Towards a Molecular Profile of Marine Turtles in the Caribbean Overseas Territories

Foreign & Commonwealth Office

defra
Department for Environment Food and Rural Affairs

This document is part of a larger publication and should be cited as:
Godley BJ, Broderick AC, Campbell LM, Ranger S, Richardson PB (2004) 10. Towards a Molecular Profile of Marine Turtles in the Caribbean Overseas Territories. In: An Assessment of the Status and Exploitation of Marine Turtles in the UK Overseas Territories in the Wider Caribbean. pp 223-236. Final Project Report for the Department of Environment, Food and Rural Affairs and the Foreign and Commonwealth Office.

The full report is hosted in PDF format at the Project website: http://www.seaturtle.org/mtrg/projects/tcot/finalreport/

This project was implemented by the Marine Turtle Research Group (University of Exeter in Cornwall, UK), the Marine Conservation Society (UK), and Duke University (USA) in association with the Cayman Islands Department of Environment, Cayman Turtle Farm, and University of Cardiff (UK). This initial consortium was expanded to include a large number of organisations across the Overseas Territories.

10. Towards a Molecular Profile of Marine Turtles in the Caribbean Overseas Territories.

Contents

10.1. Summary and Recommendations

Summary

The genetics component of TCOT involved collection of genetic samples on a vast scale from nesting and feeding populations of green turtles (*Chelonia mydas*), hawksbills (*Eretmochelys imbricata*), loggerheads (*Caretta caretta*) and leatherbacks (*Dermochelys coriacea*) from Anguilla, the British Virgin Islands, the Cayman Islands, Montserrat and the Turks and Caicos Islands. A total of 383 samples were successfully analysed (following 530 analysis runs), including 112 from nesting females/hatchlings and 271 from foraging turtles. A fragment of the mitochondrial DNA control region was sequenced and the haplotype of each sample was determined through alignment against known sequences.

Hawksbill nesting populations in Anguilla, the British Virgin Islands (BVI), Montserrat, and Turks and Caicos (TCI) were described for the first time, and were found to exhibit six different haplotypes, two of which were previously undescribed. In the Cayman Islands we found 7 haplotypes among the Cayman Turtle Farm green turtle breeding population, and 3 among the wild nesting population. The loggerhead nesting population in the Cayman Islands exhibited 2 known haplotypes and one new one. The feeding green turtle populations at all TCOT sites combined exhibited a total of 9 different haplotypes, including two previously undescribed. The hawksbill feeding populations exhibited 14 haplotypes, 4 previously undescribed.

We compared our results with haplotype distributions for nesting and foraging ground populations in the Caribbean region and the Atlantic Ocean basin to attempt to establish possible qualitative links between sites. An overview is also presented regarding additional green turtle, hawksbill and loggerhead samples from Bermuda that have been collected by the Bermuda Turtle Project and analysed by Dr. Peter Meylan of Eckert College, USA.

A total of 446 samples are still pending analysis from Anguilla, BVI, Cayman, Montserrat and TCI. Leatherback sample analysis will be carried out in the near future in collaboration with the Dr. Peter Dutton of the Southwest Fisheries Science Center, USA.

Recommendations

10.1.1. It is recommended that sample sizes for both nesting and feeding populations are increased at all sites.

In the IUCN MTSG Techniques Manual (1999) Fitzimmons *et al.* recommended that: *"Sampling sizes for mixed stock assessment depend on the number of candidate source populations and the level of differentiation between nesting colonies. A typical feeding ground population should include at least 100 individuals."* Given the low genetic variability observed among certain species (such as green turtles, for example) we recommend that feeding ground studies should attempt to maximise sample sizes, to include as many as 200 individuals wherever possible. Samples of foraging populations can be part of ongoing inwater monitoring/research protocols but should also be part of the recording process, which we highly recommend as part of all remaining marine turtle fisheries.

10.1.2. We recommend that as many as possible of the potentially contributing rookeries in the region be described and their level of differentiation be carefully assessed.

Baseline rookery descriptions for mixed stock assessments should be based on larger sample sizes than what has been available to date for most populations. In addition, we recommend that a concerted effort be made to sample the greatest possible number of potentially contributing baseline rookeries worldwide. For nesting rookeries in the Caribbean OT's which are all critically reduced, sampling will be limited to as many possible individuals that can be accessed in the population, with the acknowledgement that this will confound the likelihood of finding definitive answers as to which foraging grounds are used by the remnant populations nesting in the Caribbean OT's.

10.1.3. International coordination should be strengthened.

In order for the genetic results to be applied most effectively in management and conservation efforts of regional marine turtle populations a regional view must be taken. Coordinated international efforts in conservation are essential in the management of such migratory species. Through a better understanding of distribution and migratory behaviour of sea turtle populations provided through genetic studies, management strategies will be more targeted and efficient in future.

10.2. Introduction to the Use of Molecular Markers in Marine Turtles

Habitat use, philopatry, mating behaviour and migratory patterns play an important role in determining population structure, and molecular techniques have proved effective in gaining insights in these behaviours so difficult to observe in sea turtles (Allard *et al.* 1994; Bass *et al.* 1996; Bowen *et al.* 1995; Bowen *et al.* 1996; Dutton 1996; FitzSimmons *et al.* 1997; Karl *et al.* 1992; Meylan *et al.* 1990; Norman *et al.* 1994). Sea turtles maintain fidelity to the nesting site of birth (natal homing), which leads to strong genetic differentiation of nesting populations over time (Meylan *et al.* 1990). This variability can be detected via sequencing of the fast-evolving mitochodrial DNA (mtDNA) control region, that can provide levels of resolution appropriate to phylogeographic studies based on matrilineal inheritance (Bowen *et al.* 1992; Encalada *et al.* 1996; Lahanas *et al.* 1994; Lahanas *et al.* 1998).

In addition, mitochondrial DNA polymorphisms can be used as genetic markers, linking turtles in genetically-mixed foraging aggregations to their nesting beach origins. Recent genetic studies have demonstrated that hawksbills and green turtles recruit to foraging grounds from multiple

nesting beaches (Bass *et al.* 1996; Bass 1999; Bowen *et al.* 1996). This implies that foraging aggregations may originate from nesting beaches in a range of jurisdictions. Without knowledge of migratory patterns, the effects of threats on nesting beaches, foraging grounds, and migratory corridors cannot be evaluated for populations (Bowen 1995).

