Mitochondrial DNA Diversity and Population Structure in Marine Fish Species from the Gulf of Mexico

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Variation in mitochondrial DNA (mtDNA) was examined among 693 red drum (*Sciaenops ocellatus*), 300 black drum (*Pogonias cromis*), and 421 red snapper (*Lutjanus campechanus*) sampled from several localities in the Gulf of Mexico. The number of mtDNA genotypes (haplotypes) observed were: 99 in red drum, 37 in black drum, and 68 in red snapper. Variation in mtDNA haplotype frequencies among localities in all three species was not significant, although two mtDNA haplotypes in black drum appeared to be clinally distributed. Maximum-parsimony analysis and phenetic clustering of mtDNA haplotypes and of samples in each species revealed little evidence of phylogeographic structuring. These data indicate that gene flow among localities in each species is sufficient to preclude genetic divergence. Spatial autocorrelation analysis of mtDNA haplotype frequencies revealed an isolation-by-distance effect in red drum and black drum, and indicated that migration between neighboring estuaries or bays in black drum may be less frequent than in red drum. Spatial autocorrelations in red snapper were negative in all distance classes, suggesting little migration even between adjacent localities. Differences in intrapopulational mtDNA diversities were found in all three species, suggesting that geographic differences in effective female population size may occur within each species.

Les variations de l'ADNmt (ADN mitochondrial) on été examinées chez 693 tambours ocellés (*Sciaenops ocellatus*), 300 grands tambours (*Pogonias cromis*) et 421 vivaneaux (*Lutjanus campechanus*) échantillonnés dans plusieurs localités du golfe du Mexique. Le nombre de génotypes d'ADNmt (haplotypes) observé dans chacune des espèces a été le suivant : 99 dans tambours ocellés, 37 dans grands tambours et 68 dans vivaneaux. La variation des fréquences d'haplotypes d'ADNmt entre les localités chez les trois espèces n'était pas statistiquement significative, bien que deux haplotypes observés chez le grand tambour aient semblé présenter une variation clinale. L'analyse du maximum de parcimonie et le regroupement phénétique des haplotypes d'ADNmt et d'échantillons de chacune des espèces n'ont donné que peu d'indices de structure phylogéographique. Ces données indique que le flux génique entre les localités dans chacune des espèces est suffisant pour écarter la divergence génétique. L'analyse d'autocorrélation spatiale des fréquences des haplotypes d'ADNmt a révélé un effet d'isolement par la distance chez le tambour ocellé et le grand tambour, et indiqué que la migration entre les baies ou les estuaires voisins chez le grand tambour peut être moins fréquente que chez le tambour ocellé. Les autocorrélations spatiales chez le vivaneau étaient négatives pour toutes les classes de distance, ce qui indiquerait qu'il y aurait peu de migration entre les diverses localités. Des différences touchant la diversité intrapopulation de l'ADNmt ont été observées dans le cas des trois espèces, ce qui donnerait à entendre qu'il existerait des différences géographiques en ce qui a trait à la taille réelle de la population de femelles dans chacune des espèces.

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Over the past few years, we have carried out studies of spatial and temporal genetic variation among several economically important marine fish species from the northern Gulf of Mexico (Gulf). The central objective of the research has been to determine whether each species is subdivided into discrete subpopulations or stocks. The need for information on stock structure relates to resource allocations and development of fishery regulations on a regional basis. Should separate stocks exist, fishery units could be managed regionally, providing opportunities to adjust regulations to unique needs of subpopulations and resource users. Alternatively, should only a large, single stock exist, management plans could be based on the premise that policies in one region might significantly impact resources in an adjacent region. A second objective of the research has been to determine whether levels of genetic variation differ within the species studied. Information on genetic variation is of interest relative to the concept that levels of genome-wide variation affect probabilities of population survival and fitness (Soulé 1980; Frankel and Soulé 1981).

