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Journal
Genetics, 124(2)

ISSN
0016-6731

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Publication Date
1990-02-01

DOI
10.1093/genetics/124.2.397

A Genetic Discontinuity in a Continuously Distributed Species: Mitochondrial DNA in the American Oyster, *Crassostrea virginica*

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Manuscript received June 19, 1989
Accepted for publication October 10, 1989

ABSTRACT

Restriction site variation in mitochondrial DNA (mtDNA) of the American oyster (*Crassostrea virginica*) was surveyed in continuously distributed populations sampled from the Gulf of St. Lawrence, Canada, to Brownsville, Texas. mtDNA clonal diversity was high, with 82 different haplotypes revealed among 212 oysters with 13 endonucleases. The mtDNA clones grouped into two distinct genetic arrays (estimated to differ by about 2.6% in nucleotide sequence) that characterized oysters collected north *vs.* south of a region on the Atlantic mid-coast of Florida. The population genetic "break" in mtDNA contrasts with previous reports of near uniformity of nuclear (allozyme) allele frequencies throughout the range of the species, but agrees closely with the magnitude and pattern of mtDNA differentiation reported in other estuarine species in the southeastern United States. This concordance of mtDNA phylogenetic pattern across independently evolving species provides strong evidence for vicariant biogeographic processes in initiating intraspecific population structure. The post-Miocene ecological history of the region suggests that reduced precipitation levels in an enlarged Floridian peninsula may have created discontinuities in suitable estuarine habitat for oysters during glacial periods, and that today such population separations are maintained by the combined influence of ecological gradients and oceanic currents on larval dispersal. The results are consistent with the hypothesis that historical vicariant events, in conjunction with contemporary environmental influences on gene flow, can result in genetic discontinuities in continuously distributed species with high dispersal capability.

THIS study was motivated by molecular-genetic observations made previously on another coastal marine invertebrate, the horseshoe crab *Limulus polyphemus* (SAUNDERS, KESSLER and AVISE 1986). In that species, a phylogenetic "break" in maternally inherited mtDNA (sequence divergence about 2%) distinguished all individuals that were sampled north *vs.* south of a region in northeastern Florida (Figure 1). This result was surprising, because horseshoe crabs have a continuous coastal distribution, as well as a trilobite larval stage which could provide a means of gene flow between populations. SAUNDERS, KESSLER and AVISE (1986) raised two classes of hypothesis that might account for this pattern of genetic divergence in Florida: (1) deterministic factors, such as historical restrictions on gene flow in the northeast Florida area, or a steep cline in selection pressures influencing mtDNA genotype frequencies; or (2) stochastic processes, involving random extinction of maternal lines. NEIGEL and AVISE (1985) showed by computer simulation that discontinuities in a genealogy can arise in continuously distributed species along a linear habitat (such as a coastline) when gene flow is uniformly restricted across a species range.

Two kinds of distributional data on genotype frequencies should allow distinction between these possibilities. First, under the stochastic scenario, little concordance should exist among the geographic patterns of alleles at unlinked loci; but deterministic factors should have molded the histories of many genes (both nuclear and cytoplasmic) in a geographically concordant fashion. Data available for nuclear genes in *Limulus* are equivocal on this matter–limited samples from Atlantic and Gulf coasts showed only mild allozyme frequency shifts (SELANDER *et al.* 1970). Second, under the stochastic scenario, little concordance should exist among the geographic patterns of alleles among different species; but vicariant biogeographic events and gene flow restrictions should affect distributions of neutral alleles of ecologically similar species in a geographically similar fashion.

Here we test the stochastic *vs.* vicariant biogeographic hypotheses in marine invertebrates by examining mtDNA differentiation in the American oyster, *Crassostrea virginica*. This oyster was chosen for comparison to *L. polyphemus* because the two species inhabit similar estuarine environments along the Atlantic and Gulf coasts, and have been the subject of

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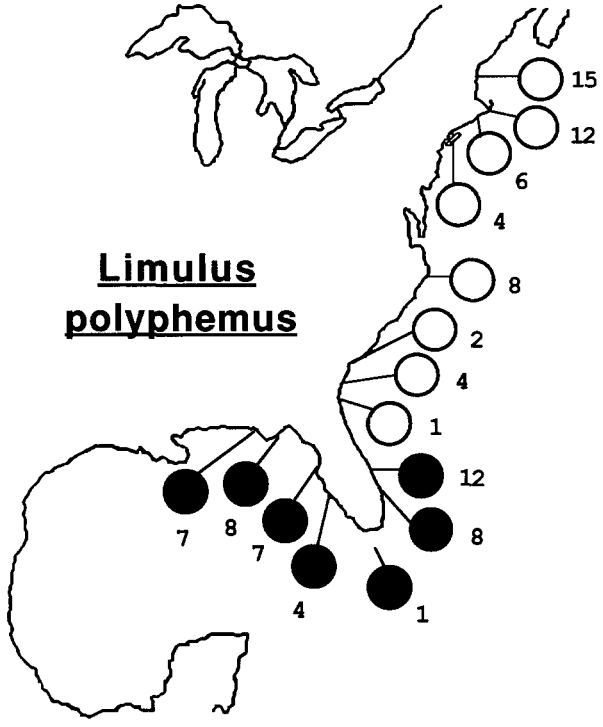