Genetic studies are key to unravelling the fundamentals of marine turtle distribution and population dynamics. Studies in the Atlantic involving or focussing on the Caribbean have been undertaken or are underway on hawksbill turtles (Bass 1999; Bass *et al.* 1996; Diaz-Fernandez *et al.* 1999, Engstrom *et al.* 2002), green turtles (Bass & Witzell 2000; Bowen *et al.* 1992; Encalada *et al*. 1996; Lahanas *et al.* 1994; Lahanas *et al.* 1998), loggerhead turtles (Bowen *et al.* 1994; Encalada *et al.* 1998; Engstrom *et al.* 2002) and leatherback turtles (Dutton 1999).

10.3. Background to Analyses within TCOT

At the time of the TCOT project launch, work was already underway in Bermuda and Cayman. This led to TCOT molecular work on hardshelled sea turtles being carried out in 3 ways.

1. Samples from **Anguilla, British Virgin Islands, Montserrat and Turks and Caicos Islands** were analysed by Dr. Angela Formia and Prof. Mike Bruford at Cardiff University (CU).

2. Samples from the **Cayman Islands** were already subject to a preliminary investigation by Janice Blumenthal (then Eckerd College, USA) and Dr. Peter Meylan (Eckerd College and Bermuda Turtle Project). This work was extended greatly and additional analyses were carried out by J. Blumenthal at CU under the supervision of Dr. Formia and Prof. Bruford as part of TCOT.

3. Samples from **Bermuda** have already been subject to extensive and ongoing analysis by Dr. Peter Meylan and colleagues. They opted to continue working independently but have kindly contributed a section to this report for the sake of completeness.

4. **Leatherback turtle samples** have been routed to Peter Dutton of the US National Marine Fisheries Service, Southwest Fisheries Science Centre, La Jolla California, USA. Samples will be analysed as part of PD's global leatherback genetics project. Samples and data will be stored or repatriated to OT partners upon request. Ten leatherback samples collected in Anguilla (6) and BVI (4) from nesting females have yet to be analysed.

Here we summarise the sampling and analysis, which have been undertaken to date and we give an intimation of the level of data likely to be available in the near future. Possible qualitative links are given but should be treated with great caution as not all possible links are likely and increased sampling will be needed to allow maximum likelihood and bayesian analyses of the relative contributions of different source populations to foraging areas. Sampling during TCOT has exceeded expectations and we already have an excess of samples in hand. Additional funds have been given by Defra to run an additional 200 samples in FY 2004/05. It is expected that by Spring 2005, we will be closer to giving more quantitative answers regarding possible linkages between nesting populations and foraging grounds/harvests.

Rigorously analysed results will subsequently be made available in the peer-reviewed literature in scientific publication authored by key personnel in the UK and UK OTs. Please contact Angela Formia <formiaa@cardiff.ac.uk> for updates regarding TCOT genetics other than for Bermuda. For Bermuda we refer interested parties to Peter Meylan <meylanpa@eckerd.edu>.

Photo 10.1. Taking a tissue sample from a juvenile hawksbill turtle (Photo CIDoE).

10.4. Samples from Anguilla, British Virgin Islands, Cayman Islands, Montserrat and Turks and Caicos Islands

(Text contributed by Angela Formia and Janice Blumenthal)

10.4.1. Methodology

Samples were collected from a wide variety of sources, such as nesting females, hatchlings recovered from nests, animals captured as part of turtle fisheries, dead strandings of whole individuals or old carapaces, and uncooked meat in restaurants. Each sample consisted of a tissue biopsy

		Anguilla	BVI	Cayman	Montserrat	TCI	Total
Nesting Samples	Farmed Green	0(0)	0(0)	48(52)	0(0)	0(0)	48(52)
	Wild Green	0(0)	0(0)	4(51)	0(3)	0(0)	4(54)
	Hawksbill	2(2)	2(2)	0(0)	3(4)	1(1)	8(9)
	Loggerhead	0(0)	0(0)	48(69)	0(0)	0(0)	48(69)
	Hybrid	0(0)	0(0)	4(4)	0(0)	0(0)	4(4)
	Subtotal	**2(2)**	**2(2)**	**104(176)**	**3(7)**	**1(1)**	**112(188)**
Foraging/Harvest	Green	16(16)	21(23)	0(0)	2(8)	17(18)	56(65)
	Hawksbill	4(5)	66(68)	103(149)	4(9)	38(46)	217(279)
	Loggerhead	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	Subtotal	**20(21)**	**87(91)**	**103(149)**	**6(17)**	**55(64)**	**271(342)**
Total		**22(23)**	**89(93)**	**207(325)**	**9(24)**	**56(65)**	**383(530)**

Table 10.1. The number of samples successfully analysed as part of TCOT. (Numbers in parentheses are the number of sample analysis runs that were undertaken to obtain data).

(skin whenever available), approximately 5 mm in diameter, taken from the neck or flipper and causing minimal disturbance to live animals. All samples were stored at ambient temperature in 20% w/v DMSO in saturated NaCl.

Samples were collected by a network of researchers and field assistants throughout the study area, and posted (with all appropriate CITES permits) to Cardiff University for laboratory analysis. A total of 530 sample runs were carried out in Cardiff between the project start date and March 2004, yielding data for 383 samples (i.e. pieces of tissue), including 89 from BVI, 9 from Montserrat, 56 from TCI, 22 from Anguilla, and 207 from Cayman (see below regarding analysis failure rates). Additional sampling is ongoing, as we continue to build up the sample size available. Mixed stock analyses and population assignment tests will be carried out upon completion of all field-work and laboratory analysis.

