using $\Phi_{st}$ to estimate gene flow). Levels of mtDNA diversity within nesting colonies are shown in Table 4. Overall haplotype and nucleotide diversity were $h = 0.67$ and $\pi = 0.023$, respectively. These values are comparable but slightly lower than those reported for Atlantic hawksbill and green sea turtles (Bass et al. 1996; Encalada et al. 1996).

## Discussion

### Population structure

The present mtDNA survey indicates that nesting loggerheads from the surveyed region are divided into at least six demographically independent cohorts, corresponding to the nesting areas of: (1) NE Florida to North Carolina, USA; (2) southern Florida, USA; (3) NW Florida, USA; (4) Quintana Roo, Mexico; (5) Bahia, Brazil; and (6) Kiparissia Bay, Greece. The distinction between southern Florida and NEFL/NC samples corroborates the earlier mtDNA restriction analysis study (Bowen et al. 1993a), which found a separation between Georgia/South Carolina and southern Florida nesting areas. Although the present study encompasses the vast majority of the known loggerhead nesting aggregates in the Atlantic Ocean and Mediterranean Sea (together, the regions surveyed account for ~35 000 to 40 000 nesting females; Murphy and Hopkins 1984), recent evidence indicates additional population units along the coasts of Greece and Turkey (Schroth et al. 1996), and the recent discovery of a loggerhead rookery in Libya raises the possibility of another population unit in the southern Mediterranean (Venizelos 1996). Small but significant nesting aggregates are also known from the Bahamas, Cuba, and western Africa (Dodd 1988). Furthermore, the low mtDNA diversity in

**Table 3** *Caretta caretta*. *Above diagonal*: pair-wise haplotype frequency comparisons based on $\chi^2$ statistics, using the Monte Carlo procedure. Numbers in parentheses indicate *P*-values for each comparison (*NS* not significant). *Below diagonal*: migration rates ($N_m$) based on $\Phi_{st}$ estimator

| Population | NW Florida | SW Florida | SE Florida | NE Florida | Georgia | South Carolina | North Carolina | Mexico | Greece | Brazil |
|---|---|---|---|---|---|---|---|---|---|---|
| NW Florida | | 14.01 (0.001) | 15.56 (0.001) | 3.11 (0.286) NS | 6.63 (0.051) NS | 4.36 (0.317) NS | 6.02 (0.091) NS | 44.00 (~0.000) | 48.13 (~0.000) | 53.00 (~0.000) |
| SW Florida | 1.3 | | 4.18 (0.525) NS | 13.65 (~0.000) | 29.29 (~0.000) | 18.00 (~0.000) | 23.43 (~0.000) | 17.71 (~0.000) | 16.36 (0.001) | 36.00 (~0.000) |
| SE Florida | 2.1 | High | | 10.92 (~0.000) | 23.88 (~0.000) | 14.63 (~0.000) | 19.29 (~0.000) | 21.76 (~0.000) | 16.36 (~0.000) | 36.00 (~0.000) |
| NE Florida | 5.0 | 0.49 | 0.68 | | 0.33 (1.00) NS | 0.00 (1.00) NS | 0.00 (1.00) NS | 34.00 (~0.000) | 35.00 (~0.000) | 25.00 (~0.000) |
| Georgia | 3.9 | 0.33 | 0.46 | High | | 0.47 (1.00) NS | 0.66 (1.00) NS | 58.77 (~0.000) | 59.69 (~0.000) | 54.00 (~0.000) |
| South Carolina | 3.9 | 0.41 | 0.57 | High | High | | 0.00 (1.00) NS | 40.00 (~0.000) | 41.00 (~0.000) | 31.00 (~0.000) |
| North Carolina | 3.2 | 0.34 | 0.47 | High | High | High | | 48.00 (~0.000) | 49.00 (~0.000) | 39.00 (~0.000) |
| Mexico | 0.18 | 1.05 | 0.73 | 0.013 | 0.024 | 0.011 | 0.0092 | | 13.12 (0.003) | 31.00 (~0.000) |
| Greece | 0.17 | 0.95 | 0.66 | 0.0028 | 0.018 | 0.0023 | 0.0019 | 4.12 | | 32.00 (~0.000) |
| Brazil | 1.6 | 0.57 | 0.76 | 0.00 | 0.20 | 0.00 | 0.00 | 0.016 | 0.0034 | |

