Conservation Genetics (2005) 6:235–251
DOI 10.1007/s10592-004-7821-y

Historical perspectives on population genetics and conservation of three marine turtle species

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Received 22 April 2004; accepted 26 July 2004

Key words: conservation, green turtle, hawksbill, historical biogeography, loggerhead, marine turtle population genetics, nested clade analysis

Abstract

Considerable phylogeographic and population genetic research has been conducted on marine turtles. Less attention, however, has been paid to the historical patterns and processes that have led to present patterns of genetic structure, and particularly, how these populations have responded to major climatic changes in the past. To address these questions, we analyzed previously published mitochondrial haplotype data independently for three marine turtle species, the loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), and green turtle (*Chelonia mydas*). Considering all three species, we conducted analyses on a total of 657 individuals from 36 nesting beaches in the Atlantic and Mediterranean. Our results suggest that much of the contemporary genetic structure has been significantly affected by complex patterns of historical population subdivision, long-distance dispersal, and restricted geneflow. These inferences also imply that the climatic and sea level fluctuations during the Pleistocene may have had contrasting effects on genetic structure (e.g., fragmenting versus homogenizing) and on population sizes. Estimates of historical and current effective population sizes further highlight differential demographic responses across species to historical climatic cycles. Collectively, our results provide evidence for the occurrence of historical refugia through climatic cycles and complex historical metapopulation dynamics, and identify common and unique patterns of metapopulation structure across species. These historical patterns provide a basis for predictive estimates of metapopulation responses to habitat loss, population extirpation, and global climatic change.

Introduction

Population genetics and phylogeography of natural populations are intimately tied to life-history, which for marine turtles is marked by long maturation time, enigmatic dispersal characteristics, and isolated nesting habitats. The unique biology of marine turtles includes a complex life history characterized by multiple distinct phases, each with unique habitat and geographic range associations. Although marine turtle species share many life-history characteristics, patterns of genetic diversity vary among species based on life-history strategies and historical population processes (Bowen et al. 1994; Bass et al. 1996; Encalada et al. 1996). Here we reexamine and compare published data on three species of marine turtles that use Western Atlantic and Mediterranean beaches as nesting grounds, the loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), and green (*Chelonia mydas*) sea turtles. As threatened or endangered species within and outside of the United States, these species have been subjects of increased conservation efforts over the past 20 years.

The wide geographic distribution of marine turtles over their lifetime contrasts with the discrete subset of that total range over which reproductive events are localized. Marine turtle migratory behavior often consists of vast journeys spanning entire ocean basins (Carr 1987; Witzell and Banner 1980; Parmenter 1983; Mortimer and Carr 1987; Limpus et al. 1992; Bolten et al. 1998). Despite these tendencies for massive migratory events and the potential for mixing of populations, each of these three species generally displays high levels of mitochondrial genetic separation between nesting aggregations. Two key life-history traits have led to this separation, natal homing and nest site fidelity. Natal homing refers to the propensity for mature females to return to the nesting beach of their natal origin for deposition of eggs. Nest site fidelity refers to the subsequent return, year after year, to the same beach for nesting. The combination of these traits results in the localization of the same female genetic stock on the same beach generation after generation. Here, we refer to natal homing (NH) and nest site fidelity (NSF) as a complex of forces (NH/NSF) that may affect the dispersal patterns of marine turtle matrilines.

Natal homing and nest site fidelity (NH/NSF) have been demonstrated to some degree for each of the species in question. Florida loggerheads have been observed to exhibit NH/NSF on a scale of approximately 100 km (B. Bowen, personal communication). Green turtles in Florida display a higher degree of NH/NSF, often within 10 km of their previous nest deposition (Carr et al. 1978; Balaz 1980; Limpus et al. 1992). High levels of NH/NSF in hawksbills have been demonstrated in the Caribbean (Bass et al. 1996); however, movement between adjacent beaches or islands is reported in the Indian and Pacific Oceans (Diamond 1976; Limpus et al. 1983).

In addition to differences in migratory and nesting behavior across species, marine turtles occupy unique ecological niches. Loggerheads nest primarily in warm temperate regions (Pritchard and Trebbau 1984), whereas green turtles and hawksbills nest primarily in the tropics (Bowen et al. 1992; Bass et al. 1996). Efforts to conserve marine turtle species require consideration of these complex life-history traits, along with an understanding of how the population biology of these species has shaped genetic population structure through time, and will continue to do so in the future.

Here we employ an array of statistical estimates of among-population gene flow and historical population processes, including nested clade analysis (NCA; Templeton 1998), to address large-scale patterns of population history and genetic structure in three marine turtle species. Traditional interpretations of NCA need to be altered slightly in the case of marine turtles because reproduction is confined to only a small subset of their otherwise wide overall ranges. We employ NCA to infer processes centered on the geography of the reproductive portion of marine turtle populations (i.e., nesting beaches), which drastically contrasts with inferences regarding their overall distribution. Inferences of population fragmentation must be modified from the traditional interpretation derived from terrestrial systems, because in the case of marine turtles, fragmentation of nesting colonies occurs when NH/NSF sufficiently constrains geneflow between nesting populations. In addition to NCA, we employ neutrality test statistics, analyses of relationships between geographic distance and population genetic differentiation, and observed distributions of pairwise nucleotide differences, to infer historical patterns of population dynamics and differentiation. We compare estimates of historical patterns of population contraction and effective population size to integrate inferences of historical demography with phylogeography. Overall, our goal was to exploit the overlap in inference capability across statistical methods to formulate broad cross-validated hypotheses for historical patterns and processes, decreasing the biases or shortcomings of any single analytical method (e.g., Knowles and Maddison 2002; Masta et al. 2003). We believe that such robust historical perspectives represent a useful resource for constructing long term goals for marine turtle conservation, given the insight it provides on historical and ongoing patterns of geneflow and its predictive potential under scenarios of habitat loss, population extirpation, and global climatic change.

Methods

In this study, previously published data on the same portion of the mitochondrial control region (~400 bp) was analyzed for three marine turtle species. Loggerhead turtles (*Caretta caretta*) were evaluated using data from Pearce (2001) and

Laruent et al. (1998). This combined dataset includes 417 individuals from 20 populations in Florida, Mexico, Brazil, and the Mediterranean. Hawksbill turtles (*Eretmochelys imbricata*) were evaluated using data from Bass et al. (1996) including 93 individuals from 7 populations in the Caribbean and Brazil (excluding hawksbill/loggerhead hybrids). Green turtle (*Chelonia mydas*) populations were evaluated using data from Encalada et al. (1996) including 147 individuals from 9 populations on the east and west coasts of the Atlantic Ocean and the Mediterranean.

