Investigating sea turtle migration using DNA markers

John C Avise and Brian W Bowen

University of Georgia, Athens and University of Florida, Gainesville, USA

The past year has seen a further marshaling of genetic evidence for 'natal homing' in several species of marine turtles, a phenomenon wherein females, upon reaching sexual maturity, exhibit a propensity to return to nest at or near the respective beaches upon which they hatched some two or more decades earlier. This genetics-based inference stems from the strong spatial patterning observed in mitochondrial DNA lineages among nesting sites. Rookery-specific mitochondrial DNA markers are now being employed to monitor the natal sources of individuals captured at other phases of the life cycle, and the genetic findings have important conservation ramifications.

Introduction and background

The adult component of the life cycle in most marine turtles includes a long-distance migratory circuit between resident foraging grounds and nesting locales (Fig. 1). The migratory circuit can be hundreds or even thousands of kilometers in length and may be negotiated many times by each adult female during the several decades of her reproductive lifetime. Most previous information concerning migratory behavior in sea turtles did not come from direct observations (spatial and temporal scales in the oceanic environment are too great), but rather from recoveries of physical tags fastened to adult females on nesting beaches (e.g. see [1]). In the three species studied most intensively (green turtle, *Cheloninae mydas*, loggerhead turtle, *Caretta caretta*, and hawksbill turtle, *Eretmochelys imbricata*), these tagging studies have shown that nearly every female returns faithfully to a given nesting beach, and that different individuals are faithful to different rookeries. Because of logistic limitations inherent in tagging studies (e.g. short retention time of metal or plastic tags), however, questions remained about whether the nesting site of an adult female was also her natal site, and about the rookery origins of turtles on feeding grounds and migratory routes. Recently, much progress on these issues has come from studies of naturally occurring genetic tags carried within the mitochondrial genomes of sea turtles.

Molecular genetic studies of marine turtles began in earnest with a 1989 publication focusing on the evolutionary origins of the isolated Ascension Island nesting colony [2]. Green turtles nesting at Ascension Island proved to be cleanly distinct from those from most other Atlantic rookeries with respect to mitochondrial DNA (mtDNA) restriction sites, yet closely related to them in matriarchal phylogeny (which mtDNA genotypes register by virtue of their maternal transmission through pedigrees). Subsequent genetic surveys extended the mtDNA restriction fragment length polymorphism (RFLP) analyses to green turtle rookeries around the world [3,4] and included analyses of RFLP markers in the nuclear genome ([5], see also [6]). In the near future, additional study of nuclear genetic markers (including microsatellite DNAs) should further illuminate male-mediated gene flow and the mating systems of sea turtles [7*]. In this review, however, we will focus on available mtDNA data, which have special relevance to the female migratory puzzle and to rookery demographics.

The findings of early mtDNA studies have led to the view that green turtle rookeries (or regional assemblages of nearby rookeries) are normally distinct matrilineally from one another (exhibiting large or fixed differences in mtDNA genotypic frequencies), yet remain closely related evolutionarily (retaining high similarities in mtDNA nucleotide sequence). The two logical implications are that adult females usually return to natal sites for nesting (otherwise, extensive inter-rookery exchange of matrilines would have blurred the distinctions between rookeries in mtDNA genotypic frequency) and that conspecific rookeries are allied closely phylogenetically, perhaps through colony turnover via occasional extinction and colonization processes (otherwise, mtDNA molecules in long-isolated rookeries should have accumulated much larger sequence differences) [8*]. In other words, green turtle rookeries tend to be strongly isolated from one another over ecological timescales because of the propensity for natal homing by females, yet weakly differentiated over evolutionary timescales because of a

Abbreviations

mtDNA—mitochondrial DNA; RFLP—restriction fragment length polymorphism.
'concerted' colony evolution stemming from 'mistakes' in natal homing that mediate occasional inter-site movement of matriline.

In the past year, these conclusions have been extended through further mtDNA analyses to additional green turtle populations and to other sea turtles species. Also, for the first time, mtDNA analyses have been used to assess the genetic composition of marine turtle populations on feeding grounds and migratory routes. The mtDNA genotypes that show large frequency differences between rookeries provide excellent molecular markers for deducing the natal origins of juvenile and adult turtles captured during non-nesting phases of the life cycle.