FIGURE 1.—Geographic frequencies of the two major mtDNA clonal assemblages observed in the horseshoe crab, *Limulus polyphemus* (from SAUNDERS, KESSLER and AVISE 1986). Numbers refer to sample sizes at various locales.

allozyme surveys. Further, both species have changed little in morphology over tens of millions of years (STEBBINS 1966), necessitating molecular genetic characterizations to assess geographic population structure. In this report, we will: (1) document that oysters exhibit a mtDNA phylogeographic pattern similar to that of horseshoe crabs; (2) review the geologic and climatic history affecting post-Miocene estuarine environments; and (3) integrate the environmental, phylogenetic, and life-history information into a phylogeographic hypothesis for Crassostrea that may apply generally to coastal marine species in the southeastern United States.

LIFE HISTORY BACKGROUND

Adult American oysters are sessile, eurythermal and euryhaline molluscs inhabiting estuarine environments from the Gulf of Saint Lawrence to the Yucutan Peninsula. The species has a tendency toward protandrous hermaphroditism: first-time spawners are predominantly males, while older age classes often change to females. Simultaneous production of eggs and sperm by an individual is known but rare, and hence self-fertilization is minimal (GALTSOFF 1964). Spawning takes place several times during the summer months, with both sexes synchronously releasing gametes into the water column. Sperm and eggs remain viable for up to 5 and 24 hr, respectively. Fertilization is external, and successful zygotes develop into veliger larvae which may swim and drift in the water column for 2–3 weeks. Although there is some evidence for larval retention mechanisms within estuaries (MANN 1988), veligers provide the potential for high dispersal (KORRINGA 1952).

Sediment inhibits veliger settlement, and suitable hard substrate is no doubt a limiting factor for oyster numbers in many areas. Settled oysters require salinities between about 10 and 34 parts per thousand. In bays with high salinity, populations are increasingly challenged by marine predators (*e.g.*, *Urosalpinx cinerea*), competitors (*e.g.*, *Ostrea equestris*), and disease; while at river mouths and heads of estuaries, heavy mortality can be induced by sediment loads and low salinities from heavy rainfall (GALTSOFF 1964; HOFSTETTER 1983; RAY 1987). However, freshwater flow also provides an influx of nutrients supporting the primary production of organic matter utilized by the suspension-feeding oyster. Indeed, *C. virginica* has been used as a paleoecologic indicator of brackish water habitats over the 65 million years of its recognized tenure on earth (HEDGEPATH 1957).

MATERIALS AND METHODS

A total of 232 oysters was collected between May and August, 1988, from 14 locales from Canada to Texas: Prince Edward Island, Canada ($N = 19$); Narrangansett Bay, Rhode Island ($N = 20$); Mobjack Bay, Virginia ($N = 18$); Skidaway, Georgia ($N = 11$); Sapelo, Georgia ($N = 22$); Jacksonville, Florida ($N = 12$); Cape Canaveral, Florida ($N = 11$); West Palm Beach, Florida ($N = 11$); Miami, Florida ($N = 12$); St. Petersburg, Florida ($N = 23$); Panama City, Florida ($N = 21$); Grand Isle, Louisiana ($N = 11$); Galveston, Texas ($N = 19$); and Brownsville, Texas ($N = 22$). Mantle tissue containing the gonads was dissected and utilized in CsCl-gradient isolations of closed-circular mtDNA, as described by LANSMAN *et al.* (1981) with the following modification. When sodium dodecyl sulfate was added to lyse mitochondria, we encountered a severe viscosity problem, likely due to mucins secreted by oyster connective tissue (GALTSOFF 1964). This difficulty was overcome by heating the resuspended mitochondrial pellets in a 55–60° water bath for 30–60 min. The concentration of EDTA was also doubled to 20 mM to reduce degradation by DNAses.

Purified mtDNA was digested with 18 restriction endonucleases under conditions recommended by the supplier (New England Biolabs). Fragments were radioactively end-labeled with [^{35}S]dNTP(s) (DROUIN 1980), and then electrophoresed through 1.0–1.8% agarose gels. Bands were visualized by autoradiography, and sizes were determined by comparison to a one-kilobase ladder purchased from Bethesda Research Laboratories.

Each unique mtDNA gel profile produced by a given enzyme was designated by an uppercase letter: "C" for the most common pattern in Atlantic Coast; and, when the pattern differed from that in the Atlantic, "X" for the most common profile in the Gulf of Mexico. Of the 18 enzymes employed, five (*Bam*HI, *Bst*EII, *Kpn*I, *Pst*I, and *Sst*II) produced only zero or one mtDNA cut in our assays, and with the exception of the use of *Bam*HI and B*st*EII in the mapping experiments, are not considered further. The remaining 13 endonucleases were utilized on 212 oysters, whose compos-

