

Genetic composition, population structure and phylogeography of the loggerhead sea turtle: colonization hypothesis for the Brazilian rookeries

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Abstract The loggerhead sea turtle, *Caretta caretta*, is the most common species of sea turtle nesting in Brazil and is listed as endangered by the IUCN. Our study characterizes the genetic structure of loggerheads in Brazil based on mitochondrial DNA control region variability and presents a hypothesis for the colonization of Brazilian rookeries. We analyzed 329 samples from Brazilian rookeries and an oceanic foraging ground, and we compared our results with previously published data for other loggerhead populations. Brazilian rookeries had four haplotypes, none

of which have been reported for rookeries outside Brazil. Six haplotypes were found in the foraging aggregation. The presence of the CC-A4 haplotype at all sampled sites and the low nucleotide diversity suggest a common origin for all rookeries, with CC-A4 being the ancestral haplotype of the Brazilian populations. The occurrence of three haplotypes in the foraging aggregation that are known only from rookeries outside of Brazil is consistent with the trans-oceanic migratory behavior of loggerheads. Our results indicated that the colonization of Brazilian rookeries probably occurred from the southern USA stock. This recent colonization most likely followed a north to south route along the Brazilian coastline, influenced by the Brazilian warm current. Our results further suggest the existence of two genetic population units of loggerheads in Brazil and corroborate natal homing behavior in loggerheads.

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Introduction

The loggerhead sea turtle, *Caretta caretta*, is widely distributed in tropical and temperate waters around the world (Pritchard and Trebbau 1984) and is listed as endangered by IUCN (2008). As with other sea turtle species, the loggerhead life cycle consists of developmental stages that are segregated spatially and temporally, involving marked changes in diet and habitat. Juveniles are believed to spend their first years drifting passively in ocean current systems or in floating sargassum rafts (Carr 1986; Bolten 2003). Older juveniles subsequently shift to coastal foraging

habitats (Carr 1987). After reaching sexual maturity at about 30 years of age (Snover 2002), adults undertake reproductive migrations that range from tens to thousands of kilometers (Meylan 1982; Limpus et al. 1992). Tagging and genetic data indicate that loggerhead turtles exhibit maternal philopatry to the natal site (Bowen 2003).

The Brazilian nesting population of loggerheads is one of the largest in the world, after the super-aggregations at Masirah, Oman, and eastern Florida, USA (Marcovaldi and Chaloupka 2007). This species is the most abundant sea turtle species along the Brazilian coast. The nesting beaches range from the north of Rio de Janeiro state (southeast coast) to Sergipe state (northeast coast); nesting density is greatest on beaches of Bahia state (Marcovaldi et al. 2005). Loggerheads have a long history of exploitation in Brazil. Prior to 1980 nearly all eggs laid along the coast were removed, and most nesting females were taken for meat (Marcovaldi et al. 2005). The establishment of Projeto TAMAR (The Brazilian Sea Turtle Conservation Program) by the Brazilian government in 1980, and the enactment of full legislative protection of all sea turtle species in 1986 have contributed significantly to the improving status of the Brazilian loggerhead stock (Marcovaldi and Chaloupka 2007). However, in more recent years, loggerheads have become exposed to other hazards such as coastal development (Marcovaldi et al. 2006), marine debris (Bugoni et al. 2001), and incidental capture in coastal gillnet and pelagic longline fisheries operating in southern Brazilian waters (Soto et al. 2003; Kotas et al. 2004; Sales et al. 2008). Protection of the Brazilian loggerhead stock is of great importance for the global conservation of this species.

Genetic analyses have been used worldwide to investigate genetic diversity and rookery structure, phylogeography, foraging ground composition, rookery contributions to foraging aggregations, migratory patterns, natal homing behavior, taxonomic relationships, paternity, and hybridization in sea turtles (Bowen and Karl 2007; Bowen et al. 2007; Bjorndal and Bolten 2008). A phylogeographic survey with 176 samples from rookeries in Greece, Brazil, South Africa, Oman, Japan, Australia, and USA demonstrated the existence of two primary mtDNA lineages in loggerheads. Both lineages are found in both Atlantic-Mediterranean and Indian-Pacific basins, probably due to the ability of this temperate-adapted species to migrate around southern Africa (Bowen et al. 1994). On the basis of a molecular clock for marine turtles calibrated at 0.2–0.4% per million years (Avice et al. 1992), the deepest bifurcation in the loggerhead mtDNA phylogeny would be around 2–4 million year old (Bowen et al. 1994). The absence of a clear matrilineal separation between these oceanic basins could be explained by gene flow around southern Africa that perhaps occurred through the last 20,000 years. These ocean basins were relatively isolated

by geography and climate in the Pleistocene. During interglacial periods, the expansion to higher latitudes was possible because of warmer temperatures. An alternative explanation is that major mtDNA lineages have been retained in both ocean basins for several million years and recent inter-oceanic exchange of mtDNA haplotypes has resulted in the similarity of haplotypes in separate oceans (Bowen et al. 1994).