DNA was extracted from each sample according to standard protocols (Allen *et al.* 1998; Milligan 1998). A fragment of the mtDNA was amplified using Polymerase Chain Reaction (PCR) and specific primers for each species: LTCM1 and HDCM1 for green turtles (Allard *et al.* 1994), TCR5 and TCR6 for loggerheads (Norman *et al.* 1994) and LTEi9 and H950 for hawksbills (Abreu-Grobois, unpublished). The fragments obtained varied in length between approximately 400 and 850 base pairs. Negative controls were used to test for contamination. PCR products were cleaned and sequenced in both directions using Big Dye Terminator chemistry (Applied Biosystems) and analysed with an automatic sequencer. Sequences were then aligned and edited using appropriate software and matched against previously published haplotypes. Any new haplotypes were assigned a preliminary nomenclature with the initials TCOT. Haplotype designations for green turtles and loggerheads were based on identifications summarised at http://accstr.ufl.edu/genetics.html. Hawksbill designations were assigned based on a haplotype classification by Abreu-Grobois. Where previously published haplotypes were based on shorter fragments than those amplified during this study, we indicate the old nomenclature in brackets below. Resequencing of these shorter haplotypes is in progress and we refer interested parties to Abreu-Grobois at <alberto.abreu@ola.icmyl.unam.mx>. Hawksbill haplotype designations presented here are, therefore, preliminary and possible nomenclature errors will be investigated and addressed as part of the full analysis of the TCOT genetic sample base.

10.4.2. Anguilla

i) Green turtle (see table 10.2)

Nesting	***Foraging***
Samples tried: 0	Samples tried: 16
Data generated: 0	Data generated: 16
Samples pending: 0	Samples pending: 35

ii) Hawksbill turtle (see tables 10.3 and 10.4)

Nesting	***Foraging***
Samples tried: 2	Samples tried: 5
Data generated: 2	Data generated: 4
Samples pending: 0	Samples pending: 17

10.4.3. British Virgin Islands

i) Green turtle (see table 10.5)

Nesting	***Foraging***
Samples tried: 0	Samples tried: 23
Data generated: 0	Data generated: 21
Samples pending: 0	Samples pending: 35

ii) Hawksbill turtle (see tables 10.6 and 10.7)

Nesting	***Foraging***
Samples tried: 2	Samples tried: 68
Data generated: 2	Data generated: 66
Samples pending: 1	Samples pending: 60

10.4.4. Cayman Islands

i) Green turtle (see tables 10.8 and 10.9)

The Cayman Turtle Farm was established in 1968, and has released over 30,000 headstarted turtles into Cayman waters. Recent papers document the long-term survival and reproductive contributions of turtle farm releases (Bell & Parsons 2002; Bell *et al.* submitted; Wood & Wood 1993). An assessment of genetic identity is necessary in order to evaluate impacts of releasing farmed turtles on wild populations, with additional relevance to issues of captive breeding and reintroduction of endangered species.

Farm Turtles	***Wild nesting population***
Samples tried: 52	Samples tried: 51
Data generated: 48	Data generated: 4*
Samples pending: 91	Samples pending: 85

*(Failed samples were degraded tissue collected from dead, non-emergent hatchlings and embryos).

Foraging
Partners in the Cayman Islands are approaching collection of sufficient samples to accurately characterize origins of foraging green turtles in the Cayman Islands. TCOT has facilitated collection of genetic samples in the Cayman Islands, and continued funding will permit sequencing of these samples in hand. A total of 35 foraging samples are in hand, but yet to be analysed.

ii) Hawksbill turtle (see table 10.10)

Nesting
Hawksbill nesting is reported in historical accounts (Lewis 1940) and Wood & Wood (1994) and Aiken *et. al.* (2001) documented several occurrences of hawksbill nesting. However, hawksbill nesting has not been recorded in 2001-2004, and is now believed to have been extirpated (C. Bell (CIDoE) pers. comm. 2004).

Foraging
Samples tried: 149
Samples completed: 103
Samples pending: 58

iii) Loggerhead turtle (see table 10.11)

Nesting
Samples tried: 69
Samples completed: 48
Samples pending: 53

iv) Hybrid samples
Early anecdotal accounts of hybrid turtles were collected in Cayman and summarized in Lewis (1940), though in recent years, Carr (1967) and Wood & Wood (1994) found no evidence hybridization in the Cayman Islands. However, in 2002, the Cayman Islands Department of Environment documented two nests on Seven Mile Beach, from which hatchlings displayed intermediate diagnostic morphological characteristics (variable numbers of prefrontal scales and lateral scutes). Based on morphological evidence, these hatchlings were believed to be *Chelonia mydas* x *Caretta caretta* hybrids. Sequencing of the mitochondrial DNA control region revealed *Caretta caretta* mtDNA sequences, while identity of the male parent awaits confirmation via analysis of single-copy nuclear (scn) DNA markers. Additionally, a juvenile, believed to be *Eretmochelys imbricata* x *Caretta caretta* based on intermediate morphological characteristics was sampled by the Department of Environment in 2002. Sequencing revealed a mitochondrial DNA sequence typical of *Caretta caretta.* Previously, *Chelonia mydas* x *Caretta caretta* hybrids have been documented in Australia, Japan, and Brazil, while *Eretmochelys imbricata* x *Caretta caretta* hybrids have been documented in Japan, Brazil, and the USA (references reviewed in Seminoff *et al.* 2003).

10.4.5. Montserrat

i) Green turtle (see table 10.12)

Nesting	***Foraging***
Samples tried: 3	Samples tried: 8
Data generated: 0	Data generated: 2
Samples pending: 0	Samples pending: 0

ii) Hawksbill turtle (see table 10.13 and 10.14)

Nesting	***Foraging***
Samples tried: 4	Samples tried: 6
Data generated: 3	Data generated: 4
Samples pending: 0	Samples pending: 0

Three additional samples collected in Montserrat failed to amplify and species identification could not be confirmed. Overall, the 15 failed samples from Montserrat were taken from poor quality tissue, 9 from dead and decayed hatchlings and 2 from dead stranded adults. DNA fragments of sufficient size and quality could not be amplified despite attempting several extraction and PCR protocol variations.

Photo 10.2. Juveniles gathered from foraging sites for tissue sampling (Photo P. Richardson).

10.4.6. Turks and Caicos Islands

i) Green turtle (see table 10.15)

Nesting	*Foraging*
Samples tried: 0	Samples tried: 18
Data generated: 0	Data generated: 17
Samples pending: 0	Samples pending: 0

ii) Hawksbill turtle (see table 10.16)

Nesting	*Foraging*
Samples tried: 1	Samples tried: 46
Data generated: 1	Data generated: 38*
Samples pending: 0	Samples pending: 0

*(The 8 failed samples were taken from poor quality tissue, including the inner surface of carapaces and deteriorated muscle tissue; DNA fragments of sufficient size and quality could not be extracted).

