

# Investigating sea turtle migration using DNA markers

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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.

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## 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, *Chelonia 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.

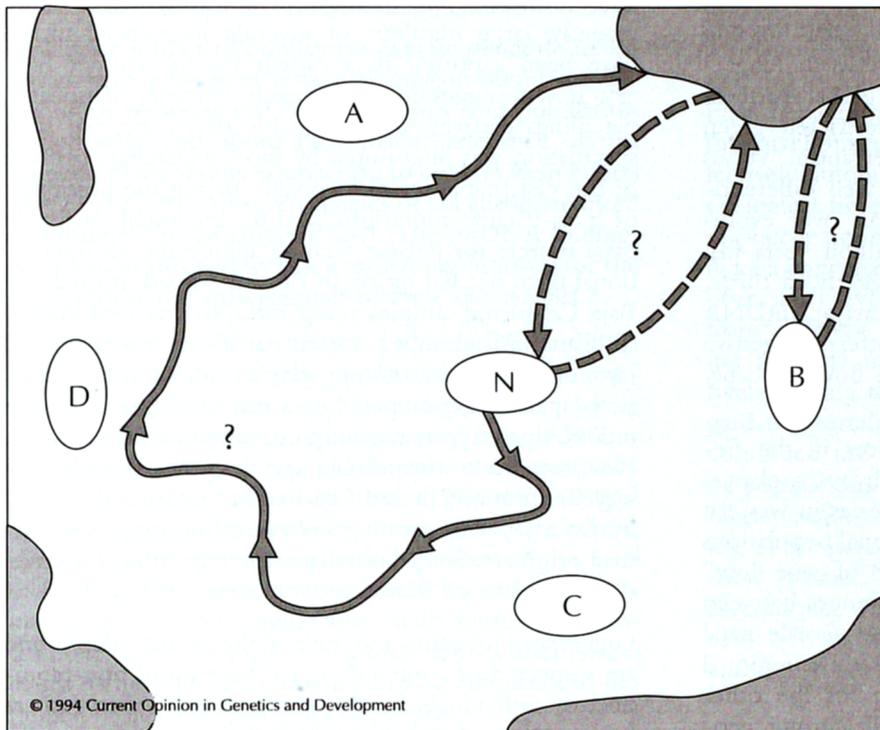
Molecular genetic studies of marine turtles began in earnest with a 1989 publication focusing on the evolutionary origins of the isolated Ascension Island nesting colony [2]. Green turtles nesting at Ascension Island proved to be cleanly distinct from those from most other Atlantic rookeries with respect 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.



**Fig. 1.** Simplified life cycle of a typical marine turtle female. After hatching on a sandy beach in the natal rookery (N), the hatchling enters the pelagic realm, in which she may remain for several years (route indicated by solid line) before recruiting onto an adult foraging area (shaded) that may be far distant from the natal rookery. At intervals of typically 2–5 years, an adult female migrates from the foraging location to a nesting location (possible routes indicated by dashed lines), and after laying several batches, each of ~100 eggs, over a two month period, returns to a marine foraging site. From direct tagging studies, it had already been well documented that an adult female is normally faithful to a particular rookery in successive nesting seasons. Recent mtDNA genetic analyses have shown that the rookery to which a female is fidelic as an adult is also likely to have been her natal site. Genetic analyses have also indicated which among the candidate rookeries contribute juveniles and adults to particular feeding grounds and migratory routes. Other rookeries (A–D) are shown.

'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

differences in mtDNA genotypic frequency [12,13\*], again consistent with a propensity for natal homing by females. One interesting contrast between loggerhead and green turtles concerns the global patterns of phylogeography. In the tropically restricted green turtle, rookeries from Atlantic–Mediterranean versus Indian–Pacific ocean basins belong to well differentiated mtDNA clades, indicating isolation over evolutionary timescales of perhaps 1.5–3 million years [4]. In the more temperately distributed loggerhead turtle, however, genotypes representing two major mtDNA clades are shared between oceans, in a pattern suggestive of recent inter-oceanic matrilineal gene flow, probably around southern Africa.

In the hawksbill turtle, strong differences in the frequency of mtDNA haplotypes distinguish two rookeries in northeastern Australia from two rookeries in western Australia, “demonstrating that these regional populations are not connected by significant amounts of gene flow” [14\*]. A lack of detectable genetic differences between rookeries within either area suggests that female natal homing in this species occurs normally on a regional rather than local scale (as may be true for the other sea turtle species as well, e.g. see [12]). Strong geographic differentiation in mtDNA haplotype frequencies has been demonstrated also for hawksbill turtle rookeries in the Caribbean [15].

### 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 whale products currently available in the Japanese market-place 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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  5. Karl SA, Bowen BW, Avise JC: **Global population structure and male-mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analyses of anonymous nuclear DNA regions.** *Genetics* 1992, **131**:163-173.
  6. Gyuris E, Limpus CJ: **The loggerhead turtle *Caretta caretta* in Queensland: population breeding structure.** *Aust Wildl Res* 1988, **15**:197-209.
  7. FitzSimmons NN, Moritz C, Moore SS: **Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution.** *Mol Biol Evol* 1995, in press.

