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Testing Dispersal Hypotheses in Foraging Green Sea Turtles (*Chelonia mydas*) of Brazil

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Abstract

Testing theories of dispersal is challenging in highly migratory species. In sea turtles, population size, geographic distance, natal homing, and ocean currents are hypothesized to affect dispersal. Little is known, however, about these mechanisms in sea turtles foraging along the South American coast. Green sea turtles feeding at Ubatuba (UB, $n = 114$) and Almofala (AF, $n = 117$), Brazil, were sequenced at the mitochondrial DNA (mtDNA) control region (486 bp) and genotyped at 7 microsatellite loci to test dispersal hypotheses. Fifteen mtDNA haplotypes were revealed, including a previously undescribed sequence, and the average observed heterozygosity (H_o) was 76.4%. Overall short-term temporal differences were not detected, and differentiation was less pronounced in microsatellite than in mtDNA analyses. Mitochondrial results reveal significant differentiation between the Brazilian feeding grounds and most other Atlantic groups, whereas microsatellites uncover similarities to some of the geographically closest populations. Ubatuba and Almofala are mixed stocks, drawn primarily from Ascension, with lesser contributions from Surinam/Aves and Trindade. Costa Rica is also a significant source of individuals feeding at AF. The results are consistent with a model of juvenile natal homing impacted by other factors. Effective protection of turtles foraging along the extensive Brazilian coast may enhance breeding populations thousands of kilometers away.

A fundamental and challenging research priority in conservation biology is to investigate dispersal of endangered organisms. Elucidating linkages among groups is important for comprehensive protection and understanding population biology. Determining such relationships, however, can be especially challenging in highly migratory species or in those that are hard to observe. This difficulty can be exacerbated when individuals from different areas mix during subsequent life stages. Harvest, bycatch, or other factors affecting these mixtures can also impact distant and possibly vulnerable source populations. Assessing the effects of harvesting individuals originating from different natal areas is particularly relevant in fisheries management and led to the development of mixed stock analysis (MSA) techniques. In this approach, molecular markers are used to trace contributions of genetically differentiated source populations to a mixed harvest. These methods were first developed for the management of salmon fisheries (Grant et al. 1980; Pella and Milner 1987; Pella and Masuda 2001) and are now employed to address similar questions in other migratory species including marine chelonians (Bowen 1995).

Green sea turtles (*Chelonia mydas*) are globally endangered (Seminoff 2004; IUCN 2006), are highly migratory (Hirth 1997), and forage in mixed aggregations drawn from various rookeries or nesting beaches (Bass et al. 1998, 2006; Lahanas et al. 1998; Bass and Witzell 2000; Luke et al. 2004). Adults undertake breeding migrations between feeding grounds (FGs) and rookeries that may be widely geographically separated (Hirth 1997). Mating generally occurs offshore of the nesting beach and also during reproductive migrations (FitzSimmons, Limpus, et al. 1997; FitzSimmons, Moritz, et al. 1997). Many females return to nest in the area of their birth, a behavior known as natal homing (Carr 1967). This process contributes to the genetic differentiation among rookeries required for MSA (Bowen 1995). After hatching from eggs deposited on nesting beaches, green sea turtles disperse into the ocean (Hirth 1997). Young turtles may drift passively with currents during the subsequent oceanic phase until,

as juveniles, they recruit from the pelagic zone to coastal FGs (Hirth 1997).

Genetic, mark–recapture, and satellite telemetry research can provide information about insufficiently understood links among sea turtle populations. The naturally high mortality of young turtles, long generation times, and logistic difficulties, however, have in most cases precluded the use of tagging or telemetry in tracking a hatchling to its eventual FG. Genetic studies have been key in illuminating such relationships. The genetic composition of FGs may be related to rookery size (Bass et al. 1998; Lahanas et al. 1998), geographic distance (Bass and Witzell 2000), ocean currents (Luke et al. 2004), or juvenile natal homing (Luke et al. 2004; Bass et al. 2006). In the last process, young postpelagic sea turtles are hypothesized to move toward the site of their birth to forage (Norrgard and Graves 1996; Rankin-Baransky et al. 2001; Engstrom et al. 2002; Witzell et al. 2002; Bass et al. 2004; Bowen et al. 2004). Ocean currents may impact dispersal by influencing the movements of young turtles (Luke et al. 2004; Bass et al. 2006). Alternately, if either population size or geographic distance drives FG composition, MSA estimates are expected to be proportional to numbers of nesting females or distance from source rookeries, respectively.

This study tests hypotheses of dispersal using mitochondrial DNA (mtDNA) control region sequences and microsatellite genotypes of green sea turtles foraging at Ubatuba (UB) and Almofala (AF), Brazil. UB is a juvenile developmental habitat (Gallo et al. 2006), whereas turtles of various sizes are found at AF (Marcovaldi MA and Marcovaldi GG 1999; Lima et al. 2003). These FGs are high-priority sites for conservation and research in Brazil, where green sea turtles forage along the extensive coastline (Marcovaldi MA and Marcovaldi GG 1999). The research fills an important gap in green sea turtle FG studies by both increasing geographic representation and considering insights from multiple loci. It is well established that results from any single marker may not reflect organismal characteristics, and recent analysis of loggerhead sea turtles indicates a comprehensive approach is recommended for these chelonians as well (Bowen et al. 2005). The goals of this study are to 1) determine the genetic composition at multiple loci of 2 green sea turtle FGs in Brazil; 2) assess genetic differentiation between these FGs and other Atlantic populations, as well as among years and seasons at each study site; 3) elucidate the natal origins of turtles foraging at UB and AF; and 4) consider effects of population size, geographic distance, natal homing, and ocean currents on FG composition.

