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POPULATION STRUCTURE OF THE BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) AS DETERMINED BY RESTRICTION ENDONUCLEASE ANALYSIS OF MITOCHONDRIAL DNA

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ABSTRACT

Restriction fragment length polymorphisms of mitochondrial DNA (mtDNA) were used to test for population subdivision in the bottlenose dolphin (*Tursiops truncatus*). Atlantic and Pacific dolphin mtDNA samples exhibited distinctly different haplotypes (approximately 2.4% sequence divergence), indicating a lack of gene exchange. Within the Atlantic Ocean, mtDNA samples from the Gulf of Mexico and the Atlantic Coast were also found to be distinct, with a sequence divergence of approximately 0.6%. The Atlantic Coast–Gulf of Mexico dichotomy is consistent with patterns of genetic variation from other marine and coastal organisms from this region, and supports the hypothesized role of biogeographic events in promoting the divergence of these and other forms. Regional differentiation was identified along the Atlantic Coast, whereas low sequence divergences among haplotypes and consistent haplotype frequencies across populations suggested considerable gene exchange among Gulf of Mexico populations. A highly divergent haplotype found in two individuals from two localities in the Gulf of Mexico is best explained by dispersal from either a distinct offshore Gulf stock or an unsampled Atlantic Coast stock. Additional samples are required to test for the existence of a distinct offshore race and, if it exists, to identify its distribution and contribution to population structure.

Key words: bottlenose dolphin, *Tursiops truncatus*, mitochondrial DNA, restriction fragment length polymorphism.

The bottlenose dolphin is abundant in tropical and warm temperate areas worldwide and occasionally strays into colder waters as well. Historically, it has

Table 1. Locality data, tissue type, and sample sizes (n) for this study.

Locality	Tissue	n
Gulfport, MS[a]	blood	21
	liver	1
Destin, FL	blood	2
	liver	1[b]
St. Petersburg, FL	blood	5
Sarasota, FL	liver	1
Miami, FL	liver	1
Indian River, FL	blood	4
Daytona Beach, FL	liver	1
Cumberland Island, GA	liver	1[c]
Roanoke Island, NC	liver	1
Gulf of California, MX	blood	2
Pacific Ocean, N of Australia	liver, heart, kidney, muscle	11

[a] This locality includes samples from the Mississippi Management area (Gulfport, Mississippi Sound, and Mobile Bay).

[b] Total DNA was also isolated from blood of this individual and is included in the count above.

[c] Because of the poor quality of tissue, only total DNA could be isolated from this individual.

base recognizing restriction endonucleases: *Hinf*I, *Hin*PI, *Mbo*I, *Msp*I, *Rsa*I, *Sau*96I, *Scr*FI, and *Taq*I. The resulting fragments were end-labelled with α^{32}P dNTPs, separated by electrophoresis through agarose (1.0–1.5%) and polyacrylamide (4.0%) gels, and visualized by autoradiography as described in Dowling *et al.* (1990). Fragment sizes were determined by their relative electrophoretic mobilities in comparison with fragments of known size (*Hin*dIII digested bacteriophage lambda DNA and *Hae*III digested bacteriophage ϕX RF DNA) that were included in each gel. Fragments as small as 50 base pairs (bp) were detected in these analyses.

Blood samples—The small amounts of blood available from live animals (10–20 ml) made isolation of sufficient quantities of intact circular mtDNA impractical. Therefore, transfer-hybridization was used to visualize cleavage fragment patterns. Total DNAs were prepared by combining a phenol : chloroform extraction protocol (Hillis *et al.* 1990) with ultracentrifugation (Dowling *et al.* 1990). We found that numerous phenol extractions failed to produce DNA that could be efficiently digested by restriction endonucleases, but that sufficiently clean DNA was obtained when an ultracentrifugation step was added.

Buffy coats (white cells and platelets; usual volume was 0.5–1.0 ml) provided by collectors were resuspended in STE (10 mM NaCl, 100 mM EDTA, 10 mM Tris, pH 7.5) to a final volume of 4.5 ml. Proteinase K (0.05 ml from a 20 mg/ml stock solution, final concentration 100 μg/ml) and 20% SDS in water (0.5 ml, final concentration 0.5%) were added, and the mixture was incubated at 55°C for 2 h to lyse membranes. Following incubation each sample

was extracted once with an equal volume of phenol (equilibrated with Tris-HCl, pH 7.5), followed by extraction with an equal volume of chloroform: isoamyl alcohol (24:1). DNA was precipitated by addition of 1/10 volume 3 M sodium acetate (pH 8.0) and 2 volumes of cold 95% ethanol followed by incubation at −20°C for approximately 16 h. After centrifugation, precipitated DNA was resuspended in 3 ml of TE (10 mM Tris, 100 mM EDTA, pH 7.5). Ethidium bromide (EB; from a 10 mg/ml stock solution, final concentration 1 mg/ml) and cesium chloride (solid CsCl to a final density of 1.55 g/ml) were added, and samples transferred to ultracentrifuge tubes. Each tube was filled to the top with a CsCl stock solution (density of 1.55 g/ml), sealed, and mixed. Tubes were placed in a Beckman 65VTi rotor and centrifuged at 21°C for approximately 16 h at 55,000 rpm. Purified total DNA samples were collected by bottom puncture, and EB and CsCl removed by extraction with isopropyl alcohol and dialysis as described in Dowling *et al.* (1990).