Another study, based on mtDNA control region sequences of 249 Atlantic and Mediterranean loggerhead turtles from 10 major nesting areas, defined six demographically independent groups: (1) North and South Carolina, Georgia and northeast Florida, USA, (2) southern Florida, USA, (3) northwest Florida, USA, (4) Quintana Roo, Mexico, (5) Bahia, Brazil, and (6) Peloponnesus Island, Greece (Encalada et al. 1998). The significant differentiation among the regional nesting aggregates was consistent with natal homing behavior. The lack of differentiation between North Carolina and northeast Florida was attributed to the recent colonization of these warm temperate coastlines after the Wisconsin glaciation. Thus, Encalada et al. (1998) concluded that climate, natal homing, and rare dispersal events defined the loggerhead biogeographic scenario.

In the present study, we assessed the population genetic composition of Brazilian loggerhead rookeries and foraging aggregates through the analysis of sequences of the mtDNA control region. We also used data from Atlantic-wide loggerhead populations to understand the genetic structure of rookeries and to provide a phylogeographic scenario that can account for the colonization of the Brazilian loggerhead rookeries.

Methods

During the nesting seasons (September–March) of 1996/1997, 2003/2004, 2004/2005 and 2005/2006, tissue samples were collected from 204 individual female loggerheads at rookeries in Rio de Janeiro ($N = 64$), Espírito Santo ($N = 50$), Bahia ($N = 39$), and Sergipe ($N = 51$) states. Samples were collected with 6 mm disposal biopsy punches. We also sampled 125 individual loggerheads captured at Elevação do Rio Grande (ERG) as incidental take in the longline fishery. ERG is a seamount chain located ca. 800 km off the south coast of Brazil that rises to within 350 m of the sea surface. Similar to the Azores Archipelago in the North Atlantic (Bolten et al. 1998), ERG is an important foraging ground and oceanic developmental habitat for immature loggerheads in South Atlantic waters (Marcovaldi et al. 2006; Sales et al. 2008).

Tissue samples were collected by biologists from Projeto TAMAR-ICMBio. All animals were tagged on the

front flippers with Inconel tags (National Band and Tag Co. style 681) to avoid re-sampling individual turtles.

Genomic DNA extraction was performed according to a modified protocol from Damato and Corach (1996). The mtDNA control region was amplified using primers LCM15382 (5'-GCT TAA CCC TAA AGC ATT GG-3'; Abreu-Grobois et al. 2006) and H599 (5'-TGC ACG GCC AAT CAT TTT GAA CGT AG-3'; Laurent et al. 1998), according to the conditions described in Shanker et al. (2004). The amplified fragment of about 800 bp was purified using GFXTM PCR DNA and gel band purification kit (GE Healthcare), following manufacturer's instructions. Direct DNA sequencing was performed with the ET Dye terminator cycle sequence kit (GE Healthcare) and analyzed in a MegaBace 1000 automated sequencer (GE Healthcare).

For each PCR amplicon, a 627 bp consensus sequence was produced by the CAP3 sequence assembly program (Huang and Madan 1999) and BioEdit sequence alignment editor version 7.0.1 (Hall 1999). Mitochondrial haplotypes of 380 bp fragment length were classified by comparison with known loggerhead mtDNA control region haplotypes of the Atlantic and Mediterranean already deposited at the DNA database of the Archie Carr Center for Sea Turtle Research (ACCSTR 2008).

The software package ARLEQUIN version 3.1 (Excoffier et al. 2006) was used to estimate haplotype and nucleotide diversity (Nei 1987; Excoffier and Slatkin 1995). Wright's fixation index of population subdivision (pairwise F_{ST} ; Excoffier et al. 1992), computed with 10,000 random permutations, and exact tests of population differentiation (Raymond and Rousset 1995), computed with 10,000 steps in the Markov Chain and 1,000 dememorization steps, were both carried out in ARLEQUIN 3.1 to assess population differentiation. Pairwise F_{ST} enabled us to estimate the average gene flow per generation between rookeries (Nm), which is the product of the effective population size N and the migration rate m , using the equilibrium relationship for haploid data: $F_{ST} = 1 / (2Nm + 1)$. Analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used to examine genetic structuring among rookeries and among different groups of regional rookeries, through the determination of F -statistics and significance of P values computed with 10,000 permutations. Estimates of past population expansion were made using the mismatch distribution of mtDNA haplotypes (Schneider and Excoffier 1999), and Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997) estimators. The mismatch analysis describes the distribution of pairwise nucleotide differences among DNA sequences based on a model of sudden population expansion (Rogers and Harpending 1992; Harpending et al. 1998; Schneider and Excoffier 1999). It assumes that population growth or decline will reveal a genetic signature (unimodal distribution)

different from that observed with a constant population size (multimodal distribution; Rogers and Harpending 1992). The sum of square deviations (SSD) between the observed and the expected distribution and the raggedness index r of the observed distribution of the mismatch classes (Harpending 1994) were computed as test statistics under the null hypothesis of population growth. Tajima and Fu neutrality tests also give signatures of population expansion when their estimators present significantly negative values (Tajima 1989; Fu 1997). Correlation between genetic (pairwise F_{ST}) and geographic distance (km) matrices was tested with a Mantel nonparametric permutation test (Mantel 1967) with 1,000 replications. The geographic distances were obtained with Google Earth 2007 considering continental contours. In all tests that required estimates of sequence divergence, we used the Tamura-Nei model of nucleotide substitutions (Tamura and Nei 1993).