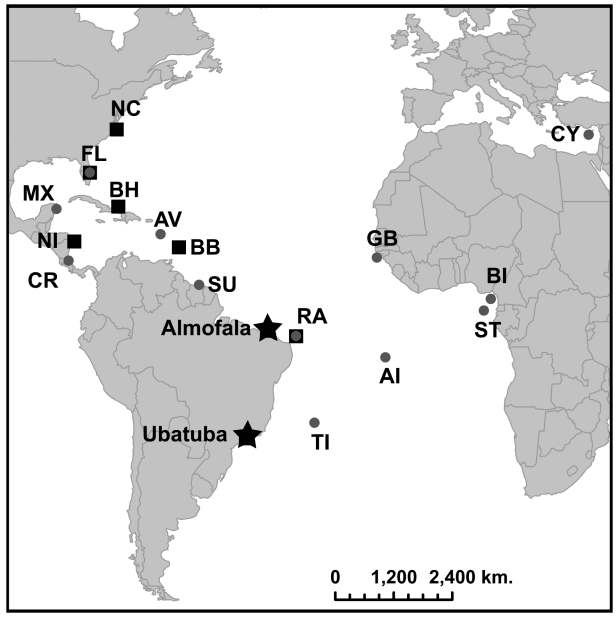

Figure 1. The UB and AF study sites and other *Chelonia mydas* groups previously subject to genetic analysis. References and abbreviations for other FGs, symbolized by squares, are as follows: Bahamas (BH; Lahanas et al. 1998), Nicaragua (NI; Bass et al. 1998), Florida (FL; Bass and Witzell 2000), Barbados (BB; Luke et al. 2004), North Carolina (NC; Bass et al. 2006), and Rocas Atoll (RA; Bjorndal et al. forthcoming). Rookeries considered possible sources of turtles foraging at UB and AF, symbolized by circles, were Hutchinson Island, FL; Aves Island, Venezuela (AV); Matapica, Surinam (SU); Quintana Roo, Mexico (MX; Encalada et al. 1996); Lara Bay, Cyprus (CY; Encalada et al. 1996; Kaska 2000); Tortuguero, Costa Rica (CR; Encalada et al. 1996; Bjorndal et al. 2005); Ascension Island, UK (AI); Poilão, Guinea Bissau (GB; Encalada et al. 1996; Formia et al. 2006); Bioko Island, Equatorial Guinea (BI); São Tomé (ST; Formia et al. 2006); Trindade Island, Brazil (TI; Bjorndal et al. forthcoming); and Rocas Atoll, Brazil (RA; Encalada et al. 1996; Bjorndal et al. forthcoming).

Materials and Methods

Sampling and Laboratory Procedures

Samples were collected using standard protocols (Dutton 1996) from turtles foraging at UB and AF, Brazil (Figure 1). Sterile, disposable 5-mm AcuPunch biopsy punches (AcuDerm Inc., Fort Lauderdale, FL) were employed, and samples were stored in a 20% dimethylsulfoxide buffer saturated in salt (Amos and Hoelzel 1991). Projeto TAMAR-IBAMA (The Brazilian Sea Turtle Conservation Program) biologists sampled tissue from the flippers of green sea turtles incidentally captured by local coastal artisanal fishers and released alive or to a lesser extent from dead stranded turtles. Samples were obtained at UB (n = 114) each month from July 1998 to February 2000 (except for November and December 1999) and at AF, n = 117) from April 2000 through July 2002 (except for September and October 2000). In temporal analyses, samples were grouped into tropical winter (April–September) and summer (October–March) seasons (UB: $n_{\text{season 1}} = 28$, $n_{\text{season 2}} = 44$, $n_{\text{season 3}} = 30$, $n_{\text{season 4}} = 12$; AF: $n_{\text{season 1}} = 9$, $n_{\text{season 2}} = 13$, $n_{\text{season 3}} = 33$, $n_{\text{season 4}} = 21$, $n_{\text{season 5}} = 41$), the most recent of which were then paired for testing among years. All sampled turtles were juveniles, measuring on average 42 cm curved carapace length at UB (range: 32–75 cm) and 48 cm at AF (range: 29–80 cm). The largest turtles were well under the average breeding size in the region (Hirth 1997), addressing

Table 1. Molecular diversity at 7 microsatellite loci in foraging green sea turtles of Brazil

				UB (n = 114)				AF (n = 117)			
Locus	Designed in	Reference	Tag	A	Range	H_o	H_e	A	Range	H_o	H_e
Cm3	*Chelonia mydas*	FitzSimmons et al. (1995)	FAM	14	152–204	0.614	0.597	16	152–204	0.603	0.645
Cm58	*Chelonia mydas*	FitzSimmons et al. (1995)	NED	11	122–148	0.770	0.782	11	124–148	0.786	0.805
Cm72	*Chelonia mydas*	FitzSimmons et al. (1995)	FAM	33	220–298	0.974	0.945	35	220–308	0.932	0.948
Cm84b	*Chelonia mydas*	FitzSimmons N (personal communication)	NED	16	180–214	0.780	0.871	19	180–216	0.845	0.896
Cc7	*Caretta caretta*	FitzSimmons (1998)	HEX	13	162–218	0.823	0.832	19	162–218	0.821	0.886
Klk314	*Lepidochelys kempii*	Kichler et al. (1999)	FAM	5	103–115	0.667	0.625	7	103–115	0.607	0.652
Or7	*Lepidochelys olivacea*	Aggarwal et al. (2004)	FAM	8	218–244	0.737	0.712	8	218–244	0.741	0.745

The locus name, species the primers were designed in, reference, and 5′ fluorescent label used are indicated. The sample size (n), number of alleles (A), allele size ranges, observed (H_o), and expected (H_e) heterozygosities are also shown for each FG.

the concern that transient adults migrating through the area to breed might be confused with resident foraging turtles (Limpus and Reed 1985).

