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Molecular evolution and population genetics of Greater Caribbean green turtles (*Chelonia mydas*) as inferred from mitochondrial DNA control region sequences

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Abstract

The molecular evolution and population genetics of migratory green turtles (*Chelonia mydas*) in the Greater Caribbean were examined with mitochondrial DNA (mtDNA) control region I sequences. A total of 488 base positions (bp) per individual were aligned for 44 individuals from four nesting populations in Florida, Costa Rica, Aves Island (Venezuela), and Surinam. Twelve sequence polymorphisms were detected, representing ten transitions, one transversion, and one 10-bp repeat. Sequence analyses of within- and between-population diversity revealed a deep divergence between western and eastern Caribbean nesting colonies and an inverse relationship between reproductive female population size and mtDNA diversity. In small populations, genetic admixture was important to maintaining high diversity, whereas larger populations appear to have experienced historical bottlenecks or resulted from founder effects. Mitochondrial DNA sequences of the control region offer an order of magnitude greater resolution than restriction site data for addressing questions about mtDNA variation, both within and between populations of green turtles.

Introduction

Field studies of green turtles (*Chelonia mydas*) in the Western Atlantic have documented the remarkable natural history of this species (Carr & Hirth, 1962; Carr, 1967; Carr & Carr, 1974; Carr, Carr & Meylan, 1978), demonstrating that mature females often cycle great distances between specific nesting beaches and feeding grounds. At least part of the migratory behavior may be a consequence of natal homing, in which females return to nest on the same beach where they hatched (Carr, 1967). One expected outcome of natal homing is that differences in maternally inherited traits should accumulate among populations as reproductive females are partitioned by nesting sites (Bowen, Meylan & Avise, 1989). This prediction has been corroborated by comparative restriction site studies of mitochondrial DNA (mtDNA) (Meylan, Bowen & Avise, 1990; Bowen *et al.*, 1992), an extranuclear genomic system with a primarily maternal mode of inheritance in vertebrates (Avise, 1991). More recently, Allard *et al.* (1994) applied mtDNA control region sequences to similar problems in green turtle biology.

The complex and well documented natural history of green turtles makes them excellent candidates for studies of geographic differentiation, population diversity, and migration. As documented by Allard *et al.* (1994), direct sequencing of the mtDNA control region provides greater resolution for addressing questions about population structure in *Chelonia mydas* than does restriction site analysis of the entire mitochondrial genome. Moreover, PCR technologies facilitate the rapid and economical accumulation of additive sequence data and have become the method of choice among molecular evolutionists (Bernatchez & Danzmann, 1993). The application of these and other methods to mtDNA sequences have been particularly useful in understanding the molecular evolution and genetics

of many vertebrate species (Brown, 1986; Moritz & Brown, 1987; Brown, Beckenbach & Smith, 1993; Dillon & Wright, 1993; Helm-Bychowski & Cracraft, 1993; Tamura & Nei, 1993; Allard *et al.*, 1994; Sang *et al.*, 1994; Stewart & Baker, 1994; Wenink, Baker & Tilanus, 1994). Finally, the greater population structure detail afforded by sequence data will be invaluable to conservation efforts for green turtles.

The goals of the present investigation are therefore to use direct sequencing of the control region to examine the molecular evolution and population genetics of *Chelonia mydas* in the Greater Caribbean. This new information is combined with orthologues for other western Atlantic colonies (Allard *et al.*, 1994), and then coupled and compared with available restriction site data (Bowen *et al.*, 1992). Finally, the natural history of this species and the paleogeography of the region are integrated with the mtDNA information to provide greater resolution of green turtle population biology. Given these different lines of evidence, a hypothesis is developed to explain the pattern of mtDNA diversity within and between populations of this endangered migratory species.