A network of haplotypes was constructed using statistical parsimony (Templeton et al. 1992). The method, implemented by TCS version 1.21 software (Clement et al. 2000), links first haplotypes with the smaller number of differences as defined by a 95% confidence criteria and identifies the most probable ancestral haplotype according to coalescent theory (Castelloe and Templeton 1994).

For these analyses, in addition to our data, we used previously published mtDNA data from rookeries in Mexico (Quintana Roo), USA (Florida northern and southern Gulf of Mexico, Florida southern and northern Atlantic coast, Georgia, South Carolina, North Carolina and Dry Tortugas), Greece (Kiparissia Bay), and Turkey [compiled by Bowen et al. (2004)]. We also used data from foraging grounds in USA (Texas, Florida northern and southern Gulf of Mexico, Florida southern and northern Atlantic coast, Georgia, South Carolina, North Carolina, Virginia and northeast USA), Azores, Madeira, and Mediterranean (Lampedusa, Gimnesias Islands, Pitiüses Islands, northeastern Spain, western and eastern Italy) (Bolten et al. 1998; Bowen et al. 2004; Carreras et al. 2006; Reece et al. 2006). For comparisons of genetic composition, the samples from the Azores and Madeira foraging grounds were combined because haplotype frequencies were not significantly different (Bolten et al. 1998). For phylogeographic analyses, the USA rookeries were combined into two groups, as suggested by Bowen et al. (2004): (1) southern USA, composed of Florida northern Gulf of Mexico (FL-NG), Florida southern Gulf of Mexico (FL-SG), Florida southern Atlantic coast (FL-SA) and Dry Tortugas (DT), and (2) northern USA, composed of Florida northern Atlantic coast (FL-NA), Georgia (GA), South Carolina (SC) and North Carolina (NC).

We also performed a mixed stock analysis (MSA) to estimate the relative contributions of different rookeries to the ERG foraging aggregation. We used the Bayesian algorithm with a Markov Chain Monte Carlo (MCMC)

estimation procedure implemented in BAYES (Pella and Masuda 2001). We used six baseline stocks [Brazil, Mexico, USA, Greece, Turkey and Australia; compiled by Bowen et al. (1995, 2004)] to run two MCMC chains of size 200,000, one chain per baseline stock with a starting point of 0.90 for the first and 0.02 for the others. Convergence of MCMC estimates to a desired posterior probability was assessed using the Gelman-Rubin shrink factor (Gelman and Rubin 1992), increasing the MCMC size until all values obtained were less than 1.2. The ERG composition was estimated from the mean of two chains after 100,000 burn-in steps.

Results

Genetic composition and diversity

Four distinct loggerhead control region haplotypes were observed among the 204 turtles sampled from Brazilian rookeries: CC-A4 (86.3%), CC-A24 (6.4%), CC-A25 (0.5%), and CC × LO (6.8%) (Fig. 1; Table 1). The CC × LO haplotype, only found in Sergipe, was attributed to specimens considered hybrids because they have the typical *Lepidochelys olivacea* mtDNA haplotype, but the external morphology of loggerheads (64.29%) or a mixture between loggerheads and *L. olivacea* (35.71%). CC × LO haplotypes were not included in the determination of standard diversity

indices. Six distinct haplotypes were found among the 125 loggerhead turtles sampled from the foraging aggregation at Elevação do Rio Grande (ERG): CC-A2 (10.4%), CC-A4 (47.2%), CC-A11 (15.2%), CC-A33 (14.4%), CC-A34 (12%), and CC-A35 (0.8%) (Fig. 1; Table 1). For comparisons, data from other Atlantic and Mediterranean rookeries and foraging aggregations are shown in Table 1. Brazilian rookeries have three unique loggerhead haplotypes that have not been observed at other rookeries (Table 1). Evaluating the 380 bp sequences, 54 polymorphic sites were found for all Brazilian haplotypes, including CC × LO, corresponding to 39 transitions, seven transversions and nine indels (supplementary Table S1, Supplementary Material online). Although more differences were observed in the longer 627 bp sequences, no new haplotypes were distinguished with the longer sequences (data not shown).