Each total DNA sample was characterized with the following restriction endonucleases: *Hinf*I, *Hin*PI, *Mbo*I, *Rsa*I, *Scr*FI, and *Taq*I. Restriction fragments were separated by electrophoresis through 1.0–1.5% agarose gels. DNA was transferred from the gel to a nylon filter (GeneScreen Plus, NEN) by capillary action as described in Dowling *et al.* (1990), except that the transfer was performed under alkaline conditions (0.4 M NaOH, 0.6 M NaCl; Reed and Mann 1985). Following transfer, the filter was placed in neutralizing solution (0.5 M Tris-HCl, pH 7.0, 1.0 M NaCl) and then dried in an oven at 65°C for 20 min (Dowling et al. 1990). The dried filter was placed in 10 ml of prehybridization solution (2× SSC, 1% SDS, 0.5% nonfat dry milk) for 2–4 h at 65°C to reduce background contamination, then hybridized in 10 ml of hybridization solution (radioactive DNA—see below, 2× SSC, 1% SDS, 0.5% nonfat dry milk, 10% dextran sulfate) at 65°C for approximately 18 h. Purified bottlenose dolphin mtDNA (prepared from solid tissue as described above) was used to prepare a radioactive probe to visualize mtDNA restriction fragments. Purity of the probe was essential as even small amounts of contaminating nuclear DNA produced considerable background contamination, some in the form of discrete fragments. The radioactive probe was prepared using the random priming reaction described in Feinberg and Vogelstein (1983), except that all four α^{32}P dNTPs were used. Following hybridization, nonspecifically bound probe DNA was removed by soaking the filter in a series of wash solutions (twice, each time for five minutes, in 2× SSC, 0.1% SDS at 21°C, and twice, each time for one-half to one hour, in 0.1× SSC, 0.1% SDS at 65°C). The washed filter was blotted dry, wrapped in plastic wrap, and used to expose autoradiographic film (Kodak XAR). Fragment sizes were estimated by comparison with end-labelled fragments of known size (*Hin*dIII digested bacteriophage lambda DNA) that were included in each gel. Only restriction fragments larger than approximately 550 bp were included in the analysis.

Statistical analysis—Distinctive restriction fragment patterns for a specific enzyme were identified alphabetically (for purified mtDNA) or numerically (for total DNA). Letters/numbers were assigned by order of discovery and do not reflect similarity of patterns. The composite haplotype for each individual rep-

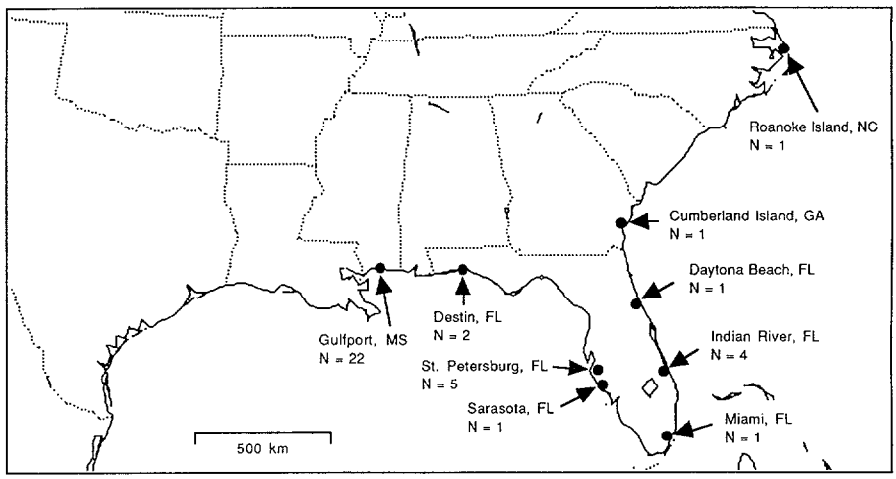

Figure 1. Sample sizes and collection localities for Atlantic bottlenose dolphins. Sample sizes include both total DNA and purified mtDNA samples analyzed (see Table 1 for actual numbers of each kind of sample).

resented fragment patterns for all enzymes, indicated by a string of eight letters for purified mtDNA and six numbers for total DNA.

Frequencies of specific composite haplotypes were used to calculate nucleon diversity ($\hat{h}$, Nei and Tajima 1981). This measure is analogous to average heterozygosity and provides an estimate of the level of mtDNA genotypic diversity within populations. The distribution of variation among populations was quantified using the program HAPLOID (Weir 1990), which uses haplotype frequency data to calculate θ (analagous to F_{st}). Estimates of the mean and standard deviation of θ were obtained by jackknifing across populations (Weir 1990).