The samples from UB (n = 114) and AF (n = 117) were analyzed at the Molecular Systematics Laboratory of the American Museum of Natural History (AMNH). A DNeasy Tissue Kit was used for DNA extractions, following the manufacturer's instructions for animal tissues (Qiagen Inc., Valencia, CA). An automated sequencer was employed for sequencing and genotyping (Applied Biosystems model 3700 or 3730). Control region primers LTCM2 and HDCM2 (Lahanas et al. 1994) were used to amplify and sequence 486 bp of the mtDNA control region, using standard conditions and negative controls. These primers are longer extensions of the LTCM1 and HDCM1 pair originally designed by Allard et al. (1994). The program SEQUENCHER 3.1.2 (Gene Codes Corporation, Ann Arbor, MI) was used for sequence alignment. Mitochondrial haplotypes were classified following the standardized nomenclature of the Archie Carr Center for Sea Turtle Research (ACCSTR). Microsatellite loci were amplified using a multiplex polymerase chain reaction (PCR) kit (Qiagen Inc.) following the manufacturer's instructions. The 7 loci genotyped (Table 1) were as follows: Cm3, Cm58, Cm72 (FitzSimmons et al. 1995), Cm84b (shorter version; FitzSimmons N, personal communication), Cc7 (FitzSimmons 1998), Klk314 (Kichler et al. 1999), and Or7 (Aggarwal et al. 2004). PCR products were sized according to the ROX 500 size standard (ABI Prism, Foster City, CA). GENESCAN 3.1 (ABI Prism) was used for data processing, and GENOTYPER 3.7 (ABI Prism) determined the allele length.

Data Analysis: mtDNA Sequences

Genetic Diversity and Differentiation

The program ARLEQUIN 3.01 (Excoffier et al. 2005) was employed to estimate Nei's (1987) haplotype diversity (h) and nucleotide diversity (π). A minimum spanning network based on statistical parsimony was constructed with TCS 1.21 (Clement et al. 2000) to determine relationships among the new and previously described haplotypes at UB. Exact tests of population differentiation (Raymond and Rousset 1995a) were employed to assess differences among years and seasons at each site, as well as between the study FGs and other nesting grounds or FGs from the published literature (Figure 1 and the references therein; Table 2). These tests were carried out using a Markov chain length of 10 000 steps with 1000 dememorization steps implemented by ARLEQUIN (Excoffier et al. 2005).

Natal Origins of Foraging Turtles

MSA was carried out using Bayesian methods implemented by the program BAYES (Pella and Masuda 2001). Bayesian analysis is suitable when sample sizes are small or when there are rare haplotypes (Pella and Masuda 2001). This method also allows for incorporation of ecological data such as source population size (Bolker et al. 2003; Bass et al. 2004, 2006; Okuyama and Bolker 2005). Two MSAs were carried out for each FG, the first with equal prior probabilities for each rookery (MSA_1), and in the second approach (MSA_2), priors were weighted to reflect the number of nesting females at each possible source following Bass et al. (2004). Population size data (Table 2) were obtained from Bellini et al. (1995), Seminoff (2002, 2004), and Formia et al. (2006). Atlantic or Mediterranean green sea turtle rookeries described in the literature were considered possible sources for turtles foraging in Brazil (Figure 1 and the references therein; Table 2). As noted by Chapman (1996), the MSA should include all adequately described potential sources to avoid error. The BAYES program requires that sequences not found in any of the source samples be removed; thus, the few rare haplotypes unique to the FGs (described below) were excluded. The analyses were performed until Gelman and Rubin diagnostics confirmed convergence of the chains to the posterior density, with most shrink factors close to 1.0 and all less than 1.2 (Pella and Masuda 2001). In each analysis, the first halves of the chains were discarded as burn-in, or dememorization steps, and estimates were based on the second halves of the chains only (Pella and Masuda 2001).

Chi-square tests and linear regression were used to investigate whether population size or distance was associated with mean MSA estimates from the rookeries contributing to the Brazilian FGs. The effect of population size was analyzed by calculating the numbers of turtles that would be expected at each FG under 2 conditions: 1) equal source contributions or 2) contributions proportional to population size. These

Table 2. Green sea turtle mtDNA control region haplotypes detected at UB and AF compared with other Atlantic groups from the published literature, with population size and geographic distance estimates used in this study also shown

