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Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology

John C. Avise

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Mitochondrial (mt) DNA data on the comparative phylogeographic patterns of 19 species of freshwater, coastal, and marine species in the southeastern U.S.A. are reviewed. Nearly all assayed species exhibit extensive mtDNA polymorphism, although still orders-of-magnitude less than predicted under neutrality theory if evolutionary effective population sizes of females are similar to current census sizes. In both the freshwater and marine realms, deep and geographically concordant forks in intraspecific mtDNA phylogenies commonly distinguish regional populations in the Atlantic versus Gulf Coast areas. These concordant phylogeographic patterns among independently evolving species provide evidence of similar vicariant histories of population separation, and can be related tentatively to episodic changes in environmental conditions during the Pleistocene. However, the heterogeneity of observed genetic distances and inferred separation times are difficult to accommodate under a uniform molecular clock. Additional population genetic structure within geographic regions is evidenced by species-specific shifts in frequencies of more closely related mtDNA haplotypes, and by high frequencies of private haplotypes in some species. The magnitude of local population structure appears partially related to the life history pattern and dispersal capability of a species.
The mtDNA results indicate that conspecific populations can be structured at a wide variety of evolutionary depths. The deeper subdivisions in an intraspecific phylogeny reflect the major sources of evolutionary gene pool diversity within a species, while the shallower molecular separations evidence more recent population subdivisions that can be related to comparative dispersal and gene flow patterns. These molecular findings are relevant to the understanding of biogenetic diversity, and carry implications for conservation biology.

J. C. Avise, Dept of Genetics, Univ. of Georgia, Athens, GA 30602, USA.

Over the past several years, our laboratory has conducted geographic surveys of mitochondrial (mt) DNA variability within a number of marine and freshwater species in the southeastern United States. These studies had a variety of immediate objectives, such as characterization of the genetic status of threatened or endangered species (Atlantic sturgeon – Bowen and Avise 1990; seaside sparrow – Avise and Nelson 1989), examination of the evolutionary genetic consequences of an unusual, catadromous life cycle (American eel – Avise et al. 1986), appraisal of levels of genetic variation in "living fossils" (horseshoe crab – Saunders et al. 1986; American oyster – Reeb and Avise 1990), assessment of cytonuclear associations in a hybrid zone (bluegill sunfish – Avise et al. 1984, Asmussen et al. 1987), or examination of atypical molecular features of mtDNA variation (menhaden fish – Avise et al. 1989; diamondback terrapin, Lamb and Avise, unpubl.). To date, my students and I have assayed genetic variation in some 19 freshwater, coastal, and marine species in this geographic region.

Here these results will be summarized in the context

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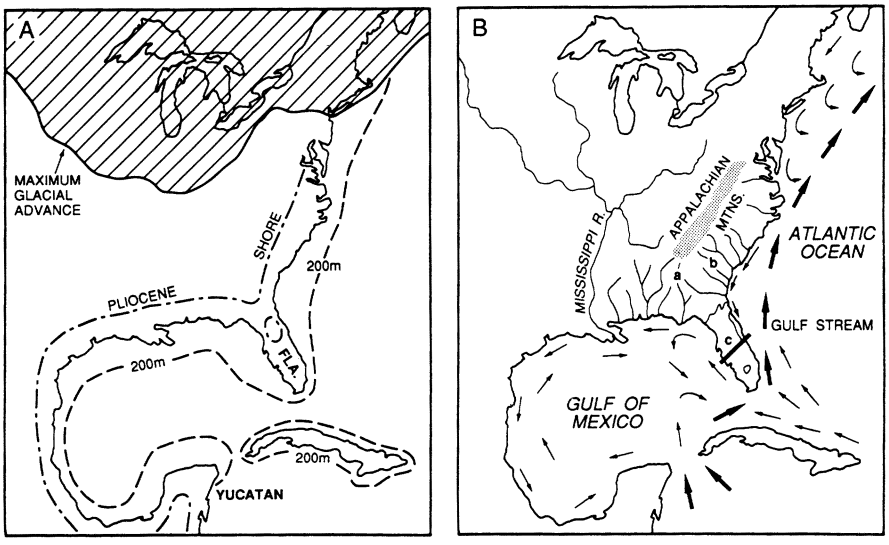

Fig. 1. A) Selected historical physiographic features of the eastern U. S. Shown are the current shoreline, the high sea stand of the Pliocene (somewhat higher than any Pleistocene interglacial shorelines), and the 200 m depth contour (probably slightly outside the exposed land areas of the Pleistocene glacial maxima). Also shown is the maximum extent of Pleistocene glacial advance. B) Selected contemporary physiographic features of the southeastern U. S. Shown are trends in marine currents (including the Gulf Stream (heavier arrows)), major river drainages entering the Gulf of Mexico and Atlantic (including the Apalachicola (a) and Savannah (b) drainages), and the Appalachian mountains. Also indicated by the bar (c) is the approximate transitional zone between temperate and tropical faunal elements along the east and west coasts of peninsular Florida.

of a comparative appraisal of geographic population structure. Despite the diverse array of species represented in these surveys, several striking and unanticipated trends in mtDNA variation and phylogeny have emerged. Perhaps our empirical experience with the evolutionary genetic structure of a regional fauna will also provide some general lessons on the kinds of contributions molecular genetic data can (and cannot) make to the concerns of "stock" assessment, population management, and conservation biology.

Several recent reviews have thoroughly summarized the major features of mtDNA evolution in higher animals (Wilson et al. 1985, Avise 1986, 1987, Avise et al. 1987a, Ferris and Berg 1987, Moritz et al. 1987, Shields and Helm-Bychowski 1988, Harrison 1989), and these should be consulted for necessary background. In general, mtDNA evolves rapidly and exhibits extensive polymorphism within most species. MtDNA also exhibits uniparental (maternal) inheritance, without known recombination between molecules from different female lines. Thus even within interbreeding populations, mtDNA lineages are genetically isolated from one another, such that any observed homologies in structure presumably result from historical connection in a matriarchal genealogy. The phylogenies of mtDNA haplotypes within a species can normally be estimated by reasonable evolutionary criteria such as cladistic analysis or parsimony, and the results compared to expectations of theoretical demographic models to make inferences about population histories (Avise 1989a). Caution must be exercised in drawing such population inferences, since any gene tree (such as that provided by mtDNA) represents only one realization of the multigene process of lineage sorting through an organismal pedigree (Ball et al. 1990). Nonetheless, the phylogenetic content in a mtDNA gene tree, when interpreted in conjunction with the observed geographic distributions of mtDNA clades, provides one picture of the "phylogeographic" past of a species (Avise et al. 1987a, b), and thereby adds a historical perspective to population structure and intraspecific evolutionary process.

The evolutionary theatre of the southeastern U.S.A.

The southeastern U.S.A. is of special biogeographic interest because of both historical and contemporary climatic and geologic influences on its biota. Some of the major physiographic features of the region are summarized in Fig. 1. In the marine realm, the Florida peninsula currently protrudes southward into subtropical waters (25–28°N), and separates temperate faunas into sometimes allopatric units on the Atlantic Coast and Gulf of Mexico. Thus the east and west coasts of Florida are well-recognized zones of transition between temperate and tropical adapted forms, with the southern ranges of many temperate species terminating in the approximate regions of Cape Canaveral and Naples, respectively (Briggs 1974). Other "temperate" species

Table 1. Ranges and salient life history characteristics of the marine, coastal, and freshwater species in the southeastern U.S.A. considered in this paper.