Haplotype diversity (h; Nei 1987), nucleotide diversity (π; Nei 1987), and the average number of nucleotide differences (k; Tajima 1983) for each species and per population were estimated in DnaSP v4.0 (Rozas et al. 2003). The number of migrants (N_m) was estimated for each species (according to Nei 1973) and F_{st} values between populations were estimated (based on Hudson et al. 1992) in DnaSP. Geographic distance and F_{st} values between populations were plotted to investigate correspondence between geographic and genetic distance across populations. Linear regressions were fit to F_{st} versus geographic distance plots of groups of populations for each species to estimate correlation statistics. The overall significance of correlations between F_{st} and geographic distance matrices (per species) was tested using one-tailed mantel tests with 10,000 permutation replicates implemented using the Poptools v2.5.9 (Hood 2003) software supplement in MS Excel. To test the hypothesis that control region variation per species does not differ from neutral expectations, Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1989) tests of neutrality were conducted in DnaSP.

We calculated the effective female population sizes (N_{ef}) for each species from the formula theta (θ) = $2N_{ef}v$ (Tajima 1993). Theta (per DNA sequence) was estimated from the infinite-site equilibrium relationship between the number of segregating sites and the sample size (Watterson 1975), implemented in DnaSP. To solve this equation, we estimated v (= sequence length [m] × the mutation rate per generation [μ]). We used an approximation of the generation time at 30 years for each species (L. Ehrhart, P. Pritchard, personal communication), and the mutation rate estimated by Encalada et al. (1996) for the control region of green turtles at ~2% per million years. Pairwise haplotype mismatch distributions (Rogers and Harpending 1992; Rogers 1995) under a model of constant population size and a model of population growth/decline were performed in DnaSP to identify patterns of historical population expansion or contraction. Based on models of population growth/decline, estimates of the time since the last major population contraction were calculated from tao (τ). The number of years since population contraction was estimated from the equation $\tau = 2\ \mu t$, where t is the number of generations since population contraction. The N_{ef} prior to population contraction was estimated from θ initial (θi), following the calculations for N_{ef} above.

Evolutionary relationships among haplotypes within species were inferred by constructing 95% plausible parsimony networks in TCS v1.13 (Clement et al. 2000) based on statistical parsimony (Templeton 1998). For these analyses, gaps in alignment were treated as a 5th character, multiple base gaps were coded as a single character/event, and cladograms were estimated for each species independently. Statistical parsimony networks estimated in TCS were used to construct the nesting design for NCA analyses (Templeton 1998). Nested cladograms were constructed by hand based on a haplotype network following the guidelines of Templeton et al. (1992) and Templeton (1998). Since all species analyzed are marine, we used GIS software (ArcView v3.2, ESRI) to estimate the shortest over-water (oceanic) distance between all sampled sites and used these distances to construct a distance matrix between sites for each species (e.g., Fetzner and Crandall 2003). The nested clade design was used along with geographic location, designated by the constructed distance matrix, to analyze geographic associations among hierarchically nested clades using the program GeoDis v2.0 (Posada et al. 2000). Statistical significance was calculated by comparison with a null distribution generated from 10,000 random permutations of clades against sampling localities. The results of GeoDis analyses were interpreted based on the revised inference key provided by Templeton (2004).

Results

Loggerheads

Ten mitochondrial control region polymorphisms were documented on Florida, Mexico, Brazil, and

Mediterranean beaches. Overall loggerhead haplotype diversity, although moderately high ($h = 0.5867$), was the lowest observed across the three species (Table 1). Overall nucleotide diversity ($\pi = 0.027$) and the average number of nucleotide differences ($k = 7.891$) were the highest among the three species. These relative trends in h, π, and k were observed across a majority of eastern Atlantic populations, yet contrasted with trends of low h and π in Mediterranean populations. The estimated N_m across populations of loggerheads was the highest among species ($N_m = 0.76$; Table 1). Neutrality statistics rejected neutral evolution ($D = 4.015$, $P < 0.01$; $Fs = 22.548$, $P < 0.01$; Table 1), suggesting that loggerhead control region haplotypes defy neutral patterns.

The estimate of θ from the number of segregating sites was $\theta = 2.9386$ (Table 1). This provided an estimate of the current N_{ef} for loggerheads at 64,106 (Table 1). The distribution of the pairwise nucleotide differences across loggerhead haplotypes was bimodal and independent peaks were well-differentiated (Figure la). This observed distribution differed substantially from null models assuming either constant population size or population growth/decline primarily because these models are unimodal, whereas our data were bimodal (Figure la). Based on a model of population growth/decline, a population contraction was inferred to have taken place ca. 2.8 million years ago, prior to which, the N_{ef} was inferred at 171,654 (Table 1).

The haplotype network construction in TCS identified haplotypes B and D as ancestral haplotypes (Figure 2) based on root probability density criterion (Templeton 1998). The two ancestral

Table 1. Summary of population genetic statistics for all three species sampled

Parameter	Loggerhead	Hawksbill	Green
	Caretta caretta	*Eretmochelys imbricata*	*Chelonia mydas*
Number of sequences	417	93	147
Number of sites	391	382	496
Segregating sites (s)	20	16	18
Unique haplotypes	7	17	17
Haplotype diversity (h)	0.587	0.823	0.830
Nucleotide diversity [per site] (π)	0.021	0.010	0.010
Average number of nucleotide Differences (k)	7.891	3.745	4.700
N_m	0.76	0.53	0.45
Neutrality Tests			
Tajima's D	4.01499*	0.74663	1.03341
Fu's Fs	22.548*	−2.248	0.106
Current N_{ef} Estimates			
Theta [per seq.] (θ)	3.026	2.939	3.235
Current N_{ef}	66,185	64,106	54,351
Historical Inferences			
Theta initial [per seq.] (θi)	7.848	2.297	2.908
Tao (τ)	4.341	1.427	1.793
Time since population contraction (years before present)	2,848,425	933,901	903,730
N_{ef} prior to population contraction	171,654	50,109	48,858

(* = significant at $P < 0.001$).

