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Genetic Analysis to Elucidate the Natural History and Behavior of Hawksbill Turtles (*Eretmochelys imbricata*) in the Wider Caribbean: a Review and Re-Analysis

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ABSTRACT. – **Surveys of hawksbill turtles in the Atlantic Ocean have been undertaken to examine mitochondrial DNA (mtDNA) diversity and to test hypotheses concerning female reproductive migratory behavior and the composition of foraging ground populations. Studies of nesting females indicate that a natal homing mechanism predominates and that nesting populations should be considered separate stocks. Analyses utilizing maximum likelihood algorithms have indicated that foraging populations are composed of cohorts from several regional nesting colonies. The significant haplotype frequency shifts between two foraging areas (in Puerto Rico and Cuba) indicate the contributions from regional nesting areas differ from location to location. These differences may be attributed to the proximity of nesting areas, the availability of foraging habitat, oceanographic conditions, and migratory behavior. Management practices such as head-starting also may impact the demography of foraging populations.**

KEY WORDS. – **Reptilia; Testudines; Cheloniidae; *Eretmochelys imbricata*; sea turtle; life history; genetics; mtDNA; populations; nesting; foraging; juveniles; adults; mixed stock analysis**

The hawksbill turtle, *Eretmochelys imbricata*, occurs in tropical waters of the Atlantic and Indo-Pacific regions (Márquez, 1990). Hawksbills have long been coveted for their beautiful scutes (tortoiseshell) and exploitation dates back to at least ancient Egypt. Hawksbill shell was introduced to Japan from China around AD 745–784, and the worked shell was reserved for aristocracy as a status symbol (Márquez, 1990). The working of tortoiseshell is still considered a cultural tradition in several parts of the world and the demand for hawksbill turtles persists. Between 1970 and 1986, approximately 250,000 Caribbean hawksbills were harvested for exportation to Japan (Milliken and Tokunaga, 1987).

Detailed information is not available concerning the migratory behavior of juveniles or adults. In early reports this species was considered less migratory than other marine turtles, but recent research has shown that hawksbills make migrations comparable to those of other marine turtle species (Miller et al., 1998).

What role do analyses of genetic diversity, as exhibited here with mitochondrial DNA (mtDNA), serve in attempts to understand the behavior of marine turtles? Here I will illustrate the role genetic studies can play in comprehending marine turtle natural history in general, and hawksbill turtle biology in particular. The nature of these studies illustrates the necessity of international cooperation between field biologists, wildlife managers, and governments in far-flung areas.

Genetic studies of nesting and foraging hawksbill turtles have been conducted in both the Indo-Pacific and Atlantic basins (Broderick et al., 1994; Broderick and Moritz, 1996; Bass et al., 1996; Bowen et al., 1996; Koike et al., 1996; Okayama et al., 1996). These studies are the first attempts to document the distribution of genetic diversity in this endangered animal. Most of these studies have used sequences of the control region in the mtDNA genome, with the intention of resolving genetic structure of hawksbill populations. The mtDNA data have been used to test hypotheses concerning reproductive behavior and the composition of foraging populations, and to infer patterns of migration and population structure.

Nesting Beach Studies

Bass et al. (1996) examined seven nesting locations (Fig. 1) in the Caribbean and Western Atlantic. Twenty-one mtDNA haplotypes were identified among the 103 animals sampled. Individuals possessing four haplotypes at a nesting location in Bahia, Brazil, were determined to be the result of hybridization between the loggerhead (*Caretta caretta*) and the hawksbill. Only two haplotypes (A and F) were observed at multiple nesting sites, with the 15 remaining haplotypes restricted to samples from single nesting locations. Significant shifts in haplotype frequencies were found among most nesting locations, indicating that gene flow is not sufficient to unite these populations as a single demographic entity. In addition, estimates of migration rates (M, as defined by Slatkin and Maddison, 1989) between nesting locations ranged from M = 0 to 2 average number of migrants per generation (Bass et al., 1996). Generally, M values less than 4 indicate that gene flow is too low to prevent populations from differentiating over time (Slatkin, 1987). Tests of isolation by distance also were conducted to investigate a possible relationship between distance and the estimated migration rates (Bass, 1996). Proximal nesting colonies are expected to be closely related in terms of mtDNA if coloni-