Materials and methods

This study was based on green turtle specimens from Aves Island, Venezuela (8) and Matapica, Surinam (15), used by Bowen *et al.* (1992) and Karl, Bowen and Avise (1992) in their investigations of mtDNA and nuclear DNA RFLP variation, respectively. DNA isolation and purification procedures in the current study are described therein. Using dsDNA cycle sequencing techniques outlined by Allard *et al.* (1994), each specimen was examined for heavy strand positions 86-564 which included the 5′ end of the control region and adjacent $tRNA^{PRO}$ gene, by using longer versions of primers LTCM1 and HDCM1 (LTCM2 [5′CGGTCCCCAAAACCGGAATCCTAT 3′] and HDCM2 [5′GCAAGTAAAACTACCGTATGCCAGGTTA 3′], respectively), in addition to LDCM1 (see Allard *et al.*, 1994; Fig. 1 for alignment positions and primer-annealing locations). The Aves Island and Matapica orthologues were then combined with those of ten individuals from Costa Rica and 11 individuals from Florida. These latter specimens represented the portion of data presented by Allard *et al.* (1994) containing sequences that correspond to those examined in the present study. Thus, the current study doubles the earlier work in terms of both geographic scope and the number of specimens examined.

Variation within populations was quantified by haplotype ($\hat{h}$) and nucleotide ($\hat{\pi}$) diversities (Nei, 1987; equations 8.5 and 10.6, respectively). In calculating $\hat{h}$, haplotypes were diagnosed according to both their sequence polymorphisms in the control region and restriction site differences (Bowen *et al.*, 1992) after first checking the two data sets for independence. In contrast, values of $\hat{\pi}$ were based only on control region differences to provide estimates of sequence divergence and evolutionary rates for this noncoding portion of the mtDNA genome. Estimates of $\hat{\pi}$ were corrected for multiple mutations by the method of Jukes and Cantor (1969).

An unrooted tree of haplotypes was constructed by the exhaustive search procedure of the computer program PAUP (Phylogenetic Analysis Using Parsimony [Swofford, 1990]). Haplotype frequency differences between populations were statistically compared with the G-test of independence (G_{adj}), adjusted for small sample size by Williams's correction (Sokal & Rohlf, 1981), using the sequential Bonferroni procedure suggested by Rice (1989). Sequence differentiation between populations was quantified by both total and net divergence (d_{TOT} and d_{NET}; Nei, 1987: equations 10.20 and 10.21, respectively), with their standard errors estimated as described by Nei and Jin (1989). These divergence calculations were restricted to the control region sequences and were corrected for multiple mutations by the Jukes/Cantor method.

Migration rates among populations (Nm, migrants/generation) were generated using the G_{ST} (Takahata & Palumbi, 1985) and private alleles (Barton & Slatkin, 1986; Slatkin & Barton, 1989) methods. In the former, calculations of G_{ST} followed Nei (1987: p. 191).

An evolutionary rate for the control region sequences was determined by correlating our estimates of d_{NET} with those calculated by Bowen *et al.* (1992) for restriction site data. Our rate estimates are based on the net sequence divergence (p of 0.006) between Pacific and Atlantic green turtle populations reported by Bowen *et al.* (1992) and calibrated with the closure of the Panamanian portal at 4.1 million years (MY), which presumably isolated these ancestral stocks.

Long-term, effective population sizes of females (N_{fe}) were calculated as described by Avise, Ball and Arnold (1988). In these calculations, average time (in generations) to common ancestry for two haplotypes of one population was estimated with the evolutionary rate for control region sequences (see above) and with

Table 1. Sequence polymorphisms of mtDNA haplotypes for four Greater Caribbean colonies of the green turtle (*Chelonia mydas*). Haplotypes are designated according to their control region sequences (Roman numerals) and their restriction patterns (superscript letters following Bowen *et al.* [1992]). Base positions correspond to the alignment of Allard *et al.* (1994: Fig. 1). Following position 540, haplotype VI^C possesses a 10 base-pair repeat (CCTTTGGTTG) of sites 531–540, not found in other individuals (–). This polymorphism is counted in all comparisons as a single difference.

	Base position											
Haplotype	164	167	204	321	355	436	438	481	502	504	Repeat	558
I^A	C	A	G	T	T	C	G	T	A	G	–	A
I^B	C	A	G	T	T	C	G	T	A	G	–	A
II^A	T	A	G	T	T	C	G	T	A	G	–	A
III^A	T	A	G	T	T	T	G	T	A	G	–	A
IV^C	C	G	A	C	G	C	A	C	G	A	–	G
V^C	C	G	A	C	G	C	A	C	G	A	–	A
VI^C	C	G	A	C	G	C	A	C	G	A	+	G

the assumption of a mean of 40 years/generation (based on age at sexual maturity of 30–50 years [Balazs, 1982; Frazer & Ladner, 1986]). These estimates were then expressed in terms of nesting females/year by assuming that on average, females nest every 2.5 years (Carr, Carr & Meylan, 1978; Witherington & Ehrhart, 1989). In the same way, N_{fe}s were calculated for the 15 globally distributed populations considered by Bowen *et al.* (1992) using their restriction site data and an evolutionary rate of 0.0015 mutations/site/MY calculated by dividing their p (0.006) by 4.1 MY.