Most of our studies have employed restriction enzyme site variation in mitochondrial DNA (mtDNA). Briefly, mtDNA in vertebrates is a compact, physically circular, genetically haploid molecule that is uniclonally inherited through the maternal parent (Brown 1983; Avise 1986; Wilson et al. 1985). MtDNA sequence variants are fairly easy to identify, and importantly, do not segregate and recombine during sexual reproduction. This means that effective

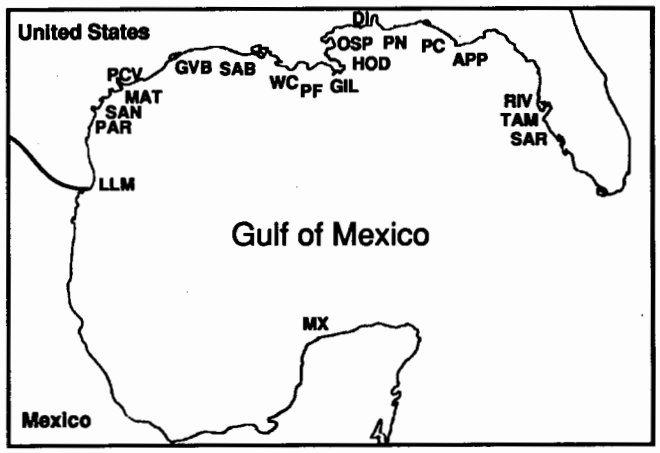

FIG. 1. Localities in the Gulf of Mexico where samples of red drum, black drum, and red snapper were procured. Acronyms are defined in Table 1.

population sizes required to measure genetic impact of population substructuring and gene flow should be at least four times smaller for mtDNA than for nuclear genes (Birky et al. 1983; Templeton 1987). Finally, vertebrate mtDNA appears to have a rapid rate of DNA sequence evolution (Brown 1983; Wilson et al. 1985). The rapid rate of mtDNA sequence change is important relative to identifying discrete subpopulations of recent origin.

In this paper, we summarize studies on geographic variation in mtDNA among three species: red drum (*Sciaenops ocellatus*), black drum (*Pogonias cromis*), and red snapper (*Lutjanus campechanus*). Brief accounts of the biology of the three species, taken primarily from Manooch (1984), Shipp (1986), and Hoese and Moore (1977), are given below. The two drum species are the largest members of the family Sciaenidae and are distributed in coastal and estuarine waters of the western Atlantic from New England through the Gulf to northern Mexico (red drum) or the Yucatan Peninsula (black drum). Both are estuarine dependent as juveniles and move offshore after 1–2 yr (black drum) or 2–3 yr (red drum). Both species are long-lived, although red drum become sexually mature at ages 3–4, whereas black drum become sexually mature at ages 5–6 (Murphy and Taylor 1989; C. Wilson, Coastal Fisheries Institute, Louisiana State University, Baton Rouge, personal communication). Both species form large, offshore schools that can migrate extensively (Matlock 1984, 1987; Swingle et al. 1984; C. Wilson, personal communication). Mass spawning in both species occurs near passes, inlets, river mouths, or bays. Tagging studies in both species have indicated that movement of juveniles among nearshore localities is limited (Matlock and Weaver 1979; Osburn and Matlock 1984; Osburn et al. 1982). No data exist on whether adults return to an area for repeated spawning. Red snapper are the most economically valuable member of the family Lutjanidae and occur in the western Atlantic from North Carolina through the Gulf to the Yucatan Peninsula. Adult red snapper are considered to be sedentary, nonmigratory, and typically associated with offshore, low- and high-relief hard bottoms (Bradley and Bryan 1974; Beaumariage and Bullock 1976). Tagging studies (Fable 1980) indicate that adult movement is limited. Spawning occurs offshore, and eggs and larvae are pelagic. Juveniles are generally found over mud–sand bottoms (Bradley and Bryan 1974; Beaumariage and Bullock 1976).

The primary purposes of this paper are to (1) demonstrate the approaches used to determine whether genetic variation in these species is spatially partitioned, and (2) illustrate that levels and patterns of mtDNA variation differ both within and among species. MtDNA data from red drum are published (Gold and Richardson 1991; Gold et al. 1993), as are part of the mtDNA data from red snapper (Camper et al. 1993).

Materials and Methods

Samples of each species were procured from several localities in the northern Gulf (Fig 1, Table 1) between 1987 and 1991. Tissues were removed from each specimen, frozen in liquid nitrogen, and stored at −80°C. Red drum and black drum were caught in nearshore estuaries and bays using gill and trammel nets, haul seines, and hook and line. Red snapper were caught by hook and line over offshore, hard-bottom substrates. Ages of all but yearling (age zero) red drum (i.e., individuals <300 mm total length) were determined from annuli on otoliths using methods described in Bumguardner (1991). Ages for black drum were not obtained. Otoliths from red snappers are currently under examination by personnel of the Texas Parks and Wildlife Department.