Estimates of sequence divergence among mtDNA haplotypes were calculated from the percentage of shared fragments (Avise et al. 1979). Relationships of haplotypes were obtained from sequence divergence estimates using the unweighted pair group method (UPGMA, Sneath and Sokal 1974).

Results

Purified mtDNA—MtDNA was isolated from solid tissues of 17 individuals from the Pacific and Atlantic Oceans (11 and 6, respectively), including the Gulf of Mexico (Fig. 1, Table 1). Treatment of each sample with the eight restriction enzymes (for one example, *see* Fig. 2) produced an average of approximately 168 fragments per individual (range = 162–176). The mtDNA molecule was 16,346 ± 216 base pairs in length.

Nucleon diversity was high in both oceans, as seven of eleven Pacific and all six Atlantic individuals exhibited different mtDNA haplotypes ($\hat{h}$ = 0.89 and 1.0, respectively; see Table 2 for haplotypes). Such high levels of variability are

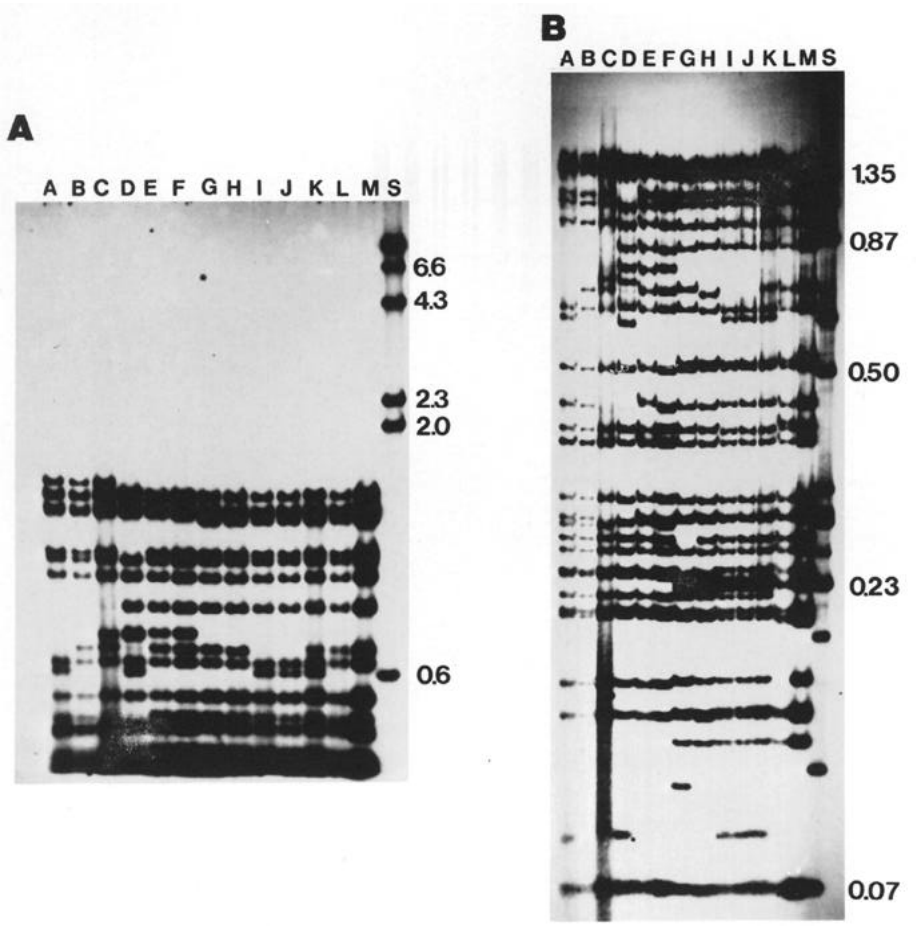

Figure 2. Autoradiogram of bottlenose dolphin mtDNAs digested with the restriction enzyme *Hinf*I. Resulting fragments were end-labelled and separated by electrophoresis through 1.5% agarose (A) and 4.0% polyacrylamide (B) slab gels. Samples are identified as follows (see Table 2 for specimen number): A— TT659; B— TT453; C— T1; D— T3; E— T4; F— T2; G— TH2; H— TAD; I— TH5; J— TH6; K— CF5; L— TH24; M— CF3. S identifies the size standard (*Hin*dIII digested bacteriophage lambda DNA on A and *Hae*III digested bacteriophage øX174RF DNA on B) with sizes of some of the fragments (in kilobases) to the right.

not surprising. Brown (1980) was able to discriminate each of 21 human mtDNAs with 11 restriction endonucleases, and Avise *et al.* (1989) reported high nucleon diversity values (range = 0.473–0.998) in a variety of animals, including marine organisms.

