Global phylogeography of the leatherback turtle (*Dermochelys coriacea*)

Peter H. Dutton[1]*, Brian W. Bowen[2], David W. Owens[3], Ana Barragan[4] and Scott K. Davis[5]

[1] NOAA-NMFS, Southwest Fisheries Science Center, P.O. Box 271, La Jolla, CA 92038, U.S.A.
[2] Department of Fisheries and Aquatic Sciences, University of Florida, 7922 NW 71st Street, Gainesville FL 32653-3071, U.S.A.
[3] Biology Department, Texas A&M University, College Station, TX 77843, U.S.A.
[4] Laboratorio de Tortugas Marinas, Departamento de Biologia, Facultad de Ciencias, UNAM, Circuito Exterior, Ciudad Universitaria, Mexico D.F. 04510, Mexico
[5] Department of Animal Sciences, Texas A&M University, College Station, TX 77843, U.S.A.

Abstract

Analyses of mitochondrial (mt) DNA control region sequences from 175 leatherback turtles *Dermochelys coriacea* from 10 nesting colonies revealed shallow phylogenetic structuring of maternal lineages on a global scale. Eleven haplotypes were observed, and mean estimated sequence divergence, $p = 0.00581$, is much lower than the deepest nodes reported in global mtDNA surveys of the green turtle *Chelonia mydas*, loggerhead *Caretta caretta*, and ridley turtles *Lepidochelys* spp. The leatherback turtle is the product of an evolutionary trajectory originating at least 100 million years ago, yet the intraspecific phylogeny recorded in mitochondrial lineages may trace back less than 900 000 years. The gene genealogy and global distribution of mtDNA haplotypes indicate that leatherbacks may have radiated from a narrow refugium, possibly in the Indian–Pacific during the early Pleistocene glaciation. Analysis of haplotype frequencies revealed that nesting populations are strongly subdivided globally ($F_{ST} = 0.415$), and within ocean basins ($F_{ST} = 0.203–0.253$), despite the leatherback's highly migratory nature. Within the Atlantic significant differences in haplotype frequency distributions and *Nm* values < 2 are observed in pairwise comparisons between St. Croix (U.S. Virgin Islands) and mainland Caribbean populations, and between Trinidad and the same mainland populations. These findings provisionally support the natal homing hypothesis for leatherback turtles, although several proximal nesting populations were indistinguishable, suggesting recent colonization or less precise natal homing behaviour than documented for other marine turtle species. The evidence of natal homing, manifested on ecological time scales, may be erased in some populations by rapid rookery turnover resulting from climatic fluctuation and the ephemeral nature of nesting habitat on a geological time scale. The evolutionary effective population size (N_e) is estimated from mtDNA data to be between 45 000 and 60 000, a value that exceeds current global census estimates of 26 000 to 43 000 adult females.

Key words: leatherback turtles, mitochondrial DNA, phylogeography, natal homing, conservation genetics

INTRODUCTION

The leatherback turtle, *Dermochelys coriacea*, is the sole surviving species of an evolutionary lineage (Dermochelyidae) that diverged from other turtles during the Cretaceous or Jurassic Period, 100–150 million years ago (Zangerl, 1980). The other six extant species of marine turtles belong to the family Cheloniidae (see Dutton *et al.*, 1996), and are thought to have evolved from a common ancestor during the middle Tertiary, 35–50 MYA (Zangerl, 1980). The evolutionary history of the genus *Dermochelys* is poorly understood due to the paucity of fossil material (Wood *et al.*, 1996). Recent evaluations of cheloniid turtle phylogeography, conducted primarily with mitochondrial (mtDNA) data, have demonstrated that evolutionary partitions within species and genera can be interpreted in terms of climate, geography, and migratory behaviour (Bowen & Karl, 1996). In particular, tropical (or warm temperate) habitat preference appears to be a key factor in limiting movement of cheloniid species between Atlantic and Indian–Pacific Oceans (Bowen *et al.*, 1992, 1994, 1998).

*All correspondence to: Peter H. Dutton, NOAA-NMFS, Southwest Fisheries Science Center, P.O.Box 271, La Jolla, CA 92038, U.S.A. E-mail: peterd@caliban.ucsd.edu
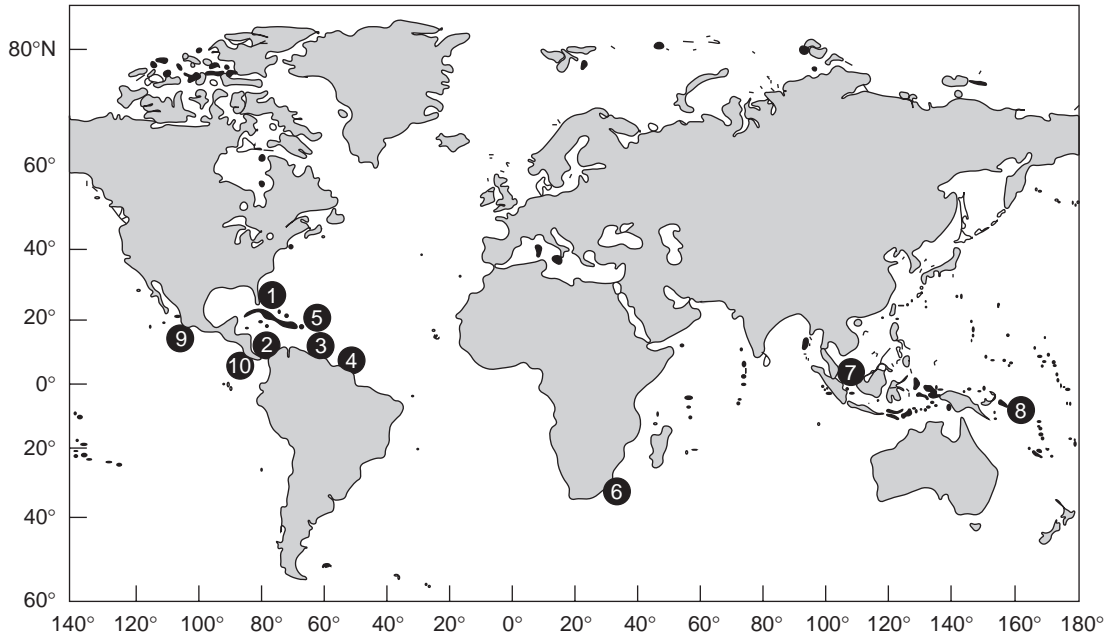
Fig. 1. Sampled nesting populations of *Dermochelys coriacea*. The numbers refer to locations given in Table 1.