10.4.7. Overview

Green turtles

Green turtle foraging samples from Anguilla, BVI, Montserrat and TCI have revealed two previously undescribed haplotypes (TCOT1 and TCOT2). Additional samples are available from individuals at Cayman, BVI, Anguilla and TCI foraging grounds, which will be analysed in future. The haplotypes exhibited by foraging individuals at all sampled sites, except Montserrat, belonged to two different and divergent haplotype groups, one which is common in the southern Caribbean and southern Atlantic rookeries (such as Suriname, Venezuela, Ascension, Guinea Bissau, Bioko, Sao Tome, Principe and Brazil), and one which is common in Mexico, Florida and Costa Rica rookeries. In other words, due to the presence of haplotypes CMA-5, CMA-8 and CMA-32 at one or several of the feeding sites analysed, we cannot exclude that rookeries in the southern Caribbean and southern Atlantic may be contributing to our Caribbean mixed aggregates, in addition to rookeries such as Tortuguero, Mexico and Florida which appear to contribute a majority of the haplotypes. Mixed stock analysis will be used to resolve the feeding stock composition based on best available data for rookery characteristics.

Vice versa, the presence of nesting haplotypes such as CMA-5 and CMA-8 in West African foraging grounds (Formia 2002) indicates that the gene flow may be occurring in both directions. In other words, rookeries in the western Atlantic such as Ascension, Suriname, Venezuela, Mexico and Brazil may be contributing individuals to African mixed stocks. It is indeed possible that future analysis of nesting green turtles in the TCOT study area may also show links with African feeding grounds.

Although green turtle rookeries throughout the Atlantic have been genetically described in several studies (Allard *et al.* 1994; Encalada *et al.* 1996; Lahanas *et al.* 1994; Lahanas *et al.* 1998), these data are currently being extended and detailed by a number of research groups. All additional nesting samples obtained at our Caribbean study sites will be valuable contributions to understanding the overall haplotype distribution of nesting populations, and thus to correctly assigning individuals at sea to their respective rookeries of origin.

Hawksbill turtle

Insufficient data are available on the haplotype composition of hawksbill turtle rookeries found in the eastern Atlantic. It is therefore not possible, at present, to assess any potential contribution from these rookeries to mixed aggregates. None of the haplotypes found in Caribbean feeding grounds or rookeries appear to belong to highly divergent haplogroups, although hawksbill haplotypes generally exhibit greater variability than green turtles (i.e. distribution not consisting of few common and many rare haplotypes).

To date, we have analysed nesting individuals from four previously unstudied hawksbill rookeries (Anguilla, BVI, TCI and Montserrat). Among these we have identified 2 previously undescribed haplotypes (TCOT3 and TCOT6). However, it must be noted that they are both equivalent to haplotype EiA011, as designated by A. Abreu-Grobois (pers. comm. 2004). However, EiA011, like many other hawksbill haplotypes, was identified based on a much shorter sequence (approximately 500 bp) than that analysed here, not including the region where TCOT3 and 6 are differentiated. Thus, we maintained here the TCOT designations until the matter can be resolved by re-sequencing of the original EiA011 samples.

We have also found 5 haplotypes previously undescribed among foraging hawksbills (TCOT3, TCOT4, TCOT5, TCOT6, TCOT7). TCOT4, TCOT5 and TCOT7 have not yet been found among known rookeries, which may be explained by small sample sizes for sampled rookeries and/or unsampled nesting sites. Unfortunately, sample sizes available are not yet sufficient to provide accurate haplotype distributions of the nesting populations at our four study sites. Statistically significant assessments of the origin of

Photo 10.3. Genetics sampling, Tortola, BVI (Photo B. Godley).

individuals in foraging grounds and harvests are contingent on thorough descriptions of potentially contributing baseline rookeries and on extended sequencing of known haplotypes to include longer fragments.

Loggerhead Turtle

Only the Cayman Islands have a notable loggerhead nesting population, although nesting is suspected by this species elsewhere in the TCOT range. The presence of loggerhead haplotypes, which may be unique to nesting beaches in the Cayman Islands, indicates that knowledge of genetic diversity in the Atlantic is incomplete for the species, and highlights the need for future research. Genetic diversity of small nesting beach populations is vulnerable due to exploitation both on nesting beaches and on foraging grounds overseas. However, the impacts of that exploitation cannot be assessed until genetic surveys are completed for small rookeries. Continued research on critically reduced populations such as the Cayman Islands will contribute to a crucial evaluation of loggerhead genetic diversity in the Caribbean.

(End of Section by Formia/Blumenthal)

10.5. Samples from Bermuda

Peter Meylan, Anne Meylan and Jennifer Gray of the Bermuda Turtle Project write:

10.5.1. Introduction

Blood or tissue samples of green turtles, hawksbills and loggerheads from Bermuda have been studied by the Bermuda Turtle Project. Preliminary results on green turtles (Engstrom *et al.* 1998) and hawksbills (Meylan *et al.* 2004) have been presented at the International Symposia on Sea Turtle Biology and Conservation. In all cases, published control region primers have been used to amplify sequences of approximately 550bp. In most cases, a sequence was generated in one direction and aligned with a published sequence for confirmation. Most of the green turtle samples were initially processed by students in Eckerd College genetics classes. These students isolated and amplified DNA from a small subsample of blood or tissue. They then cleaned the PCR product which was sent to the BEECS lab at the University of Florida for sequencing. Edited sequences were returned from UF and aligned by students who made the initial haplotype determination. Haplotype determinations were confirmed before being added to the database. Hawksbill and loggerhead samples were processed entirely by the BEECS lab.

10.5.2. Green turtles

As of December 2003, 1222 genetics samples from Bermuda green turtles have been collected. Control region sequences are now available for 128 of these, with about 30 more in various stages of completion. Among the sequenced samples are at least 10 different genotypes, seven of which match published haplotypes and three of which have not been published to our knowledge. These haplotypes represent, at a minimum, contributions from three different nesting beaches, but may represent all eight of the major green turtle nesting aggregations in the Atlantic (not including Mediterranean). Just over one hundred of these sequences represent a random sample from the available genetic material collected by the Bermuda Turtle Project. They are being used to estimate stock contribution to the green turtle aggregation in Bermuda. The remaining samples were not chosen at random but instead were selected for sequencing after we learned of an individual turtle's capture in another country. These samples, which are presumed to represent the various feeding grounds of the population, have been processed to test for patterns of dispersal from Bermuda that may be explained by genotype. Because they were not selected at random, they are not used in the mixed stock analysis.