Species	Range in southeastern U.S.A.	Relevant life history and dispersal characteristics	Status[a]
Marine and coastal species			
Am. eel (*Anguilla rostrata*)	Continuous, Atl. and Gulf coasts	Catadromous; mass spawning in tropical mid-Atl. Ocean; larvae return to coast, and juveniles reside in fresh and brackish waters	common
hardhead catfish (*Arius felis*)	continuous, Atl. and Gulf coasts	adults strong and active swimmers; mouthbrood eggs and fry	common
Oyster toadfish (*Opsanus tau*)	Atl. coast to south Fla.	adults sluggish bottom dwellers; lay demersal, adhesive eggs	intermediate-common
Gulf toadfish (*Opsanus beta*)	Gulf coast to south Fla.	same	same
Atl. menhaden (*Brevoortia tyrannus*)	Atl. coast to central Fla.	adults active pelagic feeders; spawn offshore; larvae move to estuarine feeding grounds	abundant
Gulf menhaden (*Brevoortia patronus*)	Gulf coast to south Fla.	same	abundant
Atl. sturgeon (*Acipenser oxyrhynchus*)	Atl. and Gulf coasts, but perhaps absent from South Fla.	anadromous; spawn in freshwater streams; juveniles move to coastal waters after 1–8 yr; adult movements poorly known	rare
Black sea bass (*Centropristis striata*)	Atl. and Gulf coasts, but rare in extreme south Fla.	spawn near coast; larvae move to estuarine feeding grounds	common
Am. oyster (*Crassostrea virginica*)	continuous, Alt. and Gulf coasts	adults sessile, in estuarine habitats; sequential hermaphrodites; pelagic gameters (few h.) and larvae (2–3 wk)	abundant
Horseshoe crab (*Limulus polyphemus*)	continuous, Atl. and Gulf coasts	adults slow-moving, primarily in estauries; eggs laid on sandy beaches; trilobite larvae stay in sand or water probably near shore	common
Seaside sparrow (*Ammodramus maritimus*)	Atl. and Gulf coasts, but absent from southeast Fla.	confined to salt marshes; populations in the southeast U.S.A. non-migratory	intermediate
Diamondback terrapin (*Malaclemys terrapin*)	more or less continuous, Atl. and Gulf coasts	coastal marshes, estuaries, sheltered bodies of brackish or salt water	rare-intermediate
Freshwater fishes			
Spotted sunfish (*Lepomis punctatus*)	throughout southeastern U.S.	prefers ponds, rivers, streams with heavy vegetation;	intermediate-common
Redear sunfish (*Lepomis microlophus*)	throughout southeastern U.S	similar	same
Warmouth sunfish (*Lepomis gulosus*)	throughout southeastern U.S.	similar	same
Bluegill sunfish (*Lepomis macrochirus*)	throughout southeastern U.S.	similar	abundant
Bowfin (*Amia calva*)	throughout southeastern U.S.	similar; adults protect schooling young after hatching	intermediate
Mosquitofish (*Gambusia affinis*/*G. holbrooki*	*affinis* – primarily Gulf drainages *holbrooki* – primarily Atl. drainages	livebearer; shore-hugging in lentic waters	abundant

[a] Admittedly rough and impressionistic estimates of the sizes of adult populations at the present time: rare, $N < 10^4$; intermediate, $N \cong 10^4$–10^6; common, $N \cong 10^6$–10^8; and abundant, $N > 10^8$.

are continuously distributed around south Florida at the present time.

During the ten or more glacial advances and retreats of the Pleistocene, sea level fluctuations and climatic changes no doubt had great impact on the distributions of coastal and marine species in the southeast. While the glaciers themselves never advanced beyond the north-central U.S.A., the associated climatic cooling pushed temperate populations southward, and may have increased the opportunity for contact of Atlantic and Gulf populations around south Florida. However, the glacial advances also caused drops in sea level (by as much as 150 meters) and exposed tremendous expanses of the Florida (and Yucutan) peninsulas (Fig. 1A). At such times, Florida was more arid than it is today, and presumably bordered by few of the intermediate-salinity estuaries and salt-marsh habitats favored by many coastal species. Thus during glacial advances, an enlarged Floridian peninsula may actually have contributed to a separation of some Atlantic and Gulf coast populations through creation of a rather isolated pocket of estuarine habitat in the western Gulf of Mexico.

Opposing influences on species' distributions may also have been at work during interglacial periods (such as the present), when sea levels were higher and the Florida peninsula likely bordered by more extensive estuaries and salt marshes. At such times of climatic warming, some strictly temperate species may have been increasingly separated into disjunct Atlantic and Gulf populations by the tropical conditions of south Florida, while other species that were more eurythermal and estuarine-adapted may have expanded out of the putative Gulf refugium to regain increased contact with Atlantic forms around the southern tip of the peninsula. At present, swift water currents moving out of the Gulf of Mexico contribute to the "Gulf Stream", which hugs the coast of southeast Florida and may facilitate transport of Gulf-spawned pelagic larvae into the south Atlantic (Fig. 1 B). The Gulf Stream tends to move offshore beyond the Cape Canaveral region of central Florida.

Cyclical changes in Pleistocene climates and landscapes must have influenced the distributions of freshwater biotas as well. At present, about a dozen major rivers and numerous smaller systems drain the southeastern coastal states from the Carolinas to Louisiana (Fig. 1 B). Eastern drainages enter the south Atlantic, while western drainages enter the Gulf. During the high sea-stands of the Pliocene and the moderate sea-stands of the Pleistocene interglacial periods, smaller coastal streams were likely flooded, and freshwater faunas probably isolated in the upper reaches of the larger rivers (and perhaps in lakes or rivers of the central Florida peninsula itself). Any interdrainage transfers of fish must then have occurred via headwater or lateral stream capture, as for example between the Apalachicola and Savannah drainages which now come into close contact in the southern Appalachians. Conversely, during the glacial periods which dominated much of the Pleistocene, the broader coastal plains associated with low sea levels may have increased the opportunities for coalescense of adjacent rivers near their mouths, and hence for the lateral, interdrainage transfer of freshwater species, perhaps primarily on an Atlantic versus Gulf coast regional scale. The Apalachicola drainage (which forms the southern state line between Georgia and Alabama), now represents an important boundary region between freshwater zoogeographic provinces, as judged by the large number of species whose current ranges exhibit eastern or western termini in this general area (Swift et al. 1985).

Most major rivers in the southeastern U.S.A. traverse several physiographic provinces. Originating as clear headwater streams in the southern Appalachian mountains, they enlarge and accumulate sediments as they traverse the hilly Piedmont region of red-clay soils, and finally emerge as warmer rivers crossing a broad and relatively flat coastal plain of sandy substrate. If present-day ecological selection pressures (rather than historical patterns of population connection and gene flow) were the primary moulders of mtDNA genotypic distributions, then clines in haplotype frequencies should probably parallel such strong ecological gradients within each river. However, as shown beyond, the intraspecific mtDNA phylogenies of assayed freshwater fishes generally orient in an east-west regional pattern more consistent with a primary influence of historical biogeographic forces.

The discussion above provides a brief background to the major historical and contemporary features of the physical environment that may have influenced southeastern faunas. Expanded treatments can be found in Bermingham and Avise (1986), Bert (1986), Reeb and Avise (1990), and references therein. Nonetheless, it should be understood that very little is firmly known about the physiographic history of the area, and that most historical geologic scenarios are highly speculative. For example, possible opportunities for Atlantic-Gulf biotic separations have been discussed above primarily because the genetic data suggest they exist (see beyond), rather than because were necessarily predicted a priori on physiographic grounds alone. With the large volume of molecular data now available for this regional fauna, genetic information may actually inform some of the interpretations of historical geography, rather than the usual converse.

The cast of assayed species

The species considered in this report exhibit a wide variety of population sizes, dispersal characteristics, and life history patterns (Table 1). For example, included among the marine and coastal species are the rare and anadromous Atlantic sturgeon, the common and catadromous American eel, mouthbrooding marine catfishes, demersal-spawning toadfishes, and abundant menhaden fish and oysters that produce pelagic larvae with high dispersal potential. Several of the assayed coastal species, including the menhaden, black sea bass, American oyster, horseshoe crab, seaside sparrow, and diamondback terrapin, prefer or require estuarine habitat for all or part of their life cycle. Among the assayed freshwater fishes are four common species of sunfish, live-bearing mosquitofish, and one living representative of an ancient Holostean order, the bowfin. If any shared patterns of mtDNA variability or phylogeography emerge among a significant fraction of such a heterogeneous group of species, the evolutionary forces responsible must have been of rather overriding influence.