Results

A total of 488 base positions (bp) per individual were aligned for the 44 representatives of the four Greater Caribbean populations. Using haplotype I^B as the standard (Table 1; GenBank accession number M98394), nucleotide proportions of control region I (heavy strand) were asymmetrical with adenine (0.357) and thymine (0.309) occurring most frequently, followed by cytosine (0.195) and guanine (0.137). Among these 44 orthologues, 12 sequence polymorphisms were detected, representing ten transitions (sites 164, 167, 204, 321, 436, 438, 481, 502, 504, and 558), one transversion (355), and one 10-bp repeat (CCTTTGGTTG) inserted between positions 540–541 (Table 1). Nucleotide substitutions were found throughout the 488 bp stretch, showed a transition/transversion ratio of 10:1 and an overall substitution frequency of 0.0225. Specific changes (number) were A → G (3), G → A (3), C → T (2), T → C (2) and T → G(1). The transition at site 204 corresponded to the *Hinc* II difference reported by Bowen *et al.* (1992) for haplotypes A and C. Otherwise, the polymorphisms of the two data sets were independent.

Seven haplotypes were recognized on the basis of their control region differences and restriction site polymorphisms (Tables 1 & 2). The Aves Island and Surinam colonies shared the same common haplotype (IV^C) at frequencies of 0.88 and 0.87, respectively. Two rare haplotypes (V^C and VI^C) were also detected in the Surinam colony, as was one (II^A) in the Aves Island sample. With the exception of the one individual from Aves Island, these two populations did not share any haplotypes with the Florida and Costa Rica colonies (see below).

The common haplotype of both the Costa Rica and Florida populations was II^A, which occurred at frequencies of 0.90 and 0.55, respectively (Table 2). Haplotype I^A was moderately common in the Florida sample (frequency of 0.36). With the exception of the haplotype II^A individual from Aves Island, rare haplotypes were restricted to single populations and represented by single specimens.

The most parsimonious arrangement of the seven haplotypes supported a clear break between IV^C, V^C, and VI^C versus I^A, I^B, II^A, and III^A (i.e. between those fixed to nearly fixed in the eastern [Aves Island and Surinam)] versus western [Costa Rica and Florida] populations, respectively (Fig. 1). This pronounced east/west pattern is demarcated by 9–13 mutations separating the two sets of haplotypes. In contrast, haplo-

Table 2. Haplotype frequency and estimates of $\hat{h}$ and $\hat{\pi}$ (± standard errors [SE]) for four Greater Caribbean populations of green turtles. Haplotype designations follow those in Table 1.

Haplotype	Population			
	Florida	Costa Rica	Aves Island	Surinam
I^A	4	0	0	0
I^B	1	0	0	0
II^A	6	9	1	0
III^A	0	1	0	0
IV^C	0	0	7	13
V^C	0	0	0	1
VI^C	0	0	0	1
Totals	11	10	8	15
$\hat{h}$± SE	0.6182	0.2000	0.2500	0.2572
	± 0.1038	± 0.1541	± 0.1946	± 0.1416
$\hat{\pi}$± SE	0.0011	0.0004	0.0053	0.0006
	± 0.0012	± 0.0004	± 0.0017	± 0.0004