Standard diversity indices calculated for rookeries and foraging aggregations from Brazil and elsewhere can be seen in Table 2. Brazilian rookeries have low values of genetic and nucleotide diversity, only higher than the rookery from Greece. The genetic diversity of the Brazilian rookeries decreased from north to south (Fig. 1; Table 2). Sergipe had higher values with three different haplotypes, Bahia had two haplotypes, Espírito Santo also had two different sequences, but one of them at a low frequency, and finally Rio de Janeiro had only one haplotype. When the CC × LO haplotype was included in these indices, genetic and nucleotide diversity values increased considerably due to the high proportion

Fig. 1 Map of surveyed locations on the Brazilian coast and loggerhead turtle mtDNA haplotype frequencies for rookeries of RJ Rio de Janeiro, ES Espírito Santo, BA Bahia and SE Sergipe, and for the foraging aggregation of ERG Elevação do Rio Grande, Rio Grande do Sul

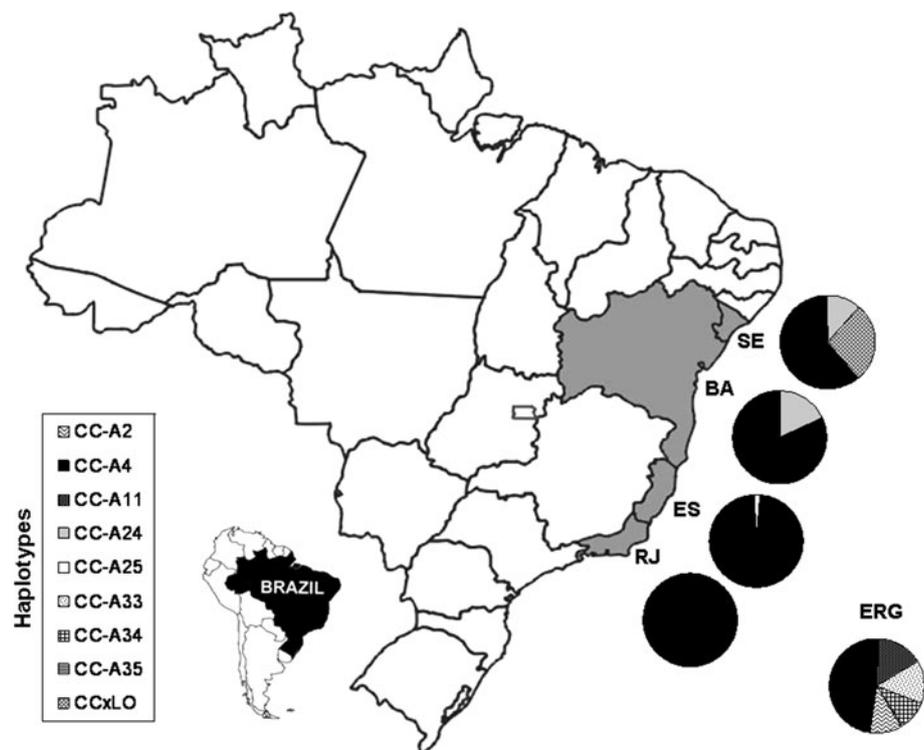


Table 1 MtDNA control region haplotypes found at rookeries and foraging grounds in the Atlantic and Mediterranean

Haplotypes	Rookeries								Foraging grounds			
	RJ	ES	BA	SE	MX	USA	GR	TR	BR	USA	AM	MT
CC-A1						198				823	60	62
CC-A2					11	103	78	19	<i>13</i>	583	50	236
CC-A3					2	6		13		66	7	22
CC-A4	<i>64</i>	<i>49</i>	<i>32</i>	<i>31</i>					<i>59</i>	1		
CC-A5						1				8		2
CC-A6							2					
CC-A7						5				23		2
CC-A8					1					4	1	
CC-A9					1	2				6		2
CC-A10					5	2	1			23	3	1
CC-A11						1			<i>19</i>	2	1	
CC-A12											1	
CC-A13										10	2	2
CC-A14						2				40	3	3
CC-A15											1	
CC-A16											1	
CC-A17											1	
CC-A18										1		
CC-A19										1		
CC-A20						1				11		
CC-A22										1		
CC-A23										1		
CC-A24			7	6								
CC-A25		<i>1</i>										
CC-A26												7
CC-A27												1
CC-A28												1
CC-A29												2
CC-A30												1
CC-A31												1
CC-A32												2
CC-A33									<i>18</i>			
CC-A34									<i>15^b</i>			
CC-A35									<i>1</i>			
CC-A44										1		
Total	<i>64</i>	<i>50</i>	<i>39</i>	<i>37^a</i>	20	321	81	32	<i>125</i>	1,605	131	347

Data from the present study (in italic) and Bolten et al. (1998), Bowen et al. (2004), Carreras et al. (2006) and Reece et al. (2006). Rookeries: *RJ* Rio de Janeiro, Brazil; *ES* Espírito Santo, Brazil; *BA* Bahia, Brazil; *SE* Sergipe, Brazil; *MX* Mexico; *USA* United States; *GR* Greece; *TR* Turkey. Foraging grounds: *BR* Brazil, Elevação do Rio Grande; *USA* United States; *AM* Azores and Madeira; *MT* Mediterranean

^a Total number of samples analyzed from Sergipe was 51, but 14 of them were CC × LO hybrids

^b Known from Australian rookeries

(~30%) of hybrids in the Sergipe population (Reis 2008). These increased values are expected since CC × LO haplotype is not a true loggerhead haplotype. The foraging aggregation (Elevação do Rio Grande) had a high haplotype diversity and a moderate nucleotide diversity compared with other Atlantic-wide foraging aggregations (Table 2).