Table 2. Comparative estimates of mtDNA variability in coastal, marine, and anadromous species surveyed from locales along the Atlantic Coast and Gulf of Mexico in the southeastern U.S.A. Also presented are estimates of evolutionary effective population sizes for females, generated from the mtDNA data under the assumptions discussed in the text.

Species (and region)	Number of			Genotypic diversity[a]	Nucleotide diversity[b]	Assumed generation length (yr)	Effective female pop. size [$N_{f(e)}$]	Reference for original data[c]
	inds.	different mtDNA genotypes	restriction sites or fragments per individual					
American eel (*Anguilla rostrata*)	109	21	78	0.54	0.0011	10	5500	1
Hardhead catfish (*Arius felis*)	60	11	57	0.47	0.0018	2	45000	2
Toadfish (*Opsanus*)								
Atlantic (*O. tau*)	43	5	50	0.58	0.0011	3	18300	2
Gulf (*O. beta*)	17	8	53	0.77	0.0033	3	55000	2
Menhaden (*Brevoortia*)								
Atlantic (*B. tyrannus*)	17	17	55	1.00	0.0316	2	800000	3
Gulf (*B. patronus*)	16	16	55	1.00	0.0099	2	250000	3
Sturgeon (*Acipenser oxyrhynchus*)								
Atlantic	21	5	68	0.64	0.0017	10	8500	3
Gulf	15	2	68	0.13	0.0000	10	50	3
Black sea bass (*Centropristis striata*)								
Atlantic	19	3	61	0.21	0.0003	3	5000	3
Gulf	10	2	61	0.22	0.0003	3	5000	3
American oyster (*Crassostrea virginica*)								
Atlantic	104	31	65	0.57	0.0014	1	70000	4
Gulf	108	51	65	0.80	0.0025	1	125000	4
Horseshoe crab (*Limulus polyphemus*)								
Atlantic	52	3	41	0.15	0.0003	3	5000	5
Gulf	47	7	39	0.89	0.0030	3	50000	5
Seaside sparrow (*Ammodramus maritimus*)								
Atlantic	21	5	89	0.36	0.0003	3	5000	6
Gulf	19	6	89	0.47	0.0004	3	6700	6
Diamondback terrapin (*Malaclemys terrapin*)								
Atlantic	25	2	74	0.08	0.0001	5	1000	7
Gulf	28	4	73	0.20	0.0003	5	3000	7

[a] $(n/(n-1))\ (1-\Sigma f_i^2)$, where f_i is the frequency of the ith mtDNA haplotype.

[b] mean $p = (n/(n-1))(\Sigma\, f_i f_j p_{ij})$, where f_i and f_j are the frequencies of the ith and jth haplotypes in a sample of size n, and p_{ij} is the estimated sequence divergence between the ith and jth sequences (Nei 1987: 256).

[c] References: (1) Avise et al. 1986, (2) Avise et al. 1987a, b, (3) Bowen and Avise 1990, (4) Reeb and Avise 1990, (5) Saunders et al. 1986, (6) Avise and Nelson 1989, (7) Lamb and Avise in prep.

Results and discussion – the evolutionary play

Most of the assayed species exhibit considerable mtDNA polymorphism, as exemplified by the values of genotypic and nucleotide diversity defined and summarized for the marine and coastal taxa in Table 2 (see Bermingham and Avise (1986) for descriptions of mtDNA variation in five of the freshwater fishes). Genotypic diversity is an observed probability that pairs of assayed individuals from a reference population differ detectably in mtDNA haplotype (regardless of the magnitude of estimated sequence divergence); nucleotide diversity gives the mean sequence divergence between all assayed individuals in the population considered. For reasons that will become apparent, these statistics were calculated separately for the Gulf of Mexico versus Atlantic populations of nearly all species.

Figs 2–4 and 6 show phenograms relating the different mtDNA haplotypes observed within each of 12 assayed species, based on UPGMA cluster analyses (Sneath and Sokal 1973) of genetic distances estimated from restriction fragment or site data. Phenograms are presented here not because they necessarily provide the best phylogenetic appraisals of mtDNA evolution, but rather because they facilitate simple visual comparisons of mtDNA haplotype distances and relationships across many species. The original references listed in Table 2 should be consulted for additional phylogenetic treatments applied to these data.

The remainder of this paper will address general conclusions to have emerged from these comparative estimates of mtDNA variability and differentiation.

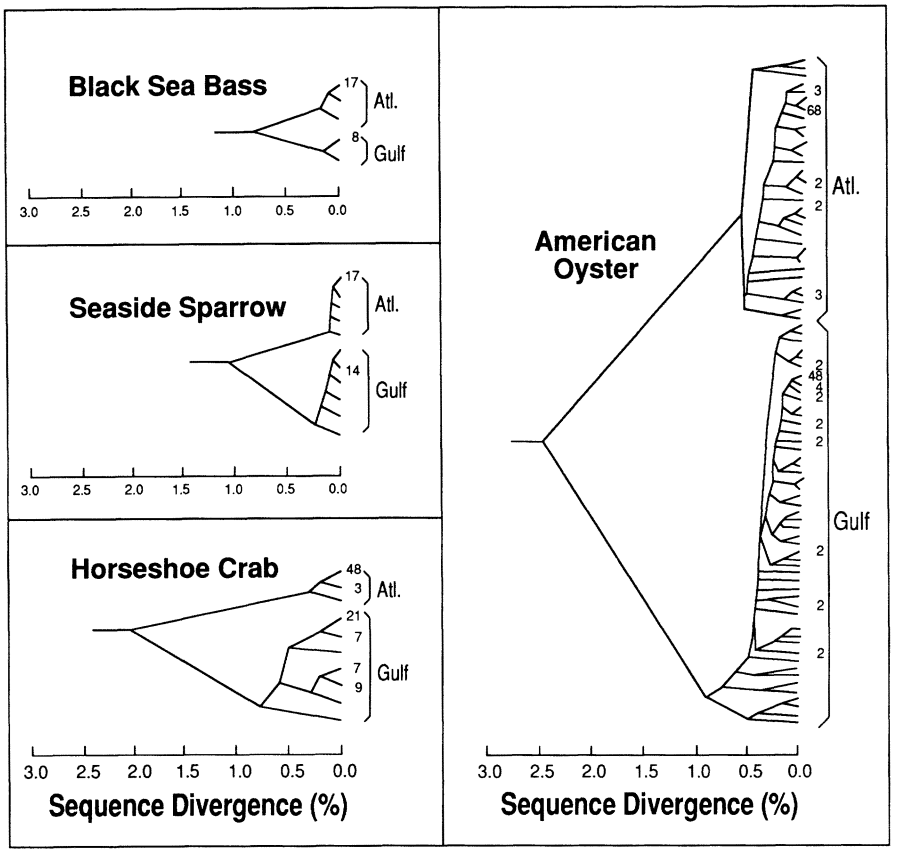

Fig. 2. UPGMA phenograms summarizing relationships among mtDNA haplotypes observed in the black sea bass, seaside sparrow, horseshoe crab, and American oyster. Numbers of individuals of various mtDNA clones are indicated to the right; terminal branches without numbers were represented by single individuals. Note that all phenograms are plotted on the same scale of mtDNA sequence divergence.