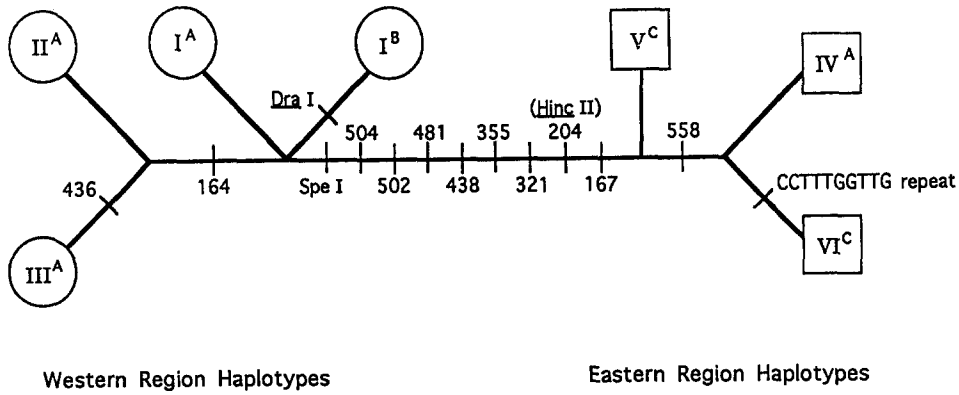

Fig. 1. Unrooted tree of haplotypes for the four Greater Caribbean populations of green turtles. This arrangement is 14 mutations in length and requires no parallel and back mutations. Hash-marks correspond to sequence changes in the control region (keyed to the polymorphic sites of Table 1) and to the restriction site differences reported by Bowen *et al.* (1992). Haplotype designations follow those in Table 1. Haplotypes are restricted to either the western (four on left) or eastern (three on right) populations, with the exception of II^A which is also represented by a single individual from Aves Island.

types within either cluster varied by only 1–3 mutations.

An identical east/west split among the four populations was supported by the estimates of sequence divergence (Table 3). The eastern and western populations differed from each other by 0.0178–0.0214 and 0.0146–0.0209 mutations/site according to d_{TOT} and d_{NET}, respectively. In contrast, the Florida colony varied from the Costa Rica sample by a d_{TOT} and d_{NET} of 0.0012 and 0.0004 mutations/position, respectively, which approached those for the Aves Island versus Surinam comparison (0.0029 and 0.0000 mutations/position). Differentiation between the eastern and western populations was therefore at least six times greater than within either region. These differences, as tested with d_{NET}, were significant (student's t-test, $P < 0.001$ in all cases).

Using the sequential Bonferonni method, significant differences in haplotype frequency existed between all pairs of populations (G_{adj}; $P < 0.05$), except for the Florida/Costa Rica and Aves Island/Surinam comparisons. A similar pattern was

Table 3. Divergences (mutations/site) and migration rates (Nm, migrants/generation) for four populations of green turtles from Greater Caribbean colonies. Above the diagonal, total and net (in parentheses) divergences are presented with their standard errors. Net divergence for the Aves Island and Surinam comparison is taken as 0.00001 since their d_{TOT} is exceeded by their mean $\hat{\pi}$ (cf. Table 2). Below the diagonal, migration rates based on the G_{ST} and private alleles (in parentheses) methods are shown.

	Population			
Population	1	2	3	4
1. Florida	—	0.0012 ± 0.0010	0.0178 ± 0.0057	0.0202 ± 0.0064
		(0.0004 ± 0.0004)	(0.0146 ± 0.0048)	(0.0193 ± 0.0063)
2. Costa Rica	0.6 (0.7)	—	0.0187 ± 0.0059	0.0214 ± 0.0067
			(0.0159 ± 0.0050)	(0.0209 ± 0.0066)
3. Aves Island	0.2 (0.1)	0.1 (0.1)	—	0.0029 ± 0.0009
				(0.0000 ± 0.0000)
4. Surinam	0.2 (0.2)	0.1 (0.1)	2.5 (3.0)	—

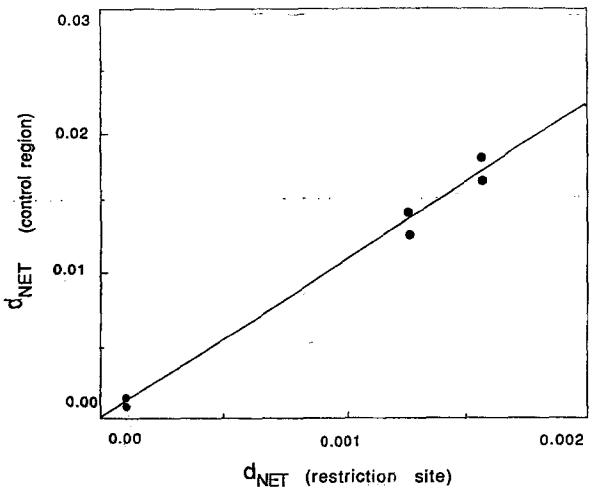

Fig. 2. Linear regression d_{NET} values, determined from the control region sequences (Table 3) and restriction site data (Bowen *et al.*, 1992).

suggested by G_{ST} and private alleles estimates of migration, both of which supported similar rates (Table 3). Except for the Aves Island and Surinam colonies (2.5–3.0 migrants/generation), all other comparisons were characterized by migration rates less than 0.6–0.7 migrants/generation.