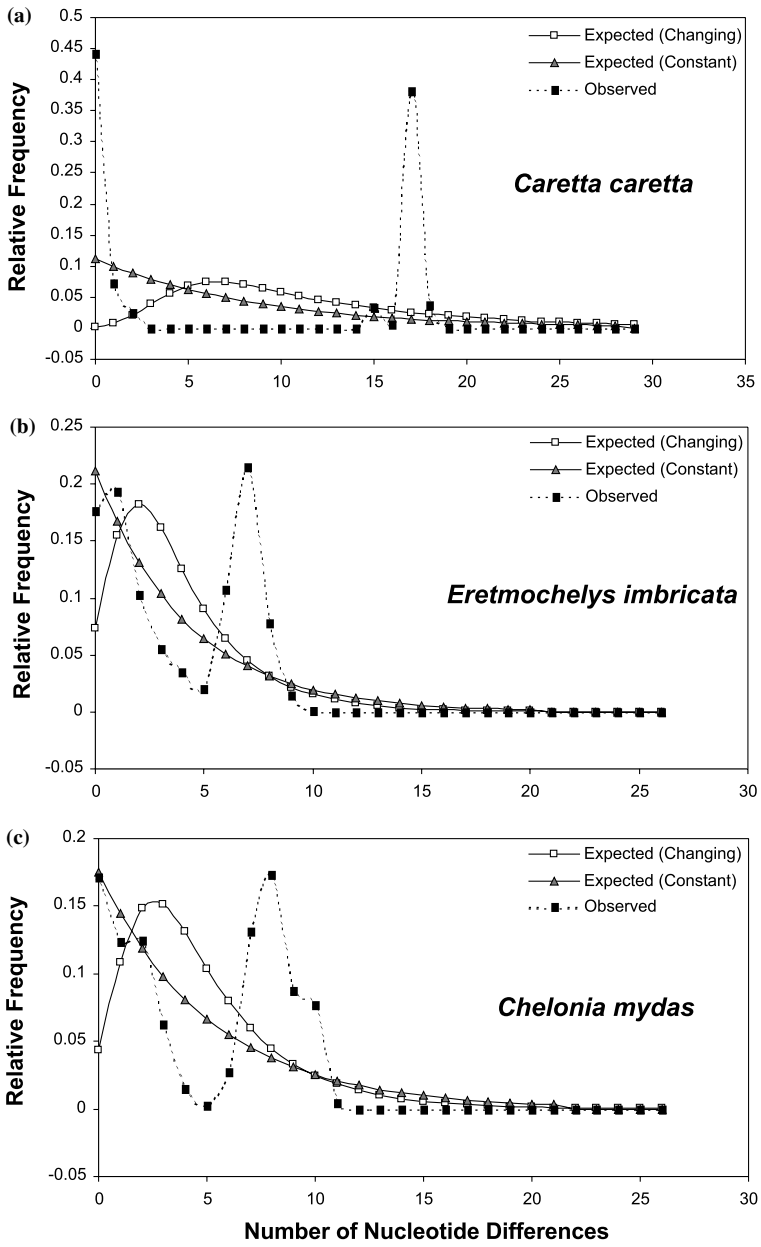

Figure 1. Distribution of observed frequencies of pairwise nucleotide differences, with frequencies expected under models of constant population size and models of population growth/decline for each species of marine turtle sampled. (a) Distribution of pairwise differences for loggerheads (*Caretta caretta*). Expected frequencies generated with $\theta i = 2.297$, θ final = infinite for constant population size model, and θ final = 1000 for growth/decline model. (b) Distribution of pairwise differences for hawksbills (*Eretmochelys imbricata*). Expected frequencies generated with $\theta i = 2.908$, θ final = infinite for constant population size model, and θ final = 1000 for growth/decline model. (c) Distribution of pairwise differences for green turtles (*Chelonia mydas*). Expected frequencies generated with ($\theta i = 7.848$, θ final = infinite for constant population size model, and θ final = 1000 for growth/decline model.

loggerhead haplotypes differed by 16 mutational steps (excluding multiple base gaps). Restricted geneflow and dispersal, possibly with limited long-distance dispersal, was inferred (clade 1–1) among all Florida and Mediterranean populations sampled, except Amelia Island (Table 2). Continuous range expansion was inferred for clade 2–1 between Brazil and all Florida sites (Table 2).

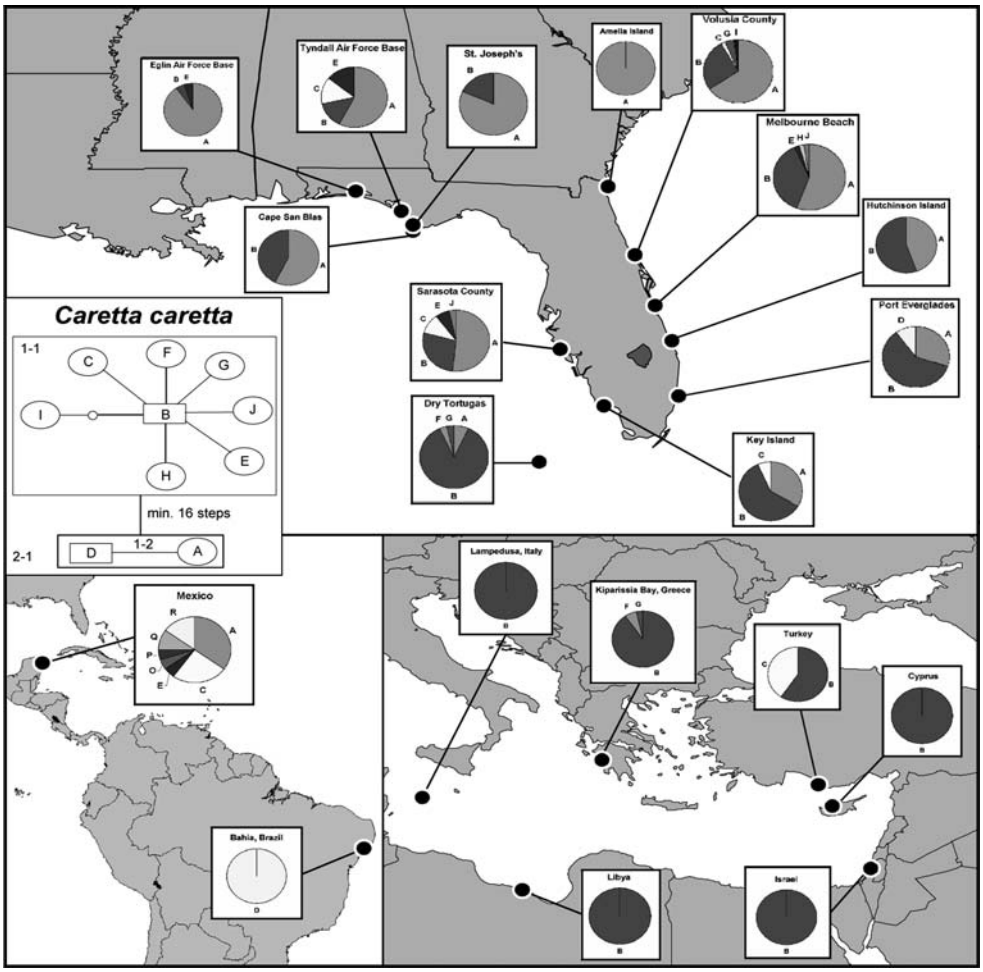

Figure 2. Map of sampled loggerhead (*Caretta caretta*) nesting populations. Haplotype frequencies per nesting population are shown in pie charts. The nested cladogram for loggerheads is also given (ovals represent sampled haplotypes, small circles represent inferred haplotypes not sampled, squares represent haplotypes inferred as ancestral by TCS).