Genetic structure

Within Brazil, pairwise F_{ST} revealed that Rio de Janeiro and Espírito Santo rookery samples were significantly different from those of Bahia and Sergipe (Table 3). No significant difference was found between Rio de Janeiro

Table 2 Standard diversity indices (mean \pm SD) calculated for rookeries and foraging grounds

	<i>N</i>	<i>h</i>	π
<i>Rookeries</i>			
Brazil ^a	190	0.1380 \pm 0.0327	0.000372 \pm 0.000606
RJ	64	0	0
ES	50	0.0400 \pm 0.0380	0.000107 \pm 0.000320
BA	39	0.3023 \pm 0.0795	0.000810 \pm 0.000957
SE	37	0.2793 \pm 0.0826	0.000749 \pm 0.000915
Mexico	20	0.6526 \pm 0.0927	0.002385 \pm 0.001930
USA	321	0.5174 \pm 0.0205	0.022985 \pm 0.011765
FL-NG	49	0.3827 \pm 0.0805	0.017651 \pm 0.009399
FL-SG	45	0.6636 \pm 0.0436	0.025408 \pm 0.013172
FL-SA	64	0.5665 \pm 0.0304	0.024627 \pm 0.012706
FL-NA	14	0	0
GA	43	0.0465 \pm 0.0439	0.002248 \pm 0.001801
SC	20	0	0
NC	28	0	0
DT	58	0.2541 \pm 0.0735	0.006824 \pm 0.004113
Greece	81	0.0728 \pm 0.0396	0.000067 \pm 0.000248
Turkey	32	0.4980 \pm 0.0391	0.001322 \pm 0.001286
<i>Foraging grounds</i>			
ERG	125	0.7140 \pm 0.0310	0.01744 \pm 0.001560
USA	1,605	0.6026 \pm 0.0078	0.024588 \pm 0.012495
AM	130	0.6450 \pm 0.0265	0.025036 \pm 0.012797
MT	347	0.5022 \pm 0.0287	0.015557 \pm 0.008235

Data from the present study (in italic) and Bolten et al. (1998), Bowen et al. (2004), Carreras et al. (2006) and Reece et al. (2006). Hybrids from the Brazilian population were not considered. *N* sample size, *h* haplotype or genetic diversity, π nucleotide diversity. *RJ* Rio de Janeiro, *ES* Espírito Santo, *BA* Bahia, *SE* Sergipe, *ERG* Elevação do Rio Grande, Rio Grande do Sul, Brazil; *Mexico* Quintana Roo; *USA* United States, Florida northern Gulf of Mexico (FL-NG), Florida southern Gulf of Mexico (FL-SG), Florida southern Atlantic coast (FL-SA), Florida northern Atlantic coast (FL-NA), Dry Tortugas (DT), Georgia (GA), South Carolina (SC), North Carolina (NC); *Greece* Kiparissia Bay; *AM* Azores and Madeira; *MT* Mediterranean

^a CC \times LO hybrids from Sergipe (*N* = 14) were not included in the calculation

and Espírito Santo ($F_{ST} = 0.005$, $P = 0.441$), or between Bahia and Sergipe ($F_{ST} = -0.026$, $P = 0.999$). Estimates of maternal gene flow (Nm) were very high between Rio de Janeiro and Espírito Santo ($Nm = 99.115$) and between Bahia and Sergipe ($Nm = \infty$), and moderate among the other rookeries (Table 3). The same pattern was observed with the exact test of population differentiation (supplementary Table S2, Supplementary Material online). These data suggest that Brazil has two loggerhead genetic population units: one represented by Rio de Janeiro and Espírito Santo (southern stock), and the other by Bahia and Sergipe (northern stock). AMOVA global F_{ST} (0.116, $P = 0.000$) indicated significant genetic structuring among the Brazilian sampled rookeries. However, AMOVA F -statistics

did not confirm this two-stock structure ($F_{CT} = 0.190$, $P = 0.352$, data not shown).

When considering Atlantic-wide rookeries, pairwise F_{ST} revealed significant differentiation among all sampled sites (Table 3). The same pattern was observed with the exact test of population differentiation (supplementary Table S2, Supplementary Material online). Estimates of maternal gene flow (Nm) were higher between Mexico and Greece ($Nm > 1$) and between Mexico and Turkey ($Nm > 2$), and lower among the other rookeries (Table 3). The higher Nm values involving Brazil were between the Brazilian rookeries and USA ($Nm > 1$). Despite non-significant AMOVA F -statistics ($P > 0.05$; data not shown), AMOVA global F_{ST} (0.599, $P = 0.000$) indicated the existence of a strong genetic structuring among the global sampled rookeries.

Haplotype relationships

A TCS network with all loggerhead haplotypes from Brazil and Atlantic-wide rookeries (Fig. 2) revealed that the rookery haplotypes from Brazil and some from USA were closely related. These USA haplotypes included CC-A1 (the most frequently detected haplotype in western North Atlantic rookeries), CC-A11 and CC-A14.