Details regarding assay of individual fish may be found in Gold and Richardson (1991). Our methods employ extraction of genomic DNA with phenol and chloroform, single digestions with (six-base) restriction endonucleases, electrophoresis in 0.8% agarose gels, Southern transfer to nylon membranes, hybridization with mtDNA probes labelled with ^{32}P-dCTP and/or ^{32}P-dATP (3000 Ci/mM) by random priming, and autoradiography. MtDNA fragments are sized by fitting migration distances to a least-squares regression line of lambda DNA-*Hin*dIII fragment migration distances. Restriction enzymes used for each species were: red drum (*Bam*HI, *Bcl*I, *Eco*RV, *Hin*dIII, *Nco*I, *Nsi*I, *Pst*I, *Pvu*II, *Sca*I, *Spe*I, *Stu*I, *Xba*I, and *Xmn*I); black drum (*Apa*I, *Bgl*II, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Nco*I, *Nhe*I, *Pst*I, *Pvu*II, *Sca*I, *Spe*I, *Ssp*I, *Stu*I, *Xba*I, and *Xmn*I); and red snapper (*Apa*I, *Bcl*I, *Dra*I, *Hin*dIII, *Hpa*I, *Nco*I, *Nhe*I, *Pvu*II, *Sca*I, *Sma*I, *Sst*I, *Stu*I, and *Xba*I). Probes used included a 9.0–9.2 kilobase (kb) fragment of red drum mtDNA inserted into a pTZ-18R plasmid (red drum and black drum) and the entire red snapper mtDNA molecule inserted into bacteriophage λ-DASHII (red snapper). All restriction sites detected in each species have been mapped using single and double digestions (Fig. 2).

Analysis of mtDNA data was facilitated by the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992). Homogeneity of mtDNA haplotype frequencies among geographic samples in each species was tested using (1) log likelihood (G) tests (Sokal and Rohlf 1969), (2) a Monte Carlo randomization (bootstrap) procedure (Roff and Bentzen 1989), and (3) V tests using arcsine, square-root transformed haplotype frequencies (DeSalle et al. 1987). V tests were carried out only on haplotypes found in four or more individuals in each species. Significance levels for multiple tests performed simultaneously were adjusted after Cooper (1968). F_{ST} values (a measure of the variance in mtDNA haplotype frequencies) were calculated using formulae in Weir and Cockerham (1984) and computer programs described in Weir (1990). Estimates of gene flow ($N_e m_f$), the effective

TABLE 1. Locations and number of individuals of three species sampled from the Gulf of Mexico. Acronyms for localities are as used in text and figures.

Locality	Red drum	Black drum	Red snapper†
Mexico			
Merida (MX)	—	—	44
Texas			
Lower Laguna Madre (LLM)	39	52	52
Redfish Bay (PAR)	38	—	60
San Antonio Bay (SAN)	—	53	—
Matagorda Bay (MAT)	—	11	—
Pass Cavallo (PCV)	31	—	—
Galveston Bay (GVB)	68	86	47
Sabine Pass (SAB)	43	—	—
Louisiana			
West Cameron (WC)	—	—	54
Port Fourchon (PF)	—	—	36
Grand Isle (GIL)	90	34	—
Black Bay (HOD)	20	—	—
Mississippi			
Biloxi Bay (OSP)	117	40	—
Alabama			
Dauphin Island (DI)	—	—	53
Florida			
Pensacola (PN)	—	—	25
Panama City (PC)	—	—	50
Apalachicola Bay (APP)	67	13	—
Riviera Bay (RIV)	69	—	—
Tampa Bay (TAM)	—	11	—
Sarasota Bay (SAR)	111	—	—
Totals	693	300	421