Pairwise geographic distances between populations plotted against pairwise F_{st} distances between populations showed a moderate overall trend suggesting isolation by distance across populations evidenced by positive correlation between F_{st} and geographic distances (overall $r = 0.443$). The mantel test comparing the geographic distance and F_{st} matrices indicated that F_{st} and geographic distances between populations were non-randomly distributed ($P < 0.05$).

Hawksbills

A majority of hawksbill control region polymorphisms (15 of 17) were unique to individual nesting colonies (as reported by Bass et al. 1996). Haplotype diversity was high in hawksbills ($h = 0.823$) although overall nucleotide diversity ($\pi = 0.010$) and the average number of nucleotide differences ($k = 3.745$) were comparatively low (Table 1). These trends were consistent across populations of hawksbills. The estimated N_m across populations of hawksbills was intermediate among the three species ($N_m = 0.53$, Table 1). Neutrality statistics did not rejected neutral patterns of evolution for hawksbill haplotypes (Table 1).

The estimate of theta ($\theta = 2.9386$; Table 1) was the lowest among species, yielding an estimate of N_{ef} for hawksbills at 54,351. The distribution of the pairwise number of nucleotide differences was bimodal and independent peaks were moderately differentiated. This observed distribution differed

Table 2. Results from nested clade analysis for loggerhead, hawksbill, and green turtle haplotype data

Nested clade	Chain of inference	Final inference
Loggerheads		
1–1	1–2–3–5–6–7	Restricted geneflow/dispersal but with some long-distance dispersal
1–2	1–2–11–17	Inconclusive outcome
2–1	1–2–11–12	Continuous range expansion
Hawksbills with unresolved loops		
1–3	1–2–11–17–4	Restricted geneflow with isolation by distance
1–4	1–2–11–17–4	Restricted geneflow with isolation by distance
2–1	1–2–11–17–4–9	Allopatric fragmentation
2–2	1–2–11–17–4	Restricted geneflow with isolation by distance
2–3	1–19	Allopatric fragmentation
3–1	1–2–11–12	Continuous range expansion
Hawksbills with estimated resolved loops		
1–1	1–2–3–4	Restricted geneflow with isolation by distance
1–4	1–2–3–5–6–7–8	Restricted geneflow/dispersal, but with some long-distance dispersal; or past geneflow followed by extinction of intermediate populations
2–1	1–2–11–17–4–9	Allopatric fragmentation
2–2	1–2–11–17–4	Restricted geneflow with isolation by distance
2–3	1–19	Allopatric fragmentation
3–1	1–2–11–12	Continuous range expansion
Green Turtles		
1–3	1–2–3–5–6–13	Long-distance colonization with subsequent fragmentation
1–7	1–2–11–12	Continuous range expansion
2–1	1–2–3–5–15–21	Long-distance colonization, possibly associated with fragmentation event
2–2	1–2–11–12	Continuous range expansion
3–1	1–2–11–17–4–9	Allopatric fragmentation

Inferences based on Templeton 2004.

substantially from null models assuming either constant population size or population growth/decline (Figure 1b). Based on a model of population growth/decline, a population contraction was inferred ca. 900,000 years ago, prior to which, the N_{ef} was inferred at 50,109 (Table 1).

The nested cladogram for hawksbills included one 3-step clade, three 2-step clades, and seven 1-step clades (Figure 3). The central haplotype (Q) was found only in Mexico. Two homoplacious loops were inferred between clades 1–1 and 1–3, and between 1–3 and 1–4. Alternative resolutions of these loops were analyzed *via* NCA to determine their potential effects on historical inferences. In all cases, the inferences of the two step clades were the same; with the exception that clade 2–2 (in one alternative) resulted in long-distance colonization rather than restricted geneflow with isolation by distance (Table 2). The majority of loop resolutions resulted in restricted geneflow with isolation by distance for clade 2–2 (Table 2). In the resolved nesting scheme, we found evidence for restricted gene flow with isolation by distance in the majority of one and two step clades (Table 2). We also found evidence for restricted geneflow/dispersal or past geneflow followed by the extinction of intermediate populations for haplotypes in Mexico, Puerto Rico, and Belize (clade 1–4). Overall, past fragmentation was inferred for Caribbean populations, with evidence for subsequent long distance colonization and range expansion in the total cladogram including all clades.

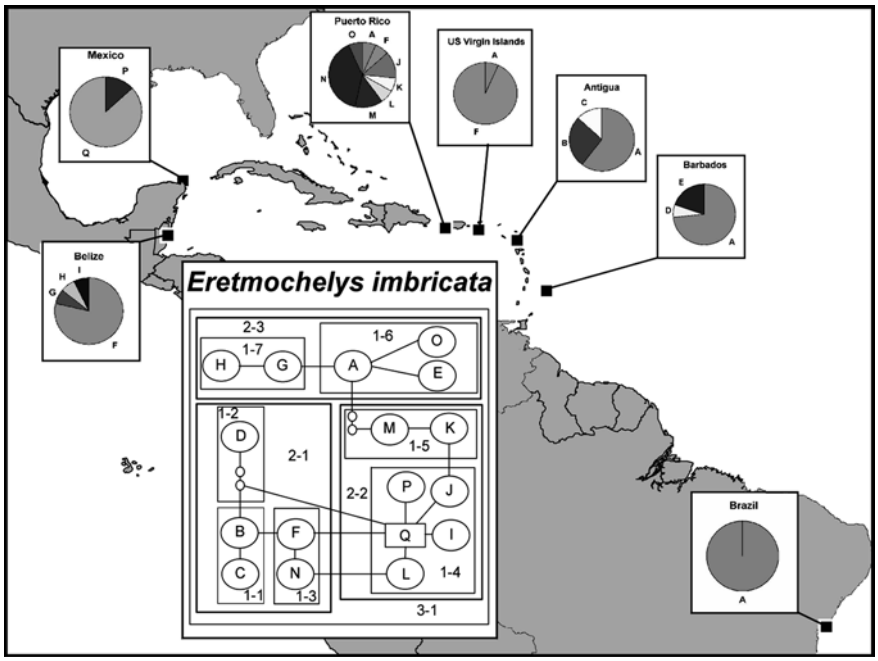

Figure 3. Map of sampled hawksbill (*Eretmochelys imbricata*) nesting populations. Haplotype frequencies per nesting population are shown in pie charts. The nested cladogram for hawksbills is also given (ovals represent sampled haplotypes, small circles represent inferred haplotypes not sampled, squares represent haplotypes inferred as ancestral by TCS).

Pairwise geographic distances between populations plotted against pairwise F_{st} distances between populations showed a moderate trend indicating isolation by distance ($r = 0.419$). The mantel test comparing geographic distance and F_{st} matrices did not indicate a significant difference between matched and randomized values ($P > 0.05$). Subdividing populations by major region resulted in a weak correlation between F_{st} and geographic distance across Caribbean populations ($r = 0.150$) and a moderately strong correlation between Brazil and Caribbean populations ($r = 0.708$).