Estimates of sequence divergence among the 13 haplotypes ranged from 0.02 to 2.62% (Table 3). Clustering of haplotypes using these distances indicated that the Atlantic and Pacific samples form discrete groups, differing in sequence divergence by approximately 2.4% (Fig. 3). Samples from the Atlantic and

Table 2. Composite mtDNA haplotypes identified from purified and end-labelled samples. Alphabetical designation is arbitrary and does not reflect the number of fragment differences between haplotypes.

Specimen	Locality	*Hinf* I	*Hin*PI	*Mbo*I	*Msp*I	*Rsa*I	*Sau* 96I	*Scr* FI	*Taq*I
Gulf of Mexico									
TT659	Gulfport, MS	A	A	A	A	A	A	A	A
TT453	Destin, FL	B	A	A	A	A	A	A	A
T1	Sarasota, FL	C	A	A	A	A	A	B	A
Atlantic Ocean									
T2	Roanoke Is., NC	E	C	D	B	C	D	A	A
T3	Miami, FL	D	B	B	A	A	B	A	A
T4	Daytona Beach, FL	E	A	C	A	B	C	A	B
Pacific Ocean (from northern Australia)									
TAD	(12°27′S, 127°25′E)	G	D	F	C	H	F	C	C
TH2	(12°18′S, 127°27′E)	F	E	E	C	F	E	C	C
TH5	(12°22′S, 127°27′E)	H	E	G	C	G	F	C	D
TH6	(12°22′S, 127°27′E)	H	E	G	E	E	F	C	D
YY2	(12°24′S, 127°17′E)	H	E	G	C	E	F	C	D
CF3	(12°51′S, 127°00′E)	J	E	G	D	D	F	C	C
CF4	(12°30′S, 127°14′E)	J	E	G	D	D	F	C	C
CF5	(12°30′S, 127°26′E)	H	E	G	C	E	F	C	D
CF6	(12°22′S, 126°59′E)	H	E	G	C	E	F	C	D
CF9	(13°03′S, 126°12′E)	J	E	G	D	D	F	C	C
TH24	(12°27′S, 127°19′E)	I	E	G	D	D	F	C	C

Table 3. Number of fragments shared (above the diagonal) and sequence divergence (below the diagonal, multiplied by 100) among mtDNA haplotypes identified from purified and end-labelled mtDNA (see Table 2). Number of fragments for each haplotype is presented on the diagonal.

	TT659	TT453	T1	T3	T4	T2	TH2	TAD	TH5	TH6	YY2 CF5 CF6	TH24	CF3 CF4 CF9
TT659	172	172	169	161	159	160	127	126	130	130	131	125	127
TT453	0.02	173	170	159	160	161	128	127	128	128	129	126	128
T1	0.17	0.15	173	159	158	159	128	127	127	127	128	126	128
T3	0.55	0.68	0.68	173	155	158	125	126	127	127	128	124	127
T4	0.71	0.68	0.78	0.92	174	158	125	126	127	127	128	124	127
T2	0.70	0.67	0.78	0.81	0.86	176	127	127	128	128	129	126	129
TH2	2.42	2.38	2.38	2.63	2.61	2.53	166	153	157	157	158	156	159
TAD	2.39	2.35	2.35	2.53	2.44	2.42	0.58	162	156	156	157	156	159
TH5	2.25	2.41	2.48	2.45	2.50	2.48	0.49	0.44	167	164	165	155	157
TH6	2.25	2.41	2.48	2.45	2.50	2.48	0.49	0.44	0.15	167	165	155	157
CF5	2.16	2.32	2.38	2.36	2.41	2.39	0.41	0.36	0.08	0.08	166	156	158
TH24	2.48	2.44	2.44	2.62	2.61	2.52	0.44	0.34	0.52	0.52	0.44	163	162
CF3	2.37	2.33	2.33	2.51	2.42	2.34	0.31	0.31	0.44	0.44	0.36	0.08	164