	FGs								Rookeries											
Haplotype	UB	AF	RA	BB	NI	BH	NC	FL	TI	AI	RA	ST	BI	GB	SU	AV	CR	MX	FL	CY
CM-A1				7		2	34	12										7	11	
CM-A2							2	1											1	
CM-A3	2	18		21	54	62	43	43								3	395	5	12	
CM-A4																	1			
CM-A5	14	28	5	13	6	10	5	3				1			13	27	32	1		
CM-A6		3	2							3		1	5		1					
CM-A7															1					
CM-A8	83	53	13	14		1	7		67	59	36	13	45	70						
CM-A9	4	3	2	1					19	1	7									
CM-A10	3	4		2						3	2									
CM-A11									1		1									
CM-A12											5									
CM-A13																				25
CM-A14																				1
CM-A15							1											1		
CM-A16		1					2											1		
CM-A17				1														2		
CM-A18							3	2										3		
CM-A20						1											2			
CM-A21		1				3											3			
CM-A22				1			2	1												
CM-A23									6											
CM-A24	2	1							1	1										
CM-A25											1									
CM-A26							2													
CM-A27							2													
CM-A28							3													
CM-A32	2	1							4		1									
CM-A33									1											
CM-A35												1								
CM-A36												1								
CM-A37												1								
CM-A38												2								
CM-A39										1										
CM-A42		2																		
CM-A44	1	1																		
CM-A45		1								1										
CM-A46	1		1							1										
CM-A55	1																			
Heteroplasmy	1																			
Total	114	117	23	60	60	79	106	62	99	70	53	20	50	70	15	30	433	20	24	26
Nesting females									3000	3709	115	90	407	2523	1814	267	26 535	1587	779	100
Distance to UB (km)									1609	3698	2481	6246	6517	7687	3422	4772	5628	6687	6827	10 481
Distance to AF (km)									2260	2907	712	5255	5449	6415	1907	3299	5022	5717	5458	8685

The literature references and abbreviations for each site are found in Figure 1.

values were compared with observed MSA estimates using chi-square tests. This analysis was carried out first with all contributing rookeries considered and then excluding the large Costa Rican rookery, which as an outlier could be a source of error. Linear regression was used to assess independence of arcsine-transformed MSA estimates and great circle distances between the Brazilian FGs and each rookery (Table 2).

Data Analysis: Microsatellite Genotypes

Genetic Diversity and Differentiation

Alleles per locus and observed and expected heterozygosities (Nei 1987) were estimated for each microsatellite locus by the program MSANALYZER 3.12 (Dieringer and Schlotterer 2003). FSTAT 2.9.3.2 (Goudet 2001) was employed to test for genotypic linkage disequilibrium among all pairs of loci with the log-likelihood ratio G-statistic and sequential Bonferroni corrections (Rice 1989). Global and per-locus exact tests of Hardy–Weinberg equilibrium (HWE) were carried out with a Markov chain method following Guo and Thompson (1992) with 5000 dememorization steps, 500 batches, and 5000 iterations per batch using GENEPOP 3.4 (Raymond and Rousset 1995b). The program STRUCTURE 2.1 (Pritchard et al. 2000) was employed to estimate the number of populations present at each FG using Bayesian methods. As recommended (Pritchard et al. 2000), 3 runs of 1 000 000 steps (with 100 000 dememorization steps) were conducted for each FG, and a model of admixture and correlated allele frequencies was assumed.

Exact tests of population differentiation were carried out as described above to assess differences between 1) years and seasons within each FG, 2) the Brazilian FGs, and 3) each FG and previously described Atlantic rookeries (Roberts et al. 2004; Naro-Maciel 2006). In rookery comparisons, concerns about differences in microsatellite data obtained in separate laboratories were addressed by genotyping all samples at the AMNH, including those previously analyzed in a global rookery study by Roberts et al. (2004; Cm3, Cm58, Cm72, and Cm84). However, MSA using these markers was not carried out because the rookery data set was inadequate as a baseline. This is primarily because the MSA assumes that all sources are included, but microsatellite data from rookeries such as Trindade are not yet available. In addition, genetic similarity between sources and mixtures can lead to MSA error (Chapman 1996), and the rookery microsatellite sample size may present a significant analytical limitation because MSA was designed for use with larger samples characteristic of fisheries research.

Results

The mtDNA Sequences

Genetic Diversity and Differentiation

At UB, 13 polymorphic sites defined 10 haplotypes (Table 2). Nine of these had been previously identified and given standardized names, and one did not match any published sequences. This new haplotype has been assigned the standardized ACCSTR designation "CM-A55" (GenBank accession number DQ294212). It differs by 1 bp from CM-A5 (Figure 2) found primarily at the Aves and Surinam rookeries, in Costa Rica, and rarely in Mexico and São Tomé (Table 2). In addition, a heteroplasmy was observed in one individual at site 164. Heteroplasmy in the green sea turtle control region has been reported previously (Encalada et al. 1996; Formia 2002), and the sequence was excluded from the analysis. The most frequent haplotypes at UB were CM-A8 (73%), found at South Atlantic and African rookeries, and CM-A5 (12%). All additional haplotypes were relatively rare (<5% each). Of these, 1 was not detected at any rookery (CM-A44), 1 was encountered among rookeries only at Ascension (CM-A46), 1 was limited to the North Atlantic (CM-A3), and the remaining 4 were restricted to Ascension and Brazilian rookeries (Table 2). Haplotype diversity (h) and nucleotide diversity (π) were below average at UB compared with other FGs (Table 3; Figure 1 and the references therein).

At AF, 19 polymorphic sites defined 13 mitochondrial haplotypes, all of which had been previously named (n = 117, Table 2). The most common were CM-A8 (45%), CM-A5 (24%), and CM-A3 (15%). All others were rare (<5%), encountered among rookeries in Brazil, Ascension, Africa, Mexico, Costa Rica, and Surinam (Table 2; Figure 1 and the references therein). The haplotypes CM-A44 (n = 1) and CM-A42 (n = 2) were found only at FGs, and haplotype diversity (h) and nucleotide diversity (π) at AF were comparatively high (Table 3).