1) Major mtDNA phylogeographic patterns are shared across species

The most striking result of our comparative studies is the remarkable degree of concordance in the major mtDNA phylogeographic discontinuities across taxa. Within each of four coastal or marine species – the black seabass, seaside sparrow, horseshoe crab, and American oyster – a fundamental split in the mtDNA gene trees cleanly distinguishes individuals from the Gulf of Mexico versus those from most Atlantic coast locales (Fig. 2). In the horseshoe crab and American oyster, mtDNA genotypes normally characteristic of the Gulf clonal assemblage also extend northward along the Atlantic coast as far as central Florida (Fig. 5). The major mtDNA subdivisions in these four species are similarly evident in other phylogenetic treatments, including Wagner parsimony analyses for which bootstrap resampling of restriction data indicates significant support for these putative clonal lineages. In the black sea bass, two taxonomic subspecies (*Centropristis striata striata* and *C.s. melana*, corresponding to Atlantic and Gulf coast locales, respectively) are conventionally recognized, but the other species had not previously been suspected of exhibiting Atlantic-Gulf distinctions.

The mtDNA data for at least two additional groups – diamond backterrapins and toadfish – can be interpreted as further provisional support for a fundamental phylogenetic distinction between Gulf and Atlantic coast populations of many species. In the terrapins, mtDNA genetic variation and divergence were atypically low, with the four variant haplotypes in the Gulf and Atlantic occurring in single individuals and usually differing from the common patterns in these respective regions by a single restriction site change. However, two mtDNA haplotypes (also differing by a single restriction site) were observed in multiple individuals such that geographic patterns could be assessed, and they exhibited an Atlantic-Gulf distribution nearly identical to those for the horseshoe crab and American oyster (Fig. 5). Among the toadfish, two common species (*Opsanus tau* and *O. beta*) are currently recognized, and these are essentially confined to the Atlantic and Gulf coasts, respectively. Since they differ dramatically in mtDNA sequence (Avise et al. 1987a, b), a mtDNA

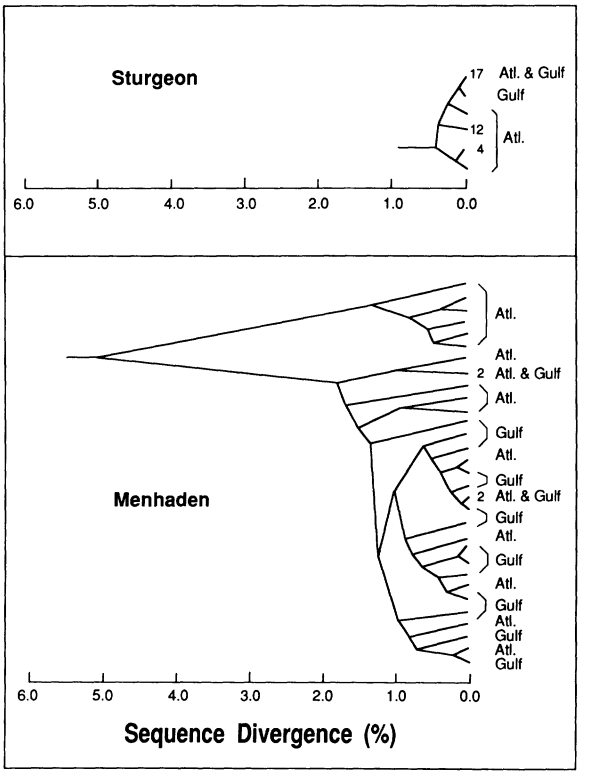

Fig. 3. UPGMA phenograms summarizing relationships among mtDNA haplotypes observed in sturgeon and menhaden. Numbers of individuals of various mtDNA clones are indicated to the right; terminal branches without numbers were represented by single individuals. Note that both phenograms are plotted on the same scale of mtDNA sequence divergence.

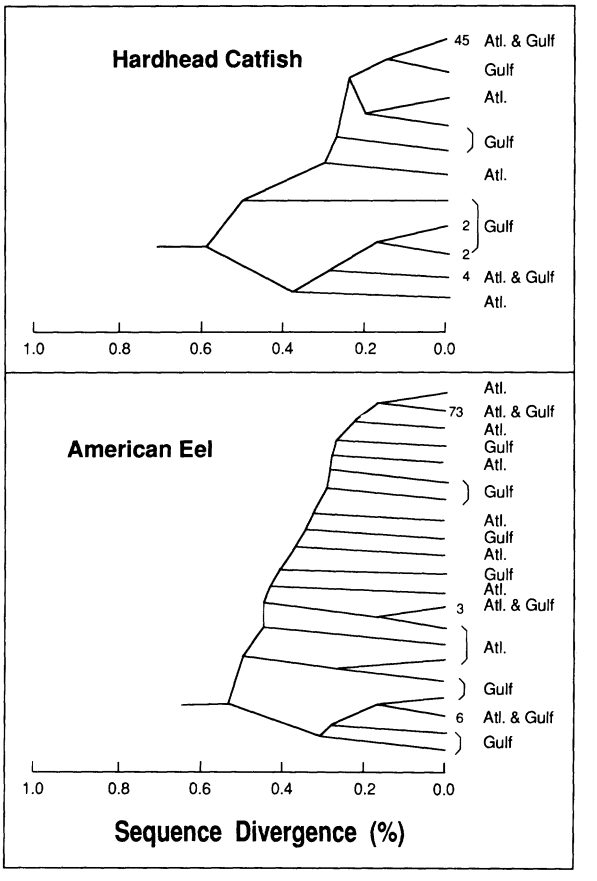

Fig. 4. UPGMA phenograms summarizing relationships among mtDNA haplotypes observed in the hardhead catfish and American eel. Numbers of individuals of various mtDNA clones are indicated to the right; terminal branches without numbers were represented by single individuals. Note that both phenograms are plotted on the same scale of mtDNA sequence divergence.

phylogeny treating the complex as a whole reveals two major mtDNA groups, again corresponding to Atlantic versus Gulf locales (Fig. 5).

In the menhaden and sturgeon, evidence supporting any putative Atlantic Gulf subdivision are more ambiguous. Two menhaden sibling species are currently recognized, *Brevoortia tyrannus* in the Atlantic and *B. patronus* in the Gulf. However, two well defined haplotype clusters in the mtDNA phenogram (Fig. 3) do not conform exactly to these two taxon assignments or geographic regions (although their representatives do differ significantly in frequency between these areas). Thus while one cluster appears confined to the Atlantic, the other contained numerous individuals collected from both Gulf and Atlantic locales. Perhaps there had indeed been an Atlantic-Gulf separation, but the two populations (or species) have not yet evolved to a status of reciprocal monophyly with respect to the mtDNA gene tree (see Avise 1986, Neigel and Avise 1986). However, since very closely related mtDNA haplotypes (identical or nearly identical at all assayed restriction sites) were observed in both Atlantic and Gulf locales (Fig. 3), recent gene flow between the two regions is also strongly implicated (Bowen and Avise 1990). In the

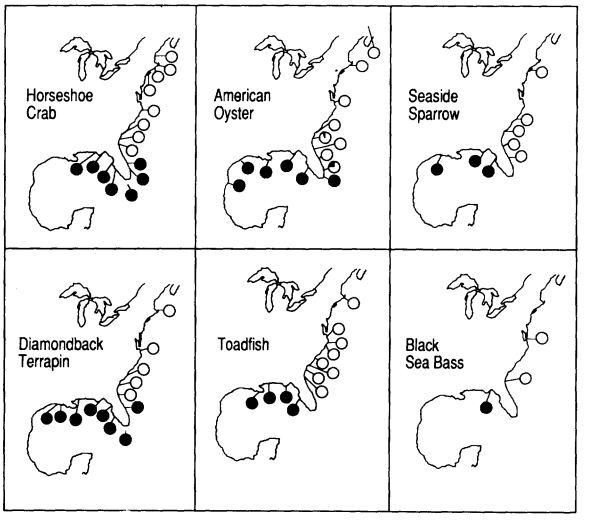

Fig. 5. Pie diagrams summarizing geographic distributions of the two fundamental mtDNA clades in various coastal and marine taxa (see Fig. 2 and text).

