GENETIC VARIATION AND GEOGRAPHIC DIFFERENTIATION IN MITOCHONDRIAL DNA OF THE HORSESHOE CRAB, *LIMULUS POLYPHEMUS*

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ABSTRACT

Restriction site variation in mitochondrial DNA (mtDNA) of the horseshoe crab (*Limulus polyphemus*) was surveyed in populations ranging from New Hampshire to the Gulf Coast of Florida. MtDNA clonal diversity was moderately high, particularly in southern samples, and a major genetic "break" (nucleotide sequence divergence approximately 2%) distinguished all sampled individuals which were north *vs.* south of a region in northeastern Florida. The area of genotypic divergence in Limulus corresponds to a long-recognized zoogeographic boundary between warm-temperate and tropical marine faunas, and it suggests that selection pressures and/or gene flow barriers associated with water mass differences may also influence the evolution of species widely distributed across such transition zones. On the other hand, a comparison of the mtDNA divergence patterns in Limulus with computer models involving stochastic lineage extinction in species with limited gene flow demonstrates that deterministic explanations need not *necessarily* be invoked to account for the observations. Experiments to distinguish stochastic from deterministic possibilities are suggested. Overall, the pattern and magnitude of mtDNA differentiation in horseshoe crabs is very similar to that typically reported for freshwater and terrestrial species assayed over a comparable geographic range. Results demonstrate for the first time that, geographically, at least some continuously distributed marine organisms can show considerable mtDNA genetic differentiation.

CONSPECIFIC populations of terrestrial and freshwater vertebrates typically exhibit considerable geographic differentiation in mitochondrial DNA (mtDNA) (reviews in AVISE and LANSMAN 1983; AVISE 1985). A common result, observed in mammals (AVISE *et al.* 1979; LANSMAN *et al.* 1983), reptiles and amphibians (WRIGHT, SPOLSKY and BROWN 1983; SPOLSKY and UZZELL 1984) and freshwater fishes (AVISE *et al.* 1984; E. BERMINGHAM and J. C. AVISE, unpublished results), is of high mtDNA clonal diversity within species, and major genetic "breaks" distinguishing some sets of populations. The geographic patterning of restriction site polymorphism argues that, for many species, dispersal and gene flow have not overridden historical influences on population subdivision presumably revealed in mtDNA phylogeny reconstruction. Well-documented exceptions to these rules involve humans and house mice (a

commensal of man), in which lack of substantive differentiation of mtDNA among worldwide collections has been hypothesized to reflect relatively recent population and range expansions (BROWN 1980; FERRIS *et al.* 1983).

Few such data on mtDNA are available for marine species. A survey of 80 restriction sites in mtDNA of the American eel, *Anguilla rostrata*, showed no genetic divergence among samples from a 4000-km stretch of North American coastline from Maine to Louisiana (J. C. AVISE *et al.*, unpublished results). This result is most likely attributable to the peculiar life history of these catadromous fishes—one that is thought to involve a single spawning population in the western mid-Atlantic Ocean—and to the subsequent widespread dispersal of larvae by ocean currents (WILLIAMS and KOEHN 1984). GRAVES, FERRIS and DIZON (1984) report no consistent differences in mtDNA genotype (about 40 assayed restriction sites) between samples of skipjack tuna, *Katsuwonus pelamis*, collected from Hawaii, Puerto Rico and Brazil. Skipjack tuna lack discrete spawning areas, and their pelagic larvae are found circumtropically. GRAVES, FERRIS and DIZON (1984) conclude that interoceanic gene flow (perhaps around the Cape of Good Hope) has been sufficient to prevent genetic differentiation of Atlantic and Pacific samples. Taken together, the above studies raise the testable hypotheses that (1) dispersal capability may be a major determinant of degree of population genetic differentiation and (2) species inhabiting the potentially more continuous marine realm may *generally* exhibit less geographic differentiation than appears typical for terrestrial and freshwater faunas.

Here, mtDNA differentiation is surveyed among geographic populations of a coastal marine species, the horseshoe crab *Limulus polyphemus*. The primary purpose is to begin an empirical appraisal of the possible consequences of life history and habitat on the magnitude and pattern of genetic differentiation in marine species. A major and unanticipated genetic "break" distinguishing northern from southern populations of the continuously distributed horseshoe crab will be documented, and alternative explanations for this result will be examined.

Throughout its range from New Hampshire to the Yucatán peninsula, *L. polyphemus* is primarily an estuarine species with "anadromous tendencies" (SHUSTER 1979). Spawning takes place from May (south) to July (north) in the intertidal zone of sandy beaches, where eggs are deposited at a depth of about 15 cm (SEKIGUCHI and NAKAMURA 1979). At about 15 days postfertilization, eggs hatch into trilobite larvae, and young crabs inhabit intertidal flats. Instars go through a series of molts into adults which are largely benthic and tend to inhabit deeper continental shelf waters until the spawning season.