Enhanced resolution of population structure using control region sequences

In the original mtDNA surveys using RFLP analyses, a few rookeries of green turtles (usually geographically proximate) appeared indistinguishable genetically; for example, the nesting populations at Tortuguero (Costa Rica) and eastern Florida were statistically alike in mtDNA genotype frequencies. Are such rookery-pairs connected by high levels of contemporary matrilineal gene flow? Or is present day gene flow low or absent, the genetic similarity instead being merely reflective of recent shared ancestry coupled with a lack of resolving power in the RFLP assays? To address this issue, portions of the rapidly evolving and hypervariable mtDNA control region (not coding for proteins) have been sequenced directly [9*]. In the Tortuguero/Florida test case, significant differences in mtDNA haplotype frequency were uncovered, suggesting previously undetected limits on contemporary matrilineal gene flow between these populations.

A panel of oligonucleotide primers has been developed recently, designed explicitly for the PCR amplification of mtDNA control region sequences in sea turtles, and has been applied in direct sequencing assays (and denaturing gradient gel electrophoresis) to studies of green turtle population structure in the Indo-Pacific region [10*]. Population-specific mtDNA markers (unique variants or frequency profiles of variants) were observed for nine of the 10 rookeries surveyed, including several groups of rookeries that had not been distinguished in restriction assays of the whole mtDNA genome [11]. In general, as increasingly refined mtDNA assays are conducted for sea turtles (or more sequence information obtained), estimated levels of matrilineal gene flow for surveyed rookeries can be expected only to remain the same or to decrease.

Matrilineal population structure in other sea turtle species

In the past year, mtDNA studies of rookery population genetic structure have been extended to two other marine turtle species and the results found to be similar qualitatively to those for the green turtle. In the loggerhead turtle, most assayed rookeries within and between ocean basins display large or fixed
Natal sources of turtles on feeding grounds and migratory routes

In February of 1994, an International Symposium on Sea Turtle Conservation Genetics was held at the University of Florida. There, several researchers presented exciting new findings on the mtDNA composition of marine turtles on feeding grounds and migratory routes. Although few of these studies are as yet published, a brief outline of salient findings is presented here to emphasize the great potential that mtDNA markers hold for reconstructing the movements of both male and female turtles during non-nesting portions of the life cycle.

Peter Lahanas and colleagues at the University of Florida presented evidence for the random recruitment of juvenile green turtles from regional rookeries to feeding grounds in the Bahamas. By comparing mtDNA haplotypes in a Bahamian feeding area to those of Caribbean and Atlantic rookeries (see [16]), they showed that a linear model incorporating the relative sizes of nesting populations could account for the observed frequency distribution of haplotypes in this feeding arena.

A second report addressed the enigma of juvenile loggerhead turtles observed great distances from known rookeries. In recent decades, thousands of juvenile loggerhead turtles have been reported off the coast of Baja California (Mexico), a remarkable finding because the nearest known rookeries for this species are in Japan and Australia (more than 10,000 km away). Even more recently, large numbers of juvenile loggerhead turtles have been captured in a North Pacific driftnet fishery. It was suggested that oceanic currents transported hatchlings passively across the North Pacific, but such speculation was discounted by most researchers because of the vast spatial scales involved. To test the possibility of trans-Pacific migrations, and to document particular natal sources for pelagic feeding aggregates, an international team headed by Brian Bowen assayed driftnet and Baja California samples using mtDNA markers known to distinguish cleanly between particular Australian and Japanese rookeries. Most pelagic samples (95%) carried Japanese genotypes, but a few (5%) possessed the mtDNA genotype seen only in Australian nest samples. Results indicate that pelagic-stage loggerhead turtles observed in the North and East Pacific are derived primarily from Japanese nesting colonies, but some juveniles may originate from Australia or perhaps from rookeries that are yet to be discovered.

Loggerhead juveniles also traverse the North Atlantic and are suspected of contributing to the pool of non-reproductive individuals in the Mediterranean Sea (where one major rookery also exists). To assess this possibility, mtDNA sequences were assayed in juvenile turtles captured in a Spanish longline fishery in the western Mediterranean [17]. A genotype endemic to West Atlantic rookeries was observed at high frequency (22%) among sampled Mediterranean juveniles, subsequently leading to a maximum likelihood estimate that ~57% of the individuals feeding in the western Mediterranean came from West Atlantic nesting beaches (with the remainder being derived presumably from Mediterranean nesting sites). In Australia, a similar comparison of mtDNA genotypes in hawksbill turtles captured on feeding grounds against those in known rookeries led to the conclusion that individual foraging areas can support hawksbill turtles from distant breeding populations [14*].