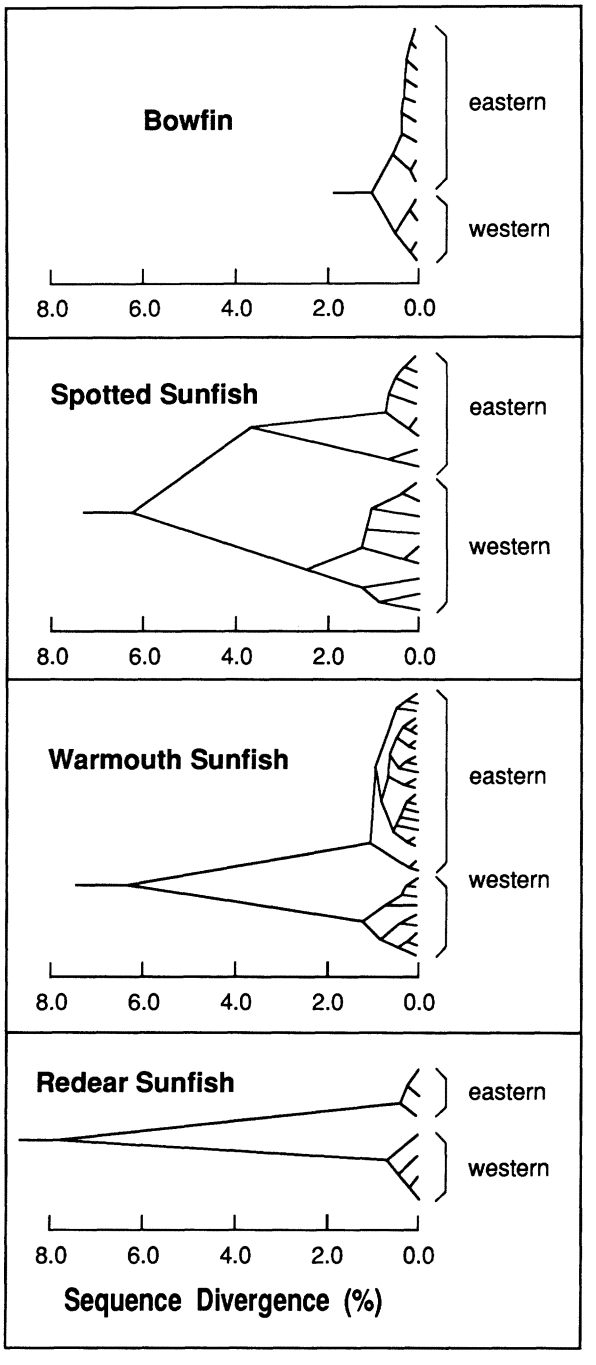

Fig. 6. UPGMA phenograms summarizing relationships among mtDNA haplotypes observed in the bowfin, spotted sunfish, warmouth sunfish, and redear sunfish. Note that all dendrograms are plotted on the same scale of mtDNA sequence divergence.

sturgeon, recognized Atlantic and Gulf coast subspecies (*Acipenser oxyrhynchus oxyrhynchus* and *A.o. desotoi*, respectively) again differed significantly in mtDNA haplotype frequencies. The most common genotype was observed along both coasts (Fig. 3), suggesting a recent historical connection between these populations.

Two species – the American eel and hardhead catfish – showed no evidence for an mtDNA phylogenetic subdivision between Atlantic and Gulf (Fig. 4). Results might reasonably be attributable to high effective gene flow between these areas. American eels presumably spawn in the western tropical mid-Atlantic ocean (the Sargasso Sea), and larvae disperse largely by passive transport to coastal areas where maturation occurs. Conventional wisdom has been that this life history pattern should result in a nearly random distribution of genotypes along the coast (Williams and Koehn 1984), a suggestion with which our mtDNA data are consistent (Avise et al. 1986). In the case of the hardhead catfish, adults are strong and active swimmers, and movement between the Atlantic and Gulf around south Florida may be considerable.

Overall, among the 10 coastal species or species-complexes surveyed (Table 2), at least five and as many as eight evidence a fundamental mtDNA subdivision involving Atlantic versus Gulf coast populations. Geographic distributions of the two major mtDNA lineages within each of six such taxa are summarized in Fig. 5.

A comparable degree of mtDNA phylogeographic concordance is exhibited among the surveyed freshwater fishes in the southeastern U.S.A. Genetic relationships among mtDNA haplotypes of bowfin and each of three sunfish species are summarized in Fig. 6. Each phenogram exhibits a fundamental split (also supported at levels greater than 95% by bootstrapping in Wagner parsimony networks – Bermingham and Avise 1986) distinguishing mtDNA lineages from eastern versus western portions of the respective species' ranges in the area. A similar pattern of mtDNA phylogeographic divergence also occurs in the bluegill sunfish, *Lepomis macrochirus* (although a full dendrogram could not be constructed because most individuals were surveyed only at selected "marker" restriction sites – see Avise et al. 1984), and in the mosquitofish (*Gambusia affinis* – *G.*

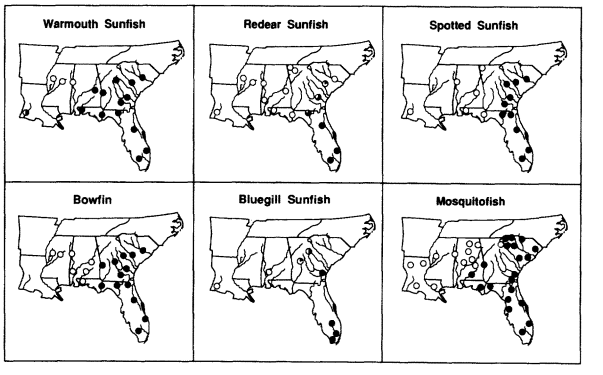

Fig. 7. Pie diagrams summarizing geographic distributions of the two fundamental mtDNA clades of various freshwater fishes (see Fig. 6 and text).

Table 3. Estimates of mtDNA sequence divergence and provisional times since separation (in millions of yr) between the western versus western assemblages of freshwater fishes, and between the Atlantic versus Gulf assemblages of those marine and coastal-restricted species whose populations clearly differ between regions.

Species	mtDNA sequence divergence		times[c] of pop. separation	
	uncorr.[a]	corr.[b]	uncorr.[a]	corr.[b]
Marine and coastal species (Atlantic vs Gulf)				
Horseshoe crab (*Limulus polyphemus*)	0.020	0.016	1.00	0.80
American oyster (*Crassostrea virginica*)	0.026	0.022	1.30	1.10
Black sea bass (*Centropristis striata*)	0.009	0.007	0.45	0.35
Toadfish (*Opsanus beta* and *O. tau*)	0.101	0.096	5.05	4.80
Diamondback terrapin (*Malaclemys terrapin*)	0.001	ca. 0.00	0.05	ca. 0.00
Seaside sparrow (*Ammodramus maritimus*)	0.011	0.010	0.55	0.50
Freshwater fishes (eastern vs western)				
Spotted sunfish (*Lepomis punctatus*)	0.062	0.044	3.10	2.20
Redear sunfish (*Lepomis microlophus*)	0.087	0.082	4.35	4.10
Warmouth sunfish (*Lepomis gulosus*)	0.063	0.056	3.15	2.80
Bluegill sunfish (*Lepomis macrochirus*)	0.085	d	4.25	d
Bowfin (*Amia calva*)	0.009	0.006	0.45	0.30

[a] Based on the mean genetic distance (p_{xy}) between mtDNA haplotypes of the two regions, x and y.
[b] Based on a correction for within-region polymorphism: $p_{corr.} = p_{xy} - 0.5\ (p_x + p_y)$, where p_x and p_y are the mean pairwise distances of mtDNA haplotypes within regions x and y, respectively.
[c] Based on a "conventional" mtDNA clock calibration reported in several vertebrate groups (1 percent sequence divergence per lineage per million years – Brown et al. 1979, Shields and Wilson 1987). However, caution is indicated because rates of mtDNA evolution have also been reported to differ in either direction from this estimate by several-fold (Moritz et al. 1987).
[d] Corrections could not be applied because only selected restriction sites were used in the geographic survey.

holbrooki sibling species complex – Scribner and Avise, unpubl.).