Demographic history

Because of the pairwise F_{ST} results (Table 3), we made mismatch distribution analyses for the two Brazilian stocks (northern: Sergipe and Bahia, and southern: Espírito Santo and Rio de Janeiro) and for the combined Brazilian stocks. Mismatch distributions were all unimodal due to the small number of haplotypes and substitutions among them (supplementary Figure S1, Supplementary Material online). Greece and Turkey also exhibited unimodal distributions while Mexico and USA had multimodal distributions (data not shown). These data suggest that Brazil has experienced recent demographic and spatial expansions, as well as Greece and Turkey, resulting from bottlenecks or founder effects.

Tajima's D and Fu's F_s are expected to be negative when genetic structure has been influenced by rapid population expansion, and this pattern is seen for Brazil ($D = -0.82072$, $F_s = -1.50394$), Brazilian southern stock ($D = -1.01327$, $F_s = -2.32952$), and Greece ($D = -1.05129$, $F_s = -0.42766$). The neutrality tests did not confirm the expansion scenario for the Brazilian northern stock and Turkey.

The Mantel test for the Brazilian rookeries indicated non-significant correlation between genetic and geographic distances ($r = 0.867$, $P = 0.122$). For all Atlantic and Mediterranean rookeries, the Mantel test suggested the isolation by distance model ($r = 0.564$, $P = 0.003$).

Table 3 Genetic differentiation (pairwise F_{ST}) between the eight loggerhead turtle rookeries (below diagonal) and estimation of the number of migrants per generation (Nm ; above diagonal)

Rookeries	RJ	ES	BA	SE	MX	USA	GR	TR
RJ		99.115	1.904	2.104	0.007	1.248	0.000	0.005
ES	0.005		2.657	3.023	0.009	1.305	0.001	0.007
BA	0.208*	0.158*		∞	0.016	1.342	0.004	0.012
SE	0.192*	0.142*	−0.026		0.016	1.355	0.003	0.012
MX	0.987*	0.983*	0.969*	0.969*		0.607	1.432	2.307
USA	0.286*	0.277*	0.271*	0.269*	0.452*		0.490	0.553
GR	0.999*	0.998*	0.993*	0.993*	0.259*	0.505*		0.486
TR	0.989*	0.987*	0.976*	0.977*	0.178*	0.475*	0.507*	

∞ means infinite

The significance of permutation test (10,000 permutations) is shown for * $P < 0.05$. Data from the present study and compilation by Bowen et al. (2004). *RJ* Rio de Janeiro, Brazil; *ES* Espírito Santo, Brazil; *BA* Bahia, Brazil; *SE* Sergipe, Brazil; *MX* Mexico; *USA* United States; *GR* Greece; *TR* Turkey

Mixed stock analysis

Nineteen individuals in the ERG foraging aggregation sample were excluded from the analysis because they represented orphaned haplotypes (18 individuals with CC-A33 and 1 individual with CC-A35). All chains consistently indicated a major contribution to the ERG foraging aggregation from Brazil (mean 59.5%) and a non-significant contribution from the USA, Mexico, and Turkey (Table 4). This result is expected due to the presence of the Brazilian exclusive haplotype (CC-A4) in 59 out of 106 individuals from the foraging aggregation used for the MSA. The second highest contribution detected by MSA was from Australia (28.5%), because haplotype CC-A34 has only been reported from Australian rookeries. Another significant contribution was also detected from Greece, due to the presence of CC-A2 among the Brazilian foraging aggregation, which is present at high frequencies in Greece. Although CC-A2 is also frequently observed in USA, the MSA takes into account that the contribution from a particular rookery should include all of the most frequent haplotypes.

Discussion

Genetic composition and haplotype relationships

Endemic haplotypes (CC-A4, CC-A24, CC-A25), found only in Brazilian rookeries, create a unique Brazilian haplotype profile (Table 1). The CC-A4 haplotype is common to all Brazilian rookeries and foraging areas (Fig. 1). This fact, associated with the Brazilian low nucleotide diversity (Table 2) and phylogenetic proximity among rookery haplotypes (data not shown), suggests a common origin, with CC-A4 the probable ancestral haplotype of Brazilian populations. The phylogenetic proximity between CC-A1 and

CC-A4 haplotypes suggests that the colonization of Brazilian rookeries could have been from the USA stock. Because the Brazilian rookeries also show low genetic diversity values (Tables 1, 2) and low divergence between those haplotypes, the Brazilian populations were probably colonized recently. As genetic diversity decreases from northern to southern Brazil, we suggest that colonization of the rookeries followed a north to south route along the coastline, probably influenced by the Brazilian warm current which flows north to south. At times of glacial retreats, loggerhead nesting and foraging habitats expanded into higher latitudes. Encalada et al. (1998) suggested that, during these interglacial periods, an equatorial lineage may have colonized northern latitudes along the Florida peninsula, which explains the existence of different phylogenetic lineages in USA. We believe that colonizations in Brazil followed the same pattern. Our hypothesis is supported by the fact that loggerheads show the propensity for occasional long distance colonization, as indicated by the widespread distribution of some haplotypes.