Forensic Identification of commercial products

Another potential application for molecular markers is the genetic identification of species and geographic sources of turtle products in retail markets. Forensic typing of turtle products (e.g., meat, eggs, and shells) by DNA analysis would be a boon to government agencies charged with the enforcement of legislation restricting trade in endangered species. Although few such applications for marine turtles are published as yet (but see [18*]), analogous genetic studies with marine cetaceans have demonstrated unequivocally the power of this approach—a substantial proportion of the whole products currently available in the Japanese marketplace derive from species that are proscribed from harvest by the Convention on International Trade in Endangered Species [19*].
Conservation implications

Molecular studies of population genetic structure in sea turtles have prompted critical discussion about the relationship between mtDNA phylogeny and population demography, and about the broader relevance of this genetics/demography connection to conservation efforts \[20^*,21^*,22,23^*.\] All seven species of sea turtle are listed as threatened or endangered \[24\]. The mtDNA findings described in this review carry several implications for the preservation and management of these species \[8^*\].

The matrilineal differentiation registered in mtDNA among conspecific nesting populations implies a considerable degree of demographic independence among rookeries with respect to reproduction. Thus, decline or loss of a given rookery is not likely to be compensated by natural recruitment of females hatched elsewhere (at least over ecological timescales germane to immediate human interests). This conclusion holds regardless of the level of inter-rookery gene flow mediated by males and the mating system and implies that, with regard to reproduction, particular local or regional nesting assemblages of sea turtles must be considered to be autonomous demographically for management purposes.

Interestingly, a contrasting perspective on conservation is provided by genetic studies at other stages of marine turtle life history. Migrational corridors and feeding grounds of sea turtles are often subject to human activities that result in a high mortality among feeding aggregates. Such anthropogenic factors include the direct harvesting of turtles for food and shell products, incidental capture in commercial fisheries directed towards other organisms, and effects of pollution and other alterations of the marine environment \[25\]. In several cases (e.g. juvenile loggerhead turtles in the North Pacific and the Mediterranean, green turtles in the Caribbean, and hawksbill turtles in northern Australia), mtDNA markers have documented the co-occurrence of individuals from multiple source colonies, such that rookeries whose individuals overlap geographically during non-reproductive periods can be impacted jointly by human activities or other environmental insults. Thus, with regard to mortality factors at non-nesting times, particular regional assemblages of rookeries should be considered to be linked demographically. Mortality of juvenile and adult turtles on feeding grounds or migratory routes could decimate multiple nesting populations simultaneously. Let us hope that this realization may serve as a strong stimulus for increased international cooperation in sea turtle conservation.

Conclusions

Because of their long generation times and oceanic habits, sea turtles are notoriously difficult to study by direct observation and, as a consequence, many aspects of their ecologies and behaviors remained poorly known. Genetic markers have helped to narrow some of these information gaps. In particular, studies of maternally inherited mtDNA have indicated a number of characteristics. First, females of several species show a strong propensity for natal homing, thereby making particular rookeries effectively autonomous with regard to reproduction over ecological timescales. Second, 'mistakes' in natal homing occur nonetheless with sufficient frequency to produce a concerted evolution among conspecific rookeries over short evolutionary timescales. Third, individuals from different rookeries sometimes overlap on foraging grounds and migrational corridors, thereby effectively linking these rookeries demographically with regard to mortality factors at non-nesting locations. The continued use of genetic markers to monitor sea turtle populations on nesting beaches, feeding grounds, and migrational corridors will not only increase understanding of the natural histories and migrational behaviors of these species, but will also aid conservation efforts by ascertaining which rookeries are impacted demographically by various causes of mortality during reproductive and non-reproductive phases of the life cycle.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

This study describes a series of PCR primers for amplification of microsatellite loci across marine turtle species and demonstrates the presence of hypervariable nuclear regions, which should have numerous applications in population genetics.

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mtDNA gene tree carries special demographic relevance for population
management for three reasons. First, in most species, females are spatially
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that they are functionally independent, as might be evidenced, for ex-
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JC Avise, Department of Genetics, University of Georgia, Athens,
Georgia 30602, USA

BW Bowen, BEECS Genetic Analysis Core, PO Box 110699,
University of Florida, Gainesville, Florida 32611, USA.