Leatherbacks, however, are partially endothermic (Frair, Ackerman & Mrosovsky, 1972; Greer, Lazell & Wright, 1973), and evidently are not confined by such climatic barriers, since they are capable of extensive migrations (> 5000 km) between tropical nesting habitat and cold-temperate feeding habitats (Pritchard, 1976; Goff *et al.*, 1994; Eckert, 1999).

A broad oceanic distribution and strong migratory capacity have been associated with intra-oceanic mixing in populations of marine fishes (Graves, Ferris & Dizon, 1984; Graves & Dizon, 1989; Scoles & Graves, 1993; Graves, 1995) and marine mammals (Dizon, LeDuc & LeDuc, 1994; Curry, 1997). However, one life history feature that may restrict dispersal in leatherback turtles, particularly in terms of maternal (mitochondrial) lineages, is the propensity to return to the same area in successive nesting seasons. Although leatherbacks range widely in temperate and boreal waters, nesting activity is almost exclusively tropical (Pritchard & Trebbau, 1984; Spotila *et al.*, 1996). The finding that female sea turtles repeatedly return to the same beach to nest in successive years has led to the widespread belief that adults return to nest on their natal beach (Carr, 1967). Hendrickson (1958) and Owens, Grassman & Hendrickson (1982) proposed an alternative hypothesis consistent with nest-site fidelity of adult females. Under the 'social facilitation' hypothesis, first-time breeders follow experienced females to a nesting beach, and if nesting is successful, imprint on that site for future nesting. While it has been a formidable challenge to test these hypotheses directly due to the difficulties of marking hatchlings for long-term studies, recent work using RFLP and sequence data from mtDNA has yielded evidence of regional genetic population structuring consistent with the theory of natal homing in the green turtle, *Chelonia mydas,* the loggerhead turtle,

Caretta caretta, and the hawksbill turtle, *Eretmochelys imbricata* (Bowen *et al.*, 1992, 1994; Broderick *et al.*, 1994; Bass *et al.*, 1996; Schroth, Streit & Schierwater, 1996). The question of natal homing is particularly intriguing for leatherbacks in the light of the unusual traits that set them apart from other sea turtles, and one that this study seeks to address by examining mtDNA control region sequences.

The control region, which contains the displacement loop (D loop), has been shown to be the fastest evolving region of the mitochondrial genome in other vertebrates (Vigilant *et al.*, 1991; Quinn, 1992), and has been useful in resolving intra-specific population structure of various marine vertebrates, including sea turtles (Allard *et al.*, 1994; Norman, Moritz & Limpus, 1994; Bass *et al.*, 1996; Encalada *et al.*, 1996, 1998), cetaceans (Hoelzel, 1991; Hoelzel & Dover, 1991; O'Corry-Crowe *et al.*, 1997; Lyrholm & Gyllensten, 1998), and pinnipeds (Maldonado *et al.*, 1995; Bickham *et al.*, 1996; Slade *et al.*, 1998). The maternal mode of inheritance (Brown, 1983) makes this molecule particularly appropriate for testing patterns of gene flow among nesting populations of sea turtles.

While movements between regional rookeries have been documented at low frequency in leatherback turtles (Eckert *et al.*, 1989), it is not known whether the smaller populations that nest in the Caribbean islands are discrete or part of the large population that nests along the northern coast of South America. It has been suggested that the turtles that nest on Trinidad, West Indies, are part of the population that nests in nearby French Guiana (Bacon, 1970), possibly including turtles that have been displaced as a result of competition for nesting sites. Over two decades of intense tagging on these nesting beaches have not resolved this question. Likewise, tagging studies at rookeries in the east Pacific

Table 1. Mitochondrial DNA sample locations and estimated rookery sizes for *Dermochelys coriacea*

Rookery location	Sample size	Sample type[a]	Rookery size (females/year)	Reference
Atlantic				
(1) Florida	10	H	< 20	B. Schroeder (pers. comm.)
(2) Costa Rica: Tortuguero and Gandoca	28	H, B	200–500	D. Chacon (pers. comm.)
(3) Trinidad: Grande Riviere	27	B	200–800	K. Fournillier (pers. comm)
(4) Guianas: Les Hattes (French Guiana) and Matapica (Suriname)	20	H, B	400–5900	Fretey & Girondot (1989); Schulz (1975)
(5) U.S. Virgin Islands: Sandy Point, St. Croix	22	B	30–119	Boulon *et al.* (1996); McDonald & Dutton (1996)
Indian–Pacific				
(6) South Africa: Tongaland	8	H	56–120	Hughes (1996)
(7) Malaysia: Terengganu	9	H	15–25	Chan & Liew (1996)
(8) Solomon Islands	8	S	< 100	D. Broderick (pers. comm.)
East Pacific				
(9) Mexico: Playa Mexiquillo	18	B,H	700–900	Sarti *et al.* (1996)
(10) Costa Rica: Playa Grande	25	B	400–1500	Spotila *et al.* (1996)

[a] H = tissue from hatchling; B = blood from nesting female; S= skin biopsy from nesting female.

have been inadequate in delineating population structure. Leatherbacks nest at several locations along the Pacific coast of Mexico and central America, with major rookeries at Mexiquillo (Mexico), and Playa Grande (Costa Rica), occurring at opposite ends of this nesting range (Fig. 1), yet the degree of isolation between these populations is unknown.

We examined the intraspecific phylogeny of *D. coriacea* with mtDNA control region sequences to determine how the unusual biological traits outlined above can shape global partitioning of genetic diversity and to elucidate aspects of the molecular evolution of this species. This study provides the first genetic appraisal of diversity and stock structure in *D. coriacea* and is motivated in part by the need to identify conservation priorities for this endangered species.

