

# Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers

(conservation genetics/mixed stock assessment/marine turtles)

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**ABSTRACT** Juvenile loggerhead turtles (*Caretta caretta*) have recently been documented in the vicinity of Baja California, and thousands of these animals have been captured in oceanic fisheries of the North Pacific. The presence of loggerhead turtles in the central and eastern North Pacific is a prominent enigma in marine turtle distribution because the nearest documented nesting concentrations for this species are in Australia and Japan, over 10,000 km from Baja California. To determine the origin of the Baja California feeding aggregate and North Pacific fishery mortalities, samples from nesting areas and pelagic feeding aggregates were compared with genetic markers derived from mtDNA control region sequences. Overall, 57 of 60 pelagic samples (95%) match haplotypes seen only in Japanese nesting areas, implicating Japan as the primary source of turtles in the North Pacific Current and around Baja California. Australian nesting colonies may contribute the remaining 5% of these pelagic feeding aggregates. Juvenile loggerhead turtles apparently traverse the entire Pacific Ocean, approximately one-third of the planet, in the course of developmental migrations, but mortality in high-seas fisheries raises concern over the future of this migratory population.

In the past five decades, loggerhead turtles (*Caretta caretta*) have been reported sporadically along the Pacific coast of the Americas from Alaska to Baja California (1–3). More recently, biologists have documented a concentration of juvenile loggerhead turtles, estimated at 10,000 individuals, off the Baja California coast in association with an upwelling zone and concentrations of the pelagic red crab (*Pleuroncodes planipes*) (4–6). These records represent a prominent gap in the scientific understanding of marine turtle distribution, as nesting concentrations for this species occur in Japan and Australia but are notably absent from the central and eastern Pacific (7, 8). The presence of juvenile turtles around Baja California has prompted suggestions that the prevailing currents transport turtles from Japanese nesting beaches to East Pacific feeding areas (9). This possibility is supported by a single tag recovery (10) but is not widely accepted because a trans-Pacific migratory circuit would greatly exceed the known geographic scale of marine turtle migrations (11). Indeed, transportation across the North Pacific basin would stand as one of the longest migrations known among marine animals.

Another recent development in sea turtle biology is the documentation of juvenile loggerhead turtles in the high-seas fisheries of the central North Pacific (9, 12). Sea turtle mortality in these fisheries is a compelling conservation concern, as annual take may exceed 4000 loggerheads in the North

Pacific drift-net fishery (9) and 16,000 for all sea turtle species in the West Pacific (13).

Do loggerhead turtles traverse the Pacific Ocean in the course of developmental migrations? To test this hypothesis, mtDNA sequence polymorphisms were screened for potential applications as genetic markers (14). Previous analyses of loggerhead turtle population structure demonstrate the presence of rookery-specific mtDNA polymorphisms and significant haplotype frequency shifts between most nesting populations (15–17). In principle, these frequency shifts may be used to indicate the origin of turtles found in distant feeding grounds or in migratory corridors (18–21). This approach was originally developed by fishery biologists, using a maximum likelihood (ML) algorithm to estimate the relative contribution of riverine salmon stocks to coastal and oceanic fisheries, based on differences in allele frequencies among breeding populations as detected by protein electrophoresis (22–24). Here the same methodology is applied to mtDNA sequence polymorphisms to reconstruct the migrations of Pacific loggerhead turtles and to determine which nesting populations are affected by high-seas fisheries.\*\*

## MATERIALS AND METHODS

Nest samples (blood, eggs, or moribund hatchlings) were collected from Mon Repos and Swain Islands in Queensland, Australia ( $n = 26$ ) and from Wakayama Prefecture and the Ryukyu Archipelago in Japan ( $n = 26$ ). Precautions were taken to ensure that only one egg, hatchling, or blood sample was taken from a particular nesting female. Soft-tissue samples (heart, liver, or muscle) were obtained from turtles drowned in the North Pacific drift-net fishery ( $\approx 35^{\circ}\text{N}$ ,  $170^{\circ}\text{E}$ – $170^{\circ}\text{W}$ ;  $n = 34$ ), and blood or tissue samples were obtained from turtles captured in the vicinity of Baja California ( $n = 26$ ). Total genomic DNA was isolated with a standard phenol/chloroform protocol and stored in Tris/EDTA buffer (25).

Control-region (d-loop) sequences offer an appropriate level of resolution for fine-scale definition of marine turtle populations (18, 26). Biotinylated versions of the primers described by Norman *et al.* (18) were used to amplify mtDNA control-region sequences with PCR methodology (27). Standard precautions, including the use of negative controls (template-free PCR reactions), were taken to guard against contamination and related problems. PCR products were purified with streptavidin-coated magnetic particles available from Promega (28). Single-stranded sequencing reactions were conducted with fluorescently labeled M13 primers (29) in a robotic work station (Applied Biosystems model 800), and the labeled extension products were analyzed with an automated

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Abbreviation: ML, maximum likelihood.