Green Turtles

Similar to hawksbills, green turtle haplotype diversity was high ($h = 0.830$; Table 1) and nucleotide diversity and the average number of nucleotide differences was comparatively low ($\pi = 0.010$, $k = 4.700$; Table 1). Haplotype and nucleotide diversity varied considerably across populations of green turtles. Mexican and Brazilian populations had the highest h while populations in Guinea Bissau, Cyprus, and Costa Rica had the lowest h. The estimated N_m across populations of green turtles was the lowest among species ($N_m = 0.45$; Table 1). Neutrality statistics did not reject neutral evolution for green turtle haplotypes (Table 1).

The estimate of theta was $\theta = 3.235$, yielding an estimate of the N_{ef} for green turtles at 654,351 (Table 1). The distribution of the pairwise number of nucleotide differences was bimodal and independent peaks were not completely differentiated. This observed distribution did not correspond well with distributions from null models assuming either constant population size or population growth/decline (Figure 1c). Based on a model of population growth/decline, a population contraction was inferred to have taken place ca. 900,000 years ago, prior to which, the N_{ef} was inferred at 48,858 (Table 1).

The nested cladogram included 18 unique haplotypes and 5 inferred unsampled haplotypes, nested as one 3-step clade, two 2-step clades, and seven 1-step clades (Figure 4). Allopatric fragmentation was inferred for the total cladogram (Table 3). Long distance colonization, possibly associated with a fragmentation event, was inferred for clade 2–1 including haplotypes from

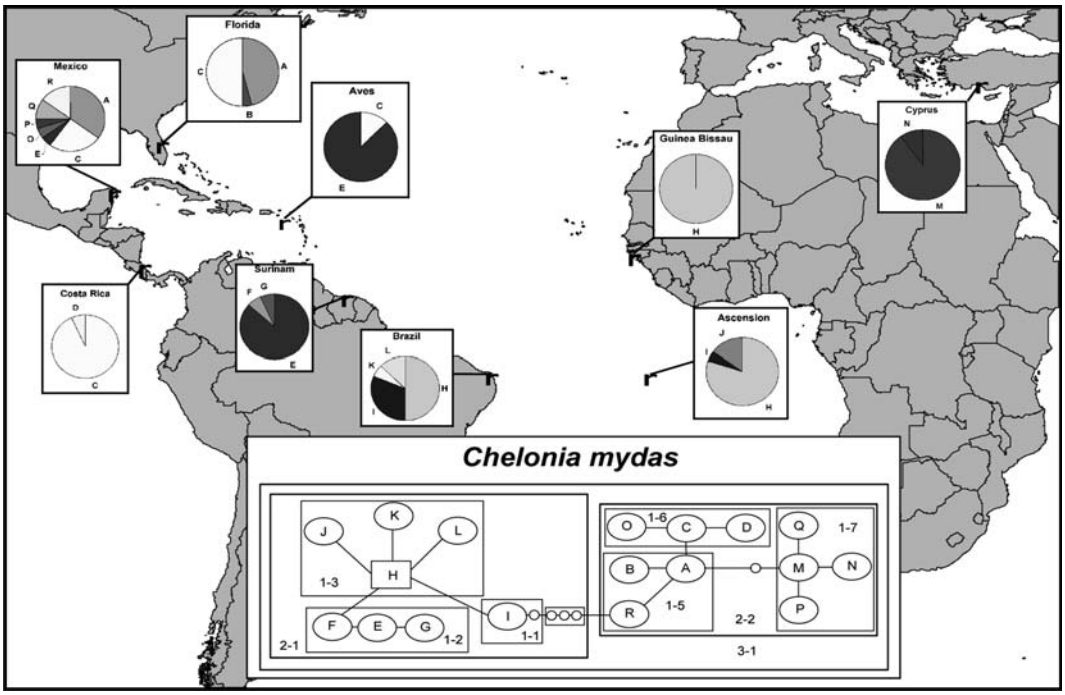

Figure 4. Map of sampled green turtle (*Chelonia mydas*) nesting populations. Haplotype frequencies per nesting population are shown in pie charts. The nested cladogram for green turtles is also given (ovals represent sampled haplotypes, small circles represent inferred haplotypes not sampled, squares represent haplotypes inferred as ancestral by TCS).

Surinam, Aves Island, Mexico, Brazil, Ascension Island, and Guinea Bissau. Long-distance colonization and subsequent fragmentation of populations in Brazil, Ascension Island, and Guinea Bissau was inferred for clade 1–3 (Table 3). Continuous range expansion was inferred for both nested clades that included Caribbean and Mediterranean haplotypes (1–7 and 2–2; Table 3).

Pairwise geographic distances between populations plotted against pairwise F_{st} distances between all populations showed a moderately correlated positive trend suggesting isolation by distance ($r = 0.546$). The mantel test comparing geographic distance and F_{st} matrices did not show significant correlation between matched values and randomized values ($P > 0.05$).

Discussion

Loggerheads (Caretta caretta)

Among the three species in this study, loggerheads were characterized by relatively low endemism, depressed genetic structure, and elevated levels of haplotype panmixia and geneflow between populations (Table 1). These data are consistent with the hypothesis that loggerheads have the lowest levels of NH/NSF among the three species studied. The relationship between F_{st} and geographic distance between populations was significantly nonrandom (based on a mantel test), although these were only moderately correlated ($r = 0.443$), implicating distance as a contributing factor determining relationships and geneflow among populations. Evidence for similar patterns was observed in NCA analyses (clade 1–1).

Results of our analyses on loggerheads in the Mediterranean and Atlantic suggest a metapopulation scenario with strong source–sink relationships among distant populations. Historical periods of climatic depression are thought to have shifted the range of suitable nesting habitat towards the equator, while simultaneously affecting dispersal patterns *via* changes in the geography of coastlines resulting from sea-level fluctuations (Nairn and Stehli 1986). Loggerheads require sand temperatures of at least 25 °C to successfully nest

(Dodd Jr 1988). Climatic depression at the Pliocene–Pleistocene border would likely have confined loggerhead nesting to southern Florida, the Caribbean, and near-equatorial regions. Thus, northern portions of Florida may not have been continuously suitable for loggerhead nesting until after the last glacial period (Hedgpeth 1954; Pearce 2001). Moreover, Pleistocene fluctuations in sea level have resulted in a tremendously dynamic Florida coastline over the last two million years (Nairn and Stehli 1986; Webb 1990). Therefore, over recent evolutionary time, Florida loggerhead populations have been continually displaced as the coastline has cyclically changed. Additionally, Mediterranean nesting grounds would have been unsuitably cold during much of the Pleistocene to harbor nesting populations of loggerheads (Bowen et al. 1994; Encalada et al. 1996).