METHODS

Field sampling

We obtained blood or tissue samples from 5 nesting locations in the western Atlantic and 5 locations in the Indian and Pacific Ocean, as described in Table 1 and Fig. 1. Sample sizes and geographic locations were dictated to a considerable degree by political considerations and permit limitations, although most major rookeries from each ocean basin are included. Most locations in this study are represented by samples from single nesting beaches. However, in the case of Atlantic Costa Rica and the Guianas (French Guiana and Suriname), samples were combined from adjacent nesting beaches (within 100 km). In both instances tagging studies have demonstrated that nesting females move between these beaches (Schulz, 1975; Pritchard, 1976; M. Godfrey, Univ. Toronto, pers. comm.; D. Chacon, ANAI, pers. comm.). The Suriname/

Guiana location included nesting beaches at Les Hattes in French Guiana (on the Suriname border) and the Wia-Wia refuge in Suriname, while the Atlantic Costa Rica location combined samples from Gandoca-Manzanillo and Tortuguero (Table 1).

Blood was collected from the dorsal cervical sinus or the hind flipper (Dutton, 1996) of nesting females for the samples from St. Croix, Suriname, Trinidad, Pacific Costa Rica and Mexico. Skin biopsies were taken from nesting females in the Solomon Islands (Dutton, 1996). The remaining samples consisted of hatchlings or embryos salvaged from nests after emergence, with care taken to ensure that no more than 1 nest per female was sampled. Packed red and white blood cells were frozen with liquid nitrogen or preserved in a lysis buffer (100 mM Tris-HCl, pH 8; 100 mM EDTA, pH 8; 10 mM NaCl; 2.0% SDS) prior to processing. Tissue samples were stored either in 95% ethanol or a 20% DMSO solution saturated with laboratory grade salt (NaCl).

Laboratory procedures

We extracted genomic DNA using the proteinase K digestion protocol of Maniatis, Fritsch & Sambrook (1982) as modified by Hillis & Davis (1986). The resulting DNA pellets were washed in ice cold 70% ethanol, redissolved in sterile water and stored at 4 °C. We used LTCM1 and HDCM1 primers, designed by Allard *et al.* (1994) for green turtles, to amplify a 496 bp fragment of the control region of mtDNA using the polymerase chain reaction (PCR) (Innis *et al.*, 1990). This fragment has been used extensively for intraspecific comparisons in other sea turtles. We amplified template DNAs in 50 µl total reaction volumes with 35 PCR cycles (Saiki *et al.*, 1988), using Amplitaq DNA polymerase (Perkin Elmer-Cetus) and a Perkin Elmer-Cetus thermal cycler with the following protocol: DNA

denaturing at 94 °C for 30 s; primer annealing at 53 °C for 60 s; and primer extension at 72 °C for 2 min. We took standard precautions to avoid contamination, including the use of positive and negative controls. Homology between the control regions of *D. coriacea* and other turtles confirmed that the PCR products were not artifacts of the amplification process (see Dutton *et al.*, 1996). We checked the size of the amplified fragments by ethidium bromide staining and electrophoresis in 1.5% agarose minigels (Maniatis *et al.*, 1982), and purified the double stranded amplified product using a PCR purification kit (QUIAGEN). Depending on yield, we sequenced 2–5 μl of the purified product directly using the Prism cycle sequencing kit (Applied Biosystems). We cleaned the labelled extension products through G50 sephadex spin-columns, or by ethanol precipitation, and analysed them with either an Applied Biosystems model 373A or 377 DNA sequencer. We sequenced both strands separately using LTCM1 and HDCM1. To confirm polymorphisms we used the following 2 leatherback-specific primers to sequence 370 bases of the light strand (DCDL4): 5′-TTATTTGCCACTAGCATAT-3′, and 413 bases of the heavy strand (DCDL5): 5′-ACAACCAGAGGCCA-GAATAAATCA-3′. Amplification and sequencing were conducted independently in different laboratories (Texas A&M University, College Station, TX, and National Marine Fisheries Service, La Jolla, CA) to confirm haplotypes.

Sequence alignment and analysis

We checked the chromatograph output from the automated sequencer for either miscalled or uncalled bases, editing and aligning sequences manually using software features available in SeqED (ABI 1992), and PAUP version 3.1.1 (Swofford, 1993). We estimated haplotype and nucleotide diversities with methods described in Nei & Tajima (1981) and Nei (1987) respectively, and used these as measures of within-population genetic variation. We calculated nucleotide sequence divergence (*p* values) between mtDNA genotypes with the Jukes–Cantor method (Jukes & Cantor, 1969). An exhaustive search of branching networks was conducted using parsimony criteria in PAUP version 3.1 (Swofford & Olsen, 1990; Swofford, 1993), and an unrooted parsimony network was constructed to elucidate the relationships between the haplotypes and to infer phylogeographic patterns.

The natal homing theory was evaluated by testing the null hypothesis that genetic variation is homogeneously distributed among nesting populations. Since leatherbacks undertake trans-oceanic migrations and females from different rookeries converge on the same forage areas (Pritchard, 1976; Boulon, 1989; Girondot & Fretey, 1996; Eckert, 1998), one would expect a lack of population subdivision in the absence of natal homing. A number of test statistics based on haplotype frequencies was used. Geographical subdivision was tested with a contingency chi-square test of independence using Monte Carlo procedures with 1000 random permutations of the original data matrix (Roff & Bentzen, 1989). Wright's fixation index of population subdivision, F_{ST} was estimated by an analysis of variance of haplotype frequencies among rookeries (Weir & Cockerham, 1984; Excoffier, Smouse & Quattro, 1992), implemented in Arlequin version 1.0 (Schneider *et al.*, 1996). Gene flow among nesting populations was estimated from F_{ST} values by the approach outlined by Wright (1951) adapted for haploid genomes using the formula $Nm = 0.5(1/F_{ST} - 1)$, where Nm is the effective number of females that migrate between populations per generation (Takahata & Palumbi, 1985). In addition, *F*-statistic analogues that incorporate information on nucleotide differences between haplotypes (Phi-statistics), were calculated to test for population subdivision using an analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992), also implemented in Arlequin version 1.0. Significance values for all analyses were obtained from 1000 permutations of the original data matrices.