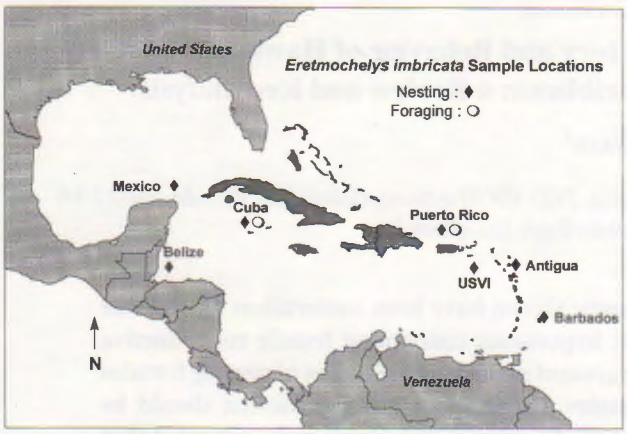

Figure 1. Map of sampling locations for hawksbill (*Eretmochelys imbricata*) nesting and foraging sites referred to in the text. One sampled nesting location (Bahia, Brazil) is not shown on this map.

zation events occur preferentially over short distances. No significant correlation was detected between migration rates and distances among nesting locations. These analyses showed no evidence that nesting colonies in close proximity tended to be more closely related.

Results from the nesting beach study indicated that at least six of the seven assayed nesting populations in the Caribbean and Western Atlantic with separations ranging from 300 to more than 7000 km (Mona Island, Puerto Rico; Jumby Bay, Antigua; Isla Holbox, Yucatan, Mexico; Barbados; Bahia, Brazil; Buck Island, St. Croix, U.S. Virgin Islands; and Gale's Point, Belize) were genetically isolated in terms of maternal lineages, and thus represented distinct stocks. This information is useful for examining models of female reproductive migratory behavior and provides a basis for estimating the nesting colony origin of turtles found at non-nesting foraging grounds.

The Republic of Cuba (1997) summarized data from Koike et al. (1996) and Okayama et al. (1996) on the genetics of nesting females from Mexico, Mona Island (Puerto Rico), and Cuba (see Table 1). They found no sharing of haplotypes between samples from these three nesting locations except for one individual in the Cuban population that carried a haplotype also found in Puerto Rico. Since the mtDNA control region was the focus of these studies, direct comparisons can be made with the results from other nesting locations in the Caribbean (Bass et al., 1996). The nesting population in Cuba contains a unique haplotype which was detected (but not assigned a place of origin) in turtles on foraging grounds off Mona Island, Puerto Rico (Bowen et al., 1996). One new haplotype was identified in an individual from Mexico, and two haplotypes found in Cuba (A and F) were also observed in nesting locations elsewhere in the Caribbean.

In general, genetic studies of nesting females indicate that natal homing (returning to nest in the region of origin) is the dominant influence on reproductive migrations in female hawksbill turtles, although rare movement between nesting colonies occurs. These findings indicate that if a population is eradicated, recolonization will not occur in a time frame relevant to management concerns. Historical records of extirpated nesting sites in the Caribbean support this hypothesis of infrequent recolonization. Investigations have found little or no current nesting at Chiriquí, Panama (Meylan, 1989), in what used to be the largest nesting colony of hawksbills in the Caribbean (Carr, 1956). Finally, the presence of significant shifts in haplotype frequencies between nesting locations, as well as the presence of endemic haplotypes, enables scientists and managers to distinguish among different genetic stocks of marine turtles and to identify the place of origin of turtles on feeding grounds.

Foraging Ground Studies

Bowen et al. (1996) reported the genetic composition of foraging juveniles captured on the reefs surrounding Mona Island, Puerto Rico. In their analysis of 41 individuals from the foraging ground, two hypotheses were tested: 1) are mtDNA haplotypes found among foraging animals drawn primarily from the adjacent nesting location at Mona Island, Puerto Rico, and 2) can the origin of marine turtles on foraging grounds be determined by maximum likelihood analysis (see Pella and Milner, 1987; Masuda et al., 1991).

Nine haplotypes were detected among the 41 animals sampled from the foraging ground. Based on a statistical test (*G*-test) of independence, foraging ground haplotype frequencies were significantly different from those of females nesting on adjacent Mona Island. In addition to the two common haplotypes detected in multiple nesting locations, haplotypes found only at single nesting locations were observed in the foraging population. The unique haplotypes

Table 1. Haplotype frequencies for hawksbill nesting locations in the Caribbean (for specific location data see Bass et al., 1996); * = data from Republic of Cuba (1997).