Geographic distributions of the two mtDNA major clonal lineages within these species or species complexes are summarized in Fig. 7. Exact positions of the geographic boundaries between clades appear to differ somewhat among species (appearing most aberrant in *Lepomis gulosus*), but typically occur near the Florida panhandle. Consistently, a stronghold of the "eastern" forms is peninsular Florida.

Other evidence further supports a fundamental phylogenetic distinction between eastern and western populations of many freshwater taxa in the region. In the largemouth bass, *Micropterus salmoides* (Philipp et al. 1981), bluegill sunfish (Avise and Smith 1974), and mosquitofish complex (Wooten et al. 1988), strong shifts in allozyme frequency at several loci reveal geographic patterns remarkably similar to those presented in Fig. 7. And as already mentioned, the Apalachicola region has been recognized as an important boundary between freshwater zoogeographic provinces as evidenced by clusters of species' distributional limits (Swift et al. 1985).

Overall, among the seven species or species-complexes of southeastern freshwater fishes genetically assayed to date, all exhibit a fundamental phylogeographic distinction of populations in eastern and Floridian drainages from those to the west. Such concordance in phylogeographic profiles across independent species strongly suggests a significant influence of historical biogeographic factors.

2) A uniform mtDNA "clock" may not exist for all species

Brown et al. (1979) first reported a high rate of mtDNA evolution – roughly 0.5–1.0% change in nucleotide sequence per lineage per million years – in primates. Similar rates were subsequently described for several other mammals, birds, and invertebrates (Wilson et al. 1985, Vawter and Brown 1986, Moritz et al. 1987, Shields and Wilson 1987). Nonetheless, the reliability and precision of molecular dating in evolution (through use of either mtDNA or nuclear DNA "clocks") remains highly controversial (e.g., Sibley and Ahlquist 1984, Britten 1986, Powell et al. 1986).

Application of a "conventional" mtDNA clock calibration (2% sequence divergence per million years between pairs of lineages) to the species considered in this report yields the estimated divergence times presented in Table 3. Among the marine and coastal species, the Gulf-Atlantic mtDNA lineage separations range from about 50 000 to 5 000 000 years before present (bp); among the freshwater fishes, the east-west mtDNA lineage separations range from 450 000 to 4 350 000 bp. Since mtDNA lineage separations can vastly predate population separations, particularly when effective population sizes are large, corrections for within-region polymorphism were also applied (Table 3, rightmost column). After such corrections, large differences remain in the estimated times of population divergence (100-fold and 10-fold among the various marine and freshwater species, respectively).

Thus while all of the phylogenetic separations appear

Table 4. Various comparative estimates of the geographic structure of mtDNA haplotypes within regional populations[1]. Freshwater and marine/coastal species are listed in rank order with respect to degree of local population structure as reflected in a "localization index" (the proportion of mtDNA haplotypes observed in multiple individuals, yet confined to either a single collection locale or to two adjacent locales). Also presented are relative estimates of *Nm*, calculated (with corrections for sample size) from estimated frequencies of: (a) all "private" haplotypes; and (b) only those "private" haplotypes present in more than one specimen. See text for further explanation and qualifications.

Species	Total no. haplotypes	"Private" haplotypes, confined to...			Localization index		*Nm*	
		1 ind.	>1 ind., 1 locale	>1 ind., 2 adj. locales	fraction	%	(a)	(b)
Warmouth sunfish	32	17	10	2	12/15	80	0.48	0.18
Bowfin	13	3	5	3	8/10	80	0.13	0.13
Spotted sunfish	17	9	5	1	6/8	75	0.33	0.08
Redear sunfish	7	2	1	0	1/5	20	0.18	0.04
Total (freshwater fish):					27/38	71	0.28	0.11
Toadfish (*beta* and *tau*)	13	8	1	2	3/5	60	0.30	0.10
American oyster	82	68	4	4	8/14	57	1.88	2.14
Hardhead catfish	11	7	1	1	2/4	50	0.33	0.08
Horseshoe crab	10	4	0	2	2/6	33	0.49	0.15
Atlantic sturgeon	6	3	1	0	1/3	33	0.42	0.17
Seaside sparrow	11	9	0	0	0/2	00	1.15	>>1
Menhaden (*tyrannus* and *patronus*)	33	31	0	0	0/2	00	2.14	>>1
American eel	21	18	0	0	0/3	00	3.04	>>1
Total (marine/coastal taxa):					16/39	41	1.22	–

[1] Black sea bass are not included in this table because too few locales were sampled; diamondback terrapins because no variant haplotypes within the Atlantic or Gulf were observed in multiple individuals; and mosquitofish because the sampling is still in progress.

to date to the late Pliocene or Pleistocene, particular times for the various species span a large range. Assuming that the sequence divergence estimates are reasonably accurate, two explanations for these discrepancies appear most likely: either (1) mtDNA evolution exhibits considerable rate heterogeneity among taxa; and/or (2) the dates of particular vicariant or dispersal events influencing population separations differed among the various species. We have some independent evidence (presented elsewhere – Avise et al., unpubl.) for the first possibility – that of mtDNA rate differences among certain major taxa. However, due to the cyclical nature of the climatic and geologic changes postulated to have influenced faunas in the southeastern U.S.A. (see above), the latter possibility may also be a major contributor to the large range of inferred separation times. Multiple episodes of glacial advance and retreat likely provided repeated opportunities for population isolation (and perhaps later coalescense), such that the times of the regional population disjunctions may truly differ among the assayed species.

3) Shallow phylogenetic structures are also evident within regions, and may be related to species-specific gene flow regimes

In addition to the deep mtDNA phylogenetic separations between regional populations, which are probably tied to vicariant Pleistocene events, many assayed taxa in the southeastern U.S.A. also show evidence of "shallower" within-region structures. These are evidenced most clearly by apparent geographic localizations of less common (and often presumably derived) mtDNA genotypes. For example, among 59 bowfin collected from 10 drainages in the eastern portion of the species' range (Fig. 7), mtDNA haplotype "2" was observed only in 4 specimens from the Cooper River, haplotype "3" only in 3 individuals from the adjacent Savannah and Ogeechee Rivers, and so on for each of 3 other mtDNA haplotypes (Bermingham and Avise 1986). Only haplotype "1" occurred throughout nearly all eastern drainages, and by several lines of evidence it represents the ancestral condition from which the localized genotypes appear to have arisen independently by one or two assayed restriction site changes (Bermingham and Avise 1986, Avise et al. 1987a, b).