For the first time, CC-A2, CC-A11, CC-A33, CC-A34 and CC-A35 haplotypes were reported for a Brazilian foraging aggregation (ERG). CC-A2 and CC-A11 have been reported from northwestern Atlantic rookeries, and CC-A2, also from Mediterranean rookeries. Haplotype CC-A34 has been reported from Australia (Bowen et al. 1995). The occurrence of these haplotypes is consistent with the transoceanic migratory behavior of loggerheads. The source rookeries for CC-A33 and CC-A35 haplotypes are unknown.

Foraging aggregations generally show higher diversity indices than rookeries (Table 2) because rookeries host philopatric females while foraging aggregations can receive individuals from many source rookeries. The occurrence of CC-A4, an endemic Brazilian haplotype, in the ERG aggregation indicates that those turtles belong to the Brazilian loggerhead genetic stock, which was corroborated by

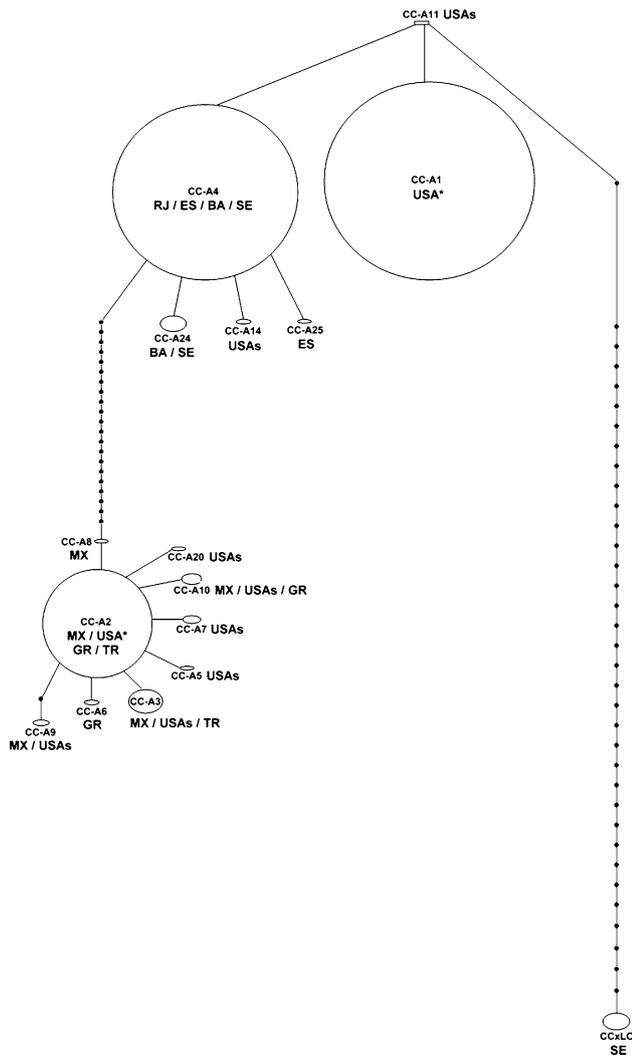


Fig. 2 TCS network of loggerhead turtle mtDNA haplotypes from Atlantic and Mediterranean rookeries. *Lines* between haplotypes represent one mutational step; *small black circles* are hypothetical haplotypes; the *rectangle* identifies the most probable ancestral haplotype according to the coalescent theory. *Circles/ovals* are approximately proportional to haplotype frequencies. *RJ* Rio de Janeiro, Brazil; *ES* Espírito Santo, Brazil; *BA* Bahia, Brazil; *SE* Sergipe, Brazil; *MX* Mexico; *USAs* southern United States; *USA** southern and northern United States; *GR* Greece; *TR* Turkey

the MSA results (Table 4). However, the ERG aggregation is a mixed stock with haplotypes from worldwide rookeries. The haplotypes CC-A33, CC-A34 and CC-A35 form an independent cluster in phylogenetic reconstructions (data not shown) and probably have a common origin. The MSA results (Table 4) must be interpreted with care. MSA assumes that all source rookeries are known, but African and Indo-Pacific rookeries have been very poorly surveyed. Thus, the proposed contributions from Australia and Greece may well derive from rookeries on the west coast of Africa or Indo-Pacific rookeries.

Genetic structure

Our results suggest the existence of two Brazilian loggerhead genetic stocks: the northern stock (Sergipe and Bahia rookeries), and the southern stock (Espírito Santo and Rio de Janeiro rookeries). Results of pairwise F_{ST} and exact tests of population differentiation indicate that these two population units exist (Table 3 and supplementary Table S2, Supplementary Material online). However, AMOVA results do not support population differentiation, and Nm values are inconclusive. Extremely high Nm values ($Nm = \infty$) were estimated between the rookeries within the two proposed stocks (Table 3). Nm values were much lower (≤ 3) between the other rookeries, but all values were greater than 1. In general, Nm values greater than one indicate that gene flow is sufficient to prevent divergence of isolated gene pools by genetic drift (Wright 1951; Birky et al. 1983; Slatkin 1987). However, Nm values based just on mtDNA data must be interpreted with caution, because estimates are derived exclusively from single haploid genealogies. So, these data should be viewed as general indicators of the magnitude of genetic exchange. We strongly recommend complementary studies based on biparentally inherited nuclear markers.