Net divergences for the control region sequences were linearly related to the restriction site data by a slope of 11.7 (Fig. 2). An evolutionary rate for the control region of 0.0176 mutations/site/MY was then obtained by multiplying the rate for restriction site data (0.0015 mutations/position/MY, using a calibration of 4.1 MY for the closure of the Panamanian portal) by 11.7.

Unexpectedly, the lowest levels of diversity, as measured by $\hat{h}$ and $\hat{\pi}$, were exhibited by the largest colonies (Costa Rica and Surinam with N_fs of 5,000–23,000 and a few thousand reproductive females/year, respectively) (Tables 2 and 4A). Their estimates of $\hat{h}$ (0.2000 and 0.2572, respectively) were between two and three times less than that for the Florida population (0.6182). Similarly, their $\hat{\pi}$ values (0.0004 and 0.0006 mutations/position) were 8–13 times less than the estimate for the Aves Island population (0.0053). In each case, these differences were significant (student's t-test, $P < 0.05$), even though N_fs for the Aves Island and Florida colonies were estimated at only 300–500 and a 'few hundred' reproductive females/year, respectively.

This inverse relationship between mtDNA diversity and N_f was consistent with the restriction site data (Table 4B). Of the seven colonies with N_fs greater than 1,000 females/year, six were fixed for single haplotypes. The one large population (Ascension Island) showing any diversity was characterized by $\hat{h}$ and $\hat{\pi}$ values of 0.056 and 0.00005, respectively. In contrast, only two of the eight colonies with N_fs of less than 1,000 females/year were fixed for single haplotypes (Cyprus and Guinea Bissau). The other six were represented by $\hat{h}$ and $\hat{\pi}$ values of 0.117–0.445 and 0.00018–0.00273 mutations/site, respectively. These differences were significant for both $\hat{h}$ and $\hat{\pi}$ (Wilcoxon two-sample test, P = 0.02 in both cases).

Table 4. Current (N_f; from Bowen *et al.* [1992: table 1]) and theoretical (N_{fe}) population sizes of reproductive females (per year) for 15 globally-distributed green turtle colonies. In A, estimates of $\hat{h}$ and $\hat{\pi}$ are based on the control region sequences (Table 2), whereas in B, they are calculated from the restriction site data (Bowen *et al.*, 1992). In both A and B, N_{fe} is estimated as described in the Materials and methods.

Population	$\hat{h}$	$\hat{\pi}$	N_{fe}	N_f
A. Control region sequences				
Hutchinson Island, Florida, USA	0.6182	0.0011	625	few hundred
Tortuguero, Costa Rica	0.2000	0.0004	227	5,000–23,000
Aves Island, Venezuela	0.2500	0.0053	3,011	300–500
Matapica, Surinam	0.2572	0.0006	341	few thousand
B. Restriction site data				
Tortuguero, Costa Rica	0.000	0.00000	—	5,000–23,000
Matapica, Surinam	0.000	0.00000	—	few thousand
Ascension Island, United Kingdom	0.056	0.00005	333	1,600–3,000
Ras Al Hadd, Osman	0.000	0.00000	—	≅ 6,000
Queensland, Australia	0.000	0.00000	—	several thousand
Michoacan, Mexico	0.000	0.00000	—	1,000–3,000
Galápagos Islands, Ecuador	0.000	0.00000	—	1,200–3,500
Ogasawara Islands, Japan	0.445	0.00083	5,533	200–400
French Frigate Shoals, Hawaii, USA	0.397	0.00033	2,200	100–500
Mopelia Atoll, French Polynesia	0.444	0.00273	18,200	few hundred
Hutchinson Island, Florida, USA	0.219	0.00018	1,200	few hundred
Aves Island, Venezuela	0.219	0.00042	2,800	300–500
Atol das Rocas, Brazil	0.117	0.00024	1,600	50–400
Pailoa, Guinea Bissau	0.000	0.00000	—	≅ 400
Lara Bay, Cyprus	0.000	0.00000	—	< 100

Discussion

The sample sizes used in this study reflect the difficulty of procuring tissue from this endangered species. However, we believe that they adequately represent the essential character of the populations examined in terms of their primary allelic components. Rare alleles influence measurements of $\hat{h}$ and $\hat{\pi}$ only slightly, and therefore have minimal effect on migration rates and N_{fe} estimates.