†Red snapper were obtained offshore from individual localities

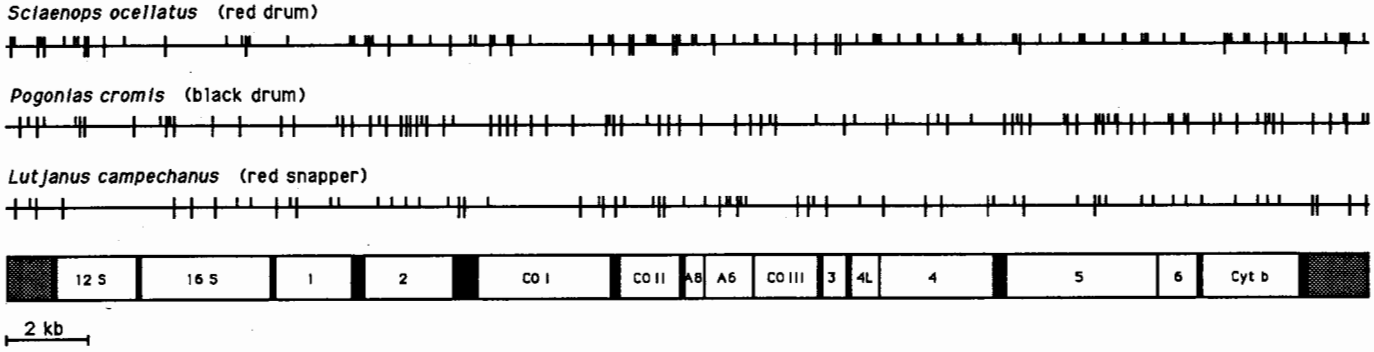

FIG. 2. MtDNA restriction enzyme maps of red drum, black drum, and red snapper. Restriction sites below the line were found in all individuals surveyed; those above the line were polymorphic. Maps are oriented to the human mtDNA gene map. Procedures used to orient maps are given in Schmidt and Gold (1992).

number of female migrants per generation, were calculated using Wright's (1943) island model modified for mtDNA, where $F_{ST} \approx 1/(2N_e m_f + 1)$.

Restriction-site presence–absence (binary) matrices for estimation of nucleotide sequence divergence and for phenetic and parsimony analyses were constructed using individual mtDNA haplotypes and individual sample localities (samples) as operational units. In the latter, restriction sites were scored as present (code 1) if they occurred within a sample, or absent (code 0) if they did not. Nucleotide sequence divergence among mtDNA haplotypes was estimated using restriction sites (Nei and Li 1979); nucleotide sequence divergence among samples (interpopulational divergence) was generated after Nei and Tajima (1981). Phenetic analysis of matrices of nucleotide sequence divergence among haplotypes and among samples was carried out using UPGMA (unweighted pair-group method using arithmetic averages) clustering (Sneath and Sokal 1973). For red drum, year-classes at each locality (sample) were pooled, because homogeneity tests revealed no significant differences between year-classes at any locality. Standard errors for nodes in phenograms were calculated after Nei et al. (1985). Maximum-parsimony analysis of restriction site presence–absence matrices representing all haplotypes and all samples in each species employed MULPARS and CONTREE options in version 3.0s of the Phylogenetic Analysis Using Parsimony (PAUP) program of Swofford (1991). All autapomorphic and symplesiomorphic characters were removed prior to PAUP runs. The strength of nodes produced from maximum-parsimony analysis was assessed by bootstrapping (Felsenstein 1985)

TABLE 2. Summary of mtDNA variation.

Parameter	Red drum	Black drum	Red snapper
Number of individuals	693	300	421
Number of mtDNA restriction sites	104	85	91
Number of mtDNA haplotypes	99	37	68
Nucleon diversity	0.95	0.78	0.75
Nucleotide sequence divergence among haplotypes (%)	0.88	0.48	0.50

TABLE 3. Results of tests for spatial homogeneity in mtDNA haplotype frequencies among localities in the Gulf of Mexico. F_{ST} is a measure of variance in mtDNA haplotype frequencies. $N_e m_f$ is effective number of female migrants per generation.

Test group	No. of localities	No. of haplotypes tested	No. of significant V tests	P[b]	Results of G-tests (P)	F_{ST}	$N_e m_f$
Red drum (86)[†]	11	24	1[a]	0.032	>0.05	−0.002	>10
Red drum (87)[†]	10	20	1[a]	0.408	≈0.01	0.008	>10
Black drum	8	9	3[a]	0.161	≈0.02	0.011	>10
Red snapper (90)[†]	3	3	0	0.851	>0.05	0.000	>10
Red snapper (91)[†]	7	10	0	0.224	>0.05	−0.001	>10

[†]Red drum from the 1986 and 1987 year-classes (determined from annuli on otoliths); red snapper sampled in 1990 and 1991.
[a]Nonsignificant when corrected for multiple tests.
[b]Probability based on 1000 bootstrap replications (after Roff and Bentzen 1989).

using two different random-number seeds and up to 500 replacements. Minimum-length parsimony networks of mtDNA haplotypes were constructed by connecting composite haplotypes in increments of single site gains or losses.