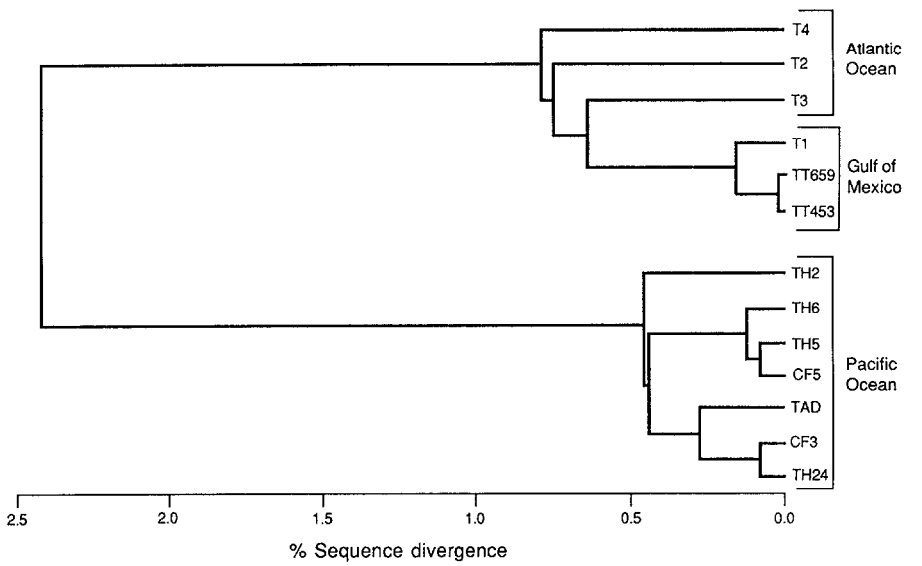

Figure 3. UPGMA phenogram summarizing relationships among mtDNA samples analyzed by end-labelling. Sample numbers are provided in Table 2.

Pacific Oceans were so distinct that no fragment patterns were shared between them.

Levels of divergence within the cluster of haplotypes from the Pacific Ocean range from 0.08 to 0.58%. Within the Atlantic Ocean cluster, the three samples from the Gulf of Mexico (Gulfport, MS; St. Petersburg and Sarasota, FL) form a discrete group (<0.2% sequence divergence) relative to the samples from the Atlantic Ocean (Fig. 3). The individual from Miami, FL, had mtDNA most similar to the Gulf of Mexico group (0.64%). Haplotypes from Roanoke Island, NC, and Daytona Beach, FL, were almost equidistant from the other four Atlantic Ocean haplotypes (0.82 and 0.86%, respectively).

Total DNA—Total DNA was isolated from blood and tissues of 35 individuals from the Gulf of Mexico, Atlantic Coast, and the Gulf of California (Fig. 1, Table 1). Digestion of each sample with six restriction endonucleases (for one example, see Fig. 4) identified eight haplotypes (Table 4), each with an average of approximately 54 fragments per individual (range = 53–56).

Results from end-labeling of purified mtDNA and transfer-hybridization of genomic DNA were compared by analysis of a sample (TT453) by both methods (Table 1). This sample provided a reference point for comparison of fragment lengths. Three of the composite haplotypes obtained from genomic DNA samples were identical to those found in samples of purified mtDNA (1 = TT453, 4 = TT659, 6 = T4), demonstrating that the results obtained by transfer-hybridization of total DNA were directly comparable to those obtained by end-labelling purified mtDNA, at least for fragments ≥550 bp.

Five mtDNA haplotypes were found among the 28 individuals sampled from

Table 4. Composite mtDNA haplotypes obtained by transfer-hybridization of bottlenose dolphin total DNA. Different fragment patterns are designated by number (1–6) for the following enzymes (in order of occurrence): *Mbo*I, *Rsa*I, *ScrF* I, *Taq*I, *Hinf* I, *Hin*PI. Numerical designation is arbitrary and does not reflect the number of fragment differences between haplotypes.

	Haplotype								
Locality	1[a] 111111	2 211111	3 112111	4[b] 111161	5 413231	6[c] 321121	7 531342	8 541152	Total
Gulf of Mexico									
Gulfport, MS	13	4	2	2	0	0	0	0	21
Destin, FL	1	0	0	0	1	0	0	0	2
St. Petersburg, FL	3	1	0	0	1	0	0	0	5
Atlantic Ocean									
Indian River, FL	0	0	0	0	0	4	0	0	4
Cumberland Is., GA	0	0	0	0	0	1	0	0	1
Pacific Ocean									
Gulf of California, MX	0	0	0	0	0	0	1	1	2
Total	17	5	2	2	2	5	1	1	35

[a] This haplotype is the same as that found in TT453 (Table 2).
[b] This haplotype is the same as that found in TT659 (Table 2).
[c] This haplotype is the same as that found in T4 (Table 2).