"Private" mtDNA haplotypes, defined here as genotypes observed in only one (or two immediately adjacent) locales, were observed in most of the assayed species (Table 4). Because of the high genetic diversities within some species, these private haplotypes were further categorized as those present in a single specimen, versus those shared by two or more individuals. "Localization indices" (the observed numbers of the latter class of private haplotypes, expressed as fractions of the total numbers of haplotypes distinguished within species) ranged from 0% in American eel, menhaden, and

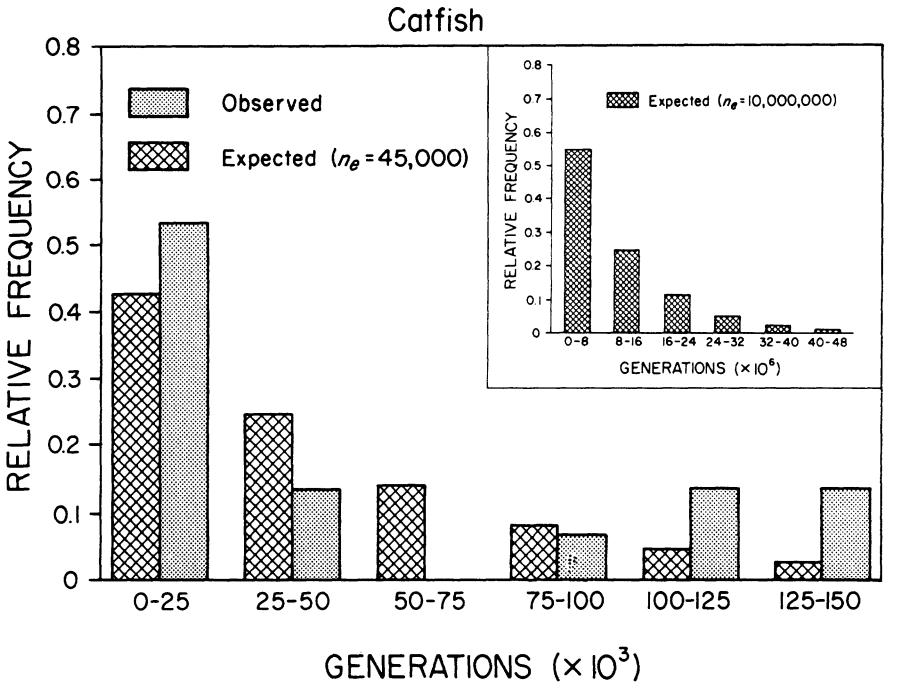

Fig. 8. Frequency distributions of times to shared ancestry of mtDNA haplotypes in the hardhead catfish. Expected distributions generated from inbreeding theory (see text) are shown for each of two values of $N_{f(e)}$: 10 000 000 (a conservative guess for the current breeding population of female catfish – inset), and 45 000 (a value which yields a mean expected divergence time equal to that inferred from the mtDNA data). The observed times were derived from the data of Avise et al. (1987b), using a conventional "clock" calibration (2% sequence divergence per million yr) and a generation length of 2 yr. Note the difference in scale along the abscissas of the inset and main graph.

seaside sparrow, to 80% in warmouth sunfish and bowfin (Table 4).

Slatkin (1985) proposed a method for estimating average levels of gene flow in a subdivided population using observed mean frequencies of private alleles within samples. The approach is based on comparisons of data with results of computer simulations, and yields estimates of *Nm*, where *N* is the local population size and *m* is the migration rate between populations. In theory, values of $Nm >> 1$ indicate high gene flow between sub-populations (such that only limited genetic divergence is expected, and frequencies of private alleles are low), while values of $Nm << 1$ indicate low gene flow (such that population structure is strong, and private alleles can sometimes reach appreciable frequency). Table 4 presents values of *Nm* (calculated by Eq. (3) in Slatkin (1985) and corrected for sample size) estimated from frequencies of: (a) all private alleles; and (b) that subset of private alleles possessed by more than 1 individual. Values range from 0.13 in bowfin to 3.04 in American eels (Table 4). The latter value is probably a severe underestimate, since all of the genotypes present in more than one eel were geographically widespread, and the *Nm* calculation was thus based solely on private alleles confined to single specimens.

Two general conclusions emerge from comparisons of these localization indices and *Nm* values. First, many species show evidence of local substructuring within the major phylogeographic regions identified previously. Second, the relative magnitudes of local population structure appear plausibly related to probable gene flow regimes of several of the species. For example, in comparison to the assayed marine/coastal taxa, the freshwater fishes tended to exhibit higher localization indices (71% versus 41%; $\chi^2 = 7.0$, $P < 0.01$) and lower mean values of *Nm* (e.g., 0.28 versus 1.22 – Table 4), as might be predicted from the more isolated nature of disjunct freshwater habitats. Furthermore, among the marine/coastal species, the highest estimated *Nm* (and a zero localization index) occurred in the American eel, a species whose catadromous life history pattern probably involves very high effective dispersal throughout the North American coastline (Avise et al. 1986). Conversely, the highest localization index occurred in the toadfish, which lay demersal eggs and are sluggish bottom dwellers.

Nonetheless, these current estimates of the magnitude of local population structure should be interpreted with considerable caution. First, the estimates apply only to female lineages, and in some species such as the American oyster, much less structure is apparent in nuclear-encoded allozymes (Reeb and Avise 1990). The reasons for such differences are unknown. Second, since our primary goal in most surveys was to assess

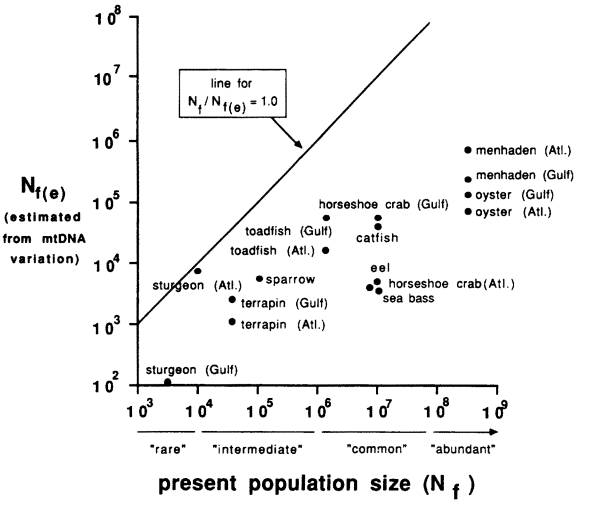

Fig. 9. Relationship between current-day census sizes (see Table 1) of assayed marine species in the southeastern U. S., and evolutionary effective sizes estimated from mtDNA haplotype diversities (Table 2). Note that both axes are in log scale. The correlation coefficient calculated between $N_{f(e)}$ and N_f is $r = 0.66$. The methods and caveats for estimating $N_{f(e)}$ and N_f are discussed in the text and Table 1.

phylogeographic structure on a broad scale, mtDNA sample sizes at particular sites were typically small, and actual frequencies of local haplotypes poorly assessed. Third, mtDNA genotypic diversities were so high in some species (Table 2) that few or no haplotypes were common. These factors greatly limit applicability of other conventional approaches to estimate gene flow (such as F_{st} – Slatkin 1987), unless the mtDNA haplotypes could be grouped reliably into more inclusive classes by the criterion of evolutionary relatedness. However, apart from the major phylogeographic disjunctions between regions, which received strong statistical support by bootstrapping in parsimony networks, most of the putative mtDNA clades within regions (Figs 2–4, 6) remain poorly defined.

Thus overall, while the available mtDNA data suggest significant phylogeographic population substructure within regions for most species, larger sample sizes and stronger characterizations of putative mtDNA micro-clades will be required for definitive conclusions about female mediated levels of historical and contemporary gene flow on local scales.

4) Evolutionary effective population sizes estimated from mtDNA diversities are correlated with, but much lower than, current-day census sizes

Using inbreeding theory as applied to neutral alleles inherited maternally, theoretical probability distributions of times to shared mtDNA haplotype ancestry can be generated as a function of the evolutionary effective size of a female population ($N_{f(e)}$). In particular, the probability that random pairs of extant mtDNA haplotypes derive from a common ancestor that existed G generations prior is given approximately by

$$f(G) \cong (1/N_{f(e)})\ e^{-(G-1)/N_{f(e)}} \quad (1)$$

(Avise et al. 1988). The geometric distributions described by Eq. (1) have mean $N_{f(e)}$ (and variance $N_{f(e)}(N_{f(e)}\text{-}1)$). For any population, or entire species with high gene flow, empirical distributions of genetic distance among mtDNA haplotype pairs (converted to sidereal time using a presumed rate of mtDNA evolution and the suspected generation length of a species) may be generated and compared to such theoretical expectations (but see Ball et al. 1990 for qualifications).