TABLE 1

MtDNA clones observed in American oysters

Clone No.	Genotypic description	No. of Individuals	Locales[a]
1	C C C C C C C C C C C C C	68	All Atlantic states
2	A A	1	GA
3	B	1	GA
4	B B	3	GA, FL
5	E	1	GA
6	A	1	GA
7	B	1	GA
8	B	1	GA
9	D	2	GA, FL
10	B B B	1	GA
11	D D	1	GA
12	B	1	VA
13	D A	1	VA
14	D	2	VA
15	B	3	CAN, VA
16	B	1	VA
17	A G	1	RI
18	B	1	RI
19	F	1	RI
20	F	1	RI
21	D B	1	RI
22	I	1	RI
23	D H	1	RI
24	D	1	FL
25	D B D D	1	FL
26	E	1	FL
27	D	1	GA
28	D B	1	GA
29	B B	1	GA
30	D	1	GA
31	E I B	1	CAN
32	X X X X X X X	48	All Gulf states[b]
33	X X X X X X X A	2	FL, TX
34	X X X Z X X Z	1	FL
35	X X Z X X X X	1	FL
36	R T X X X X X	1	FL
37	R X X X X X X	4	FL, TX
38	X X X X X X X B	2	FL, TX
39	X X X X X X Y	1	FL
40	W X X X X X X	1	FL
41	X X X X X W X	1	TX
42	X X X X X X Z	2	LA, TX
43	X X X X X X X B	1	TX
44	Z X X X X X Y	1	TX
45	R X X X Z X X X	1	TX
46	X Z X X W Y T X B	1	TX
47	X X X X Z X X	1	TX
48	X X X X X X X A	2	TX
49	X Z W X W X T X	1	TX
50	X Z X X X X X X	1	TX
51	X X X X X X W B	1	TX
52	X V X X X X X X E	1	TX
53	X S X X X X X X	1	TX
54	X Z W X W X T X	1	TX
55	X X X X X X V	1	TX
56	Z X X X X X X X	1	TX
57	X Z W X X Y T W B	1	TX
58	X X X X X X X E	1	TX
59	X X E X X X W X Q	1	TX
60	X X X X X X R	2	TX
61	V X X X X X X X	1	TX
62	X X F X X X X X S	1	TX
63	T X X X X X X X	1	FL
64	Y X X X X X X X	1	FL
65	X X Y X X X X X	1	FL
66	X X D X X X X X X D	1	FL
67	X Z Y X Y X X X	1	FL
68	X X Y X X X X X	1	FL
69	X X X X X X X F	1	FL
70	X Z G Y X X X X X	1	FL
71	Z Z X X X X X X B	2	FL
72	X X G X X X X X X	1	FL
73	X Z X X X X X X	1	FL
74	X Y X X X X X Q	1	FL
75	X X E X X X X X X	2	FL
76	X X D X X X X X X A	1	FL
77	X X X X X X X E	1	FL
78	Z R X X X X X X	1	FL
79	X X X X X X X A	1	LA
80	Q X X X X X X X	1	LA
81	S X X X X X X X	1	LA
82	X X X X X Y X	1	LA

Letters refer to digestion profiles produced by the 13 informative endonucleases, listed (from left to right) in the order given in MATERIALS AND METHODS. All entries in the table are "C," unless otherwise indicated.

[a] GA, Georgia; FL, Florida; CAN, Canada; VA, Virginia; RI, Rhode Island; TX, Texas; LA, Louisiana.

[b] Including samples in southeast Florida, as far north as West Palm Beach, plus one specimen from Georgia.

ite mtDNA genotypes were thus summarized by a 13-letter code. These enzymes (and the numbers of scored fragments in the "C" and "X" patterns) are: *Ava*I (3,5); *Ava*II (9,12); *Bgl*I (3,3); *Bgl*II (4,3); *Cla*I (2,2); *Eco*RI (2,4); *Hin*cII (8,7); *Hin*dIII (6,8); *Msp*I (11,10); *Nde*I (4,4); *Pvu*II (3,3); *Spe*I (3,3); and *Stu*I (4,4). In a few instances (74 of the 2756 enzyme-individual combinations, or 2.7%), we lacked information on an individual's genotype for 1–3 enzymes, in which case genetic divergence calculations were based on the scored subset of enzymes. The remaining 20 oysters yielded little mtDNA, and could be scored for only 1–3 endonucleases, which was nonetheless sufficient to identify them with respect to Atlantic *vs.* Gulf coast genotype (see below).

Estimates of nucleotide sequence divergence (p) between mtDNA clones were generated by the fragment approach of NEI and LI (1979), and the resulting distance matrix was clustered using the UPGMA algorithm (SNEATH and SOKAL 1973). mtDNA clonal diversity was estimated by formula 7 in NEI and TAJIMA (1981).

To provide a preliminary test for gross changes in intraspecific mtDNA gene order, restriction sites in Atlantic and Gulf clones were mapped for five enzymes (*Bam*HI, *Bst*EII, *Cla*I, *Pvu*II, and *Spe*I), using double-digestion procedures.