10.5.3. Hawksbill turtles

There are now approximately 80 genetic samples of hawksbills available from Bermuda. Control region sequences have been generated for 58 turtles that were identified as hawksbills based on morphology. However, four of these have turned out to be hawksbill X loggerhead hybrids, that is, they had mitochondrial genomes of *Caretta*. Among the remaining 54 sequences, there are at least eight haplotypes. Five of these match published haplotypes from around the Caribbean, three are unpublished to our knowledge. About half of these data are from animals that were captured alive and in good health; the other half are from stranded animals.

10.5.4. Loggerhead turtles

The stranding network at the Bermuda Aquarium has made a diligent effort over the last 10 years to take genetic samples from the few loggerheads that strand in Bermuda each year. A total of 43 samples are now available. Of these, 31 have been sequenced so far; four others are in various states of completion. This relatively small sample is highly informative, containing as many different haplotypes as the larger samples of hawksbills and greens. With a sufficiently large sample size, these results will allow us to

Photo 10.4. Samples from hatched nests are also used in analyses (Photo B. Godley).

Photo 10.5. TCOT staff sample hawksbill turtle meat at a restaurant in Providenciales, TCI (Photo S Ranger).

determine the nesting beaches of origin of the contingent of post-hatchling loggerheads that are observed in Bermuda waters and/or strand on Bermuda beaches on a nearly annual basis.

(End of section by Meylan, Meylan and Gray)

10.6. Acknowledgements

The molecular analysis is one of the major endpoints of all TCOT endeavours and in some way has involved almost every member of all project partners. For this effort we are very grateful. Individuals and organisations are acknowledged in OT-specific chapters. The TCOT staff would like to acknowledge the candid and generous collaboration of Janice Blumenthal, Mike Bruford, Angela Formia, Jennifer Gray, Anne Meylan and Peter Meylan in the production of this overview of the progress to date. We acknowledge the assistance of the UK CITES management authority at Defra. Finally, many thanks to those who offered constructive peer review which greatly helped improve its clarity of reporting: Alberto Abreu-Grobois, Vin Fleming, Jennifer Gray and Peter Meylan.

10.7. References

Aiken JJ, Godley BJ, Broderick AC, Austin T, Ebanks-Petrie G, Hays G (2001) Two hundred years after a commercial marine turtle fishery: the current status of marine turtles nesting in the Cayman Islands. Oryx 35:145-151

Allard MW, Miyamoto MM, Bjorndal KA, Bolten AB, Bowen BW (1994) Support for natal homing in green turtles from mitochondrial DNA sequences. Copeia 1994:34-41

Allen M, Engstrom AS, Meyers S, Handt O, Saldeen T, von Haeseler A, Pääbo S, Gyllensten U (1998) Mitochondrial DNA sequencing of shed hairs and saliva on robbery caps: sensitivity and matching probabilities. Journal of Forensic Sciences 43:453-464

Bass AL (1999) Genetic analysis to elucidate the natural history and behavior of hawksbill turtles (*Eretmochelys imbricata*) in the Wider Caribbean: a review and re-analysis. Chelonian Conservation and Biology 3:195-199

Bass AL, Witzell WN (2000) Demographic composition of immature green turtles (*Chelonia mydas*) from the east central Florida coast: evidence from mtDNA markers. Herpetologica 56:357-367

Bass AL, Good DA, Bjorndal KA, Richardson JI, Hillis Z-M, Horrocks JA, Bowen BW (1996) Testing models of female reproductive migratory behaviour and population structure in the Caribbean hawksbill turtle, *Eretmochelys imbricata*, with mtDNA sequences. Molecular Ecology 5:321-328

Bell CDL, Parsons J (2002) Cayman Turtle Farm Head-starting Project Yields Tangible Success. Marine Turtle Newsletter 98:5-6

Bell C, Parsons J, Austin TJ, Broderick AC, Ebanks Petrie G, Godley BJ (submitted) Some of them Came Home: The Cayman Turtle Farm Headstarting Project

Bowen BW (1995) Tracking Marine Turtles with Genetic Markers. Bioscience 145:528-534

Bowen BW, Kamezaki N, Limpus CJ, Hughes GH, Meylan AB, Avise JC (1994) Global phylogeography of the loggerhead turtle (*Caretta caretta*) as indicated by mitochondrial DNA haplotypes. Proceedings of the National Academy Science 92: 3731-3734

P**hoto 10.6.** Sampling procedures being demonstrated at the TCOT Workshop, CI (Photo P. Richardson).

Bowen BW, Abreu-Grobois FA, Balazs GH, Kamezaki N, Limpus CJ, Ferl RJ (1995) Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers. Proceedings of the National Academy of Sciences of the USA 92:3731-3734

Bowen BW, Meylan AB, Ross JP, Limpus CJ, Balazs GH, Avise JC (1992) Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46:865-881

Bowen BW, Bass AL, Garcia-Rodriguez A, Diez CE, van Dam R, Bolten AB, Bjorndal KA, Miyamoto MM, Ferl RJ (1996) Origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. Ecological Applications 6:566-572

Carr A (1967) So Excellent a Fishe. Natural History Press, NY. 248 pp

Diaz-Fernandez R, Okayama T, Uchiyama T, Carrillo E, Espinoza G, Marquez R, Diez C, Kioke H (1999) Genetic sourcing for the Hawksbill Turtle, *Eretmochelys imbricata*, in the northern Caribbean Region. Chelonian Conservation and Biology 3:296–300

Dutton PH (1996) Methods for collection and preservation of samples for sea turtle genetic studies. In: Bowen BW, Witzell WN (Eds). Proceedings of the International Symposium on Sea Turtle Conservation Genetics. US Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-396. pp. 17-24.