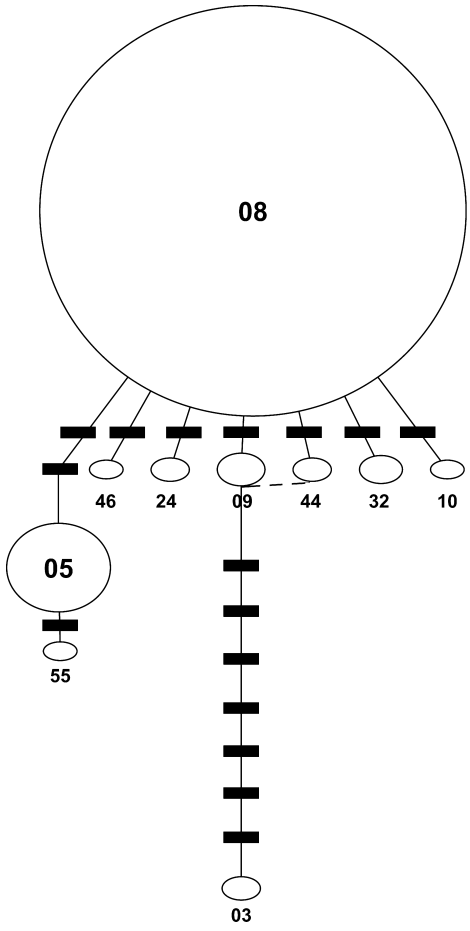

Figure 2. Minimum spanning network illustrating relationships among control region haplotypes encountered at UB. Haplotypes are named according to the ACCSTR standardized sequence designation and are prefixed by "CM-A." Substitutions of 1 bp are indicated by hash marks while the dashed line represents another possible link between haplotypes. Circles/ovals are approximately proportional to haplotype frequencies.

Exact tests revealed that FGs in the Atlantic were highly differentiated overall (exact P = 0.000) and that differences among temporal periods were not significant at either Brazilian FG. Temporal variation was not found among years (UB: exact P = 0.221, AF: exact P = 0.477) or seasons (UB: exact P = 0.595, pairwise exact P > 0.302; AF: exact P = 0.887, pairwise exact P > 0.311). The Brazilian FGs were significantly different from all others in the North Atlantic and from each other (exact P = 0.000). However, differentiation was not found between either Brazilian FG and Rocas (UB: exact P = 0.120, AF: exact P = 0.230), located in the same region.

Natal Origins of Foraging Turtles

Results of the MSAs for even and weighted priors were highly correlated at UB (r^2 = 0.935, P = 0.000) and AF (r^2 = 0.848,

Table 3. Mitochondrial control region diversity at UB and AF (in bold), as compared with other Atlantic *Chelonia mydas* FGs from the published literature (references in Figure 1)

FG	Haplotypes	Haplotype diversity (*h*)	Standard deviation	Nucleotide diversity (π)	Standard deviation	Sample size
Barbados	8	0.7734	0.0299	0.0103	0.0056	60
North Carolina	12	0.7294	0.0301	0.0053	0.0031	106
Almofala	**13**	**0.7168**	**0.0306**	**0.0067**	**0.0039**	**117**
Rocas	5	0.6443	0.0917	0.0022	0.0017	23
Florida	6	0.4855	0.0668	0.0031	0.0021	62
Ubatuba	**10**	**0.4460**	**0.0556**	**0.0020**	**0.0015**	**113**
Bahamas	6	0.3703	0.0650	0.0064	0.0037	79
Nicaragua	2	0.1831	0.0621	0.0038	0.0025	60
Average	8	0.5436		0.0050		78

To standardize comparisons with other FGs, these measures were also recalculated for FGs described in the literature (Figure 1 and the references therein) using the program ARLEQUIN (Excoffier et al. 2005). The heteroplasmy detected at UB is not included in this analysis.

$P = 0.000$). As noted in other studies, the confidence intervals in most cases were narrower for the MSA with weighted priors (Bass et al. 2004; Okuyama and Bolker 2005). The main difference between the 2 MSAs was the relative contribution of Aves and Surinam at both FGs, with Surinam playing a greater role when priors were weighted (Tables 4 and 5).

Hypotheses of single origins, equal contributions, and MSA estimates proportional to population size or geographic distance were rejected for both FGs. Both sites were significantly differentiated from all potential sources (UB: exact $P < 0.017$, AF: exact $P < 0.003$), rejecting the hypothesis of single rookery origins. Chi-square tests revealed a significant difference in mean MSA estimates from those expected

Table 4. MSA of UB green sea turtle control region haplotypes using Bayesian methods with equal priors (MSA_1) and priors weighted to reflect population size (MSA_2)