RESULTS

Individual oysters were scored on average for about 65 restriction fragments. A total of 82 distinct mtDNA genotypes was observed among the 212 specimens

TABLE 2

mtDNA clonal diversities observed in the Atlantic versus Gulf of Mexico populations of various coastal taxa in the southeastern United States

Region	American oysters	Horseshoe crabs	Seaside sparrows	Toadfish
Atlantic	0.57	0.15	0.36	0.58
Gulf	0.80	0.89	0.47	0.77
Overall	0.85	0.71	0.71	0.77

(Table 1). Considering the surveyed geographic range as a whole, the probability that two randomly chosen oysters are distinguishable in mtDNA genotype is 0.85, among the higher values reported for a species (AVISE, BOWEN and LAMB 1989). As was also true for the horseshoe crab (SAUNDERS, KESSLER and AVISE 1986) and other surveyed coastal species (see DISCUSSION), mtDNA genotypic diversity was greater in the Gulf of Mexico than in the Atlantic (Table 2).

Based on the more instructive single and double digests, the mtDNA molecule in *C. virginica* is about 17.3 kb long, typical for most metazoan animals (BROWN 1983). However, one specimen from Panama City, Florida exhibited a localized insert of about 3.0 kb, and a second specimen from Galveston, Texas, showed an addition of about 0.5 kb. These size additions were evidenced as shifts in band positions across digestion profiles of different enzymes. Similar examples of intraspecific variation in mtDNA size, which usually map to the "control" or "D-loop" region of the molecule (BROWN 1985), have been widely reported (BERMINGHAM, LAMB and AVISE 1986; HARRISON 1989; MORITZ, DOWLING and BROWN 1987).

The mtDNA genotypes are listed in Table 1 (all gel patterns are pictured in REEB 1989). One clone (CCCCCCCCCCCCC) was most common in all Atlantic Coast locales, with the exception of southeast Florida, where it was replaced by XXCXXXXXXCCCC, the most common genotype in all Gulf Coast locales. Most other variants differed from these two genotypes by single restriction sites, and were usually observed in only one or a few individuals.

The UPGMA analysis clustered the mtDNA clones into two distinct groups (Figure 2), which join in the dendrogram at a genetic divergence level of $p = 0.026$. With the exception of a single individual (from Sapelo Island, Georgia), all assayed specimens from the St. Lawrence River to Cape Canaveral, Florida clustered in one phenetic group, and all individuals from Miami, Florida, to Brownsville, Texas, clustered in the other (Figures 2 and 3). Henceforth, these will be referred to as the Atlantic and Gulf coast assemblages. Eight of the 13 endonucleases produced gel profiles diagnostic for Atlantic and Gulf assemblages (example in Figure 4). In one geographically intermediate locale, West Palm Beach, Florida, representatives of both mtDNA arrays were present in intermediate frequencies (Figure 3).

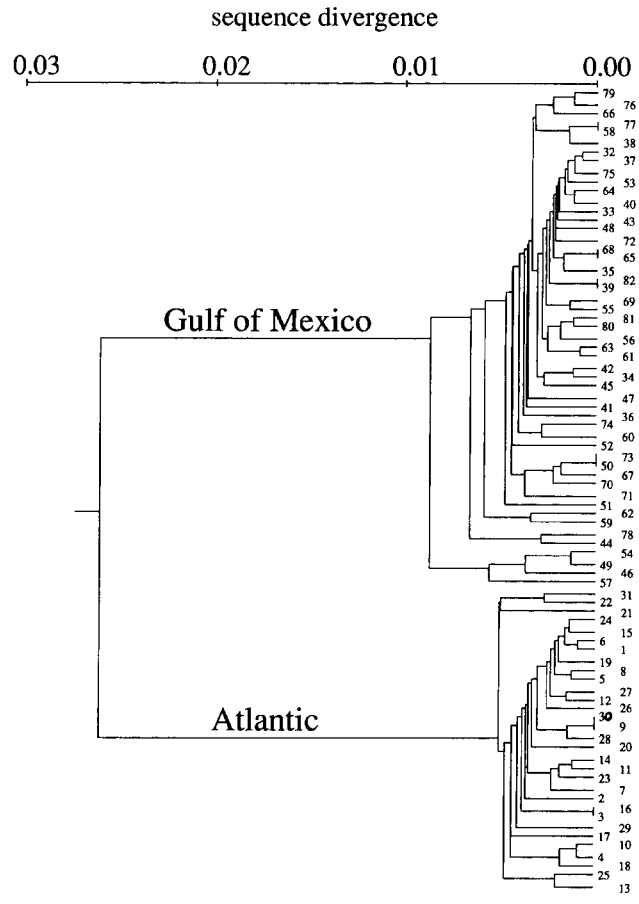

FIGURE 2.—UPGMA dendrogram for observed mtDNA genotypes in the American oyster. Numbers on the right refer to the mtDNA clones listed in Table 1.