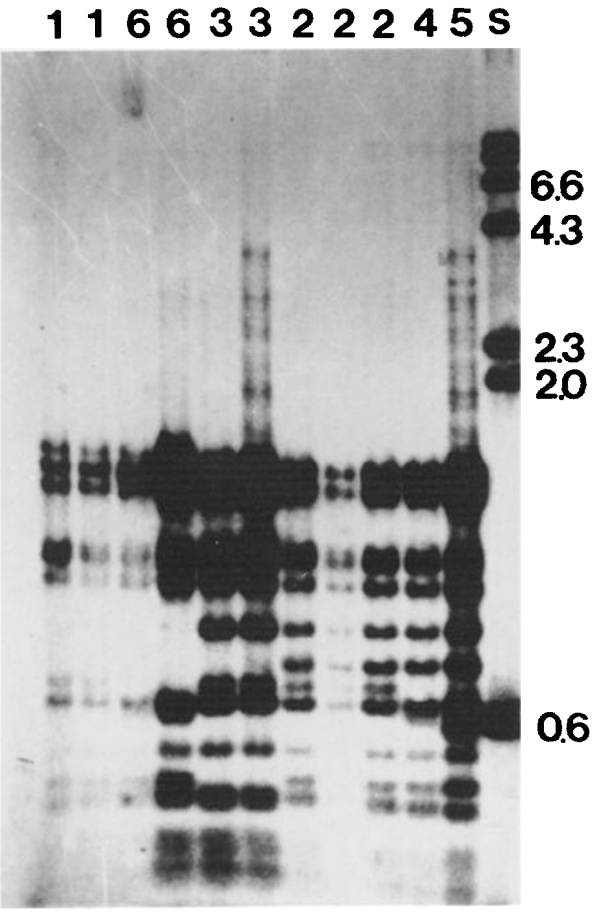

Figure 4. Autoradiogram of bottlenose dolphin mtDNAs digested with the restriction enzyme *Hinf*I. Resulting fragments were separated by electrophoresis through 1.5% agarose slab gel and visualized by hybridization with radioactively labelled bottlenose dolphin mtDNA (see Materials and Methods for details). Numbers identify *Hinf*I haplotypes (as designated in Table 4). S identifies the size standard (*Hin*dIII digested bacteriophage lambda DNA) with sizes of some of the fragments (in kilobases) to the right.

the Gulf of Mexico (Table 4). One of these was found in 61% (17/28) of the individuals. The other four were relatively rare, each occurring at a frequency of less than 20%. Despite the predominance of one haplotype, considerable nucleon diversity exists in the Gulf of Mexico ($\hat{h}$ = 0.61). Diversity values for the Gulfport and St. Petersburg populations were similar to those for the entire Gulf ($\hat{h}$ = 0.60, n = 21; $\hat{h}$ = 0.70; n = 5, respectively), while that calculated for the Destin population was higher ($\hat{h}$ = 1.00; n = 2). The population sample from the Atlantic Coast of Florida (Indian River) showed no diversity ($\hat{h}$ = 0.00; n = 4). The values for the three Florida populations (Destin, Indian River,

Table 5. Tests of population subdivision using estimates of θ. Haplotype numbers are identified in Table 4. The jackknife mean (±SD) were calculated as described by Weir (1990).

Haplotype	θ
1	−0.204
2	−0.163
3	−0.136
4	−0.136
5	0.437
Total	−0.077

Jackknifed over populations: $\theta = 0.064 \pm 0.204$.

St. Petersburg) must be regarded as provisional, because of the small sample sizes ($n \leq 5$).

To test for subdivision among Gulf of Mexico populations, the frequency of haplotypes in each population was used to calculate the F_{st} analog, θ. Four of the five haplotypes appear to be distributed equally among populations, as indicated by negative values (Table 5). The fifth haplotype (#5) appeared to be unevenly distributed among populations as indicated by a high θ value (Table 5). It was found in only two individuals (2/28 = *ca.* 7%), each from a different locality (St. Petersburg and Destin). It is noteworthy that this haplotype was not found among the large number of individuals from the Gulfport population. Overall, observed haplotypes were evenly distributed among Gulf of Mexico populations, as indicated by the distribution of θ values (0.064 ± 0.204) obtained by jackknifing across populations.

Estimates of sequence divergence among the eight haplotypes identified from total DNA samples ranged from 0.24–1.81% (Table 6). Inspection of the UPGMA results (Fig. 5) indicated that Atlantic haplotypes (Atlantic Coast and Gulf of Mexico) formed a cluster distinct from those in the Pacific (Gulf of California). However, the level of divergence between Atlantic and Pacific clusters was not as large as that observed in the previous comparison involving Atlantic and Pacific populations (Fig. 3). Within the Atlantic Ocean basin most Gulf of Mexico haplotypes form a tight cluster distinct from the Atlantic Coast samples (Indian River, FL, and Cumberland Island, GA), with the exception of haplotype 5, which is as divergent from the remaining Gulf of Mexico haplotypes (1–4) as is the Atlantic Coast haplotype (6).

Discussion

In this study, restriction fragment length polymorphisms among *Tursiops truncatus* mtDNAs were identified and used to analyze population structure. Comparison of the levels of sequence divergence between mtDNA and total DNA from the same individuals (Fig. 3 *versus* Fig. 5; T4 = 6, TT453 = 1, TT659 = 4) indicated that the use of total DNA provides higher estimates of

Table 6. Number of fragments shared (above the diagonal) and sequence divergence (below the diagonal, multiplied by 100) among mtDNA haplotypes identified from total DNA (see Table 4). Number of fragments for each haplotype is presented on the diagonal.