Stock	MSA	Mean	Standard deviation	2.5%	Median	97.5%
Florida	MSA_1	0.001	0.005	0.000	0.000	0.015
	MSA_2	0.000	0.002	0.000	0.000	0.003
Mexico	MSA_1	0.001	0.004	0.000	0.000	0.013
	MSA_2	0.001	0.003	0.000	0.000	0.007
Aves	MSA_1	0.131	0.042	0.027	0.132	0.209
	MSA_2	0.041	0.064	0.000	0.000	0.182
Costa Rica	MSA_1	0.003	0.008	0.000	0.000	0.027
	MSA_2	0.020	0.016	0.000	0.017	0.060
Surinam	MSA_1	0.009	0.026	0.000	0.000	0.101
	MSA_2	0.088	0.064	0.000	0.104	0.193
Rocas	MSA_1	0.032	0.080	0.000	0.000	0.284
	MSA_2	0.000	0.006	0.000	0.000	0.000
Trindade	MSA_1	0.144	0.102	0.000	0.135	0.373
	MSA_2	0.178	0.100	0.010	0.165	0.411
Ascension	MSA_1	0.536	0.182	0.157	0.566	0.806
	MSA_2	0.580	0.173	0.181	0.621	0.830
São Tomé	MSA_1	0.004	0.013	0.000	0.000	0.042
	MSA_2	0.000	0.003	0.000	0.000	0.000
Bioko	MSA_1	0.015	0.043	0.000	0.000	0.156
	MSA_2	0.003	0.020	0.000	0.000	0.013
Guinea Bissau	MSA_1	0.124	0.164	0.000	0.017	0.518
	MSA_2	0.090	0.150	0.000	0.001	0.496
Cyprus	MSA_1	0.001	0.003	0.000	0.000	0.009
	MSA_2	0.000	0.001	0.000	0.000	0.000

Mean values are shown with standard deviation. The 2.5% and 97.5% values indicate the upper and lower bounds of the 95% confidence interval. MSA estimates at UB are based on chains lengths of 6768 (MSA_1) and 24 920 (MSA_2) steps for each of the 12 nesting stocks.

Table 5. MSA of AF green sea turtle control region haplotypes using Bayesian methods with equal priors (MSA_1) and priors weighted to reflect population size (MSA_2)

Stock	MSA	Mean	Standard deviation	2.5%	Median	97.5%
Florida	MSA_1	0.002	0.005	0.000	0.000	0.016
	MSA_2	0.000	0.002	0.000	0.000	0.003
Mexico	MSA_1	0.008	0.013	0.000	0.002	0.043
	MSA_2	0.005	0.011	0.000	0.000	0.037
Aves	MSA_1	0.180	0.123	0.000	0.222	0.361
	MSA_2	0.054	0.106	0.000	0.000	0.318
Costa Rica	MSA_1	0.154	0.045	0.065	0.153	0.243
	MSA_2	0.176	0.041	0.098	0.175	0.261
Surinam	MSA_1	0.083	0.112	0.000	0.004	0.312
	MSA_2	0.199	0.107	0.000	0.232	0.342
Rocas	MSA_1	0.030	0.059	0.000	0.000	0.212
	MSA_2	0.000	0.006	0.000	0.000	0.000
Trindade	MSA_1	0.064	0.062	0.000	0.051	0.215
	MSA_2	0.081	0.063	0.000	0.071	0.230
Ascension	MSA_1	0.436	0.103	0.187	0.450	0.600
	MSA_2	0.466	0.089	0.259	0.476	0.615
São Tomé	MSA_1	0.004	0.014	0.000	0.000	0.037
	MSA_2	0.000	0.001	0.000	0.000	0.000
Bioko	MSA_1	0.023	0.055	0.000	0.000	0.203
	MSA_2	0.001	0.011	0.000	0.000	0.007
Guinea Bissau	MSA_1	0.018	0.047	0.000	0.000	0.177
	MSA_2	0.017	0.047	0.000	0.000	0.179
Cyprus	MSA_1	0.001	0.003	0.000	0.000	0.008
	MSA_2	0.000	0.000	0.000	0.000	0.000

Mean values are shown with standard deviation. The 2.5% and 97.5% values indicate the upper and lower bounds of the 95% confidence interval. MSA estimates at AF are based on chain lengths of 13 014 (MSA_1) and 39 138 (MSA_2) steps for each of the 12 nesting stocks.

under a model of equally contributing rookeries (UB—MSA_1: $\chi^2 = 160.743$, df = 6, $P = 0.000$; UB—MSA_2: $\chi^2 = 151.690$, df = 5, $P = 0.000$; AF—MSA_1: $\chi^2 = 153.983$, df = 8, $P = 0.000$; AF—MSA_2: $\chi^2 = 123.018$, df = 6, $P = 0.000$). Indeed, Ascension Island is clearly the major source for the Brazilian FGs in all analyses, and most distant rookeries contribute little, if at all (Tables 4 and 5). In chi-square tests, observed distributions were significantly different from those expected according to population size (UB—MSA_1: $\chi^2 = 112.297$, df = 6, $P = 0.000$; UB—MSA_2: $\chi^2 = 387.402$, df = 5, $P = 0.000$; AF—MSA_1: $\chi^2 = 732.897$, df = 8, $P = 0.000$; AF—MSA_2: $\chi^2 = 310.545$, df = 6, $P = 0.000$). As an outlier, Costa Rica could be a source of error in population size analyses; however, tests run without this major rookery produced similar results (data not shown). Linear regression revealed no significant relationship between arcsine-transformed MSA estimates and geographic distance at UB (MSA_1: $R^2 = 0.010$, $F = 0.048$, $P = 0.835$; MSA_2: $R^2 = 0.143$, $F = 0.665$, $P = 0.461$) or AF (MSA_1: $R^2 = 0.087$, $F = 0.666$, $P = 0.441$; MSA_2: $R^2 = 0.329$, $F = 2.447$, $P = 0.178$).