Green turtle control region I sequence is characterized by high levels (> 30%) of A and T and low levels of G and C, as well as G–C proportion asymmetry between heavy and light strands. These findings agree with those of previous studies which indicate that vertebrate d-loop sequences are typically G and C deficient (Saccone, Attimonelli & Sbisá, 1987; Brown, Beckenbach & Smith, 1993; Wenink, Baker & Tilanus, 1994; Sang *et al.*, 1994; Arnason, Gullberg & Widengren, 1993; Stewart & Baker, 1994).

Transition/transversion ratios in both coding and noncoding regions vary widely among vertebrates, from nearly equal (shore birds; Wenink, Baker & Tilanus, 1994) to highly biased towards transitions (up to 32:1) in birds (Edwards & Wilson, 1990), fish (Brown, Beckenbach & Smith, 1993), and mammals (Moritz, Dowling & Brown, 1987). Our finding of a 10:1 transition/transversion ratio in *Chelonia mydas* control region I sequence is therefore not unusual. One noteworthy outcome of the sequence analysis was the discovery of the 10-base tandem repeat. Although tandem duplications in the mtDNA are common in some lizards (Moritz & Brown, 1987) and some mammals (Stewart & Baker, 1994), they remain relatively uncommon in many vertebrate species. Because such repeats are generally restricted to single individuals or single populations, they are believed to be phylogenetically short-lived (Moritz & Brown, 1987). A similar 6-base repeat has also been found in one loggerhead (*Caretta caretta*) individual (B. Bowen, pers. comm.).

The current results suggest that mtDNA sequences of the control region in green turtles evolve an order of magnitude faster than the overall mitochondrial genome (Fig. 2). This finding is in agreement with other investigations which indicate a 2–15 fold increase in control region mutation rate over the entire genome (Brown, George & Wilson, 1979; Aquadro & Greenberg, 1983; Cann, Brown & Wilson, 1984; Bernatchez & Danzmann, 1993; Vigilant *et al.*, 1991; Quinn, 1992). This increased resolution was manifested in the detection of twice as many haplotypes and three times as many polymorphisms as uncovered for the same populations using RFLP data (Bowen *et al.*, 1992). It should be cautioned that finer-scale genetic data do not necessarily translate into greater geographic resolution of population structure. For example, in their study of brook charr, Bernatchez and Danzmann (1993) found approximately twice as many mutation events in a sequenced 300 bp control region segment, but the number of haplotypes revealed by this sequence was similar to that achieved for the entire d-loop using RFLP data. However in the present case, the population structure suggested by Bowen *et al.*'s (1992) analysis was brought into much sharper focus through direct sequencing of the control region.

Two major patterns for Greater Caribbean populations of green turtles emerge from the comparisons of their control region sequences. The first is that the four representative populations of this study are geographically subdivided into western and eastern groups (Costa Rica/Florida versus Aves Island/Surinam, respectively) (Fig. 1). This geographic split, weakly indicated in the RFLP analysis (Bowen *et al.*, 1992), is based on almost complete fixation for different haplotypes (the one exception involving a single individual from Aves Island [Table 2]), which vary by 9–13 mutations. Western and eastern populations differ by d_{NET}s of 0.0146–0.0209 mutations/position, values which are several orders of magnitude greater than those for within each region (Table 3). Furthermore, the two regions are characterized by migration rates (0.1–0.2 migrants/generation) that are much less than those required to offset the diversifying effects of genetic drift (Barton & Slatkin, 1986; Slatkin & Barton, 1989). RFLP data were insufficient to achieve consistent migration rates between these colonies.

Evolutionary rates calibrated from geological events must be interpreted with caution because the timing of such events may be staggered (Knowlton *et al.*, 1993), and are generally inaccurate or controversial (Bermingham & Lessios, 1993). Although we accept 4.1 MY for the uplift of Panama based on the arguments of Donnelly (1989), we recognize that other interpretations exist. Moreover, as pointed out previously (Bowen *et al.*, 1992), the closure of the Panamanian portal may not have completely isolated Pacific and Atlantic populations, with subsequent gene exchange via other routes.