Potential for long-distance dispersal of the slow-moving adults is limited. Mark-recapture experiments by several workers (BAPTISTE, SMITH and ROPES 1957; RUDLOE and RUDLOE 1981; SOKOLOFF 1978) indicate mean recovery distances of only a few (*e.g.*, 3–4) miles from the point of release and also a strong tendency for adults to remain associated with a particular harbor or estuary. Much less is known about larval dispersal. MUNSON (1899) and SOKOLOFF (1978) note that newly hatched larvae usually remain beneath the sand

TABLE 1

Composite mtDNA genotypes observed in *Limulus polyphemus*

Type	Designation[a]	Collection locale	No. of individuals[b]
1	CCCCCCCCCCC	All locales from Brunswick, GA to Dover Point, NH	48
2	CCCCCCCCCCD	Pleasant Bay, MA	1
		Long Island, NY	1
		Beaufort, NC	1
3	CCCCDCCCCCC	Sapelo Island, GA	1
4	BXCBCBXCBBC	Cape Canaveral, FL	12
		Stuart, FL	8
		Panama City, FL	1
5	BXCBCAXCBBC	Panacea, FL	2
		Panama City, FL	5
6	BXCBABXCAAC	Panama City, FL	1
7	BYCBBBXCBBB	Panacea, FL	2
		Tampa, FL	2
		Ft. Myers, FL	3
8	BXCBCBXCBBB	Panacea, FL	1
9	BYCBCBXCBBB	Panacea, FL	2
		Tampa, FL	5
		Ft. Myers, FL	1
		Islamorada Key, FL	1
10	BXCBABXCBBD	Panacea, FL	1

[a] Letter designations (from left to right) refer to digestion profiles (as diagrammed in Figure 1) for the following endonucleases: (1) *Bam*HI, (2) *Bcl*I, (3) *Bgl*II, (4) *Bst*EII, (5) *Eco*RI, (6) *Hinc*II, (7) *Hind*III, (8) *Msp*I, (9) *Nde*I, (10) *Sac*I, and (11) *Xba*I. *Pvu*II is not included in this table; the variant pattern B (Figure 1) was observed in a single individual (from Cape Canaveral, Florida).

[b] Due to exhaustion of available mtDNA, a few individuals were not scored for some enzymes. These amounted to less than 1% of the possible 1089 individual × enzyme combinations.

surface, perhaps thereby gaining protection from predators. However, RUDLOE (1979) views the trilobite larvae as "a stage specialized for dispersal with an initial 'swimming frenzy' and positive phototaxis that facilitates release from the nest" under appropriate high tides. SHUSTER (1979) reports "great swimming activity" of newly hatched larvae maintained in laboratory jars.

Many marine organisms, including a variety of fishes and invertebrates, are known to exhibit extensive long-distance movement as adults, pelagic larvae or both (*e.g.*, BURTON 1983; THRESHER 1984). Notwithstanding the possibility of occasional long-distance larval movement, along the continuum of dispersal capabilities exhibited by marine species, Limulus is probably toward the lower end. Thus, if some marine species will indeed prove to exhibit geographic patterns of mtDNA population structure comparable to that known for many freshwater and terrestrial species, Limulus is a likely and reasonable candidate for such a discovery.

MATERIALS AND METHODS

Horseshoe crabs were collected from 15 localities ranging from Panama City on the Gulf Coast of Florida to Dover Point, New Hamsphire (Table 1; Figure 2), and were

shipped live to our laboratory in Athens. MtDNA was purified in closed-circular form from fresh abdominal muscle, using procedures of CsCl-ethidium bromide gradient centrifugation (LANSMAN *et al.* 1981). Digestions were accomplished by 12 informative restriction endonucleases according to conditions recommended by the vendor, New England Biolabs. In addition, the following endonucleases were used to assay representative individuals: *Ava*I, *Bgl*I, *Cla*I, *Kpn*I and *Pst*I. These latter five enzymes were uninformative in the sense that they produced either zero or one cut in the mtDNA of assayed specimens. They will not be considered further. Digestion fragments were "end-labeled" with appropriate α-^{32}P-labeled nucleotide(s), electrophoresed through 1% agarose gels and revealed by autoradiography (BROWN 1980). Molecular weight markers were provided by a 1-kb ladder standard available from Bethesda Research Laboratories.

Estimates of nucleotide sequence divergence were calculated by both the "fragment" and "site" approaches of NEI and LI (1979). MtDNA genotypes were interconnected in a phylogenetic network by a parsimony approach of AVISE *et al.* (1979).