Microsatellite Genotypes

Genetic Diversity and Differentiation

Microsatellite analyses revealed similarities among the Brazilian FGs (Table 1). Polymorphism varied by marker, and the number of alleles was lowest at the Klk314 locus (UB: $A = 5$, AF: $A = 7$) and highest at Cm72 (UB: $A = 33$, AF: $A = 35$; Table 1). Observed heterozygosity (H_o) was lowest at Cm3 (UB: $H_o = 0.614$, AF: $H_o = 0.603$) and highest at Cm72 (UB: $H_o = 0.974$, AF: $H_o = 0.932$). Across loci and feeding areas, H_o averaged 76.4% and was essentially the same for each site (UB: $H_o = 76.6\%$, AF: $H_o = 76.2\%$). There were significant correlations among FGs in allele number ($r^2 = 0.956$, $P = 0.000$), H_o ($r^2 = 0.889$, $P = 0.001$), and H_e ($r^2 = 0.986$, $P = 0.000$). There was no evidence of linkage disequilibrium among pairs of loci after sequential Bonferroni corrections. Global tests indicated no significant departures from HWE at UB (exact $P = 0.086$) or AF (exact $P = 0.080$). Heterozygote deficit tests of individual loci revealed only one significant deviation, at Cm84b in UB (exact $P = 0.000$). Departures from HWE at Cm84 were previously reported at 3 Atlantic rookeries, possibly due to null alleles or inbreeding (Roberts et al. 2004). When this locus was not considered, the FGs remained in equilibrium (UB: exact $P = 0.843$, AF: exact $P = 0.130$).

Genetic differentiation was less pronounced at microsatellite loci than at the mtDNA control region. Because of the heterozygote deficit at the Cm84b locus, microsatellite analyses were carried out with and without this marker (data not shown); however, this did not affect the statistical significance of tests unless otherwise noted. Temporal variation was not detected at either site between years (UB: $\chi^2 = 18.584$, df = 14, $P = 0.181$; AF: $\chi^2 = 14.452$, df = 14, $P = 0.417$) or seasons overall (UB: $\chi^2 = 22.174$, df = 14, $P = 0.075$; AF: $\chi^2 = 10.633$, df = 14, $P = 0.715$). At UB, most seasonal pairwise comparisons were not significantly different, except for one pair that involved season 4, which had a relatively small sample size ($\chi^2 = 29.583$, df = 14, $P = 0.009$). Seasonal variation was not revealed in pairwise comparisons at AF ($\chi^2 < 19.209$, df = 14, $P > 0.157$). Most Bayesian clustering analyses detected more than one population at each FG; however, estimates varied between runs. Differentiation between UB and AF was not significant ($\chi^2 = 22.267$, df = 14, $P = 0.073$). UB was significantly different from most rookeries ($\chi^2 > 30.060$, df = 14, $P < 0.007$) except for Ascension ($\chi^2 = 15.889$, df = 14, $P = 0.320$) and possibly Rocas ($\chi^2 = 29.314$, df = 14, $P = 0.009$; $\chi^2_{6loci} = 19.588$, $df_{6loci} = 12$, $P_{6loci} = 0.075$). AF was not significantly different from Ascension ($\chi^2 = 20.047$, df = 14, $P = 0.129$), Surinam ($\chi^2 = 11.744$, df = 14, $P = 0.627$), or Rocas ($\chi^2 = 22.268$, df = 14, $P = 0.073$). However, this FG was differentiated from all other Atlantic rookeries ($\chi^2 > 32.217$, df = 14, $P < 0.004$).

Discussion

Juvenile Dispersal

The analysis provides insight into marine chelonian dispersal, supporting a tendency toward juvenile natal homing in foraging green sea turtles of Brazil. There are 3 expectations in this model of dispersal: 1) genetic differentiation among FGs, 2) genetic correspondence between proximate nesting and feeding areas, and 3) MSA estimates revealing greater contributions to the FGs from the closest rookeries (Bowen et al. 2004). Most Atlantic green sea turtle FGs are significantly differentiated from one another (Bass and Witzell 2000; Luke et al. 2004; Bass et al. 2006; this study). UB and AF are also genetically similar in microsatellite analyses to some of their closest described sources. Further, the largest MSA estimates tend to be from among the closest rookeries, and contributions from most distant ones are minor (Tables 4 and 5).

Although the analyses generally support a juvenile natal homing model, other factors may also affect juvenile green sea turtle dispersal in the western South Atlantic. Ocean currents may account for the disproportionately large Ascension contribution, although additional information about the pelagic life-history stage and local circulation is necessary to support this hypothesis. Natal homing and ocean currents are thought to play important roles at other regional green sea turtle FGs (Luke et al. 2004; Bass et al. 2006). Luke et al. (2004) postulate that turtles born at rookeries bathed by major currents that flow toward Barbados could form the bulk of the pool from which that FG is drawn. If this model applies to Brazil, small hatchlings from Ascension may drift with major Equatorial currents toward the South American coast, constituting a proportionally large contribution, while some turtles from other rookeries may be more likely to drift away from Brazil with prevailing currents. It is also important to note that, although regression analyses do not show a significant relationship between estimated contributions and geographic distance, this model cannot be definitively rejected. This is because complexities

of oceanic circulation and sea turtle movements may cause the actual distances traveled to differ substantially from great circle measures, impacting the usefulness of this analysis.