Location	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	γ	D11
Belize						11	1	1	1														
Mexico																2	13						
Mexico*																	33						1
Puerto Rico	1					1				2	1	1	2	6	1								
Puerto Rico*						12						1		6	1								
U.S. Virgin Islands	3					28																	
Antigua	9	4	2																				
Barbados	11			1	3																		
Brazil	4																	6	2	1	1		
Cuba*	9					1																2	

were B and Q, which have been identified only from nesting beaches in Antigua and Mexico, respectively. Two haplotypes (L and N) have been identified only in the Mona Island nesting population. The remaining three haplotypes (alpha, beta, and gamma) could not be assigned to a nesting population.

The presence of haplotypes endemic to distant nesting grounds indicated that the Mona Island foraging ground aggregation was not drawn solely from the adjacent nesting colony, but represents a mixed pool of individuals from Mona Island and elsewhere in the Caribbean. In all, contributions from six Caribbean nesting colonies were identified. It was noted that the maximum likelihood estimates were to be treated as qualitative indicators of contributions from multiple nesting locations and that testing of greater numbers of individuals from the foraging location was necessary to precisely estimate contributions from regional nesting colonies. Although the above findings were of a preliminary nature, they did suggest that increases in mortality on a foraging ground could affect nesting populations in other parts of the Caribbean, including locations far removed from the foraging ground.

Re-Analysis and Discussion of Foraging Populations

The Republic of Cuba (1997) summarized the results of foraging ground surveys from both Mona Island, Puerto Rico (n = 106) and Cuba (n = 63). With the mtDNA haplotype data (n = 12) from the nesting population in Cuba (Republic of Cuba, 1997), we can now conduct maximum likelihood analysis using this population as a possible source population. To test if the resampling of the Mona Island foraging ground (Republic of Cuba, 1997) was significantly different from the sample used by Bowen et al. (1996), a chi-square test of independence of haplotype frequencies was conducted (Sokal and Rohlf, 1981; Zaykin and Pudovkin, 1993). The two Mona Island foraging ground samples were not significantly different from each other (χ^2 = 12.7; p = 0.599), indicating no differences in feeding ground composition between samples obtained in successive years. Therefore, foraging ground haplotype frequencies from the two studies were combined, and the initial finding of Bowen et al. (1996) that the foraging ground mtDNA haplotype frequencies were significantly different from those of the adjacent nesting site was retested. Chi-square tests once again indicated that the haplotype frequency at the Mona Island foraging ground was significantly different from its adjacent nesting location (χ^2 = 67.9; $p < 0.001$). The same tests were then applied to the Cuban foraging and nesting populations. They indicated that the haplotype composition of the foraging population in Cuba was not significantly different from its adjacent nesting colony (χ^2 = 7.7; p = 0.753). This finding may indicate that the Cuban foraging population is drawn primarily from the Cuban nesting population, but does not preclude the possibility that animals from other nesting locations also forage in Cuban waters.

The expanded Mona Island data set was re-analyzed with maximum likelihood methodology (Table 2). Results indicate that several source populations contribute to the foraging ground at Puerto Rico. With the addition of the Cuban nesting population data, Cuba becomes a significant contributor to the Puerto Rico foraging population (Table 2). However, these numbers should be interpreted with caution. It would be inappropriate, for example, to say that exactly 40% of the foraging animals at Puerto Rico originate in the U.S. Virgin Islands. These maximum likelihood estimates give qualitative (rather than precise) estimates of the foraging ground composition, they are more appropriate for general hypothesis testing. The sample sizes from both the foraging and nesting locations would have to be greatly increased to obtain precise resolution. Even if sample sizes are increased, violations of the assumptions inherent in the maximum likelihood analysis may still confound the analysis (Pella and Milner, 1987). However, the initial conclusion that foraging grounds are composed of multiple stocks originating on distant nesting grounds is substantially strengthened. These data do not support the hypothesis that hawksbills are non-migratory. On the contrary, significant migration from distant nesting locations to the foraging ground is strongly indicated.

Further support for the hypothesis that foraging populations are composed of multiple nesting stocks is provided from the maximum likelihood results for the Cuban foraging

Table 2. Estimates of contributions by different nesting populations to two hawksbill foraging populations in the Caribbean, using the program GIRLSEM (Masuda et al., 1991). If a foraging population haplotype had not been identified previously at a nesting location, then the sample was excluded from the analysis. The standard deviation was calculated using the infinitesimal jackknife procedure; n = the total number of individuals used in the analysis.