Pairwise F_{ST} , exact test of population differentiation, Nm estimation, and AMOVA indicated strong genetic structuring among global rookeries (Table 3 and supplementary Table S2, Supplementary Material online). These data corroborate the natal homing behavior of loggerheads on a global scale. If female loggerheads return to natal sites for nesting, then rookeries would show pronounced differences with respect to female-transmitted genetic markers such as mtDNA (Bowen et al. 1994). However, natal homing in loggerhead turtles cannot be absolute, because new rookeries must be colonized by turtles hatched elsewhere.

Demographic history

The TCS network (Fig. 2) indicated that rookery haplotypes from Brazil are closely related to some from USA, especially from the south, suggesting that the Brazilian rookeries could have been colonized from the southern USA stock. The same pattern was supported by Nested Clade Analysis (NCA, data not shown), that indicated restricted gene flow/dispersal but with some long distance dispersal among western Atlantic rookeries (southern USA and Brazil). Encalada et al. (1998) suggested that northerly USA colonization occurred from southern USA ancient lineages during recent interglacial periods. Our data indicate that Brazilian rookeries may have had the same origin. Neutrality tests and mismatch distribution analysis (supplementary Figure S1, Supplementary Material online) suggest that the Brazilian rookeries have

Table 4 Estimated contributions of six loggerhead rookeries to the aggregation in Elevação do Rio Grande, Brazil, based on Bayesian Markov Chain Monte Carlo (MCMC) mixed stock analysis

Stock	Mean	SD	2.5%	Median	97.5%	MCMC Sample
Brazil	0.5950	0.0903	0.4610	0.5722	0.7861	81000
Mexico	0.0056	0.0155	0.0000	0.0003	0.0484	81000
USA	0.0025	0.0063	0.0000	0.0002	0.0206	81000
Greece	0.1049	0.0402	0.0031	0.1060	0.1814	81000
Turkey	0.0067	0.0178	0.0000	0.0003	0.0615	81000
Australia	0.2852	0.0913	0.1019	0.3079	0.4257	81000

Mean values are shown with SD. The 2.5 and 97.5% values indicate the upper and lower bounds of the 95% confidence interval

experienced sudden demographic and spatial expansions, resulting from bottlenecks or founder effects. Thus, Brazilian rookeries were colonized recently in loggerhead evolutionary history, which is supported by the low genetic and nucleotide diversity of the Brazilian rookeries (Table 2). We propose that this recent colonization followed a northern to southern route along the Brazilian coastline, following the Brazilian warm current, although the isolation by distance model was not supported by the Mantel test. This hypothesis is corroborated by the haplotype distribution along the Brazilian coast, because diversity decreases from north to south (Fig. 1). Neutrality tests also indicated that the Brazilian southern stock is more recent than the northern one. Maritime current dynamics have changed through the years; this colonization hypothesis should be re-evaluated considering paleogeography and paleocurrents.

Results of the Mantel test supported the isolation by distance model between western Atlantic (northern and southern USA, and Brazil) and Mediterranean (Greece and Turkey) rookeries. This model results from spatially limited gene flow with gene dispersal only between adjacent areas. However, long distance colonization is essential to explain the current global distribution of loggerhead rookeries. Despite the great migratory capacity of loggerheads, isolation by distance is a result of their philopatric behavior. As females tend to return to their natal region to nest, it is expected that new colonizations would occur in adjacent areas. We suggest that the most recently colonized rookeries are Greece and Brazil. Colonization into the Mediterranean Sea was most likely accomplished within the last 10,000 years, after the Wisconsin glaciation (Encalada et al. 1998).

Conservation concerns

The existence of two loggerhead genetic population units in Brazil, as well as evidence of extensive hybridization between loggerheads and *L. olivacea*, will influence the development and implementation of appropriate

management strategies in the country. The extent of hybridization between different species of the Family Cheloniidae must be investigated to understand the implications and causes of such events, and its impact on the genetic diversity and identity of those species. Natal homing in loggerhead females, which was corroborated by our data, means that each regional nesting population comprises an independent stock or an evolutionary significant unit (ESU). Thus, an extirpated rookery will not be reestablished over a timescale compatible with human interests. Although new nesting beaches must be occasionally colonized, the frequency of such events is low. Each ESU needs an adequate management plan, designed to meet its specific needs. There are still many sea turtle rookeries and foraging grounds that have not been sampled for genetic studies in the Atlantic and around the world. More studies are needed to fill these gaps. Due to the limited information provided by mitochondrial DNA markers, we recommend complementary analysis based on biparentally inherited nuclear markers to confirm and better understand the genetic structure and demographic history of loggerhead populations.

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