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Natal homing by an adult male green turtle at Tortuguero, Costa Rica

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Despite a proliferation of genetic studies characterizing patterns of population structure shaped by female natal homing behavior (reviewed in Bowen & Karl 2007), genetic tests of male natal philopatry remain rare given the logistical difficulty of intercepting breeding males. A further complication of testing male natal homing to breeding sites is potential lack of resolution of traditional genetic markers to discern among source rookeries. For example, the vast majority of nesting green turtles (*Chelonia mydas*) sampled in the Greater Caribbean region carries just three common 490-base pair (bp) control region haplotypes (CM-A1, CM-A3, and CM-A5), resulting in extensive sharing of genetic markers among rookeries (Bjorndal *et al.* 2005; Encalada *et al.* 1996; Ruiz-Urquiloa *et al.* 2010). This haplotype sharing is regionally common in all marine turtle species with cosmopolitan distributions (reviewed in Bowen & Karl 2007) and often prevents direct assignment of individual turtles to source rookeries.

The most elegant and comprehensive study of male natal philopatry confirmed regional natal homing by breeding male green turtles representing three genetic stocks in Australia via mtDNA frequency comparisons of both sexes at breeding grounds (FitzSimmons *et al.* 1997); however the extent to which male natal homing occurs at other green turtle rookeries globally has not been tested. On August 14, 2002, an adult male green turtle that had been killed by poachers was found on the beach at mile marker 4 and 2/8 at Tortuguero, Costa Rica, providing an opportunity to collect a tissue sample for genetic analysis. We hypothesize that this male was in the vicinity of the nesting beach at Tortuguero to mate given that no benthic foraging grounds occur in the immediate vicinity (Troëng *et al.* 2005) and the turtle was killed during the peak of the breeding season. Sequencing of the standard 490-bp mitochondrial control region fragment by the University of Florida Sequencing Core determined that the male carried haplotype CM-A5. This haplotype is the second most common among Tortuguero nesting green turtles (7.4%, Bjorndal *et al.* 2005) and is also the dominant haplotype in the eastern Caribbean rookeries of Aves Island and Matapica, Suriname (87.5% and 86.7%, respectively; Encalada *et al.* 1996) (Fig. 1). CM-A5 has also been reported from rookeries in Mexico (Encalada *et al.* 1996) and São Tome, western Africa (Formia *et al.* 2006). Therefore, it was not possible to definitively assign the rookery of origin for this male using only the 490-bp control region haplotype.

A recent analysis of the southern Greater Caribbean green turtle rookeries utilizing mitochondrial genome sequencing uncovered four mitogenomic variants of 490-bp CM-A5 that were subdivided among regions (Shamblin *et al.* 2012) (Fig. 1). The common CM-A5 variant in the Tortuguero rookery was CM-A5.1.2, which occurred in 27 of the 32 sampled nesting females carrying haplotype CM-A5. This mitogenomic haplotype was not detected in the eastern Caribbean rookeries of Galibi, Suriname (55 CM-A5 females sampled); Aves Island, Venezuela (62 CM-A5 females sampled); or Buck Island, United States Virgin Islands (45 CM-A5 females sampled) (Shamblin *et al.* 2012).

We re-amplified the control region of the male sample using primers LCM15382 (Abreu-Grobois *et al.* 2006) and CM16437

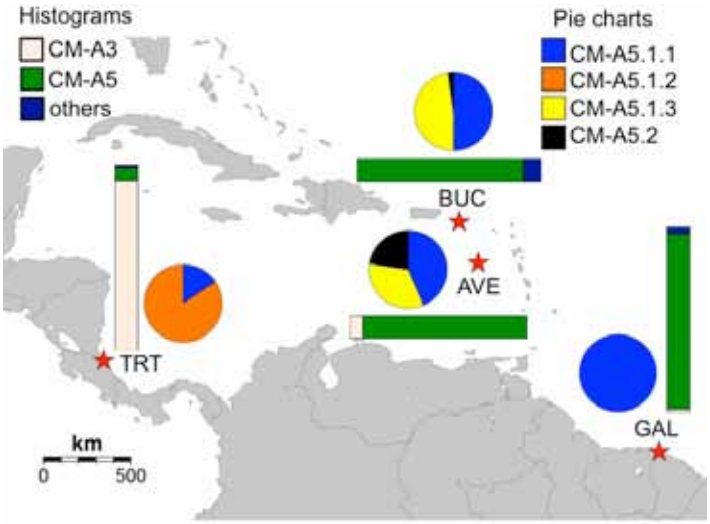

Figure 1. Distribution of 490-bp control region haplotypes (histograms) and mitogenomic CM-A5 haplotypes (pie charts) for four rookeries in the southern Caribbean modified from Shamblin et al. 2012: TRT, Tortuguero, Costa Rica; BUC, Buck Island, St. Croix, US Virgin Islands; AVE, Isla Aves, Venezuela; GAL, Galibi, Suriname.

(Shamblin *et al.* 2012). These primers amplify approximately 970-bp that fully encompass the alignment produced by LCM15382 and H950 (Abreu-Grobois *et al.* 2006) and extends the fragment approximately 150 bases downstream. The resulting fragment was sequenced with LCM15382 and internal forward sequencing primer Cm15821 (Shamblin *et al.* 2012). The 970-bp sequence matched haplotype CM-A5.1, the more common of the two CM-A5 control region variants described from the Greater Caribbean region. Following confirmation of haplotype CM-A5.1, the sample was tested at three variable positions in the mitogenome that distinguished among regional variants (see Shamblin *et al.* 2012 supplemental materials for amplification and sequencing primers for the respective fragments). Sequences of the Costa Rican diagnostic mitogenomic variable positions (10745 and 13388 based on alignments with the complete green turtle mitochondrial genome in Genbank, accession AB012104) confirmed that the poached male carried derived mutations characteristic of haplotype CM-A5.1.2 (T and C, respectively).

The presence of the endemic haplotype permitted individual assignment of this male to Tortuguero. When considered along with the Australian dataset, this result suggests that male natal homing may be the rule for green turtle populations globally.

In a test of breeding male natal homing in hawksbill turtles, the Mona Island rookery was the most likely source for the majority of breeding male hawksbill turtles captured in the vicinity of Mona Island (Velez-Zuazo *et al*. 2008). However, significant haplotype frequency differences between the sexes due to the presence of several rare haplotypes among the breeding males but absent among sampled Mona Island nesting females invoked the possibility of low level straying of breeding males to non-natal courting grounds, potentially at great distances from their natal rookeries (Velez-Zuazo *et al.* 2008). More extensive sampling is required to test the degree and scale of male natal homing among species and across regions.

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