	Foraging Populations			
	Cuba (n = 55)		Mona Island (n = 133)	
Nesting Populations	Contribution	Standard Deviation	Contribution	Standard Deviation
Belize	0.0821	0.0700	0.0344	0.0294
Mexico	0.0545	0.0306	0.1353	0.0296
Puerto Rico	0.0604	0.0308	0.1376	0.0382
U.S. Virgin Islands	0.0870	0.0929	0.4150	0.0625
Antigua	0.0483	0.0464	0.0414	0.0270
Barbados	0	0	0	0
Brazil	0	0	0	0
Cuba	0.6673	0.0850	0.2361	0.0461

populations (Table 2). Six of the eight nesting populations sampled thus far in the Caribbean and Western Atlantic contribute at detectable levels to the Cuban foraging population; however, the U.S. Virgin Islands' contribution is most likely not significant due to the large standard deviation. In addition, comparisons of these results to previous studies (Bowen et al., 1996) suggest that as more potential source populations are sampled, the maximum likelihood estimates are likely to change. There can be an increase in the number of populations contributing as was the case with the addition of the Cuban nesting location to the analysis. There can also be changes in the maximum likelihood estimates with an increase in the number of foraging animals sampled. For these reasons, I emphasize the qualitative nature of these results: that multiple nesting colonies contribute to localized foraging populations, but specific contributions are not precisely resolved.

A comparison of Cuban and Puerto Rican feeding cohorts indicates that these locations do not constitute one continuous foraging population but represent a series of demographic partitions. For example, the Cuban nesting population contributes an estimated 65% to the Cuban foraging population and 24% to the Puerto Rican foraging group. In addition, the haplotype frequencies between these foraging grounds are significantly different ($\chi^2 = 26.03$; $p = 0.002$). While turtles from separate nesting populations intermingle extensively on feeding grounds, it seems likely that turtles do not recruit randomly to these foraging locations.

Determinants of Foraging Ground Composition

Differences in the composition of Cuban and Puerto Rican foraging populations warrant more investigation. These studies will likely generate insights into the early life history and migratory habits of hawksbill turtles. At this time, several hypotheses may be forwarded to explain the significant partition between Cuban and Puerto Rican foraging cohorts.

First, there may be differences in feeding ground recruitment among different size classes. Samples collected to date encompass a wide range of size classes, but small sample sizes per size class prohibit further investigations of this question at this time.

Second, although we have not observed temporal differences in the genetic composition of nesting or foraging populations, this possibility has not been rigorously tested. Except for the nesting locations in Mexico, Puerto Rico, and the U.S. Virgin Islands, no nesting populations have yet been resampled.

A third possible hypothesis for explaining the observed differences invokes changes in life history induced by management practices (Mrosovsky, 1983; Mortimer, 1988). For example, if animals are head-started (retained in captivity after hatching and released months or years later), possible changes in their natural migratory and recruitment patterns could result. Two possible outcomes of head-starting that could explain a large contribution to an adjacent feeding location are: 1) juveniles may be more inclined to remain close to their natal beach for a period of time and then embark on migrations, or 2) they could be past their juvenile migratory stage and becoming resident near the point of release. The present data cannot differentiate between these two scenarios. Future research efforts on foraging grounds will allow tests of hypotheses concerning these determinants of foraging ground composition.

Conclusions

Investigations of hawksbill turtles with genetic markers have only recently moved beyond the preliminary stages. However, in a field where so little is known, the initial results greatly enhance our understanding of migratory behavior. Studies in both the Indo-Pacific and Atlantic Oceans have demonstrated that hawksbill nesting colonies represent distinct and identifiable genetic stocks — they should be treated as separate management units in conservation or utilization plans. Aggregations of turtles resident on surveyed foraging grounds in the Caribbean are composed of mixed stocks that recruit to these areas on a scale greater than 100 km but less than 5000–7000 km. This conclusion is based on the lack of detected contributions from the southeast Caribbean (Barbados) and Brazilian populations to the foraging grounds in Cuba and Puerto Rico. Therefore, if harvesting occurs on a foraging ground, multiple reproductive stocks will be affected. Future studies can elucidate the specific range states that contribute to a particular foraging population of hawksbill turtles. Due to long generation times, studies of hawksbills using genetic markers cannot yet determine the life history stage at which marine turtles may become "resident" in a developmental habitat (but see Laurent et al., 1998). Consequently, the data concerning both nesting and foraging populations do not preclude the possibility that residency in developmental habitats may be established for extended periods of time. The data from both nesting and foraging grounds, however, provide strong evidence that hawksbills migrate from nesting locations to other foraging habitats and back to their original nesting locations during various stages of their life. Finally, although the sampled nesting locations represent the majority of nesting populations in the Caribbean, the presence of unidentified haplotypes on the foraging grounds underscores the continuing need for additional sampling of nesting locations.

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