	1	2	3	4	5	6	7	8
1	54	52	53	53	46	48	46	46
2	0.24	53	51	51	47	49	47	47
3	0.16	0.40	54	52	46	47	45	45
4	0.16	0.40	0.32	54	45	47	47	45
5	1.27	1.01	1.27	1.46	53	46	44	45
6	1.14	0.89	1.32	1.32	1.43	56	47	47
7	1.51	1.25	1.69	1.32	1.81	1.48	56	53
8	1.51	1.25	1.69	1.69	1.62	1.48	0.46	56

divergence for the same haplotypes. This result is an artifact of the method of detection. With the analysis conditions we employed, fragments smaller than 550 bp were detectable by end-labeling, but not by transfer-hybridization. A disproportionate number of these smaller fragments are shared among haplotypes, which results in a lower estimate of divergence when they are included. Because the larger number of fragments analyzed provides a more accurate representation of the mtDNA, comparisons of end-labelled fragments give a more accurate estimate of sequence divergence.

Atlantic versus Pacific comparisons—Analysis of mtDNA restriction fragment length polymorphisms revealed considerable divergence between the Atlantic

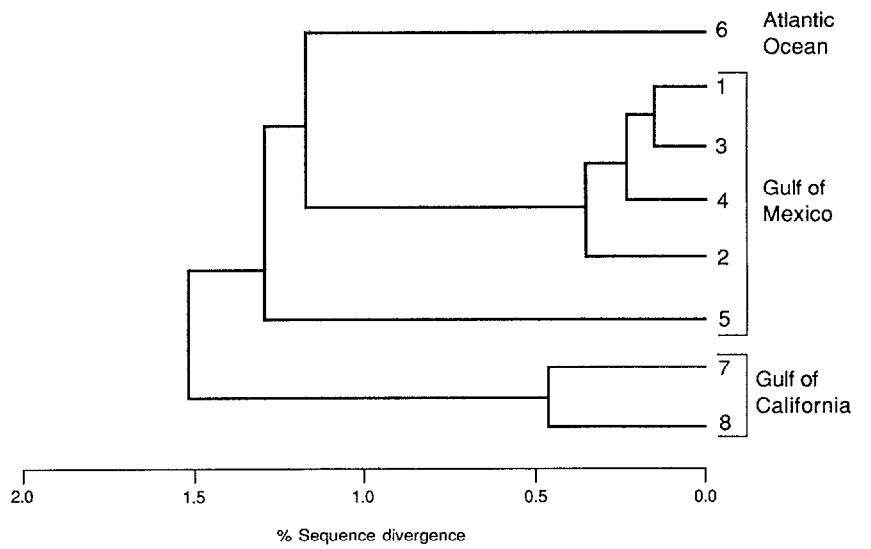

Figure 5. UPGMA phenogram summarizing relationships among mtDNA samples analyzed by transfer-hybridization. Numbers identifying haplotypes as identified in Table 4.

and Pacific population clusters. This result is similar to that found in sea turtles (Bowen and Avise 1989), but differs from studies of skipjack tuna (Graves *et al.* 1984).

For the bottlenose dolphin, high mtDNA sequence divergence between oceans (>2%) relative to within ocean divergence (<1%) suggests a historical disruption of gene flow, with limited interchange between the two oceans. A major barrier to gene flow between these oceans was produced by the emergence of the Panamanian land bridge approximately three million years ago (Marshall *et al.* 1982). However, if one applies the "standard" mtDNA clock calibration of 2% divergence per million years (Wilson *et al.* 1985) to the average divergence between Atlantic and Pacific *Tursiops,* their calculated separation occurred approximately 1.25 million years ago. Discordance between the geological and molecular estimates may be explained in three different ways: (1) The Panamanian land bridge may not have been the historical event that isolated these populations. For example, the age estimated from the mtDNA divergence corresponds to a time early in the Pleistocene, possibly implicating changes in sea level associated with glaciation as the factor leading to the isolation of these groups. (2) The date of origin for the Panamanian land bridge may be incorrect, with the emergence being much more recent than is thought. Though a formal possibility, we regard this as highly unlikely. (3) The "standard" rate of evolution, derived from primate mtDNA comparisons, may be inappropriate for *Tursiops* mtDNA. Relative rates of mtDNA evolution vary among distant (Vawter and Brown 1986) as well as among closely related taxa (DeSalle and Templeton 1988, Dowling and Brown 1989, Smouse *et al.* 1991).

Gulf of Mexico versus Atlantic Coast comparisons—Levels of divergence between mtDNA haplotypes from the Atlantic Coast and those from the Gulf of Mexico suggest considerable isolation between these regions. These data are generally consistent with genetic data obtained from a variety of coastal organisms previously studied (horseshoe crabs, Saunders *et al.* 1986; American oyster, Reeb and Avise 1990; seaside sparrow, Avise and Nelson 1989; marine toadfish, Avise *et al.* 1987*b*; stone crabs, Bert 1986, Bert and Harrison 1988; sea bass and menhaden, Bowen and Avise 1990). The commonality of patterns across these species has been used to implicate a common set of historical factors, coupled with contemporary restrictions to gene flow (Avise 1992). Reeb and Avise (1990) suggested that changes in water level during the Pleistocene may have created an isolated pocket of suitable habitat in the Gulf of Mexico, and that this isolation has been maintained by existing water current patterns.