RESULTS

Observed mtDNA digestion profiles produced by the 12 informative restriction endonucleases are shown diagrammatically in Figure 1. The following conventions have been adopted in labeling these multifragment, single-enzyme digestion patterns. The common profile observed in Limulus from the northern part of its range (see beyond) is labeled "C," and alternative profiles apparently differing from it by a single restriction site change are labeled "A," "B" or "D." Digestion profiles not related to "C" by a single restriction site change are labeled "X," and patterns one mutation step from X (observed only for *Bcl*I) are called "Y."

The criteria for single- *vs.* multiple-step mutations from a given pattern involve examination of molecular weights of observed fragments. For example, *Bst*EII pattern "B" (Figure 1) exhibits an approximately 8.9 kb fragment that is missing from *Bst*EII "C," where it is replaced by fragments of sizes 6.9 and 2.0 kb. Apparently, in evolution, an ancestral pattern *Bst*EII "C" lost a restriction site (probably due to a single nucleotide substitution) to become *Bst*EII "B." Alternatively, pattern "B" could have been the ancestral form giving rise to "C" by a restriction site gain. The single-enzyme parsimony networks are shown below their respective digestion profiles in Figure 1, where the arrows are meant to indicate direction of restriction site loss, and not necessarily direction of evolution.

[*Note:* since the intent has been to examine the population variation of mtDNA, rather than its detailed molecular characteristics, digestions were not exhaustively run through a range of gel concentrations. Thus, fragment size estimates are, in most cases, rather crude. On 1% gels, sizes of larger fragments (*i.e.*, >7 kb) are only roughly approximated, and any fragments less than $\approx$500 base pairs were not scored at all. Nonetheless, scored fragments in most mtDNA digestion profiles totalled about 14.5–16.0 kb. We suspect that mtDNA genome size in Limulus is somewhere near the low end of the range reported for higher animals (BROWN 1983).]

Geographic distributions of mtDNA genotypes revealed by nine endonucleases are shown in Figure 2. For example, *Bam*HI "C" was observed in all