Restricted geneflow with some long-distance dispersal, inferred for clade 1–1 (Figure 2), suggests that populations in Florida and Mexico may have been sources of colonization of Mediterranean populations after that area again became suitable for nesting (as suggested by Bowen et al. 1993; Encalada et al. 1998). The relationship between F_{st} and geographic distance between populations yielded moderately strong correlations only in comparisons of Mexican versus other Caribbean and Florida populations ($r = 0.744$). Collectively these data support the hypothesis that more tropical nesting grounds, including Mexico/southern Florida may have acted as Pleistocene refugia for Western Atlantic loggerhead populations.

The haplotype diversity of loggerheads shows a unique pattern relative to other species in this study, including a deep divergence between two haplotype clades in a (clades 2–1 and 2–2; Figure 2), yet otherwise shallow population genetic structure. These patterns are also evident in the distribution of pairwise differences (Figure la) in the form of well-differentiated bimodal peaks also suggesting a historical population subdivision. The significantly positive value of Tajima's D statistic (4.015) further supports the hypothesis of a substantial historical bottleneck (Tajima 1993). The strongly positive, significant value of Fu's Fs (22.548) suggests loggerhead population expansion (Aris-Brasou and Excoffier 1996; Fu 1997) following this bottleneck. Evidence for expansion is corroborated by NCA inferences of continuous range expansion throughout Florida (clade 2–1). Collectively, loggerhead data suggest that a panmictic ancient population may have become subdivided and experienced a major bottleneck, probably prior to the Pleistocene (given deep divergence between 2-step clades; Figure 2), and subsequently experienced substantial population expansion.

Unimodal models of either constant population size or population growth/decline failed to closely fit the observed frequencies of nucleotide differences among loggerhead haplotypes (Figure la). Therefore, we discuss demographic estimates derived from these models tentatively, verifying inferences with independent lines of evidence. Both frequency peaks in the plot of nucleotide differences across haplotypes are well differentiated and steep-sloped (Figure la), potentially indicative of expansion (from two sources) following a bottleneck that resulted in two population subdivisions (Slatkin and Hudson 1991; Rogers and Harpending 1992). Several authors (e.g., Aris-Brasou and Excoffier 1996; Schneider and Excoffier 1999) have shown that estimates of tao and N_{ef} prior to bottlenecks can be made without much bias (even in poorly fitting models), whereas estimates of the present-day θ (and hence N_{ef}), are commonly biased upwards. Under a population growth/decline model, a population contraction is inferred to have taken place ca. 2.8 million years ago. This estimate falls within the Late Pliocene and is generally coincident with a number of significant historical events including: (1) the final closing of the Central American Isthmus leading to major changes in nutrient load, upwelling, and current patterns in the western Atlantic and Caribbean (Allmon et al. 1996; Coates and Obando 1996; Kameo 2002), (2) the initiation of glacial cycles and sea-level fluctuations that would continue through the Pleistocene (e.g., Jansen et al. 1988; Cronin 1990), and (3) patterns of widespread extinction of western Atlantic and Caribbean invertebrates and vertebrates (reviewed by Allmon 2001). A major historical bottleneck in loggerhead populations is consistent with historical events associated with the late Pliocene and the effects we would expect these events to have on a sub-tropical nesting marine turtle. These conclusions also support those of Bowen et al. (1994) that processes of rookery extinction and recolonization over time have homogenized populations and prevented accumulation of extensive mutational separation

among nesting populations and extant haplotypes in loggerheads.

The loggerhead population is currently estimated at 21,831 nesting females in the Atlantic and Mediterranean (Ehrhart et al. 2003). These estimates are conservative due to inconsistent population surveys and limited data on remote nesting beaches. Our estimates of the current effective population size of females ($N_{ef} = 66{,}185$; based on θ) overestimate current census population sizes.

The parameters used to estimate N_{ef} (mutation rate and generation time) are admittedly rough estimates of poorly known life history and evolutionary processes of marine turtles. Additionally, the estimate of θ used to derive N_{ef} is biased by its dependence on nucleotide diversity. In the case of loggerheads, this value of θ is inflated due to the large divergence between haplotype clades (yet depressed within-clade diversity). Despite potential sources of error, our estimates of the current N_{ef} are not unreasonably high considering the dramatic population decline estimated to have occurred over the last few centuries. The long female generation time and lifespan characteristic of marine turtles would be expected to result in a substantial lag period between the timing of a recent bottleneck and the time required for a population decline to be manifest in the genetic composition of populations (see also Zhang et al. 2003). Thus, we expect estimates of present-day N_{ef} to be largely insensitive to population decline occurring in recent generations, probably since the beginning of major human impacts over the last few centuries. Estimates of N_{ef} preceding an inferred Late Pliocene bottleneck and genetic estimates of the current N_{ef} (66,185), together with recent estimates of decline that we assume has not had sufficient time to affect genetic estimates of N_{ef}, suggest that loggerheads have suffered an ongoing long-term trend of population decline since the Late Pliocene.

Hawksbills (Eretmochelys imbricata)

A high proportion of beach-specific (endemic) haplotypes were observed among populations of hawksbills. Relative to the more temperate-nesting loggerheads, hawksbills show elevated numbers of unique haplotypes, elevated haplotype diversity, and reduced overall N_m (Table 1). These trends correspond well with elevated levels of NH/NSF reported for hawksbills relative to loggerheads. In contrast to loggerheads, hawksbill populations were highly structured, and genetic distance among haplotypes did not show a comparably deep bifurcation, leading to overall lower nucleotide diversity in hawksbills.

Inferences from NCA analyses reinforce the potential for hawksbill rookeries to be genetically isolated by distance, consistent with elevated levels of hawksbill NH/NSF. Collectively, these inferences also suggest all populations except Mexico may have experienced moderate geneflow historically, and have since become allopatrically isolated, largely due to isolation by distance.

Relative to loggerheads, nesting density of the more tropical hawksbill is more focused in the Caribbean. Pleistocene effects on climate, together with changes in sea level, represent major potential forces dictating historical patterns of geneflow among hawksbill populations. Most notably, several large, shallow shelves or platforms exist in the Caribbean, which would have drastically changed the surface geography of the basin, altering overwater connectivity and the distribution of nesting and foraging habitats. Enormous expanses of area associated with these shelves or platforms are submerged between 10 and 100 m and would have formed extensive landmasses during even slight decreases in sea level during the Pleistocene (Stuart 1966; Westphal 1998). The emergence of the Nicaraguan Rise (Stuart 1966), drastically extending the NW coastline of Honduras, probably forged a broad barrier separating rookeries in northern and southern Middle America and those of the northern and southern Caribbean. The emergence of the Campeche Bank (doubling the area of the Mexican Yucatan Peninsula) and the Florida Shelf (expanding the Florida Peninsula), would have decreased the distance between Mexican and Florida rookeries, while reducing marine connectivity across the Caribbean (Stuart 1966; Nairn and Stehli 1986; Westphal 1998). As a result of increased fragmentation of the Caribbean during the Pleistocene, hawksbill populations may have become increasingly isolated, decreasing geneflow and substantially isolating matrilines. We suggest that the formation of these land barriers may explain the separation of Mexico from eastern Caribbean populations and the overall trend of high haplotype endemicity.