RESULTS

We detected 11 mtDNA haplotypes among the 10 assayed nesting populations (Table 2). We only observed nine polymorphic sites, all differing by transitions, among the 175 samples analysed. The sequence of haplotype A is deposited in GENBANK (Accession number AF121964). All other haplotypes can be related to haplotype A by the nucleotide substitutions described in Table 2. Haplotype A was fixed in the Florida, Suriname/French Guiana, and South African rookeries (Table 3). Haplotype (*h*) and nucleotide (π) diversity estimates were therefore 0 for these three populations, and ranged from *h* = 0.14 to 0.80 and π = 0.001 to 0.002 among the other sampled sites

Table 2. Mitochondrial DNA control region polymorphisms and haplotype designations for *Dermochelys coriacca* samples assayed

	Base position								
	1 1 4	1 7 9	1 9 2	1 9 3	2 2 4	2 4 7	2 7 2	3 3 8	3 7 9
Haplotype									
A	G	A	G	C	T	T	A	A	A
B	–	–	–	T	–	–	–	–	–
C	A	G	A	–	–	–	G	–	–
D	–	G	A	–	–	–	G	–	–
E	–	G	A	–	–	–	G	G	–
F	–	G	A	–	–	C	G	–	–
G	–	G	A	–	C	–	G	–	–
H	–	–	A	–	–	–	G	–	–
I	–	–	–	–	–	–	G	–	–
K	A	G	A	–	–	C	G	–	–
L	A	G	A	–	–	C	G	–	G

Table 3. Haplotype frequencies, and haplotype (h) and nucleotide (π) diversities for 10 *Dermochelys coriacea* populations
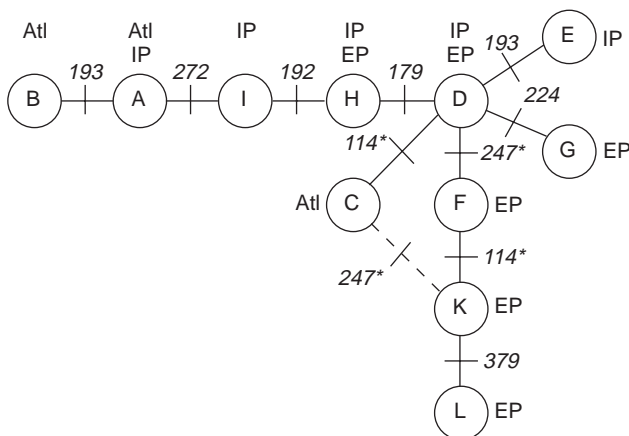
Location	Haplotype												Haplotype diversity (h) $\pm$ SEM	Nucleotide diversity (π)
	A	B	C	D	E	F	G	H	I	K	L	(n)		
Florida	10	–	–	–	–	–	–	–	–	–	–	10	0	0
Costa Rica (ATL)	26	–	2	–	–	–	–	–	–	–	–	28	0.138 $\pm$ 0.0837	0.0005
Trinidad	16	–	11	–	–	–	–	–	–	–	–	27	0.501 $\pm$ 0.0435	0.0019
Suriname/FG	20	–	–	–	–	–	–	–	–	–	–	20	0	0
St. Croix	12	8	2	–	–	–	–	–	–	–	–	22	0.589 $\pm$ 0.0663	0.0011
South Africa	8	–	–	–	–	–	–	–	–	–	–	8	0	0
Malaysia	3	–	–	2	3	–	–	1	–	–	–	9	0.806 $\pm$ 0.0889	0.0019
Solomon Islands	1	–	–	–	–	–	–	2	5	–	–	8	0.607 $\pm$ 0.1640	0.0021
Mexico	–	–	–	8	–	–	2	6	–	1	1	18	0.712 $\pm$ 0.0734	0.0017
Costa Rica (PAC)	–	–	–	14	–	1	3	4	–	2	1	25	0.663 $\pm$ 0.0920	0.0016
Overall	96	8	15	24	3	1	5	13	5	3	2	175	0.667 $\pm$ 0.0356	0.0015

(Table 3). These low nucleotide diversities reflect the shallow separations among haplotypes. We found the greatest haplotype diversity in the Malaysian population. The overall h for all populations combined was 0.667 ± 0.036, lower than the value of 0.830 reported for a comparable survey of Atlantic green turtles (Encalada *et al.*, 1996) and the value of 0.849 for Atlantic hawksbills (Bass *et al.*, 1996). All of these estimates of mtDNA variation are low relative to values reported for conspecific comparisons in other vertebrate groups (Avise *et al.*, 1987, 1992).

Sequence divergence between haplotypes ranged from 0.2% to 1.4%. The unrooted parsimony network summarizing haplotype inter-relationships (Fig. 2) demonstrates the shallow phylogenetic architecture of mitochondrial lineages in this species. It is notable that all intermediate states in the network are extant, possible evidence of reduced lineage sorting associated with recent population expansion. Some of the most divergent haplotypes were found at either extreme of the

Fig. 2. Parsimony network describing the relationships among the 11 *Dermochelys coriacea* mtDNA haplotypes. Haplotypes correspond to Table 3. Presence of haplotypes in the Atlantic (Atl), Indian-Pacific (IP), and eastern Pacific (EP) is indicated. Mutation sites are shown on the branches and asterics indicate assumed homoplasy.

geographic range in the eastern Pacific and western Atlantic (Fig. 2). Haplotype A was found in all the populations sampled in the Atlantic and Indian–Pacific, but was absent from the eastern Pacific (Table 3, Fig. 2). Two haplotypes found in Indian–Pacific populations (D and H) also occurred in the eastern Pacific populations (Table 3, Fig. 2), and four rare haplotypes (F, G, K and L) were only observed in the eastern Pacific rookeries. St. Croix (Atlantic), and the Solomon Islands (Pacific) were the only locations in which we found a unique haplotype at relatively high frequency (Table 3).