The above estimates of nucleotide sequence divergence between oyster mtDNAs are based on the assumption that nucleotide substitutions are responsible for the differences among fragment profiles, rather than genomic addition/deletions or rearrangements. Preliminary restriction site mapping (data presented in REEB 1989) yields 10 sites distributed throughout the mtDNA genome of the American oyster that align well, suggesting that major mtDNA genome rearrangements have not occurred in this species. Furthermore, mtDNA gene order is highly conserved within major taxonomic groupings of most animals (BROWN 1985; MORITZ, DOWLING and BROWN 1987) [but see PUMO *et al.* (1989) for a possible exception]. While duplications of localized portions of mtDNA are fairly common, they can usually be identified readily and hence do not confound sequence divergence estimates (for example, the redundant fragment changes from the two oysters with mtDNA additions were not counted as independent genetic changes in the sequence divergence calculations). Nonetheless, with present data we cannot entirely eliminate the

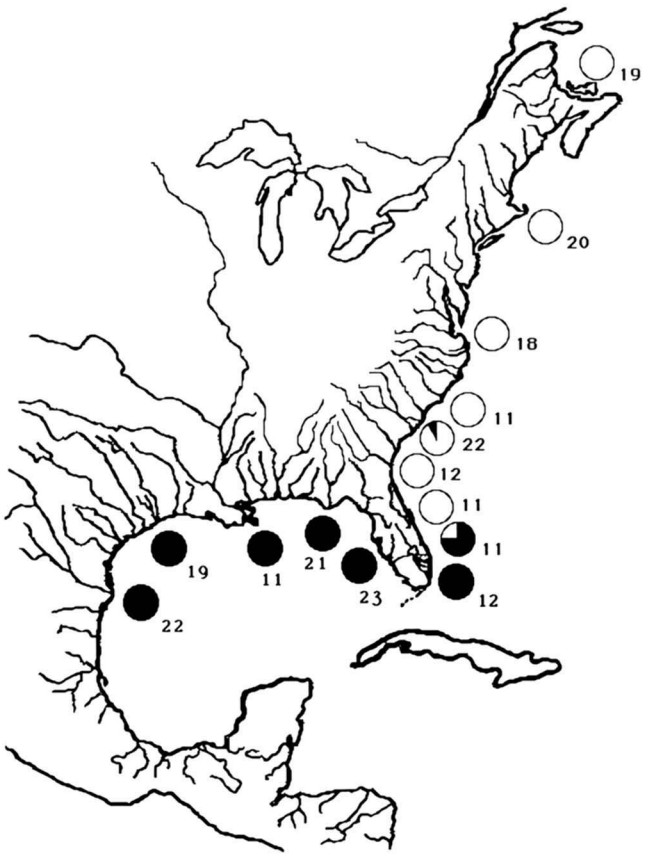

FIGURE 3.—Geographic frequencies of the two major mtDNA clonal assemblages (Atlantic *vs.* Gulf) observed in the American oysters. Numbers refer to sample sizes at various locales.

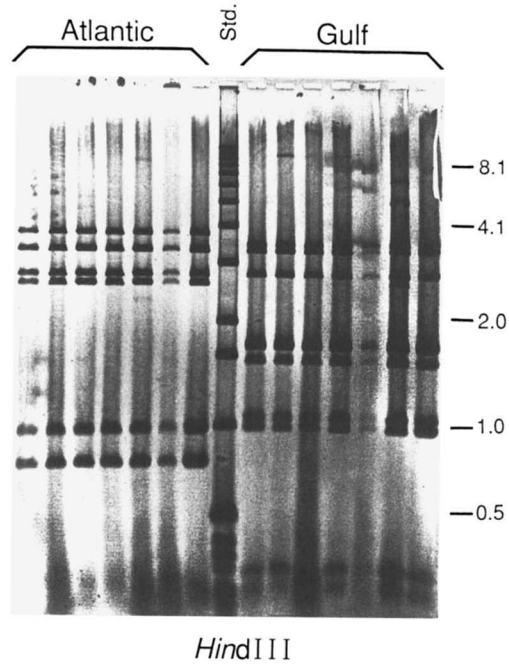

FIGURE 4.—Example of a gel profile (produced by *Hind*III) distinguishing Gulf from Atlantic assemblages of oysters. The middle lane is a molecular standard, with selected fragment sizes indicated (in kb).

possibility of smaller, localized changes in mtDNA gene order.

Estimates of mean sequence divergence between individuals within the Atlantic and Gulf assemblages of oysters were $p = 0.0014$ and $p = 0.0025$, respectively. Since there is little evidence for geographic structure of mtDNA genotypes within either area (Table 1, Figure 2), each region can provisionally be treated as a single population within which gene flow historically has been high. Using the approach of AVISE, BALL and ARNOLD (1988), such sequence divergence values can be used to estimate the evolutionary effective female population size: $N_{f(e)} = (0.5 \times 10^8)(p)$. This equation assumes a generation length of 1 year, and a "conventional" rate of mtDNA sequence divergence of about 2% per million years (BROWN, GEORGE and WILSON 1979). Under these assumptions, we estimate the $N_{f(e)}$ values for Gulf and Atlantic oysters to be 125,000 and 70,000, respectively. These values are vastly lower than current-day population sizes of American oysters. AVISE, BALL and ARNOLD (1988) have suggested that mtDNA lineages in many species have been channeled through far fewer ancestors than extant population sizes might otherwise suggest. Whether such outcomes are due to historical demographic fluctuations, or to selection favoring particular mtDNA genotypes, cannot be decided by this approach alone.

DISCUSSION

Concordance of mtDNA divergence in oysters and horseshoe crabs: The most dramatic result of this survey is the striking concordance in the pattern of mtDNA divergence in the American oyster to that previously reported for the horseshoe crab (Figures 1 and 3). Both of these continuously distributed, coastal species exhibit a major mtDNA genetic "break" distinguishing all sampled populations north versus south of the mid-Atlantic coast of Florida. Numerous restriction site changes are involved, and mean nucleotide sequence divergence between the regions is about 2.6% and 2.0% in the respective species. Both species also show a somewhat higher mtDNA clonal diversity in the Gulf of Mexico (Table 2).