We assume that cyclic patterns of climatic and sea-level change during the Pleistocene likely resulted in corresponding population contraction and expansion, affecting population sizes and extirpation/recolonization of populations, as suggested by Encalada et al. (1996) for tropically nesting green turtles. These historical population dynamics are distinct from Pliocene–Pleistocene effects on loggerheads because we expect a significant portion of the nesting range of hawksbills (more equatorial populations) to have remained constant through the Late Pliocene and Pleistocene. The non-significant neutrality statistics estimated for hawksbills (Table 1) implies that they have not experienced major contractions or expansions since, perhaps, the Late Pliocene. This is consistent with the complex genetic structure observed across hawksbill populations (cf. Bertorelle and Slatkin 1995), refuting the hypothesis of a major historical bottleneck in hawksbill populations, which would have resulted in a substantial reduction in haplotype diversity (contrary to loggerheads).

Pleistocene effects on tropical climates are incompletely resolved (e.g., Stanley and Ruddiman 1995; Hostetler and Mix 1999; Allmon 2001), although available evidence suggests that Pleistocene climatic change drastically altered climates at middle and high latitudes, while having reduced effects on low latitudes (e.g., Hostetler and Mix 1999; Liu and Herbert 2004). Also, Excoffier and Schneider (1999) have shown multiple bottlenecks and/or multiple population expansion events may obscure resolution of historical demographics from DNA sequence data (especially neutrality statistics). Therefore, despite non-significant neutrality statistics, we expect that hawksbills have undergone minor population contractions and expansions with climatic cycling during the Pleistocene. These climatic changes likely concentrated impacts on peripheral populations/rookeries at higher latitudes, with decreased impacts on lower latitude populations where effects on sea level and population connectivity (rather than temperature) predominated.

Unimodal models of either constant population size or growth/decline did not fit the distribution of nucleotide differences for hawksbills (Figure 1 b), thus inferences based on these models are tentative. The observed distribution is bimodal, implicating past population subdivision. The distinction between modes is much less dramatic than that for loggerheads, suggesting a more recent subdivision, and potentially a shorter duration of subdivision and/or a less dramatic population contraction associated with subdivision (Slatkin and Hudson 1991; Rogers and Harpending 1992; Excoffier and Schneider 1999). In contrast to loggerheads, peaks in frequencies of nucleotide differences for hawksbills are left-skewed, suggesting historical population expansion following relatively minor population contractions and subdivision (Slatkin and Hudson 1991; Rogers and Harpending 1992). This interpretation is supported by the expectation that the geographic range of suitable nesting habitat for hawksbills has expanded since the last glacial period (and during historical glacial periods in general).

We estimated a historical population contraction in hawksbills, based on estimates of tao, to have taken place ca. 900,000 years ago, which is broadly consistent with expected contractions of nesting habitat during Pleistocene glacial cycles. This time estimate corresponds well with the Mid-Pleistocene transition that is associated with increased amplitude of climatic cycles, an increased impact of cycles on tropical climates, and a change in climatic cycle periodicity (e.g., Raymo et al. 1977; Diekmann and Kuhn 2002; Liu and Herbert 2004). Alternatively, this historical estimate may represent the center of a time period over which multiple contractions and expansions took place.

The number of nesting adult hawksbills in the Caribbean and Brazil was estimated by Meylan (1999) at no more than 5000 and 600, respectively. Meylan (1999) estimated that hawksbills have experienced an 80% reduction in population size over the last three generations and that globally there were <15,000 nesting females. Our estimates of the current N_{ef} (64,106) are reconciled by those of Meylan (1999) that suggest a population of approximately 75,000 nesting females in the early 1900s. Interestingly, estimates of current N_{ef} (64,106) and N_{ef} before historical population contractions (ca. 50,000) are similar and suggest minimal net (long-term) depletion of genetic diversity resulting from Pleistocene climatic cycling.

Green Turtles (Chelonia mydas)

An intermediate number of green turtle haplotypes were endemic to single populations. A majority of

population genetic parameters and patterns for green turtles are strikingly similar (or identical) to those observed for hawksbills (Table 1, Figure 1c). These similarities are justifiable since both species are tropical nesters and are estimated to demonstrate similar levels of NH/NSF (Bass et al. 1996; Encalada et al. 1996; Laurent et al. 1998; Pearce 2001). Estimated migration among populations of green turtles was the lowest ($N_m = 0.45$), and evidence of genetic isolation of populations by distance (based on F_{st} versus geographic distance) was the strongest observed across species ($r = 0.546$).

The distribution of pairwise nucleotide differences across green turtles shows a bimodal pattern in which the peak size, shape, and mean frequencies are similar to hawksbills (Figures 1c). This suggests that, like hawksbills, Atlantic green turtle populations may have been historically subdivided, probably around the same time as were hawksbills. Estimates of tao from a model of population growth/decline suggested a population contraction in green turtles, as in hawksbills, during the Mid-Pleistocene (ca. 900,000 years ago). The nested cladogram for green turtles was nested into two 2-step clades; 2–1 comprises haplotypes possibly derived from the ancestral near-equatorial haplotype H, and clade 2–2 comprises haplotypes exclusively found in the Caribbean and Mediterranean (Figure 4). The bimodality of nucleotide differences and geographic distributions of haplotypes in these 2-step clades suggest an ancient subdivision of Atlantic green turtle populations into near-equatorial and Caribbean subpopulations.

Encalada et al. (1996) suggested climatic depressions of the Pleistocene, accompanied by decreases in sea-level (ca. 100 m), likely confined green turtles to a more narrow band of habitat straddling the equator with one refuge in the Caribbean and one in Brazil, similar to our hypothesis for hawksbills (and, in part, loggerheads). The ancestral haplotype (H) inferred by TCS for green turtles is endemic to Brazil, Ascension Island, and Guinea Bissau. Across these populations, long-distance colonization with subsequent fragmentation was inferred by NCA to explain haplotype distributions for clade 1–3 (Figure 4). Bowen et al. (1992) suggested that the geology of Ascension Island probably would have resulted in extirpation of this rookery during sea-level changes in the Pleistocene. Taken together, these lines of evidence suggest that Brazil may have historically acted as a significant source for populations along West Africa and adjacent islands. At a larger scale, these data also suggest that this near-equatorial metapopulation (especially Brazil and Guinea Bissau) may have acted as a Pleistocene refuge for Atlantic green turtles. Results from NCA further reinforce these conclusions through broad scale inferences of long distance colonization with subsequent fragmentation (clade 2–1), followed by continuous range expansion in the Caribbean (clade 2–2).