The chi-square test of haplotype frequencies revealed that nesting populations are geographically subdivided ($\chi^2 = 435.37$, $P < 0.001$). The AMOVA also revealed a high degree of genetic subdivision among rookeries, with more than 51% of the molecular variance due to partitions among rookeries ($Phi_{ST} = 0.511$, $P < 0.001$). The global F_{ST} was 0.415 ($P < 0.001$), with $F_{ST} = 0.253$ ($Phi_{ST} = 0.231$, $P < 0.001$) among Atlantic populations, and $F_{ST} = 0.203$ ($Phi_{ST} = 0.350$, $P < 0.001$) among the Indian–Pacific populations. Estimates of interpopulation gene flow were $Nm = 1.48$ among the Atlantic populations, $Nm = 1.96$ among Indian–Pacific populations, and $Nm = 0.70$ overall. Levels of gene flow greater than $Nm = 1$–4 are considered sufficient to prevent geographically isolated colonies from diverging as a result of genetic drift (Slatkin, 1987). These results are generally consistent with the natal homing hypothesis, although not all populations were genetically distinct. Pairwise comparisons within the Atlantic revealed restricted gene flow between St. Croix and the other mainland nesting populations and between Trinidad and those same populations ($P < 0.05$; Table 4). However, the mainland populations nesting in Florida and Suriname/French Guiana were indistinguishable, since both were fixed for the same haplotype (A) which also comprised 93% of the sampled Atlantic Costa Rican population, resulting in high Nm values (Table 4). Furthermore, these populations were indistinguishable from the South African rookery ($P = 0.999$, Table 4). In the east Pacific the nesting populations in Mexico and Costa Rica were also indistinguishable ($P = 0.576$,

Table 4. F_{ST} estimates and significance (P) values from 1000 permutations (below diagonal) and Nm values from Takahata & Palumbi's (1985) G_{ST} estimator modified from Wright's F_{ST} (above diagonal) among the 10 *Dermochelys coriacea* rookeries. Nm estimates designated as high include values greater than 25

	I	II	III	IV	V	VI	VII	VIII	IX	X
I Florida	–	high	1.33	high	1.44	high	0.37	0.23	0.37	0.37
II Costa Rica (Atlantic)	−0.019 $P=0.5700*$	–	1.56	24.59	1.33	high	0.59	0.22	0.31	0.32
III Trinidad	0.247 $P=0.0383$	0.243 $P=0.0040$	–	high	2.84	1.33	1.78	0.70	0.74	0.69
IV Suriname	0.000 $P=0.9990*$	0.020 $P=0.1712*$	0.346 $P=0.0010$	–	0.97	high	0.41	0.14	0.26	0.28
V St. Croix	0.258 $P=0.0131$	0.274 $P=0.0020$	0.150 $P=0.0091$	0.339 $P\sim0.0000$	–	1.43	2.53	0.23	0.92	0.84
VI South Africa	0.000 $P=0.9990*$	−0.019 $P=0.6032*$	0.274 $P=0.0393$	0.000 $P=0.9990*$	0.258 $P=0.0222$	–	0.71	0.23	0.37	0.37
VII Malaysia	0.415 $P\sim0.0040$	0.458 $P\sim0.0000$	0.219 $P=0.0201$	0.548 $P\sim0.0000$	0.165 $P=0.0211$	0.415 $P=0.0030$	–	1.60	3.44	2.72
VIII Solomon Islands	0.686 $P\sim0.0000$	0.695 $P\sim0.0000$	0.418 $P\sim0.0000$	0.785 $P\sim0.0000$	0.361 $P=0.0010$	0.686 $P\sim0.0000$	0.238 $P=0.0050$	–	1.35	1.01
IX Mexico (Pacific)	0.352 $P\sim0.0000$	0.618 $P\sim0.0000$	0.403 $P\sim0.0000$	0.658 $P\sim0.0000$	0.352 $P\sim0.0000$	−0.014 $P=0.5760*$	0.127 $P=0.0342$	0.270 $P=0.0010$	–	high
X Costa Rica (Pacific)	0.573 $P\sim0.0000$	0.609 $P\sim0.0000$	0.419 $P\sim0.0000$	0.642 $P\sim0.0000$	0.373 $P\sim0.0000$	0.574 $P\sim0.0000$	0.155 $P=0.0141$	0.331 $P\sim0.0000$	−0.014 $P=0.5760*$	–

* Not significant ($P > 0.05$).

Table 4). All Indian–Pacific populations were significantly different from each other and those in the eastern Pacific ($P < 0.005$, Table 4).

Several reports (Bowen, Meylan & Avise, 1991; Avise *et al.*, 1992; Bowen *et al.*, 1992) have demonstrated that a molecular clock for turtle mtDNA is several-fold slower than the conventional rate for vertebrate groups (e.g. Brown, George & Wilson, 1979). A provisional clock for mtDNA control region sequences in chelonids has been proposed with an initial divergence rate of about 1.2–2.4% per million years between lineages (Encalada *et al.*, 1996). Another independent clock can be obtained from data in Dutton *et al.* (1996), who report an average control region sequence divergence of 6.7% between the ridley species, *Lepidochelys kempi* and *L. olivacea*, and between Atlantic *C. mydas* and Pacific *C. mydas* and putitive *C. agassizi*. Since these two groups of species were likely to have been separated by the Panamanian land bridge 3–4 MYA, the rate of evolution for the control region is estimated at 1.7–2.2% between lineages per MY, consistent with the rate postulated by Encalada *et al.* (1996). Possible reasons forwarded for a slower clock in turtles include a long generation time (or germ cell generation time) and reduced metabolic rate relative to mammals (Avise *et al.*, 1992; Martin, Taylor & Palumbi, 1992). While the generation time of leatherback turtles is uncertain (see Rhodin, 1985; Zug & Parham, 1996), the metabolic rate in this species is comparable to other marine turtles (Paladino, O'Connor & Spotila, 1990). In the absence of clear paleontological or zoogeographic dates to calibrate rate estimates for leatherback mtDNA, the molecular clock indicated above for cheloniid control region sequences is applied. While recognizing the limitations of this provisional clock, it none the less represents a good initiation point for discussions of

evolutionary history. Based on this provisional evolutionary rate the most divergent haplotypes detected for leatherbacks ($p = 0.0141$) would coalesce to a common ancestor approximately 640 000 to 840 000 years BP.