Dutton PH (1999) Global phylogeography of the leatherback turtle (*Dermochelys coriacea*). Journal of Zoology 248:397-409

Encalada SE, Lahanas PN, Bjorndal KA, Bolten AB, Miyamoto MM, Bowen BW (1996) Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: a mitochondrial DNA control region sequence assessment. Molecular Ecology 5:473-483

Encalada SE, Bjorndal KA, Bolten AB, Zurita JC, Schroeder B, Possardt E, Sears CJ, Bowen BW (1998) Population structure of loggerhead turtle (*Caretta caretta*) nesting colonies in the Atlantic and Mediterranean as inferred from mitochondrial DNA control region sequences. Marine Biology 130:567–575

Engstrom TN, Meylan PA, Meylan AB (2002) Origin of juvenile loggerhead turtles (*Caretta caretta*) in a tropical developmental habitat in Caribbean Panama. Animal Conservation 5:125–133

Engstrom TN, Bradley WG, Gray JA, Meylan AB, Meylan PA, Roess WB (1998) Genetic identity of green turtles in Bermuda waters In: Epperly SP, Braun J (Compilers). Proceedings of the Seventeenth Annual Sea Turtle Symposium. US Department of Commerce, NOAA Technical Memorandum NMFS-SEFSC-415. pp. 50

FitzSimmons NN, Moritz C, Limpus CJ, Pope L, Prince R (1997) Geographic structure of mitochondrial and nuclear gene polymorphisms in Australia green turtle populations and male-biased gene flow. Genetics 147:1843-1854

Formia A (2002) Population and genetic structure of the green turtle (*Chelonia mydas*) in west and central Africa; implications for management and conservation. PhD Thesis, Cardiff University, UK

Formia A, Bruford MW, Broderick AC, Glen F, Godley BJ, Hays GC (unpublished-a) Genetic structure across an entire oceanic island turtle rookery: sampling and diversity in Ascension island green turtles.

Formia A, Godley BJ, Dontaine J-F, Bruford MW (unpublished-b) Mitochondrial DNA diversity and phylogeography in African green turtles (*Chelonia mydas*). Karl SA, Bowen BW, Avise JC (1992) Global population structure and male-mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analyses of anonymous nuclear loci. Genetics 131:163-173.

Karl SA, Bowen BW, Avise JC (1992) Global population structure and male-mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analyses of anonymous nuclear loci. Genetics 131:163-173

Lahanas PN, Miyamoto MM, Bjorndal KA, Bolten AB (1994) Molecular evolution and population genetics of Greater Caribbean green turtles (*Chelonia mydas*) as inferred from mitochondrial DNA control region sequences. Genetica 94:57-67

Lahanas PN, Bjorndal KA, Bolten AB, Encalada SE, Miyamoto MM, Valverde RA, Bowen BW (1998) Genetic composition of a green turtle (*Chelonia mydas*) feeding ground population: evidence for multiple origins. Marine Biology 130:345-352

Laurent L, Casale P, Bradai MN, Godley BJ, Gerosa G, Broderick AC, Schroth W., Schierwater B, Levy AM, Freggi D, Abd El-Mawla EM, Hadoud DA, Gomati HE, Domingo M, Hadjichristophorou M, Kornaraky L, Demirayak L F, Gautier C (1998). Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean. Molecular Ecology 7:1529–1542.

Lewis CB (1940) The Cayman Islands and Marine Turtles. In: Grant C (Ed.). The Herpetology of the Cayman Islands Bulletin of the Institute of Jamaica Science Series. 2: 65

Meylan AB, Bowen BW, Avise JC (1990) A genetic test of the natal homing versus social facilitation models for green turtle migration. Science 248:724-727

Meylan P, Meylan A, Gray J, Ward J (2004) The hawksbill turtle in Bermuda (abstract). In: Seminoff JA (Ed.). Proceedings of the 22nd Annual Symposium on Sea Turtle Biology and Conservation. US Department of Commerce, NOAA Technical Memorandum NMFS-SEFC-503:26

Milligan BG (1998) Total DNA isolation. In: Hoelzel AR (Ed.). Molecular Genetic Analysis of Populations, A Practical Approach. Second Edition.),IRL Press, Oxford. pp. 29-64

Norman JA, Moritz C, Limpus CJ (1994) Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles. Molecular Ecology 3:363-373

Seminoff JA, Karl SA, Schwartz T, Rosendiz A (2003) Hybridization of the green turtle (*Chelonia mydas*) and hawksbill turtle (*Eretmochelys imbricata*) in the Pacific Ocean: Indication of an absence of gender bias in the directionality of crosses. Bulletin of Marine Science 73:643-652

Wood F, Wood J (1993) Release and recapture of captive-reared green sea turtles, *Chelonia mydas*, in the waters surrounding the Cayman Islands. Herpetological Journal 3:84-89

Wood JR, Wood FE (1994) Sea Turtles of the Cayman Islands. In: Brunt MA, Davies JE (Eds.). The Cayman Islands: Natural History and Biogeography. Kluwer Academic Publishers, printed in the Netherlands. 229-236 pp

Haplotype	Nesting	Foraging
CM-A1	Florida, Mexico	Bahamas, Florida, Nicaragua, Barbados, Anguilla, BVI, TCI
CM-A3	Florida, Mexico, Costa Rica, Aves	Bahamas, Florida, Barbados, Anguilla, BVI, TCI, Montserrat
CM-A5	Mexico, Aves, Suriname, Sao Tome	Bahamas, Florida, Nicaragua, Barbados, West Africa, Anguilla, BVI, TCI, Montserrat
CM-A8	Bioko, Ascension, Guinea Bissau, Sao Tome, Principe, Brazil	Anguilla, TCI, West Africa
New (TCOT1)	Not yet identified	Anguilla

Table 10.2. Green turtle haplotypes recorded in foraging grounds in Anguilla and the nesting and foraging aggregations where turtles with these markers have previously been described (Encalada *et al.* 1996; Formia 2002; Lahanas *et al.* 1994; Lahanas *et al.* 1998; This Study).

Haplotype	Nesting	Foraging
EIA023 (Q, MXI)	Anguilla, Mexico	Anguilla, BVI, Cuba, Mexico, Puerto Rico, TCI
New (TCOT3)	Anguilla, Montserrat, TCI	Anguilla, BVI, Montserrat, TCI

Table 10.3 Hawksbill turtle haplotypes recorded in nesting turtles in Anguilla, and the nesting and foraging aggregations where individuals with these markers have previously been described (A. Abreu-Grobois pers. comm. 2004; Bass *et. al.* 1996, Bowen *et al.* 1996, Diaz-Fernandez 1999, This Study).