This bifocal (near-equatorial and Caribbean) refugia hypothesis is similar to that for hawksbills, and suggests that the distributions of these two tropically nesting species may have responded similarly to historical climatic cycles. Also, like hawksbills, the observed distribution of nucleotide differences among green turtle haplotypes was bimodal with left-skewed peaks (Figure 1c), suggesting population growth/expansion from multiple subpopulations. Considering the evidence available, we conclude that both tropical nesting species of marine turtles, hawksbills and green turtles, appear to have experienced very similar population patterns and processes over the last several million years.

Among the three species studied, historical and current population sizes of green turtles has been the most intensively studied (Seminoff 2002). The current population in the Atlantic and Mediterranean is estimated at 79,054–83,873 nesting females, compared to 43,593–94,000 nesting females, approximately 150 years ago. Our estimate of N_{ef} for green turtles (based on θ) is 54,351, well within the range of population sizes estimated to have been characteristic of the last few centuries. Corresponding with a number of close similarities observed between the population genetics of hawksbills and green turtles, estimates of the current N_{ef} (ca. 54,000) and the N_{ef} before historical population contraction (ca. 48,000) are similar. These data for both tropically nesting species suggest a minimal net (long-term) depletion of genetic diversity and effective population sizes resulting from Pleistocene climatic cycling.

Conclusions

Our findings provide strong evidence for long term, broad scale metapopulation dynamics within

marine turtles, including corroborative evidence for complex source-sink relationships among populations. Also, these relationships appear to be plastic and even reversible over time, with long-term dynamics probably driven by cycles of global climatic change. Relative levels of NH/NSF and nesting habitat preferences (i.e., temperate versus tropical) appear highly correlated with patterns of genetic population structure and inferred historical responses to climatic cycling.

Our data suggest differential effects of the Pleistocene glacial cycles across species, although the most drastic differences are observed in the effects on temperate versus tropical nesting species. Tropical species show no net long-term trend of population decline or depressed genetic diversity as a result of Pleistocene climatic change. Apparently, tropical species experienced population subdivision and possibly population contraction, yet not at a level substantial enough (in duration or severity) to result in a major genetic bottleneck. Tropical species appeared to have undergone this subdivision and possible contraction at some time around the Mid-Pleistocene, which indirectly implies they were not significantly impacted by environmental changes associated with the global onset of climatic cycling beginning in the Late Pliocene.

The temperate nesting loggerhead is inferred to have undergone substantially different population dynamics through the last several million years. This period, associated with dramatic climatic cycling, appears to have resulted in a net long-term trend of population decline and loss of genetic diversity, probably associated with an earlier and more dramatic bottleneck (in terms of duration and/or severity).

Conclusions regarding the differential patterns of response to global climatic change across species offer important insights for forecasting the impact of contemporary patterns of climatic change (i.e. global warming). In general, our findings suggest that tropical species are robust (in terms of population size and genetic diversity) to climatic change, particularly depression of global temperatures. In contrast, our data suggest that loggerheads may be negatively impacted (in population size and genetic diversity) by climatic change, although details of how elevated temperatures (rather than depressed glacial temperatures) may affect this species are unclear from our data other than that they may induce rookery decline (or extirpation) as the distribution of optimal nesting habitat shifts. Already there is evidence of temporal shifts in the median nesting day of loggerheads on the east coast of Florida (Weishample et al. 2004) consistent with similar shifts in migration and breeding patterns thought to be associated with global warming (Hughes 2000, Gitay et al. 2002, Root and Schneider 2002, Walther et al. 2002, Archaux 2003).

Present levels of genetic diversity, along with our estimates of N_{ef}, provide an optimistic perspective for conservation of marine turtles. Despite global decline in marine turtle populations resulting from several centuries of negative human impacts, the long generation time of these species has buffered rates of decline in genetic diversity. This suggests that the preservation of current levels of genetic diversity across species will rely heavily on the ability of conservation efforts to facilitate population recovery before the genetic reservoir maintained through long generation times is exhausted.

Future Research

Mitochondrial haplotype analysis has been the predominant method for analyzing population genetic patterns in marine turtles. Due to the nature of inheritance of mitochondrial haplotypes, our conclusions are limited to a matrilineal perspective of population structure and historical processes. Male-mediated gene flow has been detected in green turtles through comparisons of mitochondrial and nuclear polymorphisms (Karl et al. 1992; FitzSimmons et al. 1997; Roberts et al. 2004) although the important question remains: How reliable are mitochondrial polymorphisms at representing overall population genetics and gene flow across marine turtle populations?

Bi-parentally inherited molecular markers (e.g., microsatellite loci) have been employed in marine turtles in multiple paternity studies (Moore and Ball 2002), and polyandry and polygamy have been demonstrated (FitzSimmons 1998; Hoekert 2000; Crim 2002). Although the potential for sex-biased dispersal has been suggested by early studies and preliminary studies (Karl et al. 1992; Casale et al. 2002), FitzSimmons et al. (1997) observed a strong tendency for male philopatry in Australian green turtles, supporting a broader

population-wide interpretation and application of inferences based on matrilineal patterns of population dynamics. Pearce (2001), however, found that populations of Florida loggerheads with low mitochondrial diversity displayed normal levels of nuclear diversity.

Recently, Roberts et al. (2004) provided evidence, based on microsatellite data, that male-mediated gene flow might be more widespread than previously thought in green turtles. Rogers et al. (2004) employed four microsatellite loci, each characterized by excessive numbers of alleles and, thus, subject to high amounts of homoplasy (as discussed in Rogers et al. 2004). Based on this feature of the molecular marker employed, it is difficult to assess the accuracy of estimates of male-mediated gene flow across green turtle populations suggested by Rogers et al. (2004). To date, no study has examined an extensive number of marine turtle populations using an effective array of bi-parentally inherited molecular markers sufficient to address the relationship between nuclear and mitochondrial patterns of genetic diversity. Future studies incorporating a larger number of microsatellite loci not subject to excessive numbers of alleles per locus are required before a clear understanding of the impacts of sex-biased gene flow on marine turtle population genetics is resolved. It is possible that inferences based on mitochondrial gene polymorphisms will require revision if nuclear diversity does not correlate well with the patterns observed in mtDNA based studies.

Acknowledgements

We gratefully acknowledge Brian Bowen for sharing haplotype distributions for loggerhead turtles in Florida. We thank Llewellyn Ehrhart, John Fauth, Mathew Herron, Rachel Hirschman, Reed Noss, and Robert Ruggiero for suggestions and comments on the manuscript. This work was funded in part by a grant from the Florida marine turtle license plate grants program (03-R04).

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