DISCUSSION

Phylogeography of leatherback mtDNA lineages

Dermochelys is characterized by low genetic diversity and shallow mtDNA phylogeny compared to the chelonid sea turtles (Bowen & Karl, 1996). The deepest bifurcations observed in other chelonid sea turtles (green turtle, loggerhead turtle, hawksbill turtle, and the ridley species, *L. olivacea* and *L. kempi*) are all approx. 5–7% in control region sequences, indicating separations on the order of 2–4 million years (Bowen *et al.* 1994, 1998; Bass *et al.*, 1996; Encalada *et al.*, 1996, 1998). In every case these bifurcations are between lineages that predominate in the Atlantic–Mediterranean versus the Indian–Pacific Oceans, and timeframes correspond approximately to the closure of the Isthmus of Panama. In contrast, our data for leatherbacks indicate a recent global radiation (with all haplotypes still extant), and the estimated time separating the most divergent haplotypes (<1 MY) is more recent than for other marine turtles, even though leatherbacks represent a considerably older organismal lineage (Dutton *et al.*, 1996). Cold temperatures are not an impediment to dispersal in leatherbacks due to their ability to thermoregulate to some degree (Frair *et al.*, 1972; Greer *et al.*, 1973). Indeed, leatherbacks are known to forage in the waters off Chile as far as 47 °S (Pritchard, 1980; Frazier & Salas, 1982; Frazier &

Brito-Montero, 1990), in the Gulf of Alaska, and in proximity to pack ice along the coast of Labrador (Hodge, 1979; Goff & Lien, 1988; Goff et al., 1994). However, the physiological constraints of egg incubation effectively confine reproduction to warm tropical regions. During glacial periods nesting habitat would have been reduced, confining nesting populations to a narrow equatorial refugium. Recent extinctions of rookeries during cool periods in the Pleistocene (< 1 MYA) followed by lineage replacement during subsequent range expansion could contribute to shallow contemporary mtDNA phylogeny in this species. A second explanation invokes the possibility of selection, which prompted a replacement of ancestral mtDNA types sometime during the Pleistocene. This selection may not have acted directly on the control region, since all mtDNA loci are linked and inherited as a unit. Selection has also been proposed as a possible explanation for the low mtDNA variation in another deep diving marine vertebrate – the sperm whale *Physeter macrocephalus* (Lyrholm & Gyllensten, 1998). Given the role of mitochondria in energy production, and the physiological adaptations of leatherbacks to deep diving and cold tolerance, the notion of a selective sweep is intriguing and may merit further consideration (see Amos & Harwood, 1998).

A third explanation for more recent coalescence time is that the mtDNA mutation rate could be much slower in leatherbacks and our molecular clock calibration based on chelonid data is inappropriate. In order to account for the lower diversity in leatherbacks, the mutation rate would have to be about three or four times lower than in the other sea turtles. However, a relative rate test (Sarich & Wilson, 1973) with combined mtDNA sequence data (CytB and ND4-Leu) from Dutton et al. (1996) and Bowen, Nelson & Avise (1993), did not reveal rate heterogeneity among species, suggesting that a slowdown in leatherback mutation rate is unlikely. While we cannot discount any of these three general explanations (invoking drift, selection, or mutation) for the shallow mtDNA genealogy in leatherback turtles, we place greater confidence in the initial suggestion of population reductions and strong lineage sorting during glacial intervals. Cooler conditions may reduce the tropical nesting habitats of all sea turtles, and the tropical feeding habitats of chelonid sea turtles, but cold temperate habitats are *eliminated* on a vast scale, or displaced thousands of kilometres by glaciation. Since *D. coriacea* is the only sea turtle to feed extensively in high latitudes, glaciation may affect leatherback populations more profoundly than other sea turtles. Also, sex determination in sea turtles is temperature-dependent, and a temperature decrease of just 0.5 °C can completely masculinize hatchling production (Dutton, Whitmore & Mrosovsky, 1985). Since female-producing temperatures are higher in leatherbacks than in green turtles (Mrosovsky, Dutton & Whitmore, 1984), it is possible that cooler climate might have had a greater masculinizing effect on leatherbacks, possibly eliminating nesting populations in regions, such as the eastern Pacific, where Pleistocene temperature changes were relatively rapid and of greater magnitude (Lindberg, 1991; Kotilainen & Shakleton, 1995).

The high diversity found in the Malaysian and Solomon Islands populations taken together with the central position of haplotype D in the mtDNA parsimony network (Fig. 2), indicates that the Indian–Pacific may have been the source of the most recent leatherback radiation; eastward to the eastern Pacific and westward into the Atlantic. The finding that some haplotypes are shared between Indian–Pacific and Atlantic populations and others between Indian–Pacific and east Pacific populations further corroborates this hypothesis. We cannot rule out the possibility that this radiation began in the eastern Pacific, but consider this explanation less likely because of the greater climatic stability in the Indian–Pacific region relative to the eastern Pacific (Lindberg, 1991; Dansgaard et al., 1993). This scenario is similar to one proposed for olive ridley turtles (Bowen et al., 1998), loggerhead turtles (Bowen et al., 1994), pelagic billfish such as blue marlin *Makaira nigricans* and sailfish *Istiophorus platypterus* (Graves, 1995) in which colonization of the Atlantic is thought to have occurred from Indian–Pacific ancestral stock. An alternative hypothesis could be that haplotype D was once widely distributed and has diminished, particularly in the Atlantic, as a result of mutation to other haplotypes and lineage sorting. Moreover, the Indian–Pacific may be the center of accumulation rather than the centre of origin. We consider these alternatives to be less likely, since every intermediate in the parsimony network is still extant in at least one animal (Fig. 2), suggesting a recent radiation of lineages. More extensive sampling, including important Indian–Pacific rookeries absent from this survey (Irian Jaya, Indonesia, and Papua New Guinea) may help to test this hypothesis further, particularly since sample sizes from the three Indian–Pacific populations in this study were small. A second, more recent radiation from the Indian–Pacific into the Atlantic may have occurred as suggested by the presence of haplotype C in the Atlantic (Fig. 2, Table 3). This haplotype is more closely related to the east Pacific and Indian–Pacific haplotypes than to the other two Atlantic haplotypes (Fig. 2), and it is more parsimonious that C would have arisen from Indian–Pacific stock rather than by parallel mutation from A.

The relatively small difference between Atlantic and Pacific mtDNA lineages is consistent with the current disuse of subspecies designations for Atlantic and Indian–Pacific forms (*D. c. coriacea* and *D. c. schlegeli*, respectively). Pritchard & Trebbau (1984) suggest that any morphologically based subdivision would distinguish eastern Pacific turtles, which tend to be smaller than those in the Atlantic, Indian, and western Pacific oceans. However, this trend toward reduced size in the eastern Pacific has been noted in other sea turtles and may reflect ecological or trophic conditions rather than evolutionary processes.