Haplotype	Nesting	Foraging
EIA001 (A, CU1)	Antigua, Barbados, Brazil, Cuba, Montserrat, Puerto Rico, USVI	Anguilla, BVI, Cuba, Montserrat, Puerto Rico, TCI, CI
EIA023 (Q, MXI)	Anguilla, Mexico	Anguilla, BVI, Cuba, Mexico, Puerto Rico, TCI
New (TCOT3)	Anguilla, Montserrat, TCI	Anguilla, BVI, Montserrat, TCI

Table 10.4. Hawksbill turtle haplotypes recorded in foraging turtles in Anguilla and the nesting and foraging aggregations where turtles with these markers have previously been described (A. Abreu-Grobois pers. comm. 2004; Bass *et. al.* 1996; Bowen *et al.* 1996; Diaz-Fernandez 1999; This Study).

Haplotype	Nesting	Foraging
CM-A1	Florida, Mexico	Anguilla, Bahamas, Barbados, BVI, Florida, Nicaragua, TCI
CM-A3	Aves, Costa Rica, Florida, Mexico	Anguilla, Bahamas, Barbados, BVI, Florida, Montserrat, TCI
CM-A5	Aves, Mexico, Sao Tome, Suriname,	Anguilla, Bahamas, Barbados, BVI, Florida, Montserrat Nicaragua, TCI, West Africa
CM-A28	Unpublished	BVI, unpublished
CM-A32	Ascension and unpublished	BVI, unpublished
New (TCOT2)	Not yet identified	BVI

Table 10.5. Green turtle haplotypes recorded in foraging turtles in the BVI and the nesting and foraging aggregations where turtles with these marker have previously been described (Encalada *et al.* 1996; Formia 2002; Formia *et al.* unpublished-b; Lahanas *et al.* 1994; Lahanas *et al.* 1998; This Study).

Haplotype	Nesting	Foraging
EIA009 (c, F)	Belize, BVI, Cuba, Puerto Rico, USVI	BVI, Cuba, Mexico, Puerto Rico, TCI, CI
New (TCOT6)	BVI	Not yet identified

Table 10.6. Hawksbill turtle haplotypes recorded in hatchlings/nesting turtles in the British Virgin Islands and the nesting and foraging aggregations where turtles with these markers have previously been described (Abreu-Grobois pers. comm. 2004; Bass *et. al.* 1996; Bowen *et al.* 1996; Diaz-Fernandez 1999; This Study).

Haplotype	Nesting	Foraging
EIA001 (CU1, A)	Antigua, Barbados, Brazil, Cuba, Montserrat, Puerto Rico, USVI	Anguilla, BVI, Cuba, Montserrat, Puerto Rico, TCI, CI
EIA002 (g, alpha)	Not yet identified	BVI, Cuba, Puerto Rico, TCI, CI
EIA009 (c, F)	Belize, BVI, Cuba, Puerto Rico, USVI	BVI, Cuba, Mexico, Puerto Rico, TCI, CI
EIA020 (PR2, N)	Puerto Rico	BVI, Cuba, Puerto Rico, TCI
EIA023 (Q, MXI)	Anguilla, Mexico	Anguilla, BVI, Cuba, Mexico, Puerto Rico, TCI
EIA024 (Q, MXII)	Mexico	BVI, Cuba, Mexico, Puerto Rico, TCI, CI
EIA029 (CU3)	Cuba	Cuba, BVI
New (TCOT3)	Anguilla, Montserrat, TCI	Anguilla, BVI, Montserrat, TCI,
New (TCOT4)	Not yet identified	BVI
New (TCOT5)	Not yet identified	BVI

Table 10.7. Hawksbill turtle haplotypes recorded in foraging turtles in the British Virgin Islands and the nesting and foraging aggregations where turtles with these markers have previously been described (A. Abreu-Grobois pers. comm. 2004; Bass *et. al.* 1996; Bowen *et al.* 1996; Diaz-Fernandez 1999; This Study).

Haplotype	Nesting	Foraging
CM-A1	Florida, Mexico	Anguilla, Bahamas, Barbados, BVI, Florida, Nicaragua, TCI
CM-A3	Aves, Costa Rica, Florida, Mexico	Anguilla, Bahamas, Barbados, Florida, BVI, Montserrat, TCI
CM-A5	Aves, Mexico, Suriname, Sao Tome	Anguilla, Bahamas, Barbados, BVI, Florida, Montserrat, Nicaragua, West Africa
CM-A16	Mexico	TCI
CM-A17	Mexico	Barbados
Unpublished	Unpublished	

Table 10.8. Green turtle haplotypes recorded in the Cayman Turtle Farm and the nesting and foraging aggregations where turtles with these markers have previously been described (Encalada *et al.* 1996; Formia *et al.* unpublished-b; Lahanas *et al.* 1994; Lahanas *et al.* 1998; This Study).

Haplotype	Nesting	Foraging
CM-A1	Florida, Mexico	Bahamas, Barbados Florida, Nicaragua
CM-A3	Aves, Costa Rica, Florida, Mexico	Bahamas, Barbados Florida
Unpublished	Unpublished	Unpublished

Table 10.9. Green turtle haplotypes recorded in Cayman Islands nesting turtles/hatchlings and the nesting and foraging aggregations where turtles with these markers have previously been described (Encalada *et al.* 1996; Lahanas *et al.* 1994; Lahanas *et al.* 1998; This Study).

Haplotype	Nesting	Foraging
EIA001 (CU1, A)	Antigua, Barbados, Brazil Cuba, Puerto Rico, USVI	Anguilla, BVI, Cuba, Montserrat, Puerto Rico, TCI
EIA002 (g, alpha)	Cuba	Cuba, Puerto Rico, TCI
EIA003 (B, e)	Antigua	Mexico, Puerto Rico
EIA009 (c, F)	Cuba, Belize, Puerto Rico, USVI,	BVI, Cuba, Mexico, Puerto Rico, TCI
EIA018 (PR3, L)	Puerto Rico	Cuba, Puerto Rico
EIA024 (Q, MXII)	Mexico	BVI, Cuba, Mexico, Puerto Rico, TCI
EIA028 (b)	Not yet identified	Puerto Rico, TCI
EIA029 (CU3)	Cuba	Cuba, BVI
Unpublished	Unknown	Foraging only
New	Unknown	Cayman Islands

Table 10.10. Hawksbill turtle haplotypes recorded in foraging turtles in the Cayman Islands and the nesting and foraging aggregations where turtles with these markers have previously been described (A. Abreu-Grobois pers. comm. 2004; Bass *et. al.* 1996; Bowen *et al.* 1996; Diaz-Fernandez 1999; This Study).