On average, the western and eastern populations differ by a d_{NET} of 0.0177 mutations/site (Table 3). Using an evolutionary rate of 0.0176 mutations/site/MY for the control region, the time of divergence for these groups is placed at approximately 1 MY. If this pronounced split between western and eastern colonies is related to a major vicariance event, then similar phylogeographic patterns should be evident in other marine species as well (Avise *et al.*, 1987; Avise, 1992). However, other species do not readily fit this pattern (Briggs, 1974) and it remains unclear as to what the vicariance event might have been.

As with previous mitochondrial RFLP studies (Bowen, Meylan & Avise, 1989; Meylan, Bowen & Avise, 1990; Bowen *et al.*, 1992), mtDNA sequence variation between the two western populations (Costa Rica and Florida) and the two eastern populations (Surinam and Aves Island) offers the opportunity to test the natal homing hypothesis with the control region sequences. The significantly different haplotype frequencies (G_{adj}, $P < 0.05$) observed between these regional populations support in general the theory that females return to their natal beaches to lay their eggs, instead of randomly selecting their nesting sites (Carr, 1967). Although the more rigorous sequential Bonferroni method failed to detect significant frequency differences between the Florida and Costa Rican colonies (cf. Allard *et al.*, 1994), there were important qualitative differences (Table 2). Similarly, no significant haplotype frequency differences were found between Surinam and Aves Island populations. Lack of significant haplotype frequency differences in these cases might represent failure of the natal homing hypothesis, or more likely, lack of genetic resolution. Microsatellite data may yield the degree of resolution necessary to demonstrate significant differences between these colonies.

The second major pattern supported by the control region sequences is that larger populations of reproductive females are characterized by less mtDNA diversity than are smaller colonies, as measured by $\hat{h}$ and $\hat{\pi}$ (Table 4A). For the control region sequences, the Aves Island and Florida colonies (with N_fs of 300–500 and a few hundred reproductive females/year, respectively)

exhibit more mtDNA diversity than the larger Costa Rica and Surinam populations (5,000–23,000 and a few thousand reproductive females/year), but in different ways. The Aves Island sample is diverse because it includes one individual with a distinct haplotype (II^A), which differs from the common type (IV^C) by 11 mutations (Table 2 & Fig. 2). The distinctiveness of this individual is reflected by the large $\hat{\pi}$ value of its population (4–13 times greater than the others), but is not evident in terms of $\hat{h}$ (which does not incorporate nucleotide divergence). In contrast, the Florida colony is characterized by several haplotypes differing by only one or two mutations (Table 2 & Fig. 2). This pattern of diversity is represented by $\hat{h}$ values, but not by $\hat{\pi}$ values (because of the small differences among its haplotypes). In either case, both the Aves Island and Florida samples support the pattern that smaller populations exhibit more mtDNA diversity than larger ones.

Comparisons between expected and observed reproductive female population sizes (N_{fe} versus N_f, respectively) offer a way to evaluate whether populations are unusually low or high in terms of mtDNA diversities relative to their current sizes (Avise, Ball & Arnold, 1988). When the four western Atlantic populations are evaluated in this way, it becomes evident that both patterns apply (Table 4A). For the smaller Aves Island and Florida colonies, N_{fe}s are 2–10 times greater than their corresponding N_fs. For the larger Costa Rica and Surinam populations, the reverse holds as their N_{fe}s are many orders of magnitude less than their N_fs. Reduced genetic diversity in large populations appears to be the typical pattern (Avise, Ball & Arnold, 1988; Bowen & Avise, 1990). Nevertheless, the two larger colonies are unexpectedly low, whereas the two smaller ones are unusually high in their mtDNA diversities.