These geographic patterns of divergence are also similar to genetic configurations observed in several other coastal-restricted marine or salt-marsh species, including marine toadfish (*Opsanus tau* and *Opsanus beta*) (AVISE, REEB and SAUNDERS 1987), the diamondback terrapin (*Malaclemys terrapin*) (T. LAMB and J. C. AVISE, unpublished observations), and the seaside sparrow (*Ammodramus maritimus*) (AVISE and NELSON 1989). Each of these taxa also displays a major genetic distinction between most Atlantic and Gulf of Mexico populations, with the dividing line usually appearing along the mid-Atlantic coast of Florida. The Atlantic

mid-coast of Florida is also a long-recognized transitional zone between warm-temperate and tropical faunal assemblages, with many marine species exhibiting northern or southern distributional limits in this area (BRIGGS 1958, 1974; DEEVEY 1950). AVISE *et al.* (1987) have speculated that, when phylogenetic discontinuities occur within widely distributed species, they may tend to be concordant with the boundaries between traditionally recognized zoogeographic provinces.

According to the "conventional" calibration for rate of mtDNA evolution (BROWN, GEORGE and WILSON 1979; SHIELDS and WILSON 1987; VAWTER and BROWN 1986), the two most common mtDNA gene lineages in Atlantic *vs.* Gulf coast oysters trace to a common ancestral genotype about 1.3 million years ago (mya). Since gene lineage separations can predate population separations (NEIGEL and AVISE 1986; TAJIMA 1983), a correction for within-population variability was also applied (NEI and LI, 1979; WILSON *et al.* 1985), yielding an estimated time of separation of the two oyster populations of about 1.2 mya.

Phylogeographic history of the American oyster: The geographic concordance in patterns of mtDNA divergence between the American oyster and other coastal species strongly argues for a common set of historical factors initiating population genetic divergence, as well as contemporary restrictions to gene flow in eastern Florida. Here we review (from a more detailed summary in REEB 1989) the post-Miocene history of estuarine habitats in the southeast United States, and propose how past and present environmental conditions, coupled with the oyster's life history, may explain the mtDNA phylogeographic pattern in this species.

C. virginica is critically dependent upon the intermediate-salinity, high nutrient conditions associated with freshwater outflow (GALTSOFF 1964). Such environments in south Florida and Yucutan are limited, as evidenced by a shared carbonate (coral) depositional regime (Figure 5) correlated with the aridity of the surrounding land (SELLWOOD 1986) from which there is little freshwater or siltational input lethal to coral growth (MANN 1982). That such arid conditions also existed in these two areas in the Pleistocene is indicated by deposits of fossil benthic Foraminifera associated with hypersaline conditions (POAG 1981).

During the Pliocene, sea level was higher and climates warmer than today (STANLEY and CAMPBELL 1981). With the rise of the Isthmus of Panama about 3.5–3.0 mya (STEHLI and WEBB 1985), westerly oceanic currents that formerly connected the Atlantic and Pacific were deflected, strengthening and warming the Gulf Stream (BERGGREN and HOLLISTER 1977). Shortly after this time, climatic cooling caused glacial ice sheets to advance (SHACKLETON and OPDYKE 1978). An early mass extinction of molluscs at the Plio-Pleistocene boundary was likely the result of thermal changes in shallow water masses (STANLEY and CAMPBELL 1981; STANLEY 1986). Pulses of mollusc extinction continued between 3 and 1 mya, and cumulatively eliminated 65% of the Pliocene bivalve species in the western Atlantic. On the Atlantic coast, ice masses reached as far south as Long Island (BRAATZ and AUBREY 1987), and resulted in a southward retreat of temperate species.

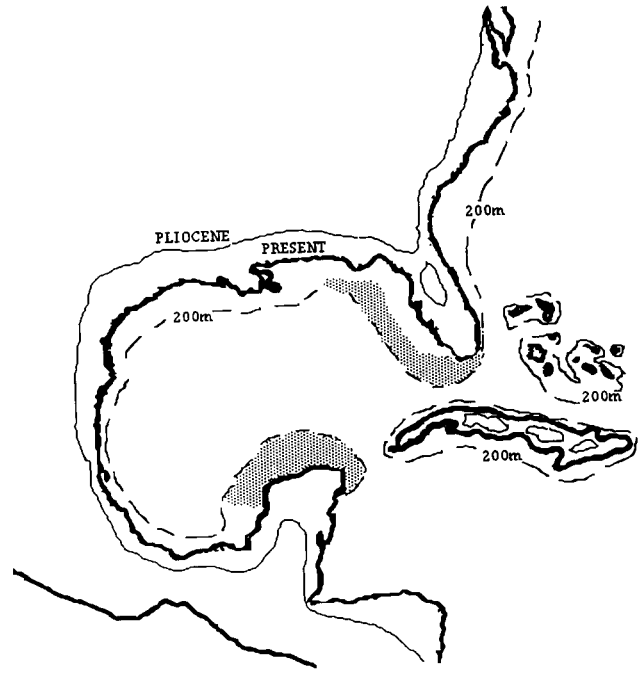

FIGURE 5.—Past environmental conditions likely to have influenced populations of the American oyster (after PETUCH 1982). The Pliocene high sea level stand is indicated (STANLEY 1986), as is the 200 meter depth contour (during the Pleistocene glaciations, sea levels may have been as much as 150 meters below present–POAG 1981). Stippled areas indicate continental coastal regions of extensive carbonate deposition (and also correspond closely to areas of deposits of Pleistocene assemblages of foraminifera indicative of historical hypersaline conditions).