Spatial autocorrelation analysis of frequencies of common mtDNA haplotypes were used in each species to determine whether haplotype frequencies at any sample locality were independent of haplotype frequencies at neighboring sample localities. The analysis was carried out using the Spatial Autocorrelation Analysis Program (SAAP) of Wartenberg (1989), and involved computation of autocorrelation coefficients (Moran's I values) as a function of geographic distance between pairs of localities. Correlograms were used to summarize patterns of geographic variation exhibited by the response surface (geographic distance) of any given variable (mtDNA haplotype frequencies). To minimize "noise" generated by low frequency haplotypes, autocorrelation coefficients were generated from reduced data sets: in red and black drum, only haplotypes found in 10 or more individuals were used (17 haplotypes in red drum and five haplotypes in black drum); in red snapper, only haplotypes found in more than six individuals were used (10 haplotypes). Two SAAP runs were carried out for each species: the first employed equal numbers of pairwise comparisons in each distance class; the second employed equal geographic distances between distance classes. Distance classes were generated by SAAP from input latitude and longitude of each sample locality.

Nucleon diversities (the probability that any two individuals drawn at random will differ in mtDNA haplotype) were calculated after Nei and Tajima (1981) and were based on the total number of mtDNA haplotypes identified. Intrapopulational nucleotide sequence diversities (the average nucleotide difference between any two individuals drawn at random) were estimated after Nei and Tajima (1981) using restriction sites (Nei and Li 1979) for all sample localities in each species.

Results

On average, red drum are the most variable in mtDNA haplotype, and individual mtDNA haplotypes in red drum are more different from one another than are individual haplotypes in black drum and red snapper (Table 2). Based on the conventional rate of evolution for vertebrate mtDNA of 0.01 substitutions·bp^{-1}·lineage^{-1}·1 000 000 yr^{-1} (Brown et al. 1979; Wilson et al. 1985), average divergence times among mtDNA haplotypes are approximately 450 000 yr (red drum) and 250 000 yr (black drum and red snapper).

Following correction for multiple tests, no significant V tests for spatial heterogeneity in mtDNA haplotype frequencies were found in any of the three species (Table 3). Prior to correction for multiple tests, two haplotypes (one in each year-class) differed significantly in frequency among samples of red drum, and three haplotypes differed significantly in frequency among samples of black drum. No consistent geographic trends (e.g., clines) in frequency were apparent for the two red drum haplotypes, suggesting that heterogeneity was due simply to random frequency variations at different localities. Two of the three black

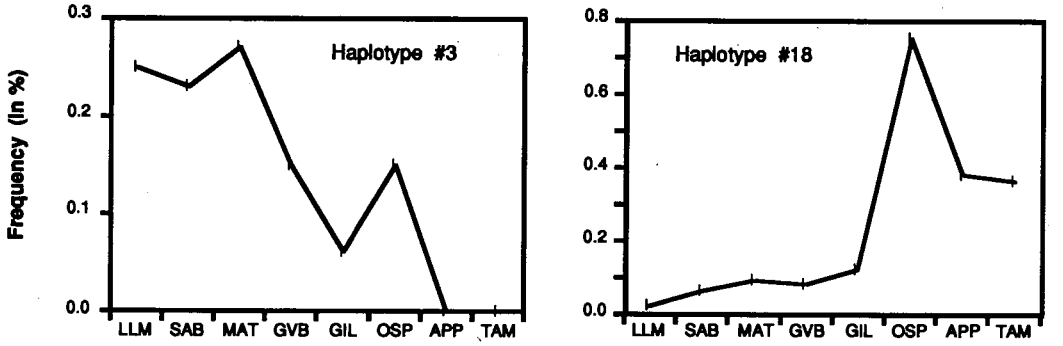

FIG. 3. Frequencies (%) of haplotypes 3 and 18 among black drum at eight localities in the Gulf of Mexico. Acronyms are defined in Table 1.