**Table 4** *Caretta caretta*. Haplotype (*h*) and nuclotide ($\pi$) diversities for Atlantic and Mediterranean loggerhead turtles (*NEFL/NC* NE Florida, Georgia, South Carolina, North Carolina)

| Population | Haplotype diversity (*h*) | Nucleotide diversity ($\pi$) |
|---|---|---|
| NEFL/NC | 0.04 | 0.0019 |
| NW Florida | 0.44 | 0.0200 |
| South Florida | 0.59 | 0.0294 |
| Mexico | 0.65 | 0.0028 |
| Greece | 0.18 | 0.0000 |
| Brazil | 0.00 | 0.0000 |
| Total | 0.67 | 0.0230 |

nesting individuals from NE Florida to North Carolina provides inadequate resolution for detecting population divisions. It is likely that additional demographic partitions exist within this region.

The sampling design of the present study, which expanded earlier geographic surveys to include samples from North Carolina, NE and NW Florida, and Mexico (as well as larger sample sizes for previously analyzed populations), allows a finer resolution of the phylogeographic discontinuity between the NEFL/NC and southern Florida nesting areas; this break evidently exists in the region between Cape Canaveral and Jacksonville, Florida. The location of this phylogeographic break along the coast of Florida is concordant with two other lines of evidence: (a) field studies indicate relatively continuous loggerhead nesting along the southern coast of Florida from Dade to Brevard Counties, with a seemingly sharp decrease in nest density at Cape Canaveral; and (b) similar phylogeographic separations have been found for a number of coastal organisms (Avise 1992; Karl and Avise 1992; Lamb and Avise 1992).

The population structure found in the present survey is in apparent agreement with the predominant pattern revealed by long-term tagging studies, i.e., most nesting females return to the same nesting beaches in successive nesting seasons, but tag returns also indicate a low incidence of relocation between nesting populations (Bjorndal et al. 1983; Dodd 1988). For example, out of thousands of loggerhead turtles tagged in the southeastern USA, a handful of turtles originally found nesting in Georgia were recaptured nesting in southern Florida (see Bowen et al. 1993a). The transfer of a few loggerhead turtles between nesting locales is perhaps reflected in the overall higher estimates of migration rates between pairs of loggerhead rookeries than between Atlantic green turtle rookeries (Encalada et al. 1996; present study, Table 3). While natal homing behavior predominates, these rare relocations might indicate an ability to colonize newly opened nesting habitats over longer evolutionary time scales, and/or a condition of nonequilibrium resulting from recent colonization events (see below).

## Evolutionary history of Atlantic and Mediterranean loggerhead nesting colonies

The habitat requirements of this poikilothermic marine reptile indicate that the recent evolutionary history of Atlantic and Mediterranean loggerhead colonies is strongly linked to the climatic and geological history of the region. Although the distribution of loggerhead nesting extends into the temperate zones, viable nesting conditions require temperatures above 25 °C for successful incubation. Thus, nesting habitats in North Carolina, South Carolina, Georgia, northern Florida and the Mediterranean were probably not occupied during glacial episodes including the Wisconsin glacial period, which ended approximately 10 000 years BP. Subsequently, at times of glacial retreats (interglacial periods), new nesting and feeding habitat opened, allowing colonization into higher latitudes. Based on the mtDNA haplotype distributions and phylogeny, we advance the following colonization scenario.

During periods of glacial maxima of the Pleistocene, loggerhead lineages could have been maintained in regions of climatic stability along tropical equatorial latitudes. Subsequently, during interglacial periods, with the expansion of viable loggerhead nesting and feeding habitats into higher latitudes, an equatorial lineage (precursor of haplotype A) may have colonized northern latitudes (for example into Caribbean localities). Haplotype A is mentioned here because of its close relationship with haplotype D (the only haplotype observed in the tropical South Atlantic), as indicated in the parsimony network (Fig. 1). From this area, consequent colonizations may have occurred in a northerly direction along both sides of the Florida peninsula: (1) along the east coast of Florida, and (2) along the west coast into the Gulf of Mexico. One possible outcome of this colonization pathway would be decreasing haplotype diversity in recently colonized (more northerly) nesting areas, as haplotypes were sorted through a series of colonization bottlenecks. The low diversity of NEFL/NC rookeries is consistent with this expectation (Table 4). A separate transplantation of haplotypes (precursors of haplotype B) into the West Atlantic could also have occurred; based on the phylogeny of Indo-Pacific and Atlantic haplotypes, Bowen et al. (1994) suggest that precursors of haplotype B might have been of recent Indo-Pacific ancestry, and could have invaded the Atlantic via southern Africa. Colonization into the Mediterranean Sea was most likely accomplished within the last 10 000 years (after the Wisconsin glaciation), and included the transplantation of haplotype B observed in southern Florida and Yucatan. The widespread distribution of this haplotype illustrates the propensity of *Caretta caretta* for occasional long-distance colonization.