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

8. Bowen BW, Avise JC: **Conservation genetics of marine turtles.**  
 • In *Conservation genetics: case histories from nature*. Edited by Avise JC, Hamrick JL. New York: Chapman and Hall; 1995: in press.

This paper summarizes genetic findings on sea turtles in the context of conservation and management efforts for these threatened species.

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

The authors utilized mtDNA control region sequences to re-examine a pair of rookeries that had been indistinguishable in conventional RFLP assays.

10. Norman JA, Moritz C, Limpus CJ: **Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles.** *Mol Ecol* 1994, 3:363–373.

The authors develop a set of oligonucleotide primers specific for the PCR amplification of mtDNA control region sequences in marine turtles and use these to generate sequence data for assessing matrilineal population structure in Indo-Pacific rookeries of the green turtle.

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This paper extends earlier conclusions about natal homing in green turtles to a second sea turtle species. The study also presents a global mtDNA phylogeography for the temperately distributed loggerhead turtle and compares results to those for the tropically and sub-tropically restricted green turtle (the populations of which were structured globally in accord with geographic and climatic boundaries between oceanic basins).

14. Broderick D, Moritz C, Miller JD, Guinea M, Prince RIT, Limpus CJ: **Genetic studies of the hawksbill turtle: evidence for multiple stocks and mixed feeding grounds in Australian waters.** *Pacific Conserv Biol* 1994, 1:123–131.

This paper presents the first evidence for population genetic structure and natal homing in the hawksbill turtle and also provides genetic evidence that particular foraging areas can support hawksbill turtles from distant breeding populations.

15. Bass AL: **Conservation genetics of the hawksbill turtle, *Eretmochelys imbricata*, in the Caribbean and West Atlantic [MSc Thesis].** Baton Rouge: Louisiana State University; 1994.

16. Encalada SE: **Phylogeography and conservation genetics of the green turtle (*Chelonia mydas*) in the Atlantic Ocean and Mediterranean Sea [MSc Thesis].** Gainesville: University of Florida; 1994.

17. Laurent L, Lescure J, Excoffier L, Bowen BW, Domingo M, Hadjichristophorou M, Kornarakis L, Trabuchet G: **Genetic studies of relationships between Mediterranean and Atlantic populations**

**of loggerhead turtle *Caretta caretta* (Cheloniidae). Implications for conservation.** *C R Acad Sci III*, 1993, 316:1233–1239.

18. Encalada SE, Eckert SA, Bowen BW: **Forensic applications of mitochondrial DNA markers: origin of a confiscated green turtle.** *Marine Turtle News* 1994, 66:1–3.

The authors of this brief note used mtDNA genotypes to determine that a captive green turtle that had been transported through the Panama Canal and abandoned in the Port of San Francisco was derived from a nesting population in the South or East Atlantic.

19. Baker CS, Palumbi SR: **Which whales are hunted? A molecular genetic approach to monitor whaling.** *Science* 1994, 265:1538–1539.

This paper describes one of the most compelling of available case studies illustrating the power of molecular forensic identification of the geographic and species source of endangered animal products in commercial markets. Although the empirical study dealt with cetaceans (whales and dolphins), it provides a model for similar efforts that might be contemplated for marine turtles.

20. Avise JC: *Molecular markers, natural history and evolution.* New York: Chapman and Hall; 1994.

This book provides an introduction to the wide variety of molecular methods and data sets available in population genetics and evolution and offers a collection of empirical case histories in which molecular markers have been used to address biological problems ranging from micro- to macro-evolutionary.

21. Avise JC: **Mitochondrial DNA polymorphism and a genetics–demography connection of conservation relevance.** *Conserv Biol* 1994, in press.

A mtDNA genealogy represents only a minuscule fraction of an organismal phylogeny. Nonetheless, this paper presents an argument that an mtDNA gene tree carries special demographic relevance for population management for three reasons. First, in most species, females are spatially associated with newly produced young. Second, dispersal and gene flow are often gender biased. Third, matrilineal differentiation implies demographic independence among populations, regardless of the magnitude of male-mediated gene flow.

22. Moritz C: **Applications of mitochondrial DNA analysis in conservation: a critical review.** *Mol Ecol* 1994, 3:403–413.

23. Moritz C: **Defining 'evolutionary significant units' for conservation.** *Trends Ecol Evol* 1994, 9:373–375.

This paper formalizes an important conceptual distinction between 'evolutionary significant units' (historically isolated sets of populations, as would be evidenced, for example, by differing genetic characteristics at multiple independent nuclear and cytoplasmic loci) and 'management units' (populations connected by such low levels of gene flow that they are functionally independent, as might be evidenced, for example, by significant differences in allele frequencies even in the absence of long-term historical isolation). These distinctions, highly relevant to conservation biology, parallel distinctions drawn previously between evolutionarily 'deep' versus 'shallow' population structures (see [20]).

24. IUCN (World Conservation Union): *1994 IUCN red list of threatened animals.* Edited by Groombridge B, Mace G. Gland, Switzerland: IUCN; 1993.

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