Haplotype	Nesting	Foraging
B	Florida, North East FL – NC, Mexico, Greece-SE Mediterranean	Chiriqui Lagoon Panama, Eastern Atlantic
J	Mexico, Greece-SE Mediterranean	Chiriqui Lagoon Panama, Eastern Atlantic
Novel Haplotype	Cayman Islands	Not yet identified

Table 10.11. Loggerhead haplotypes recorded in nesting turtles/hatchlings in the Cayman Islands and the nesting and foraging aggregations where turtles with these markers have previously been described (Encalada *et al*. 1998; Engstrom *et al.* 2002; Laurent *et al.* 1998; This Study).

Haplotype	Nesting	Foraging
CM-A3	Florida, Mexico, Costa Rica, Aves	Bahamas, Florida, Barbados, Anguilla, BVI, TCI, Montserrat
CM-A5	Mexico, Aves, Suriname, Sao Tome	Bahamas, Florida, Nicaragua, Barbados, West Africa, Anguilla, BVI, TCI, Montserrat

Table 10.12. Green turtle haplotypes recorded in foraging turtles from Montserrat and the nesting and foraging aggregations where turtles with these markers have previously been described (Encalada *et al.* 1996; Formia 2002; Formia *et al.* unpublished-b; Lahanas *et al.* 1994; Lahanas *et al.* 1998; This Study).

Haplotype	Nesting	Foraging
EIA001 (CU1, A)	Antigua, Barbados, Brazil, Cuba, Montserrat, Puerto Rico, USVI	Anguilla, BVI, Cuba, Montserrat, Puerto Rico, TCI, CI
New (TCOT3)	Anguilla, Montserrat, TCI	Anguilla, BVI, Montserrat TCI

Table 10.13. Hawksbill turtle haplotypes recorded in nesting turtles from Montserrat and the nesting and foraging aggregations where these markers have previously been described (A. Abreu-Grobois pers. comm. 2004; Bass *et al.* 1996; Bowen *et al.* 1996; Diaz-Fernandez 1999; This Study).

Haplotype	Nesting	Foraging
EIA001 (CU1, A)	Antigua, Barbados, Brazil, Cuba, Montserrat, Puerto Rico, USVI	Anguilla, BVI, Cuba, Montserrat, Puerto Rico, TCI, CI
New (TCOT3)	Anguilla, Montserrat, TCI	Anguilla, BVI, Montserrat TCI

Table 10.14. Hawksbill turtle haplotypes recorded in foraging turtles from Montserrat and the nesting and foraging aggregations where these markers have previously been described (A. Abreu-Grobois pers. comm. 2004; Bass *et al.* 1996; Bowen *et al.* 1996; Diaz-Fernandez 1999; This Study).

Haplotype	Nesting	Foraging
CM-A1	Florida, Mexico	Bahamas, Florida, Nicaragua, Barbados, Anguilla, BVI, TCI
CM-A3	Aves Costa Rica, Florida, Mexico	Anguilla, Bahamas, Barbados, BVI, Florida, Montserrat, TCI
CM-A5	Aves, Mexico, Sao Tome, Suriname	Anguilla, Bahamas, Barbados, BVI, Florida, Montserrat, Nicaragua, TCI, West Africa
CM-A8	Ascension, Bioko, Brazil, Guinea Bissau, Principe, Sao Tome	Anguilla, TCI, West Africa
CM-A16	Mexico	TCI

Table 10.15. Green turtle haplotypes we recorded in foraging turtles from the Turks and Caicos Island and the nesting and foraging aggregations where turtles with these markers have previously been described (Encalada *et al.* 1996; Formia 2002; Formia *et al.* unpublished-a; Formia *et al.* unpublished-b; Lahanas *et al.* 1994; Lahanas *et al.* 1998; This Study).

Haplotype	Nesting	Foraging
New (TCOT3)	Anguilla, Montserrat, TCI	Anguilla, BVI, Montserrat, TCI

Table 10.16. Hawksbill turtle haplotypes recorded in hatchling/nesting turtles from the TCI and the nesting and foraging aggregations where turtles with this marker have previously been described (A. Abreu-Grobois pers. comm. 2004; Bass *et al.* 1996, Bowen *et al.* 1996, Diaz-Fernandez 1999, This Study).

Haplotype	Nesting	Foraging
EIA001 (CU1, A)	Antigua, Barbados, Brazil, Cuba, Montserrat, Puerto Rico, USVI,	Anguilla, BVI, Cuba, Montserrat, Puerto Rico, TCI, CI
EIA002 (g, alpha)	Not yet identified	BVI, Cuba, Puerto Rico, TCI, CI
EIA003 (e, B)	Antigua	Mexico, Puerto Rico, TCI, CI
EIA009 (c, F)	Belize, BVI, Cuba, Puerto Rico, USVI	BVI, Cuba, Mexico, Puerto Rico, TCI, CI
EIA020 (PR2, N)	Puerto Rico	BVI, Cuba, Puerto Rico, TCI
EIA023 (Q, MXI)	Anguilla, Mexico	Anguilla, BVI, Cuba, Mexico, Puerto Rico, TCI
EiA024 (Q, MXII)	Mexico	BVI, Cuba, Mexico, Puerto Rico, TCI, CI
EIA028 (b)	Not yet identified	Puerto Rico, TCI, CI
New (TCOT3)	Anguilla, Montserrat, TCI	Anguilla, BVI, Montserrat, TCI
New (TCOT7)	Not yet identified	TCI

Table 10.17. Hawksbill turtle haplotypes recorded in foraging turtles from the TCI and the nesting and foraging aggregations where turtles with these markers have previously been described (A. Abreu-Grobois pers. comm. 2004; Bass *et al.* 1996; Bowen *et al.* 1996; Diaz-Fernandez 1999; This Study).