\*\*The sequence (haplotype A) reported in this paper has been deposited in the GenBank data base (accession no. U22261).

Table 1. Variable nucleotide sites that define three haplotypes observed in Pacific loggerhead populations, based on 350 bp of the mtDNA control region

Haplotype code	Site position						
	35	96	118	187	228	243	322
A	G	A	G	A	G	G	T
B	G	G	A	G	A	G	T
C	A	G	G	A	A	A	C

Table 2. Distribution of Pacific loggerhead haplotypes at nesting grounds and pelagic feeding areas

Location	Genotype		
	A	B	C
Australian nesting areas	26	0	0
Japanese nesting areas	0	23	3
North Pacific drift-net fishery	1	28	5
Baja California feeding area	2	19	5

DNA sequencer (Applied Biosystems model 373A) in the DNA Sequencing Core at the University of Florida. PCR products from 6–11 individuals per location were sequenced in both directions. Subsequent samples were sequenced in one direction and compared with known genotypes. Samples that matched existing genotypes were collated for population analysis, whereas ambiguous or new genotypes were sequenced in the opposite direction to assure accuracy.

To estimate the contribution of each nesting area to Baja Californian feeding grounds and North Pacific drift-net captures, we used the conditional ML algorithm in the program GIRLSEM (30). The haplotype frequencies in drift-net samples and Baja California samples were compared with a *G* test of independence (31) to determine whether these samples may be drawn from a single pool of haplotypes.

**RESULTS AND DISCUSSION**

Comparisons of a 350-nt sequence from the mtDNA control region revealed three genotypes in nest samples that differ by 4- to 5-nt substitutions (Table 1). Haplotype A is observed only in Australian nest samples, and haplotypes B and C are observed only in Japanese nest samples (Table 2). This fixed difference between Pacific nesting areas allowed mtDNA polymorphisms to be effectively used as genetic “tags,” indicating the origin of pelagic juveniles in the North and East Pacific Ocean.

Thirty-three of 34 drift-net samples and 24 of 26 Baja Californian samples matched the haplotypes observed only in Japanese nest samples (Table 2). One drift-net sample and two Baja Californian samples match the haplotype seen only in Australian nest samples. The frequencies of haplotypes in the

drift-net fishery and Baja California feeding area are not significantly different based on a *G* test of independence, consistent with the hypothesis that these are cohorts of a single migratory population. By the same criteria, the fixed difference in haplotype frequencies between nesting areas is highly significant ( $P < 0.001$ ).

The ML analysis with GIRLSEM (30) indicates that Japanese nesting beaches contribute 97% ( $\pm 5\%$  SD) of turtles captured in the drift-net fishery and 92% ( $\pm 7\%$  SD) of the Baja California population. By the same criteria Australia contributes 3% ( $\pm 5\%$  SD) to drift-net captures and 8% ( $\pm 7\%$  SD) to Baja California populations. Essentially identical results were obtained with unconditional ML algorithms in the programs UCON (30) and SHADRACQ (ref. 32; unpublished data).

The observation of a putative Australian haplotype in the North Pacific is surprising. Perhaps a fraction of Australian neonates are transported into the North Pacific. However, confidence intervals on ML estimates encompass a 0% contribution of Australian turtles to drift-net samples, invoking the possibility that this result is an artifact of sampling variance. Perhaps the “Australian” genotype exists at low frequency in Japanese nesting aggregates but escaped detection in this survey. It is also possible that diminutive and as-yet-unsampled nesting populations in New Caledonia or elsewhere contribute incrementally to North Pacific feeding aggregates.

The conclusion that loggerhead turtles traverse the Pacific Ocean is contingent, in part, on the premise that this species does not nest in substantial numbers in the East Pacific. Older scientific reports of loggerhead nesting in this region are clearly based on misidentified olive ridley turtles (*Lepidochelys olivacea*) (7). More recent surveys have documented nesting by four marine turtle species along the west coast of the Americas