Effective population size

Dermochelys coriacea represents a deep evolutionary lineage in terms of organismal evolution, with a very short evolutionary history inscribed in mtDNA sequences. Evolutionary effective population size (N_e) can be calculated from observed genetic diversity, as equivalent to the time (in generations) to shared common ancestry (Avise, Ball & Arnold, 1988). We estimate this coalescence time to be 640 000–840 000 years BP, corresponding to an N_e of 45 700 to 60 000 (assuming a generation time of 14 years for *D. coriacea* (Zug & Parham, 1996)). New nesting populations may be founded by one or a few females (Bowen *et al.*, 1992), a colonization strategy that would reduce the number of matriarchal lineages. Even in established populations, high variability in reproductive success between individuals might reduce N_e as fewer adults contribute gametes to successive generations. This effect of sweepstakes recruitment has been reported for several aquatic and marine species with extremely high fecundities, such as shellfish and widely distributed marine fish, where N_e calculated by genetic criteria are typically an order of magnitude lower than current day census sizes (see Avise, 1994; Grant & Bowen, 1998). Strong population subdivision should enhance the retention of divergent lineages (and therefore increase N_e and coalescence time). However, the physical factors that facilitate geographic isolation and buffer gene genealogies from extinction in freshwater fish and terrestrial species are mostly absent from the marine environment. Global populations of pelagic fish may be nearly panmictic, with the exception of billfishes and mako sharks, *Isurus oxyrinchus,* that appear to show fidelity to spawning grounds (Graves, 1995). In contrast to marine fish, recent genetic studies of cetaceans have documented population subdivision associated with matrilineal social organization and female philopatry to breeding grounds (Baker & Palumbi, 1995; O'Corry-Crowe *et al.*, 1997). In humpback whales, *Megaptera novaeangliae*, mtDNA variation is high and corresponding N_e is also relatively high (Baker *et al.*, 1994). Leatherbacks, and sea turtles in general, appear to be somewhat intermediate between fish and cetaceans in terms of mtDNA diversity. Like cetaceans, they have subdivided populations due to female philopatry, and yet, like fish, their high fecundity, and presumed high variance in reproductive success (sweepstakes recruitment), is conducive to rapid lineage sorting.

In 1982 Pritchard (1982) estimated the world population of leatherback turtles at perhaps 115 000 adult females. Many major nesting colonies have declined precipitously in the last two decades (Chan & Liew, 1996; Sarti *et al.*, 1996; Spotila *et al.*, 1996), but this decline may be too recent to affect N_e. Sadly, the N_e calculated from mitochondrial data is now similar to the current census size, estimated to be between 26 000 and 43 000 (Spotila *et al.*, 1996). For the evolutionary processes considered here, it is sufficient to note that the mtDNA diversity observed on a global scale in *Dermochelys* is low because the mtDNA genealogy coalesces in recent evolutionary time (< 1 MY) to a single female lineage and that the current population declines, if persistent, may further reduce this diversity.

Population structure and the natal homing theory

The finding of population subdivision in this study provisionally supports the theory of natal homing for leatherbacks. In the Pacific, for instance, leatherbacks undertake trans-oceanic migrations and individuals from both eastern Pacific and western Pacific nesting populations converge in pelagic areas of the north Pacific, and in the southeast Pacific, off the coast of Chile (Dutton, Balazs & Dizon, 1999). Despite this mixing of animals from different nesting stocks in forage areas, the segregation of maternal lineages between eastern and western Pacific rookeries suggests that natal homing occurs. However, the evidence is not entirely clear-cut, since several rookeries, including ones in different ocean basins (Atlantic and Indian) were indistinguishable (Table 4). In these cases it is difficult to refute either hypothesis (natal homing versus social facilitation) (see Taylor & Dizon, 1996), since a lack of observed population subdivision could be explained by low genetic diversity or relatively recent common ancestry. It is possible, for instance, that the South African rookery recently shared the same ancestor (Haplotype A) as rookeries in the western Atlantic and that insufficient time has elapsed for these populations to diverge, despite the predominance of natal homing. Alternatively, these instances of genetic homogeneity could indeed be due to ongoing or periodic gene flow, which would suggest a weak natal homing tendency in leatherbacks. Irrespective of the reason, these somewhat mixed results for leatherbacks pose a caveat to the strict natal homing model that is becoming generally accepted for sea turtles (Bowen & Avise, 1995). The leatherback turtle is separated from other species of sea turtle by over 65 million years and may have developed quite different solutions to the challenges of reproduction and terrestrial nesting. Pritchard (1976) has suggested that leatherback turtles may seek a particular beach type rather than a specific locale – females of this large species require deep access routes to the nesting beaches and typically nest on open sand areas of steep high energy beaches along coastal margins (Whitmore & Dutton, 1985). Such beaches are noted for instability, so that some flexibility in nest site selection may be a prerequisite for successful reproduction (Mrosovsky, 1983; Eckert, 1987; Tucker, 1989). The beaches along the Guianan coast for example, tend to go through cyclic patterns of erosion and accretion, the net effect of which is a migration of sandbars several kilometres long in a westward direction along the coast. Schulz (1975) has noted an approximate 10 year cycle in the pattern of beach movement along the Suriname Coast.