An unusual feature of these loggerhead mtDNA data is that the most divergent haplotypes (haplotypes A and B, Fig. 1) are present in turtles that nest on the same West Atlantic beaches. Using a provisional molecular clock of 0.2 to 0.4% divergence per million years calibrated for the testudines (Avise et al. 1992; Bowen et al. 1993b), and taking into account the approximate sixfold increase in resolution evident in the control region sequence data, the two primary Atlantic/Mediterranean loggerhead mtDNA lineages coalesce at around 2.1 to 4.2 million years ago. The distribution of haplotypes suggests that loggerhead turtles are active colonizers over relatively short evolutionary periods. Dispersal, natal homing behavior, climatic, and geological factors all have discernible roles in shaping the phylogeography of loggerhead turtles in the Atlantic basin and Mediterranean Sea.

## Conservation implications

A major component of management programs for the protection of endangered and threatened species is the ability to determine the level of isolation among geographically separated populations. Thus, significant haplotype frequency differences among populations can aid in defining management units (MUs), and in identifying the appropriate geographic scale for monitoring (Moritz 1994). In the case of the loggerhead turtle, the significant haplotype frequency differences among Atlantic/Mediterranean nesting aggregates indicate that at least six independent management units can be resolved for conservation purposes (it is likely that at least a few additional MUs exist in the Mediterranean, Brazil, and in the region between northern Florida and North Carolina). As is the case with other species of sea turtles, genetic separations imply demographic partitions by which nesting populations will persist or perish without significant input from other nesting aggregates (Avise 1995). In other words, while females are able to colonize appropriate habitats over evolutionary time scales, over the shorter ecological time frames relevant to wildlife management, strong natal homing behavior apparently precludes depleted rookeries from being replenished by immigration from other extant colonies.

The present study has defined the genetic demographic composition of loggerhead nesting populations in the Atlantic/Mediterranean system. This is of particular relevance for regional management given the geographic scale of loggerhead nesting in the southeastern USA (which may represent the second largest loggerhead nesting aggregate in the world). Throughout this region, it has not been possible to resolve population units by any other than genetic means (morphological, environmental, etc.). Notwithstanding the possibility of further population subdivisions in geographic areas of suspected recent evolutionary origin (NEFL/NC region), the genetic partitions observed with mtDNA allow a relatively precise definition of management units which otherwise might have gone undetected. For example, the NE Florida nesting aggregation is affiliated with the Georgia, South Carolina, North Carolina population, not with other Florida populations. Not

surprisingly, sea turtle nesting populations do not recognize political boundaries.

It is anticipated that the data presented here will have several applications in the resolution of loggerhead migration patterns and life cycles. In particular, these data can be used to resolve the composition of feeding areas (Sears et al. 1995; Bowen et al. 1996). Wildlife managers know that loggerhead turtles are killed in oceanic and coastal fisheries, but generally do not know which rookeries are impacted by this mortality. The genetic markers generated in this study can be used to identify rookery cohorts on distant feeding grounds and thereby to assess the impact of commercial fisheries on loggerhead turtles (Bolten et al. 1998). Finally, a thorough understanding of loggerhead population structure necessitates the investigation of the paternal input for this species. Male loggerheads are known to migrate to courting grounds near nesting rookeries but little else is known about their reproductive behavior. Do males also exhibit natal site fidelity? Studies based on maternally inherited mtDNA should be complemented with the analysis of nuclear loci (anonymous loci or microsatellites) to elucidate the complete population structure of Atlantic loggerhead turtles.

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