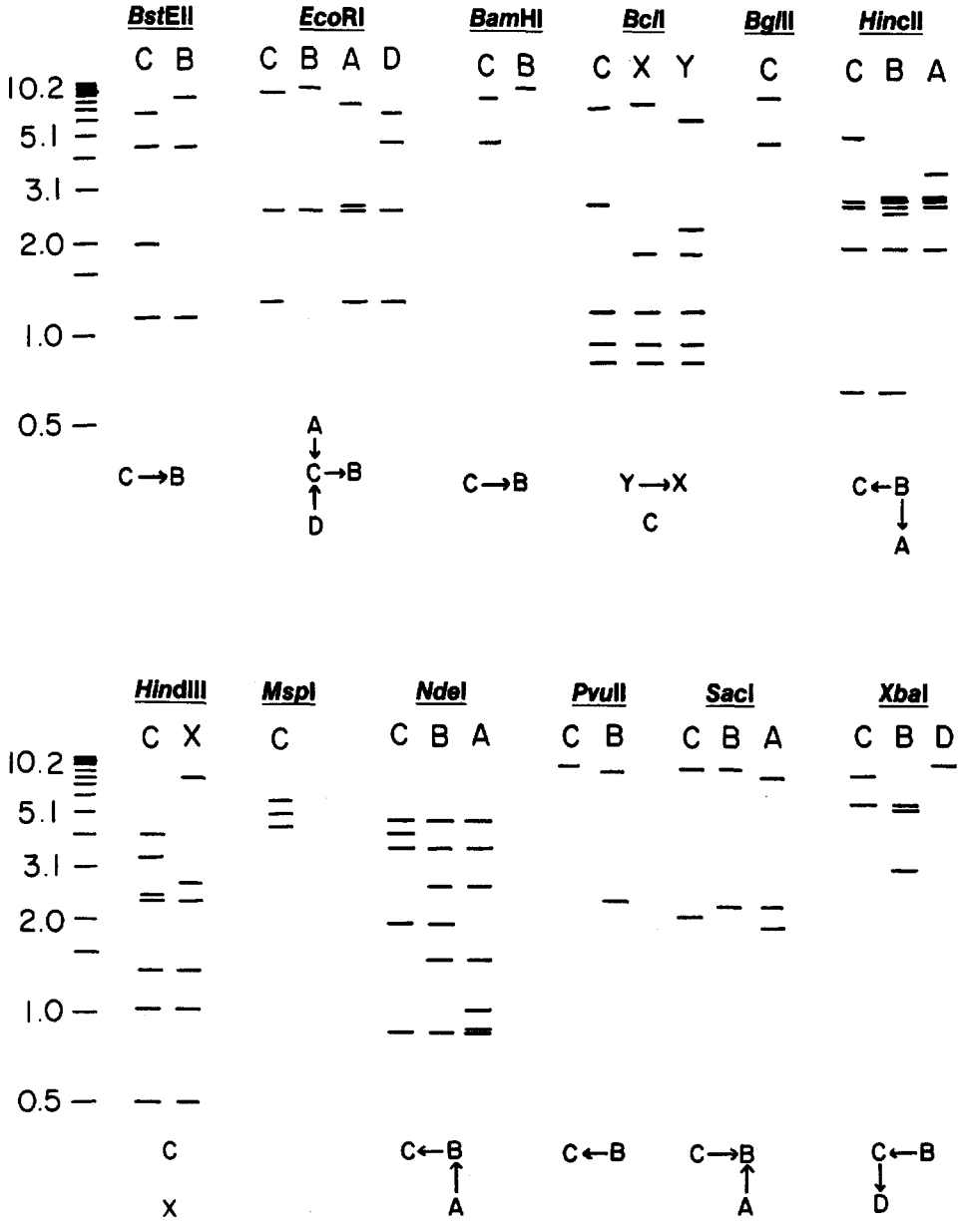

FIGURE 1.—Diagrammatic representations of multifragment mtDNA digestion profiles in *L. polyphemus*. Also shown are networks of evolutionary relationships among the profiles, where arrows indicate direction of site loss (and not necessarily direction of evolution). Letters not connected by arrows apparently differ by two or more restriction site changes. Sizes (in kilobase pairs) of selected fragments in a 1-kb ladder standard are indicated to the left.

51 assayed Limulus collected from Brunswick, Georgia, to Dover Point, New Hampshire, and *Bam*HI "B" was observed in all 47 assayed Limulus from Cape Canaveral to Panama City, Florida. Geographic distributions of genotypes vir-