In most of the other organisms studied, the point of differentiation between Atlantic and Gulf haplotypes occurs midway up the east coast of Florida. For the bottlenose dolphin, however, the transition point appears to occur further south, near the tip of the Florida peninsula. This shift in position may reflect the way water current patterns impose isolation. For many organisms with pelagic larval phases (like oysters), the current may form a formidable barrier to movement and the distribution of mtDNA variation will closely track overall current patterns. In the case of highly agile organisms, such as bottlenose dolphins, water currents are not likely to physically prevent dispersal. Rather, dolphins

may be tracking current-entrained food resources (Shane et al. 1986), and, in so doing, incidentally reducing gene exchange between Atlantic and Gulf populations.

Within basin comparisons—Small sample sizes prevent population genetic analysis (*i.e.*, calculation of *F*-statistics) of collections from the Atlantic Coast of the United States; however, it is possible to examine population structure by contrasts of within and among population divergences. Levels of divergence among mtDNAs from individuals collected along the Atlantic Coast (from the tip of the Florida peninsula to North Carolina) indicate considerable subdivision among certain regions (Fig. 3). Samples obtained from Florida (Indian River, Daytona Beach) and Georgia (Cumberland Island) were identical (Tables 2 and 4) suggesting relatively recent gene interchange among these locations. However, samples from this general region and two others (from Miami, FL, and Roanoke Island, NC) were distinct from each other (0.8–0.9% sequence divergence). The high level of divergence among these three sets of Atlantic Coast populations relative to that among populations within the Gulf of Mexico or the Pacific Ocean (<0.2% and <0.5%, respectively) suggests a lack of gene flow among these regions. We considered the possibility that these high divergences might be an artifact induced by the use of tissues from stranded dolphins. MtDNAs from Miami and Roanoke Island were obtained from individuals that had been stranded and died; it is therefore possible that those individuals were not residents at these localities. On the other hand, Sarasota, Cumberland Island, and Daytona Beach mtDNA samples were also isolated from stranded individuals, yet their haplotypes were very similar or identical to those found in known resident animals captured at nearby localities (St. Petersburg and Indian River). Therefore, it seems likely that the relatively high level of sequence divergence among Atlantic Coast populations reflects local variation and not the influence of random stranding of non-resident individuals.

Unlike the Atlantic Coast, analysis of mtDNA from several Gulf of Mexico populations provided only limited evidence of subdivision. Virtually all Gulf individuals possessed highly similar or identical mtDNAs (<0.2% from end-labelled samples, <0.4% from transfer-hybridization). Analysis of haplotype frequency data also suggested that there was considerable gene flow among populations, as *F*-statistics indicated that most mtDNA haplotypes are distributed evenly among populations. Larger samples from more localities are needed for a more accurate quantification of population subdivision.

The only apparent exception was haplotype 5, which was as divergent as the Atlantic Coast haplotypes from all other Gulf haplotypes. Its high level of sequence divergence from other Gulf haplotypes and its presence at widespread localities suggest that this mtDNA haplotype was likely derived by infrequent invasion of individuals from elsewhere. Haplotype 5 was found in only two females, one collected near Destin, FL, in 1966, and the other captured near St. Petersburg, FL, in 1971 (S. Ridgway, personal communication). Because females disperse less frequently than males (Wells *et al.* 1980), these individuals are not likely to be closely related (*i.e.*, mother-daughter, sisters, etc.). Therefore,

the disjunct distribution of this haplotype may be due to separate dispersal events from a distinctive source population.

Previous workers (reviewed in Leatherwood and Reeves 1982) have suggested the existence of distinct onshore and offshore populations of bottlenose dolphin in the Atlantic and Pacific Oceans. Perhaps the unusual haplotype noted above was contributed by individuals from an offshore stock. Alternatively, it may be derived from Atlantic populations not sampled in our survey. The level of divergence of this haplotype from those of the Atlantic Coast and the Gulf of Mexico (Fig. 3 and 5) is consistent with an Atlantic Coast origin. Further sampling is necessary to identify the location of origin for this distinctive haplotype, to test for the existence of a distinct offshore race, and to identify its distribution and contribution to population structure.

The lack of mtDNA divergence among Gulf populations (as indicated by low sequence divergences and consistency of haplotype frequencies across populations) appears to conflict with previous ecological and behavioral studies of dolphin populations in the Gulf of Mexico. These studies have identified stable, resident populations in which females are highly philopatric and males are more likely to disperse (Wells *et al.* 1980, Shane *et al.* 1986). Under these conditions, a rapidly evolving matrilineal marker like mtDNA is expected to identify significant differences among populations. The observed discrepancy is likely not real, as low levels of migration are sufficient to prevent population differentiation (Hartl and Clark 1989). Dolphins are long-lived and, therefore, infrequent exchange among resident groups would be sufficient to prevent differentiation but difficult to detect in typical observational studies.

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