One example of such comparisons is presented in Fig. 8. In the hardhead catfish, mean mtDNA sequence divergence between assayed individuals was $p = 0.0018$ (Table 2). Under a conventional mtDNA evolutionary rate of 2% sequence divergence per million years, this value translates into an estimated mean time of mtDNA haplotype separation of about 90 000 years, or 45 000 catfish generations (assuming a generation length of 2 years). Fig. 8 shows that the agreement between the observed and expected frequency distributions of times to shared haplotype ancestry is reasonably good for $N_{f(e)} = 45\,000$. For the hardhead caffish, this estimate of evolutionary effective population size is vastly lower (by more than 200-fold – see inset to Fig. 8) than the present-day size of the female population (N_f), which conservatively might include 10 000 000 individuals (Avise et al. 1988). To the extent that any local population structure exists within the species (Table 4), and may have buffered some mtDNA lineages against extinction (Avise et al. 1984), the disparity between N_f and $N_{f(e)}$ becomes even more dramatic.

In general, evolutionary effective population sizes estimated from mtDNA genetic distances are usually vastly smaller than present-day census sizes. Using the data presented in Tables 1 and 2, Fig. 9 summarizes the relationship between N_f and $N_{f(e)}$for the 10 marine and coastal species considered in this paper. Since Gulf versus Atlantic populations of many of the assayed species exhibit sharp mtDNA phylogenetic distinctions, calculations were conducted separately for these regional populations. Overall, among the 18 comparisons attempted, $N_{f(e)}$ was consistently lower than N_f, usually by 1–3 orders of magnitude. Nonetheless, a reasonably strong correlation ($r = 0.66$) between $N_{f(e)}$ and N_f across species was also evident (Fig. 9).

Possible explanations for the discrepancy in magnitude between $N_{f(e)}$ and N_f include: (1) a much slower pace of mtDNA evolution than is generally accepted; or (2) periodic decreases in the numbers of females through which surviving mtDNA lineages have been transmitted. The latter could be due either to: (2a) general historical demographic considerations, such as large variances in progeny survival among females, fluc-

tuations and relative bottlenecks in female population size, and periodic extinctions (and subsequent recolonizations) of local demes; or, (2b) the occasional appearance of selectively advantageous mtDNA variants, which might sweep through populations and"cleanse" the non-recombining mtDNA genome of much preexisting genetic variability via hitchhiking of neutral markers to the selected mutations.

By hard criteria, we cannot decide between these competing possibilities on the basis of existing mtDNA variability alone. However, similar reductions of $N_{(e)}$ relative to N have been reported previously (in many other species) on the basis of allozyme variation (Nei and Graur 1984). Thus whatever processes are involved (rate decelerations, positive directional selection at specific loci, and/or general demographic factors) probably apply to nuclear genes as well. The observed correlation between $N_{f(e)}$ and N_f (Fig. 9) is not predicted under the hypothesis of a deceleration in mtDNA evolutionary rate in particular species, nor by occasional positive selection, but it is generally consistent with historical demographic influences such as proportional fluctuations in population size.

If mtDNA variabilities do indeed reflect historical demographic conditions primarily, rather than idiosyncratic episodes of selection directed at mtDNA per se, they may provide useful evidence on comparative levels of overall genetic variation among species, or among stocks under management. For example, among the 18 estimates summarized in Table 2 and Fig. 9, the sturgeon population in the Gulf (which is currently very small) had the lowest estimated mtDNA variability and $N_{f(e)}$, while the abundant menhaden and oysters had the highest such values. Several authors have suggested that magnitude of genetic variability may significantly influence the probability of a population's survival over ecological or evolutionary time, and that reliable assessments of genome-wide variation can be based on samples of various molecular genetic characters (e.g., O'Brien and Evermann 1988, Quattro and Vrijenhoek 1989, Vrijenhoek et al. 1985, Wildt et al. 1987, but see also Lande 1988). The correlation of mtDNA diversity with population size (Fig. 9) suggests that mtDNA variation may reflect demographic conditions whose influence should extend to nuclear loci as well.

Conclusions and relevance to conservation biology

These molecular-based perspectives on biogenetic diversity carry, I believe, several implications for the fields of conservation biology and population management. It is now abundantly clear that most species should not be viewed as monotypic entities, but rather as a series of geographically differing populations with a hierarchical and sometimes deep genetic and historic structure. Conservation of this geographic and historical diversity of gene pools may be facilitated by the following management guidelines:

1) Limiting unnecessary transplantations

While many biologists now recognize that introductions of exotic species can cause irreparable harm to regional biodiversity by forcing extinctions of native species, they have been slower to appreciate the problems that can stem from transplantation and mixing of genetic "stocks" within species. Indeed, many public and private management agencies actively promote geographic transplantations from one area to another within a species range, for purposes such as bolstering local population sizes, introducing "desirable" genetic traits into a region, or increasing local heterozygosity. Unfortunately, several undesirable consequences may also arise from such geographic transplantations, including: (a) the possibility of disease or parasite spread; (b) the irretrievable loss of the rich historical genetic records of populations (an analogy in the field of anthropology might be the uncontrolled mixing of artifacts between archaeological sites); and (c) inevitable erosion of the overall genetic diversity within a species, much of which we now recognize to be generated and maintained by geographic isolation. Transplantations may sometimes be justified, as in the case of reintroduction of a native species to its former range where it had been extirpated by man. However, I suggest that in general, the burden of proof in any proposed transplantation program should rest on advocates of this strategy, rather than on those who would question the desirability of transplantations on the grounds cited above.

2) Taxonomic reassessments

Taxonomic assignments inevitably shape perceptions of biological diversity. Therefore, it is disconcerting that many subspecies and species descriptions trace to very limited information, often gathered in the last century, on the distributions of a small number of (usually morphological) traits with unknown genetic basis. Yet once a Latin binomial or trinomial is in the literature, the group of organisms to which it refers almost automatically assumes an aura of reality that may or may not be commensurate with its true evolutionary distinctiveness. Given the overriding importance of taxonomy on biodiversity recognition and management, increased attention should be devoted to taxonomic assessments (from molecular as well as other data). Elsewhere, I have summarized case histories in which existing species or subspecies taxonomies provided poor management guidelines in endangered species programs (Avise 1989b). Population managers should therefore keep an open mind regarding taxonomic realignments, particularly for populations within that vast majority of species that have remained poorly studied.

3) Regional reserves

The job of providing complete molecular genetic or other evaluations of all species that will be threatened with extinction in the next century is daunting. In the absence of such complete information, important actions nonetheless may be taken. For example, molecular data on the fauna of the southeastern U.S.A. suggest that particular areas are geographic centers for a substantial fraction of regional, intraspecific biogenetic diversity. These include the Florida peninsula in the freshwater and terrestrial biotic realm, and the Gulf of Mexico in the marine realm. These provinces, and their boundaries with western and Atlantic provinces, respectively, have also been recognized previously by a different type of biogeographic data – concentrations of species' distributional limits (Avise et al. 1987a, b). Such concordant lines of evidence for these centers of biodiversity already give ample support for special efforts to preserve the integrity of these regional faunas. Such efforts might include the designation of regional biological preserves, as well as imposition of severe limitations on the artificial transplantations of populations or species from one biogeographic region to another. While such regional perspectives on genetic diversity cannot hope to capture the idiosyncratic population structures and subdivisions of all species, they should provide general guidelines to managers, particularly as natural environments continue to decline and decisions of conservation "triage" become inevitable.

In conclusion, the phylogenetic content of mtDNA sequences has proved particularly useful in assessing evolutionary differentiation and historical demography within and among populations of a species. The remarkable phylogeographic concordance among numerous freshwater fishes and marine/coastal taxa in the southeastern U.S.A. exemplifies the utility of this molecular approach in identifying major evolutionary genetic stocks among the conspecific populations of a regional fauna. Additional phylogeographic substructures within regional populations indicate more recent restrictions to gene flow, and can be related tentatively to species-specific life history patterns and dispersal characteristics. By adding a phylogenetic perspective to species demographies and population structures, data from mtDNA and other molecular methods can contribute to a deeper understanding and appreciation of gene pool diversity.

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