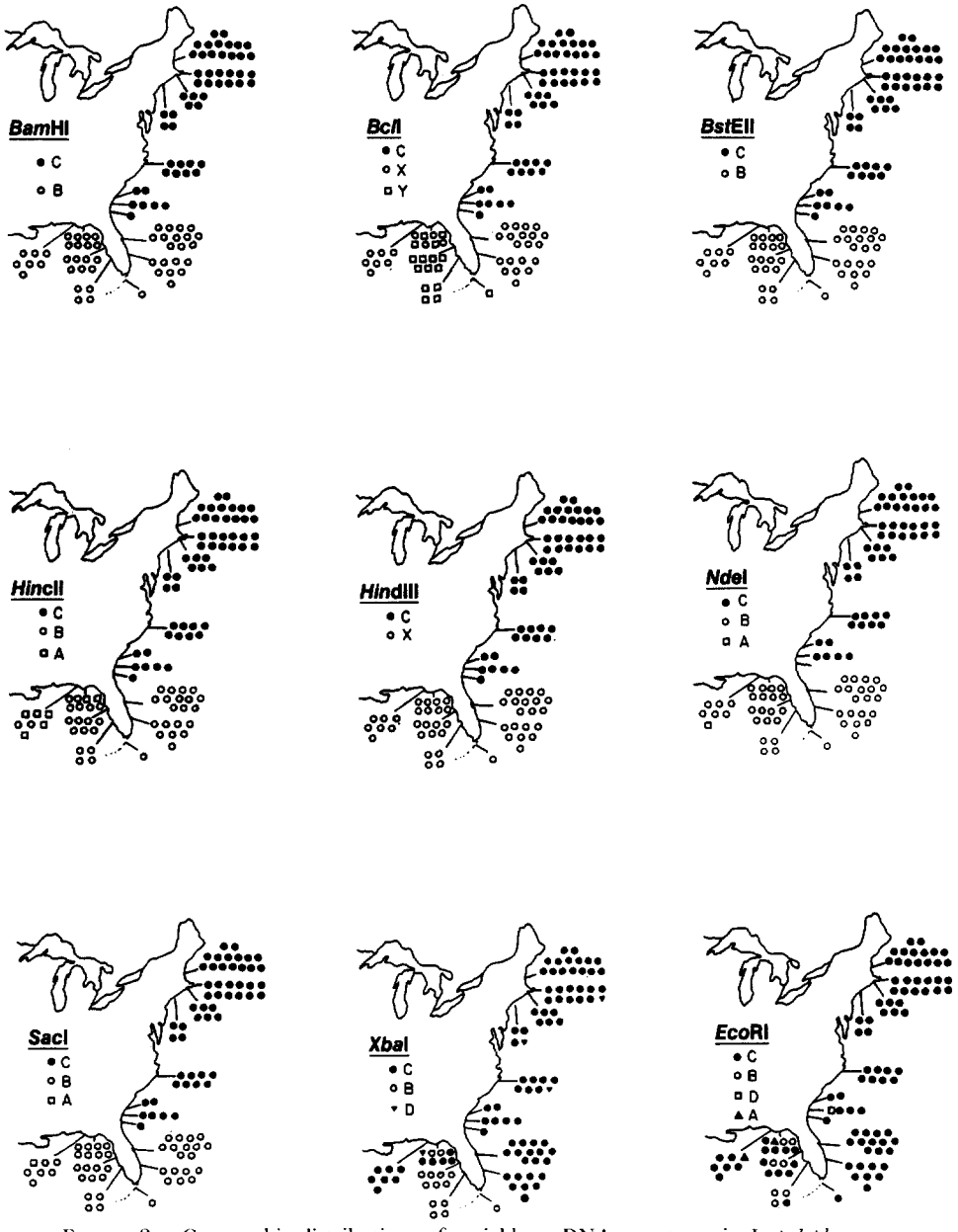

FIGURE 2.—Geographic distributions of variable mtDNA genotypes in *L. polyphemus*.

tually identical to this were observed for *Bst*EII, *Hin*dIII, *Nde*I and *Sac*I (Figure 2). Thus, in each case, diagnostic genotypes distinguished northern (Georgia to New Hampshire) from southern (Cape Canaveral to Panama City) horseshoe crabs.

Other enzymes generally confirmed the northern vs. southern genetic break and also provided additional information. For *Bcl*I (Figure 1), pattern "C" was

observed in all northern Limulus, and patterns "X" and "Y" in southern Limulus, the latter being confined to samples from Panacea, Florida, to the Florida Keys (Figure 2). *Xba*I "B" was similarly observed only from Panacea to the Keys. *Eco*RI "B" was confined to the central region of the assayed Gulf Coast area, and *Hinc*II "A" to the more western Gulf Coast region (Figure 2). Other mtDNA genetic variants occurred in one or a few individuals.

One potential concern is whether the concordant north-south breaks revealed by several endonucleases might be attributable to a single molecular genetic event, such as a large-scale deletion (or addition), or perhaps a genome rearrangement involving multiple restriction sites. This is most unlikely for several reasons: (1) some enzymes (most notably *Bgl*II and *Msp*I) showed identical multifragment digestion profiles in all individuals, indicating conservation of genome size and major arrangement; (2) some fragment patterns distinguishing northern from southern samples differed by a single restriction site (*Bam*HI, *Bst*EII, *Hinc*II, *Nde*I, *Sac*I), whereas others required at least two restriction site differences (*Bcl*I, *Hin*dIII); (3) among the diagnostic profiles involving single-site changes, some involved a site gain in going from north to south (*Hinc*II, *Nde*I), whereas others involved a site loss in that same direction of comparison (*Bam*HI, *Bst*EII, *Sac*I); and (4) patterns around the major north-south genetic break were not always perfectly concordant (thus, *Xba*I "C" characteristic of northern Limulus was also present in Cape Canaveral, Stuart, Panacea and Panama City, Florida). Overall, many apparently independent mutations are responsible for the dramatic divergence observed between the northern and southern Limulus.

The data can also be considered in composite. Table 1 lists all observed mtDNA genotypes by multiple letter designation. A total of ten clonal genotypes was observed, seven of which (numbers 4–10) occurred in the southern Limulus populations. These composite genotypes were connected into a parsimony evolutionary network (Figure 3), initially drawn without reference to collection site. Clonal types 1–3 (which represent the northern form) occupy adjacent positions in the network (as they do in geography), and the same can be said for the array of seven genotypes observed in the south. As expected, the north-south genetic break already evident from maps of the single endonucleases (Figure 2) is also apparent in the overall parsimony network (Figure 3).

Table 2 shows the observed number of mutation steps distinguishing pairs of mtDNA clones (above diagonal) and also shows the number of inferred mutation steps taken from the parsimony network (below diagonal). The total number of "direct count" mutation steps (295) is slightly lower than the total inferred number (305), indicating some evolutionary convergence or reversal of restriction sites, as has been reported in other such surveys of mtDNA (*e.g.*, LANSMAN *et al.* 1983). Using PRAGER and WILSON'S (1978) index, we calculate the homoplasy value to be 3.4, a level indicating some mild distortion in the network. One obvious digestion profile contributing to this distortion (from inspection of Figure 2) involves the *Xba*I "D" pattern, which was observed in scattered locales in the otherwise highly divergent northern and southern Lim-

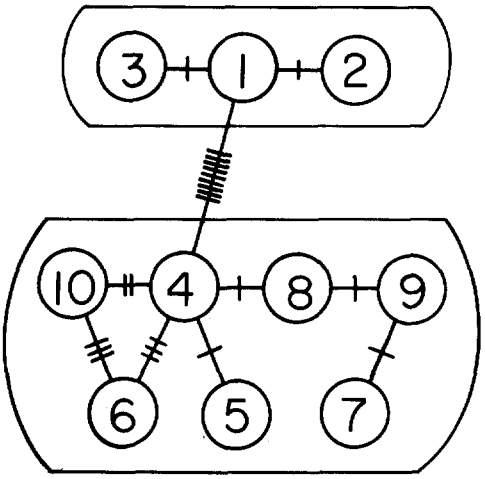

FIGURE 3.—Parsimony network interconnecting mtDNA genotypes (labeled as in Table 1) in *L. polyphemus*. Solid lines crossing branches indicate numbers of restriction site changes occurring along a path.