Associated with the glacial advances and retreats were changes in sea level. Although poorly understood, sea levels during the Pliocene were generally much higher than they are today (Figure 5), greatly truncating the Florida peninsula (PETUCH 1982). During some Pleistocene interglacials, sea levels were also higher than at present (RICHARDS 1936, 1938), although they never reached the high stands of the Pliocene. At such times, the Florida peninsula was likely less of an obstacle to gene flow between temperate-adapted populations inhabiting the south-Atlantic and Gulf coasts (AVISE 1989; BERT 1986; BERT and HARRISON 1988; STANLEY 1986). Conversely, during glacial maxima, sea levels dropped 100 to 150 meters (BLOOM 1971; POAG 1981), exposing tremendous expanses of the Florida and Yucutan peninsulas (Figure 5), and likely creating an isolated marine

FIGURE 6.—Typical marine surface currents during the late summer (after BUMPUS 1973 and SVERDRUP, JOHNSON and FLEMING 1942).

pocket in the Gulf of Mexico (PETUCH 1982). Evidence from fossil pollen suggests that during some glacial periods, the climate in the southeastern United States was more arid than today (WATTS 1975, 1980), while that in the western Gulf of Mexico region remained more mesic (MORTON and PRICE 1987; PETERSON *et al.* 1979). According to KENNETT (1982), during the last one million years some 90% of the time has been spent in the basic glacial climatic mode. The coastline around the massive, arid Florida peninsula likely constituted unfavorable habitat for oysters, and provided a formidable barrier to gene flow between formerly connected populations along the Atlantic and Gulf coasts.

At the present time, swift currents of the Gulf Stream hug the coast of southeast Florida as they are channeled through the Florida Straits (Figure 6). The predominant flow of water is northward to the mid-coast of Florida, where the Gulf Stream moves offshore (ATKINSON *et al.* 1983). Thus gametes or larvae of Gulf coast oysters may occasionally be swept up the east Florida coast, accounting for the observed presence of "Gulf" mtDNA genotypes at West Palm Beach (Figure 3). To the north of Cape Canaveral, nearshore water currents are occasionally southward from Georgia (Figure 6), particularly during the autumn months when surface currents are pushed by strong northeast winds (BUMPUS 1973; WEBER and BLANTON 1980). These currents may carry late-spawned oyster larvae southward toward central Florida.

In summary, we propose that the present pattern of marine currents (perhaps in conjunction with selection pressures associated with water-mass differences) maintains a genetic distinction between Atlantic and Gulf oyster populations that was initiated by environmental changes in the late Pliocene and Pleistocene resulting in the vicariant population separation of *C. virginica* around Florida. Thus the observed pattern of mtDNA differentiation in the American oyster can reasonably be accounted for by a specifiable combination of historical and contemporary environmental factors and their interaction with the life history features and ecological requirements of the species.

A contrasting picture from allozymes: BUROKER (1983) examined electrophoretic variation in proteins encoded by nuclear genes in American oysters sampled from Massachusetts to Texas. With the exception of one aberrant population in extreme south Texas, all populations exhibited high overall genetic similarity (NEI's I = 0.962–0.997). Furthermore, allozyme frequencies at polymorphic loci exhibited little interlocality variation (for 51 electromorphs, mean F_{st} = 0.039). Results were attributed to high gene flow due to "the rather long planktonic stage of larval development, since this species has the ability to disperse zygotes over great distances when facilitated by tidal cycles and oceanic currents" (BUROKER 1983). B. WEIR, W. ANDERSON and B. SCHAAL (personal communication) found a similar pattern of little allelic differentiation among American oyster populations at five polymorphic allozyme loci, although they did note a significant shift in the frequencies of GOT2 electromorphs along the mid-coast of Florida. A comparable shift in LAP1 electromorph frequencies was also observed at the Mississippi River. Nonetheless, the overall picture from both allozyme surveys is an absence of dramatic genetic divergence in the American oyster throughout its range.

In an island model of equilibrium population structure, the expectation is for little divergence in allele frequencies when $N_e m \gg 1$, where m is the migration rate among subpopulations of effective size N_e (SLATKIN 1985). From the mean F_{st} values for allozyme loci observed by BUROKER (1983), we estimate $N_e m$ in oysters as follows (SLATKIN 1985): $N_e m = (1/F_{st} - 1)/4 = 6.2$. This value is consistent with BUROKER's (1983) conclusion of high gene flow. In contrast, the mtDNA data indicate that there is almost no mitochondrial exchange between the Atlantic and Gulf coast assemblages of oysters.