An integrated approach considering results of demographic and tracking research is recommended when interpreting genetic data, and the major results from the Brazilian FGs are consistent with such studies. Satellite tracks and tag returns have long shown links between Ascension and northern Brazil (Mortimer and Carr 1987; Meylan 1995; Luschi et al. 1998), and microsatellite analyses reveal similarity of both FGs and Ascension. There is evidence from tag returns of movement between AF and Costa Rica (Lima and Troeng 2001), and adults tagged at Trindade have been recovered in Brazil (Marcovaldi et al. 2000). Surinam and Aves together contribute about 13% of the turtles foraging at UB and approximately 25% of those at AF. Although the relative importance of these rookeries at the Brazilian FGs varies depending on the MSA approach, mark–recapture and microsatellite data both suggest a lesser role for the small Aves rookery than for Surinam. Microsatellites indicate differentiation of the FGs and Aves and similarity between Surinam and AF. Further, links between adults nesting in Surinam and feeding in northern Brazil are well known (Schulz 1975; Pritchard 1976; Meylan 1995), whereas tag returns connecting Aves to Brazil are relatively rare (Meylan 1995).

There are some issues that warrant further investigation and highlight methodological caveats. Although MSA assumes that all the sources have been included and are well described, some nesting areas may be insufficiently characterized. The presence of many FG-specific haplotypes is an indication of inadequately described rookeries. Encouragingly, few haplotypes were unique to the Brazilian FGs. The accuracy of population size estimates may also affect these analyses. Further, some of the confidence intervals around the mean MSA estimates are relatively wide, and these values are therefore considered general indicators (Bass et al. 2006). The contribution of Guinea Bissau to UB, for example, is not supported by any demographic data, and estimates from other African rookeries are negligible (Table 4). Guinea Bissau is fixed for the CM-A8 haplotype commonly found throughout the South Atlantic, presumably due to a recent colonization event (Formia et al. 2006). This could be a source of error because assignment accuracy is affected, among other factors, by population differentiation (Chapman 1996), shared haplotypes (Bowen et al. 2004), and historical processes (Bass et al. 2006).

Genetic differentiation was more pronounced in mitochondrial than in microsatellite analyses. Similar results in rookery studies have been attributed primarily to the homogenizing influence of gene flow detectable at nuclear loci (Karl et al. 1992; FitzSimmons, Moritz, et al. 1997; Roberts et al. 2004). In some areas, gene flow is thought to occur during spatially overlapping reproductive migrations (FitzSimmons, Limpus, et al. 1997; FitzSimmons, Moritz, et al. 1997) and may be mediated by males (Karl et al. 1992; Roberts et al. 2004). If foraging juveniles originate from genetically discrete rookeries, the FGs should be differentiated from any single source in mtDNA analyses (Chapman 1996), as was the case in Brazil. At nuclear loci, a significant heterozygote deficit due to the Wahlund effect (Wahlund 1928) is expected in mixtures, and multiple populations should consistently be revealed in Bayesian clustering analyses (Pritchard et al. 2000). Instead, Bayesian estimates of the number of populations at each FG varied somewhat between runs. Pritchard et al. (2000) addressed a similar issue by increasing the number of loci analyzed. In simulations of Atlantic and Mediterranean green sea turtle anonymous nuclear gene data, sample sizes close to 100 or larger were insufficient to detect significant deviation from HWE in mixtures (Chapman 1996). Other research using similar numbers of markers and reporting modest to low population differentiation also detected few significant deviations from Hardy–Weinberg expectations in mixtures (Rosel et al. 1999; Hansen et al. 2000). Lastly, the validity of certain microsatellite markers may be limited in some cases, as a global study of green sea turtle rookeries revealed size homoplasy, possible null alleles or inbreeding, and potential deviation from commonly assumed models of microsatellite evolution (Roberts et al. 2004).

Temporal Analyses

The finding of overall temporal genetic stability at the Brazilian FGs was consistent with results from other areas and has methodological implications. Variation among seasons was also not detected in loggerhead sea turtles foraging in the southeastern United States of America (Bass et al. 2004) or in green, loggerhead, or hawksbill turtles nesting in Costa Rica (Bjorndal et al. 2005), Japan (Hatase et al. 2002), or Puerto Rico (Velez-Zuazo X, personal communication), respectively. Seasonal changes in capture numbers and juvenile body length, however, were reported at UB (Gallo et al. 2006), and significance values in microsatellite analyses of this site are not high. Temporal variation could contribute to differentiation among FGs (Bass et al. 2006) and violates the assumption of stability in MSAs. Considering also that study time periods were limited and green sea turtles in particular are known for marked fluctuations in nesting numbers (Heppell et al. 2003), continued attention to the issue is warranted in future research.

Conservation Implications

The need for international collaboration in sea turtle management is well established due to transboundary migrations such as the ones revealed in this study. Egg harvest occurs at several rookeries, and bycatch of marine chelonians in fisheries is a prevalent danger throughout the region. Threats may also impact ecosystems in which sea turtles are prevented from fulfilling their ecological roles (Bjorndal and Jackson 2003). At UB and AF, coastal artisanal fisheries incidentally capture many sea turtles, although some of these activities do not currently result in high mortality (Marcovaldi MA and Marcovaldi GG 1999; Gallo et al. 2006). In the

recent past, however, consumption of marine turtle meat was more frequent. This take included large juveniles, a size class particularly important for sea turtle population growth (Crouse et al. 1987; Heppell et al. 2003). The strong conservation and education program established in response has brought about a reversal in harvest. Thus, conserving juvenile sea turtles foraging along the extensive Brazilian coast complements other conservation efforts and ultimately leads to the protection of rookeries thousands of kilometers away.

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