TABLE 2

Number of restriction site differences distinguishing mtDNA composite genotypes in *Limulus polyphemus*

	1	2	3	4	5	6	7	8	9	10
1	—	1	1	9	10	12	11	10	10	11
2	1	—	2	10	11	13	12	11	11	10
3	1	2	—	10	11	13	12	11	11	12
4	9	10	10	—	1	3	3	1	2	2
5	10	11	11	1	—	4	4	2	3	3
6	12	13	13	3	4	—	5	4	5	3
7	12	13	13	3	4	6	—	2	1	4
8	10	11	11	1	2	4	2	—	1	3
9	11	12	12	2	3	5	1	1	—	4
10	11	12	12	2	3	3	5	3	4	—

Above diagonal, counted differences from digestion profiles; below diagonal, inferred differences from the parsimony network in Figure 4. MtDNA genotypes are numbered as in Table 1.

ulus mtDNA assemblages. Very likely, the loss of a restriction site converting the common Limulus *Xba*I "C" pattern to the *Xba*I "D" pattern has occurred more than once in the course of evolution.

Among sampled restriction sites, *at least* nine mutation steps are required to account for the differences between the northern and southern forms of horseshoe crabs (clones 1 and 4, Figure 3). Using either the "fragment" or "site" approaches of NEI and LI (1979), nucleotide sequence divergence between these two genotypes is approximately $P = 0.020$. If BROWN, GEORGE and WILSON'S (1979) molecular clock calibration can be applied to Limulus, mtDNA clones 1 and 4 (representing the common genotypes of the northern and southern horseshoe crabs) last trace to a common ancestor roughly 1 million yr ago. This is a very provisional estimate.

DISCUSSION

The overall pattern and magnitude of mtDNA differentiation in marine horseshoe crabs is very similar to that typically reported for freshwater and terrestrial species assayed over a roughly comparable geographic range. For example, in the pocket gopher, *Geomys pinetis*, sampled across the southern United States, mtDNA clonal diversity was high and geographically patterned, with a major genetic break ($P \approx 0.03$) distinguishing populations in eastern Georgia and the Florida peninsula from those in western Georgia and Alabama (AVISE *et al.* 1979). In the horseshoe crab, considerable mtDNA clonal diversity exists (Table 1), and a major genetic break ($P \cong 0.02$) distinguishes populations in Georgia northward from those in Florida's Atlantic and Gulf coasts. These results demonstrate for the first time that, geographically, at least some continuously distributed marine organisms can show considerable mtDNA genetic differentiation. The mtDNA approach, which has proven to be powerful in identification of genetic stocks of terrestrial and freshwater faunas, also appears to be a strong method for revealing intraspecific differentiation in the marine realm.

On the other hand, a single mtDNA clone (type 1) occurred in 48 of 52 Limulus collected from Georgia to New Hampshire, and the remaining four horseshoe crabs each differed from clonal type 1 by a single restriction-site change. Had the survey been confined to this northern region, a very different conclusion might have been reached—that mtDNA (and population) divergence was minimal. However, some mtDNA clones are also very widespread in terrestrial species. For example, in the deer mouse *Peromyscus maniculatus*, a single mtDNA genotype predominated in populations ranging from Idaho to Arizona (LANSMAN *et al.* 1983). In that species, major mtDNA differentiation ($P \approx 0.05$) did distinguish several other sets of populations across North America. These examples emphasize how geographic scale and sampling design can influence perception of population genetic differentiation.

An earlier survey of allozyme variation in *L. polyphemus* gave some hint that Atlantic and Gulf coast populations are genetically divergent. SELANDER *et al.* (1970) assayed 64 specimens from four localities: Massachusetts, Virginia and two sites on the Gulf Coast of Florida. Of nine polymorphic loci, seven showed consistent although small differences in allele frequencies between the Atlantic and Gulf Coast sites, leading the authors to report a "...striking regional uniformity in allele frequencies at all polymorphic loci and interregional variation at most loci." RISKA (1981) measured 16 external morphological characteristics in horseshoe crabs, from Cape Cod, Massachusetts, to the Gulf of Mexico, and found that "one-half to three-fourths of the variance of morphological characters appears to be attributable to differences among the means of local population samples. Certainly Limulus cannot be said to lack geographic variation by this criterion." RISKA (1981) did not note a particularly great distinction between his single Gulf Coast sample and those from the northern part of the range.