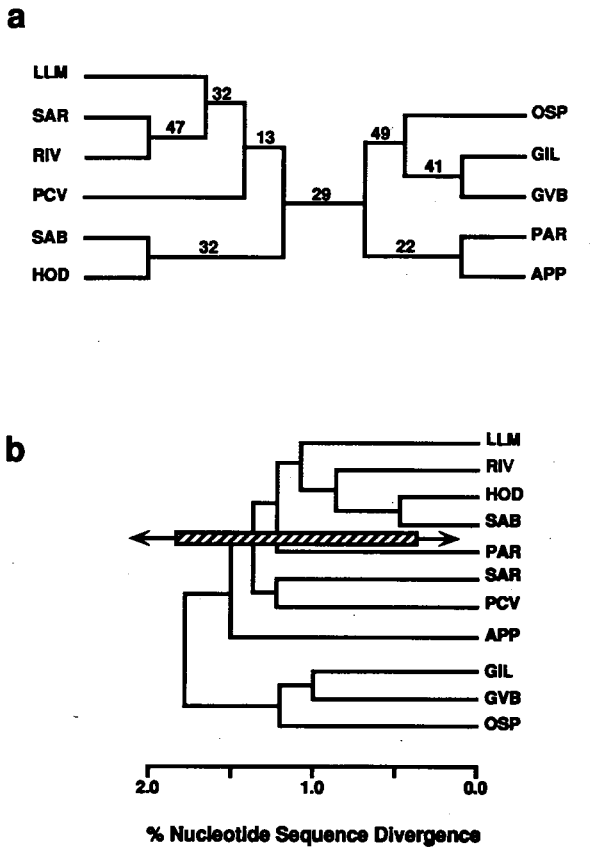

FIG. 4. (a) Strict (unrooted) consensus tree produced by maximum-parsimony analysis of presence–absence restriction-site matrix of red drum samples. Numbers along branches indicate proportion of times a branch (clade) was distinguished in bootstrap analysis. Branch lengths do not represent the number of character state changes. (b) UPGMA cluster analysis of (uncorrected) nucleotide sequence divergence (in percent) among samples of red drum. Hatched bar is standard error of the node it overlies. Acronyms are defined in Table 1.

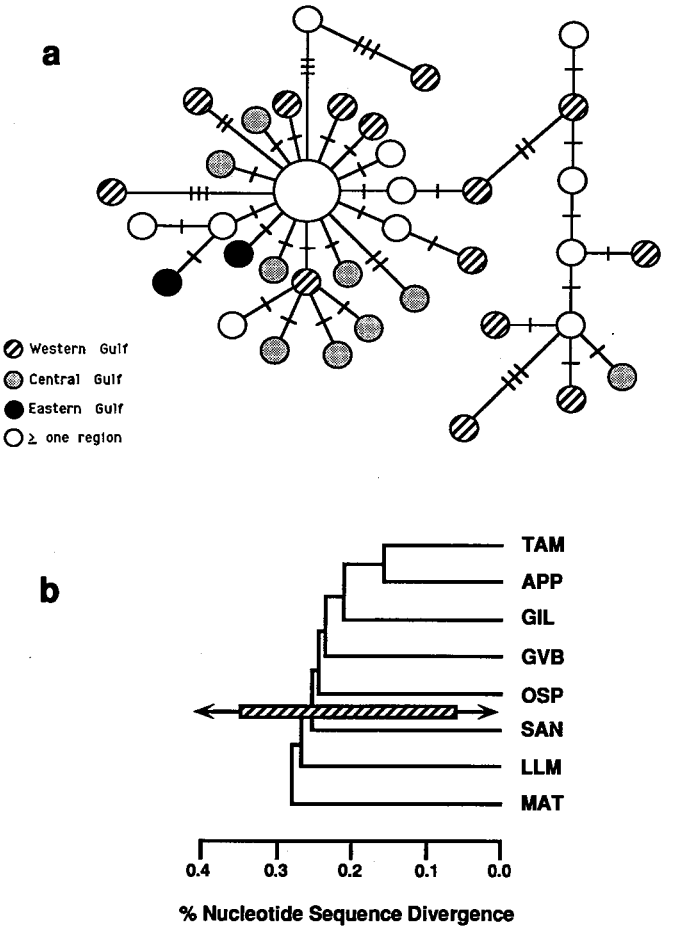

FIG. 5. (a) Minimum-length parsimony network of 37 mtDNA haplotypes in black drum. Branches connecting haplotypes are proportional in length to restriction site changes (hatch marks) required to connect adjacent haplotypes. Western Gulf includes samples from Texas; central Gulf includes samples from Louisiana and Mississippi; eastern Gulf includes samples from Florida (Table 1). (b) UPGMA cluster analysis of (uncorrected) nucleotide sequence divergence (in percent) among samples of black drum. Hatched bar is standard error of the node it overlies. Acronyms are defined in Table 1.