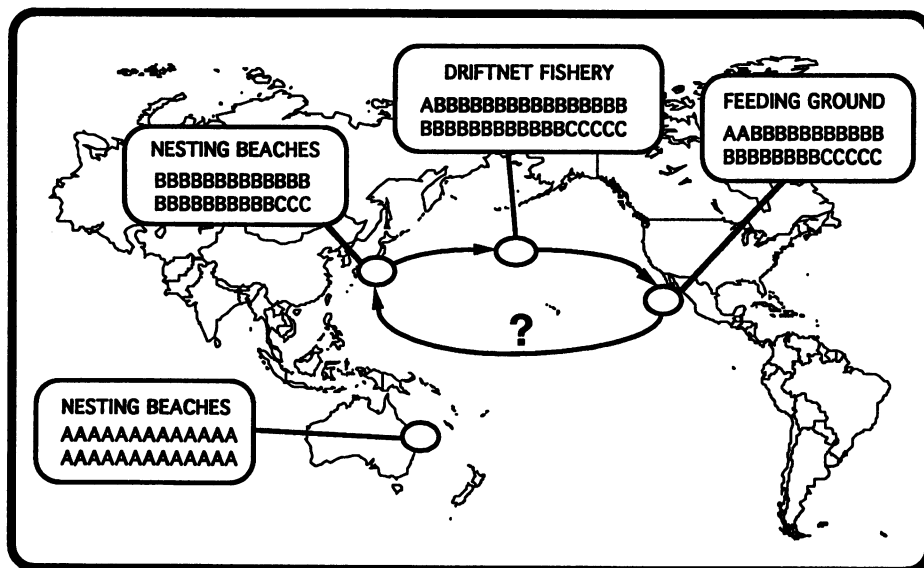


FIG. 1. Collecting locations and mtDNA haplotype distribution on Pacific nesting beaches and pelagic developmental habitats. Arrows indicate a simple version of the hypothesized migratory route between West Pacific nesting beaches and East Pacific feeding habitat. Genetic markers support the hypothesis that juvenile turtles migrate from Japan to Baja California via the easterly North Pacific Current. Turtles may return to adult habitats in the West Pacific via the westerly Northern Equatorial Current. Additional currents in the central and eastern Pacific may also be involved, and developmental migrations may include more than one circuit around the North Pacific Basin.

but have uncovered no evidence of loggerhead reproduction (33). If loggerhead nesting occurs in the East Pacific, it is likely to be in very low numbers (33).

The affiliation of central North Pacific and Baja California feeding areas with Japanese nesting populations is consistent with the hypothesis that the Kuroshio and North Pacific Currents transport loggerhead neonates from the coast of Japan to developmental habitats in the East Pacific (9, 10) (Fig. 1). Less certain is the route that loggerheads may use to return to the West Pacific. In the Atlantic, juvenile loggerhead turtles from nesting beaches in the southeast United States are believed to feed in the eastern Atlantic and Mediterranean (20, 34). The North Atlantic gyre apparently mediates migrations to these feeding grounds, as well as the return to adult feeding and nesting habitats in the western Atlantic (34). In the Pacific, the westerly North Equatorial Current may facilitate remigration from Baja California to the West Pacific (Fig. 1). The presence of loggerhead turtles in the near-equatorial waters of Pacific Colombia (33) can be construed as evidence for this scenario, and it would be instructive to survey mtDNA polymorphisms in this population. However, such explanations are speculative at present. If loggerhead turtles in the East Pacific return to nesting beaches in the West Pacific, their migratory route remains a mystery.

The ML analysis of mtDNA sequence polymorphisms demonstrates the power of molecular genetic markers for resolving migratory pathways and feeding-ground composition in marine turtles (17–21). Genetic markers are likely to find additional applications in the forensic identification of commercial products derived from marine turtles (35) and other endangered species (36).

The demographic link between West Pacific nesting beaches, East Pacific feeding habitats, and North Pacific fisheries invokes a prominent issue in marine conservation. In this case an endangered species captured on the high seas (by fishing fleets registered in Japan, Korea, Taiwan, the United States, and elsewhere) is derived from nesting beaches in Japan and possibly Australia, and nurtured in developmental habitat within the coastal waters of Mexico. How does stewardship over these turtles extend to high-seas fishing grounds? The 1982 United Nations Convention on the Law of the Sea maintains that biological resources on the high seas are the domain of the countries that sustain these species in developmental habitats (37). Furthermore, the 1983 United Nations Convention on the Conservation of Migratory Species (also known as the Bonn Convention) prohibits taking endangered species during migrations on the high seas (38). Under the principles established in these United Nations conventions, Japan, Mexico, and Australia may have authority to regulate the capture of loggerhead turtles in Pacific fisheries (37).

How severely do high-seas fisheries affect loggerhead turtle populations? A recent moratorium on drift-net fisheries in the North Pacific has alleviated this source of concern, but a long-line fishery has replaced the drift nets as the primary source of mortality. At present it is uncertain whether the Japanese nesting population(s), estimated at 2000–3000 females, can sustain a fishery mortality of several thousand juveniles per year (9). The number of nesting females in Queensland, Australia, has declined by 50–80% to perhaps 1000 females in the last decade (39), such that the loss of even a few hundred individuals could threaten the survival of this population. Large-scale oceanic fisheries may pose a significant threat to Pacific loggerhead turtles (40). Wildlife managers in Japan, Mexico, and Australia, along with appropriate international agencies, must define acceptable limits to the mortality of loggerhead turtles in high-seas fisheries.

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