The most striking result of our mtDNA survey in Limulus is the major genetic break distinguishing northern from southern populations. The area

of divergence, evidenced by 7 of the 12 informative endonucleases, occurs somewhere between Cape Canaveral, Florida, and Brunswick, Georgia. Horseshoe crabs are more or less continuously distributed through this region. What then could account for the dramatic and rather sudden mtDNA genotypic transition? Potential explanations can be grouped into two major categories: deterministic and stochastic.

Deterministic evolutionary forces: It is tempting to speculate that some particular and potentially identifiable evolutionary force(s) are responsible for the Limulus population divergence in the northeast Florida region. Such deterministic possibilities might include historical and/or contemporary influences on dispersal patterns, such as ocean currents, water masses and salinity gradients, that might reduce or eliminate gene flow across the north Florida inshore area. Another deterministic possibility is that natural selection pressures acting on mtDNA genotypes may differ dramatically from north to south of this area. Although most mutational changes in mtDNA are thought to be selectively neutral [see discussions in BROWN (1983) and AVISE (1985)], the uniparental (maternal) transmission of mtDNA serves to tightly link all of its genes and allows hitchhiking neutral markers to be carried to high frequency by any selectively advantageous mutants with which they happen to be associated.

There is no obvious topological or geologic "barrier" between north Florida and south Georgia. An important river, the St. Johns, does empty into the ocean near the mtDNA transition zone, but it is very unlikely to be a significant hurdle to gene flow. Limulus is tolerant of estuarine conditions, and in any event, many such rivers have mouths within the surveyed range of Limulus but are not similarly associated with zones of mtDNA divergence.

Water mass differences associated with a major transporting ocean current, the Gulf Stream, may provide a more plausible explanation for the pattern of genetic divergence in Limulus. The Gulf Stream generally parallels the Atlantic coast from south Florida to Cape Hatteras (North Carolina). However, it moves much farther offshore above Cape Canaveral, creating the nearshore "South Atlantic Bight" region in northern Florida, Georgia and South Carolina. Recent satellite photographs and physical oceanographic measurements dramatically show the temperature and water mass differences associated with the western boundary of the Gulf Stream as it contacts continental shelf waters (LEE 1983; HANEY and MCGILLIVARY 1985). Perhaps this water mass boundary, which would be experienced by inshore species in the area of Cape Canaveral, provides selection differences and/or dispersal barriers leading to the pattern of mtDNA differentiation observed in Limulus.

Many marine zoogeographers and faunal specialists including malacologists and ichthyologists have recognized the northeast Florida area as transitional between warm-temperate and tropical marine assemblages (see review, BRIGGS 1974). For example, ABBOTT (1957) considered a temperate "Appalachian Province" for mollusks to extend down the northern third of Florida; and BRIGGS (1958) noted that distributional limits of many fish species terminate in northeastern Florida. As stated by BRIGGS (1974), "In regard to the fauna

of the inner shelf, Cape Kennedy [now Cape Canaveral] is an intermediate point in a rather lengthy area of change and is also the site of an upwelling and movement of cooler waters near shore. Since we know that many species terminate their ranges here, it seems reasonable to consider it as the most likely boundary [between faunal provinces]."

Because the intraspecific mtDNA break in Limulus corresponds closely in geographic position to the boundary region between inshore temperate and tropical marine faunas in the western North Atlantic, it seems possible that the same deterministic evolutionary forces (related to selection pressures and/or gene flow barriers associated with water mass differences) may be responsible for both patterns. A similar geographic correspondence between zoogeographic province (as identified by species' distribution limits), and intraspecific divergence in broadly distributed species (as revealed by mtDNA genotype), has been observed in freshwater fishes of the southeastern United States (E. BERMINGHAM and J. C. AVISE, unpublished results). Here, historical patterns of river drainage interconnection presumably account for both phenomena.

For Limulus, further speculation about these possibilities is perhaps unwarranted. One reason for reluctance to wholeheartedly adopt a zoogeographic province explanation is that other classically recognized boundaries between faunal provinces or subprovinces, which occur at Cape Hatteras, Cape Cod and, perhaps, Naples, on the Gulf Coast of Florida (BRIGGS 1974), are not similarly paralleled by breaks in mtDNA genotype in horseshoe crabs. Furthermore, with our current state of knowledge, another explanation that requires no particular determinism may also be compatible with the mtDNA patterns observed.