drum haplotypes, however, were clinally distributed: frequencies of haplotype No. 3 were high in the eastern Gulf and low in the western Gulf, whereas frequencies of haplotype No. 18 showed the reverse pattern (Fig. 3). Bootstrap probabilities (after Roff and Bentzen 1989) indicated significant heterogeneity among samples from the 1986 year-class of red drum, and significant G tests were found among samples from the 1987 year-class of red drum and among samples of black drum (Table 3). In no case was a significant

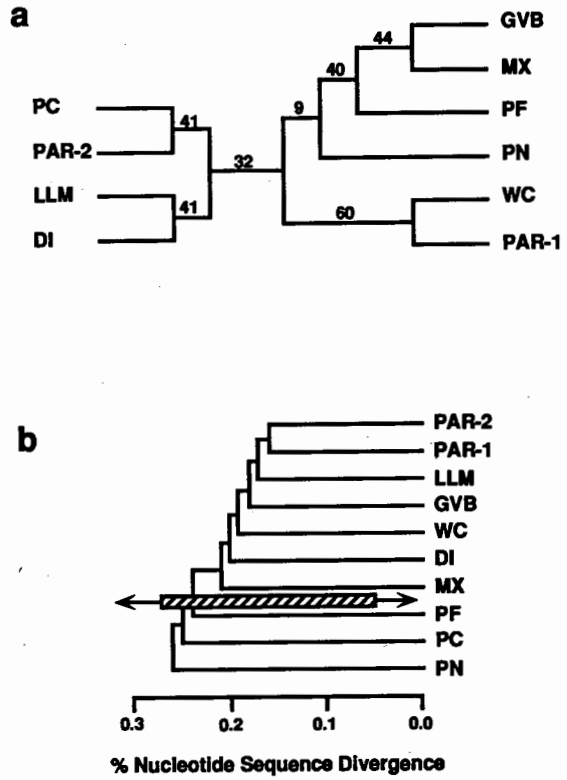

FIG. 6. (a) Strict (unrooted) consensus tree produced by maximum-parsimony analysis of presence–absence restriction-site matrix of red snapper samples. Details are the same as in Fig. 4. (b) UPGMA cluster analysis of (uncorrected) nucleotide sequence divergence (in percent) among samples of red snapper. Hatched bar is standard error of the node it overlies. Acronyms are defined in Table 1. PAR-1 and PAR-2 refer to samples from Port Aransas, Texas, collected in 1990 and 1991, respectively.

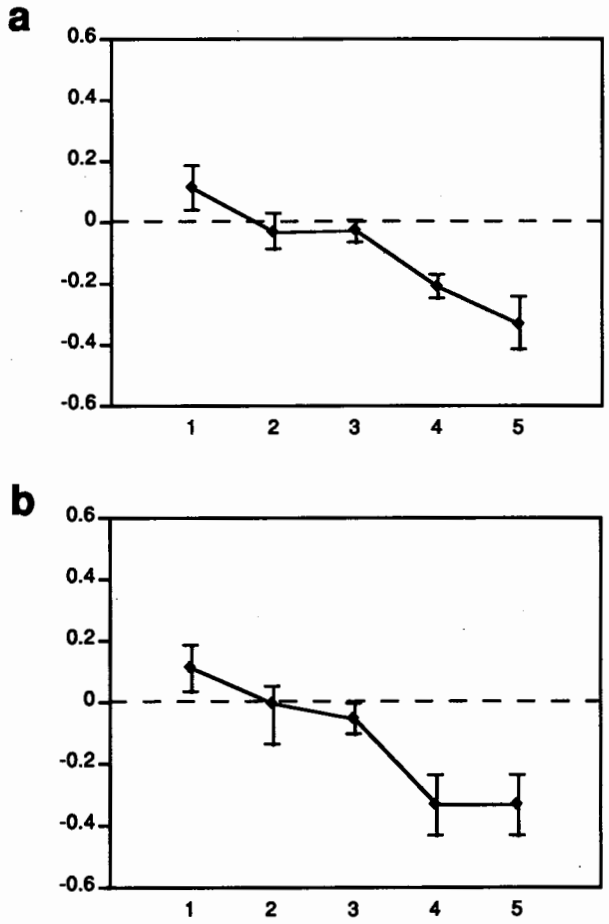

FIG. 7. Correlograms based on spatial autocorrelation analysis of mtDNA haplotype frequencies in red drum. Abscissas: distance classes 1–5 (left to right). Ordinates: mean autocorrelation coefficients (Moran's I values) for each distance class. Bars about each mean value represent one standard error on either side of a mean. (a) Equal frequencies–distance class. (b) equal distances between distance classes.

bootstrap probability and significant G-test value obtained for the same test group.