If Pritchard's suggestion about females seeking a particular beach type is correct, then the amount of

movement among the highly ephemeral mainland nesting colonies might be qualitatively different from the movement among island nesting beaches (by their very nature, the island beaches cannot move very far). Do the mtDNA data support this prediction? While the estimated level of gene flow within ocean basins in this study is generally higher than would be expected under a strict natal homing scenario, it is notable that in the Atlantic the St. Croix population has a unique haplotype (B) at high frequency (36%, Table 3). Furthermore, haplotype C was observed at 41% in Trinidad, while this haplotype was rare or not detected in the other Atlantic populations. The suggestion that turtles nesting on Trinidad are part of the larger mainland population is therefore not supported by the mtDNA data. The significant differences in haplotype frequency distributions and the Nm values < 2 that were found in pairwise comparisons between St. Croix and mainland Caribbean populations, and between Trinidad and the same mainland populations, are comparable to studies on green and hawksbill turtles (Bass *et al.*, 1996; Encalada *et al.*, 1996) and support a natal homing scenario for these island populations. This is consistent with recent findings that nest site fidelity in Caribbean populations are higher than previously thought in leatherbacks, and that most females monitored over a 15-year period consistently return to St. Croix to nest (McDonald & Dutton, 1996). Although tag loss is known to be a problem in this species (McDonald & Dutton, 1996), there has not been a single observation of migration between nesting beaches in the Guianas and Trinidad (400–800 km apart), despite several years of beach monitoring, while 22 of the 25 turtles identified as remigrants at Gandoca (Atlantic Costa Rica) in 1995 had previously nested elsewhere along the coast, as far away as Colombia (D. Chacon, ANAI, pers. comm.), a distance of approx. 800 km. Perhaps the imprinting cues necessary for natal homing are not sufficiently strong to resolve these mainland beaches, particularly since the mainland coasts are so dynamic, while the islands represent more distinctive (and more discrete) nesting habitat to a leatherback. This would explain why the estimated number of migrants is greater between the coastal populations than between those of the Caribbean islands and mainland. In the Pacific the lack of differentiation between the Costa Rican and Mexican rookeries, over 1500 km apart, may also indicate that the factors that facilitate precise natal homing are curtailed along the region's coastline and that ongoing nest site relocation is sufficient to homogenize mainland rookeries. It is harder to argue for recent common ancestry, since unlike the Atlantic, where mainland populations are essentially characterized by a single common haplotype (A), the eastern Pacific rookeries have several haplotypes, including rare ones, at almost identical frequencies (Table 3). Given the stochasticity of lineage sorting, it is unlikely that two populations would maintain this pattern of diversity in the absence of ongoing gene flow. When one considers that there is sporadic nesting along the coast between Mexico and Costa Rica (Sarti *et al.*, 1996), it seems more plausible that these rookeries are geographic extremes at either end of a continuous metapopulation and that the regional homogeneity is maintained by imprecise natal homing.

Implications for conservation

Several leatherback nesting populations have declined sharply over the last 20 years and this species has been the focus of concerted international conservation efforts. One of the most dramatic population declines has been seen at Terengannu, Malaysia, from about 2000 nesting females per year through the early 1960s to fewer than 20 females in 1995 (Chan & Liew, 1996). Such precipitous reductions in the size of breeding populations can lead to bottleneck effects with associated loss of genetic variability. Although a single bottleneck with a subsequent rapid recovery of population size does not necessarily lead to a serious reduction in genetic variability, extended population reductions are a perennial concern to wildlife managers (Nei, Maruyama & Chakraborty, 1975; Frankel & Soulé, 1981; Allendorf, 1986; Fuerst & Maruyama, 1986, Lande & Barrowclough, 1987). Based on mtDNA data, loss of variation does not appear to have occurred yet in the Malaysian population; some of the the highest haplotype and nucleotide diversities in this study were among the samples from Malaysia. Since sea turtles probably have a long reproductive life (over 50 years) and high fecundity, it is possible that genetic variation may be preserved providing that the population decline can be reversed within a few generations. This appears to be the case for another sea turtle – Kemp's ridley, *L. kempi*. The sole nesting population for this species was reduced from over 40 000 adult females in the 1950s to a few hundred females within a 15-year period, but nesting has steadily increased in the last four years, presumably in response to strong protective measures taken by Mexico and the United States (Marquez *et al.*, 1996). Because the bottleneck was brief the Kemp's ridleys appear to retain pre-exploitation levels of genetic diversity (Kichler, 1996). Although recent increases in numbers of female leatherbacks nesting in St. Croix (Boulon, Dutton & McDonald, 1996) and South Africa (Hughes, 1996) are reason for optimism, the Malaysian population has not shown signs of recovery, and other Pacific populations continue to decline at an alarming rate (Chan & Liew, 1996; Sarti *et al.*, 1996; Spotila *et al.*, 1996).

The results of this study suggest that natal homing instincts may not be as rigid in leatherbacks as in other sea turtles. Perhaps this bodes well for the conservation of this species, as it allows more flexibility to exploit new reproductive habitat and to recolonize beaches where populations have been extirpated. While a metapopulation approach (Levins, 1970; Murphy, Freas & Weiss, 1990; Gliddon & Goudet, 1994) to conservation of the chelonid species may have limited applications, since

each individual nesting region may contain unique maternal lineages that are isolated from other populations by strict natal homing (Bass *et al.*, 1996), it may be feasible to adopt this kind of approach for leatherbacks. The question remains as to whether recolonization would occur over a time frame that is relevant to current recovery plans (National Marine Fisheries Service & U.S. Fish and Wildlife Service, 1998). The increased number of leatherback nestings in Florida, U.S.A., over the last few years, from occasional rare sightings before 1985 to over 100 nests in 1994 (Conley & Hoffman, 1987; B. Schroeder, NMFS, pers. comm.) may represent such colonization. If this is the case, it will be important to identify and protect migratory corridors between nesting beaches and common forage areas (Morreale *et al.*, 1996), to facilitate colonization.

The conservation priority given to different nesting populations perhaps should not be based solely on their size. For instance, loss of populations in St. Croix and Trinidad would eliminate most of the detected mtDNA variation in the Atlantic region, although these populations represent less than 10% of the nestings in the Atlantic rookeries surveyed in this study. However, the negative ramifications of loss of mtDNA diversity are uncertain, and variation at this one locus may not reflect the level of diversity at nuclear loci involved in adaptation or individual fitness. Nevertheless, given the evidence of rapid population turnover in this species, preservation of several nesting habitats within each region might be the best line of defence against regional extinction. Our results suggest the evolutionary history of this species is one of extinction and recolonization, and perhaps their ability to colonize rapidly (relative to other sea turtles) is one reason why this species has persisted through previous population bottlenecks. The mtDNA markers identified in this study provide the first genetic tool for studying leatherbacks. More detailed studies including samples from additional rookeries and using multiple nuclear markers, such as microsatellites in combination with the mtDNA data, will undoubtedly provide further insights into the evolution and population genetics of this species.

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