Stochastic differentiation: NEIGEL and AVISE (1985) used computer simulations to study the effects of random lineage extinction and survival on distributions of mtDNA genotypes. The models addressed a different issue (the phylogenetic relationships of mtDNA across speciation events), but were developed in a manner that also appears highly relevant to our current discussion. In the models, a program (PARDIS) generated a vector of mtDNA genetic distances (measured in time units since lineages traced to a common female ancestor) for individuals in a large population. This population was assumed to exhibit density regulated growth and a Poisson distribution of female offspring per mother. The output of the program in any generation consists of distance values between adjacent individuals in the evolutionary tree. As the tree is being developed, individuals are allotted openings that can be interpreted as positions along a linear habitat (such as a shoreline). The most important assumptions of the models, for current purposes, are that a single continuously distributed population is involved, that there have been no lateral leapfroggings of lineages (equivalent to an assumption that movements and gene flow are very small compared to the total length of habitat occupied) and that deterministic factors, such as selection or zoogeographic barriers, are absent.

Figure 4 (top) shows a typical simulation result involving a population with $N = 2458$. The vast majority of geographically adjacent individuals exhibit

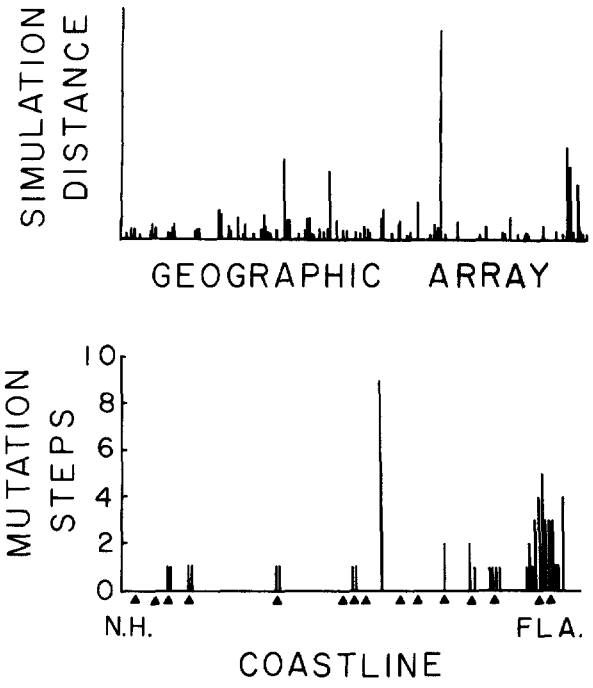

FIGURE 4.—*Above*: genetic distances between adjacent individuals along a linear habitat in a computer-simulated population with greatly limited gene flow (from NEIGEL and AVISE 1985). *Below*: observed genetic distances (measured in restriction site changes) between geographically "adjacent" horseshoe crabs in the current study.

small mtDNA distances, indicating that they have recently shared a female ancestor. Occasionally, however, two adjacent individuals along the continuous habitat exhibit huge distances, a major mtDNA genetic break, due to their chance membership in "deeply divided" branches of the phylogenetic tree. Note that a corresponding break in nuclear genotype would not necessarily be expected, because matings and sexual reproduction have the effect of anastomosing lineages.

Figure 4 (bottom) plots the empirical data for Limulus in a similar format. The coastline from New Hamsphire to Florida has been linearized, with collection sites (indicated by solid triangles) located at proper scale. Observed numbers of mutation steps (restriction-site changes) between geographically "adjacent" individuals in the collections are indicated. Most such distances are zero, reflecting shared membership in a mtDNA clone, but occasionally, larger genetic distances are involved. The major genetic break (nine mutation steps) distinguishes the southernmost Georgian Limulus in our sample from the northernmost Floridian horseshoe crab. The figure is also useful in pointing out the higher mtDNA clonal diversity observed in the Gulf Coast samples (see the right region of the figure).

Qualitatively, the observed patterns of mtDNA divergence in Limulus, including a major genetic break, are little different from those generated by stochastic population processes for a species with limited gene flow in a con-

tinuous habitat (Figure 4). This does not necessarily mean that deterministic forces have not played a role in the Limulus mtDNA divergence, but it does mean that we must be cautious in inferring deterministic processes solely from distributional patterns, striking as those patterns appear to be for horseshoe crabs.

How might these deterministic and stochastic possibilities be further evaluated? Several possibilities exist. First, a finding of nuclear genome divergence in Limulus in the north Florida region would not necessarily be expected under stochastic models, but would be predicted if selective or zoogeographic barriers to gene flow were responsible. Second, a finding of concordant placements of genetic divergence in other marine species would be a likely consequence of many deterministic, but not stochastic, scenarios. Finally, additional fieldwork on Limulus, as well as on modifications of the computer models, might allow a more realistic appraisal of the extent to which assumptions of the stochastic models truly apply to the particular life-history attributes of horseshoe crabs.

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