The high N_{fe} estimates for small populations appear to contradict the neutral gene theory (Kimura, 1979). To explain the greater diversity of the smaller Florida population, Allard *et al.* (1994) suggested that this colony was the product of admixture, resulting from recent and/or ongoing immigration from different neighboring sources. Data presented here strengthen this hypothesis with respect to the small Aves Island colony. The two haplotypes in this population, IV^C and II^A, are shared with Surinam and western colonies, respectively, and their observed frequencies on Aves Island are inversely correlated with geographic distance from the source area. As importantly, haplotypes II^A and IV^C differ by 11 mutations representing east and west lineages, and types with only a few differences are absent from this sample. Using an evolutionary rate of 0.0176 mutations/site/MY, the Aves Island colony with its two distinct haplotypes and $\hat{\pi}$ of 0.0053 mutations/position (Table 2) is estimated to be older than 300,000 years. Not withstanding sources of error associated with rate calibration (see above), this age for the colony is regarded as unlikely because of the geological instability of Aves Island (Parsons, 1962). Given these different lines of evidence, the existence of two distinct haplotypes in the Aves Island colony is best attributed to genetic admixture, with contributions from both western and other eastern populations.

The generality of the pattern that larger and smaller populations of females are characterized by unexpectedly lower and higher levels of mtDNA diversity, respectively, is supported by the restriction site data for 15 globally distributed colonies of green turtles (includes the four examined in this study) (Table 4B). Out of seven colonies with N_fs greater than 1,000 reproductive females/year, six show no haplotype variation, whereas only two of the eight with N_fs less than 1,000 exhibit no mtDNA diversity. Estimates of $\hat{h}$ and $\hat{\pi}$ for the one larger population with variation (Ascension Island) are less than those for all six of the smaller ones possessing more than one haplotype. Furthermore, comparisons of N_{fe} and N_f for each population indicate that, as before (see above), this pattern is due to the larger colonies being unusually low and the smaller ones unexpectedly high in terms of mtDNA diversities.

A corollary of the natal homing hypothesis states that occasional mistakes occur whereby females select nesting sites other than their natal beaches (Carr, 1967). These accidents are regarded as critical because in a changing environment, they ensure that new beaches will become colonized by green turtles. The importance of this corollary is now restated in terms of why smaller colonies of green turtles possess more mtDNA diversity than expected, and large colonies, less. The occasional mistakes in nesting fidelity represent opportunities for admixture in small populations where the impact of immigration is greatest. In contrast, the challenge presented by the larger populations (where the impact of occasional immigration is reduced) is to explain unusually low levels of mtDNA variation. One possible explanation can be obtained from earlier studies of genetic variation in natural populations (Nei & Graur, 1984; Avise, Ball & Arnold, 1988). These studies have shown that natural populations are often characterized by unexpectedly low diversity, presum-

ably because of recent or historical bottlenecks. Low genetic diversity could also result from founder effects. Whether through the disappearance of an historic nesting beach forcing females to colonize new ones, or the occasional site infidelity, the result could be the founding of a new nesting colony by a single female. Because populations increase exponentially whereas mutations accumulate arithmetically at very slow rates, large, genetically homogeneous colonies are inevitable. Such phenomena would have a particularly potent effect on mtDNA diversity in green turtle populations due to their long generation time (30–50 years), reproductive cycle (2–3 years), and relatively slow rate of mtDNA evolution compared to other vertebrates (Avise *et al.*, 1992; Bowen, Nelson & Avise, 1993). Moreover, this explanation is consistent with the biology of green turtles because of their dependence on ephemeral shoreline environments for nesting (Carr, 1967) and occasional nesting error (Carr, Carr & Meylan, 1978). Indeed, based on effective population sizes and neutrality theory, Bowen *et al.* (1992) suggested that most nesting colonies are probably considerably less than 40,000 yrs old.

The findings presented here have important conservation implications for green turtles. The inverse relationship observed between genetic variability and population size suggests that small colonies are as important as large ones in maintaining mitochondrial diversity. To the extent that they may represent incipient or future colonies, small populations function as genetic reservoirs in the event of extinction of larger colonies. As such, small nesting colonies should receive protection equal to that of large colonies.

The major conclusions of this study, the deep divergence between eastern and western nesting colonies, the importance of admixture and founder effects on genetic diversity, and support for natal homing, all relate to the maternal component of green turtle colonies and not necessarily to the males. Restriction-fragment analysis of nuclear DNA (with its biparental mode of inheritance) has suggested that males constitute an important avenue of gene flow among green turtle colonies (Karl, Bowen & Avise, 1992). Recombination of nDNA therefore may provide for genetic homogeneity among green turtle populations within the Greater Caribbean region despite the divergence of matrilineal mtDNA.

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