As briefly summarized below, two general classes of hypothesis might account for this difference between the allozyme and mtDNA results:

1) *The discrepancy between the mitochondrial and nuclear genomes is artifactual rather than real due to*:

A) *Hidden nuclear variation.* Many electromorphs are known to harbor "hidden" variation that can be uncovered by varying the assay conditions (*e.g.*, COYNE 1976). According to this hypothesis, such studies applied to oysters would reveal additional protein variants whose frequencies would clearly distinguish Atlantic and Gulf populations.

B) *Balancing selection at allozyme loci.* Perhaps some form of balancing selection operates to maintain similarities in electromorph frequencies in Atlantic and Gulf oysters despite strong barriers to gene flow. For example, heterogeneity in local environmental conditions has been shown to balance particular allozyme polymorphisms in other mollusc species (KOEHN, ZERA and HALL 1983; HILBISH and KOEHN 1985). In addition, MITTON and GRANT (1984) have argued that associations between growth rate and multilocus electrophoretic heterozygosity, reported in several species including oysters (KOEHN and SHUMWAY 1982; SINGH and ZOUROS 1978; ZOUROS, SINGH and MILES 1980), is due to heterozygote advantage involving loci encoding the assayed allozymes.

C) *Low rates of allozyme evolution.* Perhaps the rates of evolution (in terms of detectable amino acid replacements) are too slow at allozyme loci to have permitted detection of the Atlantic-Gulf genetic distinction. Under this hypothesis, nuclear divergence would be present in more rapidly evolving nuclear regions, such as at pseudogenes or silent positions in protein-coding loci.

2) *The discrepancy between the mitochondrial and nuclear genomes is real, due to*:

A) *Differences in dispersal of male and female gametes.* Presumably, mitochondria are inherited maternally in oysters, as they are in other higher animals (AVISE and VRIJENHOEK 1987; GYLLENSTEN, WHARTON and WILSON 1985). In principle, the lower population differentiation registered by allozymes could result from a higher level of sperm-mediated gene flow between Gulf and Atlantic regions than is true for eggs. (For example, the smaller oysters in southern Florida might reproduce disproportionately as males, so that there might be a greater movement of nuclear than mitochondrial genes from the Gulf into the south Atlantic.)

B) *Environmental selection on mtDNA.* Perhaps the mtDNA haplotype frequencies have responded to different selection pressures between the Atlantic and Gulf. The genetic break along the Atlantic mid-coast of Florida generally coincides, for example, with water temperature differences to which mtDNA genotypes might be differentially adapted. However, the environmental selection hypothesis cannot account readily for the similar placement of mtDNA breaks among a number of species, ranging from salt-marsh birds and terrapins to oysters.

C) *Differences in historical lineage sorting.* In a dioecious population with a 1:1 sex ratio, and with all else equal (such as the mean and variance in progeny survival between the sexes), an autosomal nuclear gene is expected to have a fourfold higher evolutionary effective population size (N_e) than does uniparentally inherited mtDNA (BALL, NEIGEL and AVISE 1990; NEI 1987). (In a monoecious population, the difference in effective population size between a nuclear and a cytoplasmic locus is reduced to twofold for simultaneous hermaphrodites) (BIRKY, MARUYAMA and FUERST 1983). Under a neutral model, the number of generations required for haplotype lineages to sort to a status of reciprocal monophyly in two isolated populations descended from a polymorphic ancestor population is about 2–4 times the relevant effective population size (NEIGEL and AVISE 1985). Thus on average, such lineage sorting takes longer for a nuclear gene than for mtDNA. Perhaps the time of population separation in oysters has been sufficient to allow the mtDNA lineages to have sorted to reciprocal monophyly, but insufficient for a comparable sorting of nuclear haplotypes. However, the lineage sorting model does not predict that the retained allozyme alleles should occur in similar *frequencies* in the Atlantic and Gulf populations.

D) *Interaction of factors.* Because the effective population size for mtDNA ($N_{f(e)}$) is smaller than that for autosomal nuclear genes (N_e), genetic drift should be more effective in changing mtDNA haplotype frequencies, all else being equal. Thus for isolated populations, changes in mtDNA frequency due to genetic drift might be more rapid than for nuclear haplotypes; or similarly, the intensity of balancing selection required to homogenize haplotype frequencies would be greater for mtDNA than for nuclear genes.

Although we cannot yet reach firm conclusions about the reason(s) for the contrasting geographic patterns exhibited by allozymes and mtDNA in the American oysters, several testable hypotheses exist. The growing study of geographic concordancies and discordancies among gene genealogies (AVISE 1989) is fertile ground for the further integration of ideas from molecular and theoretical population biology.

G. BOOTH, B. BOWEN, M. CHATRY, W. JUDSON, S. KARL, G. NAKAMURA, R. WALKER and G. ZAMURA, JR., kindly provided oyster specimens. W. FULKS provided excellent assistance with the laboratory work, and M. BALL and T. YOUNG assisted with the computer analyses. We thank WYATT ANDERSON for comments on the manuscript, and ED CHIN for advice and encouragement. Work was supported by National Science Foundation grants BSR-8603775 and BSR-8805360.

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Communicating editor: J. R. POWELL