Estimates of F_{ST} ranged from near zero among samples of red drum from the 1986 year-class and red snapper sampled in both 1990 and 1991 to 0.011 among samples of black drum (Table 3). Estimates of $N_e m_f$ (the effective number of female migrants per generation) were greater than 10 in all test groups (Table 3). The estimates of F_{ST} and $N_e m_f$ indicate little genetic subdivision and occurrence of significant gene flow among all test groups.

The strict consensus "gene tree" and phenogram produced from red drum haplotype matrices revealed no evidence of phylogeographic structuring or spatial cohesion of haplotypes. The strict consensus tree produced from the matrix of samples (Fig. 4a) indicated little support for phyletic cohesion of geographically proximate samples. In the UPGMA-derived phenogram of samples (Fig. 4b), standard errors of most nodes were greater than the distance between the first and last nodes, effectively collapsing all nodes and indicating that red drum samples are not strongly differentiated genetically.

One of many possible minimum-length parsimony networks of 37 haplotypes found in black drum (Fig. 5a) required 47 steps. All clades or clusters revealed by maximum-parsimony analysis or UPGMA clustering of haplotype restriction site matrices were linked in the minimum-length parsimony network. Individual haplotypes in the network are identified by occurrence in the western Gulf (samples from Texas), central Gulf (samples from Louisiana and Mississippi), or eastern Gulf (samples from Florida). No clades or clusters of black drum haplotypes occur solely in any of the three regions (i.e., western, central, or eastern Gulf), indicating little evidence of phylogeographic structuring of haplotypes. In the UPGMA-derived phenogram of samples (Fig. 5b), standard errors of most nodes were greater than the distance between the first and last nodes, effectively collapsing all nodes and indicating that black drum samples are not strongly differentiated genetically.

The strict consensus gene tree and phenogram produced from red snapper haplotype matrices revealed no evidence of phylogeographic structuring or cohesion of haplotypes. The strict consensus tree produced from the restriction site matrix of samples (Fig. 6a) indicated little support for phyletic cohesion of geographically proximate samples. In the UPGMA-derived phenogram of samples (Fig. 6b), standard errors of most nodes were greater than the distance between the first and last nodes, effectively collapsing all nodes and indicating that red snapper samples are not strongly differentiated genetically.

In red drum, mean Moran's I values were positive in the first distance class, near zero in the second and third distance

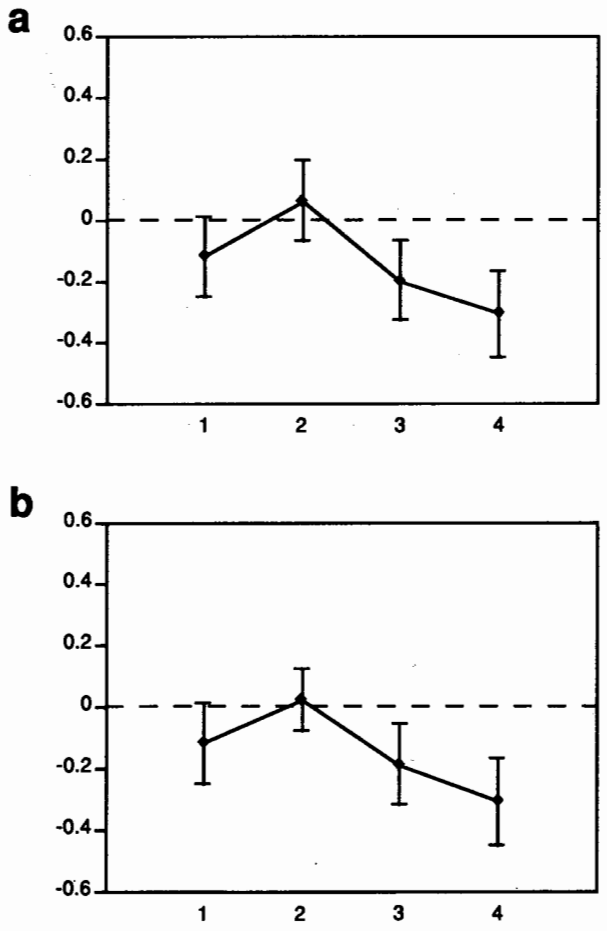

FIG. 8. Correlograms based on spatial autocorrelation analysis of mtDNA haplotype frequencies in black drum. Details are the same as in Fig. 7.

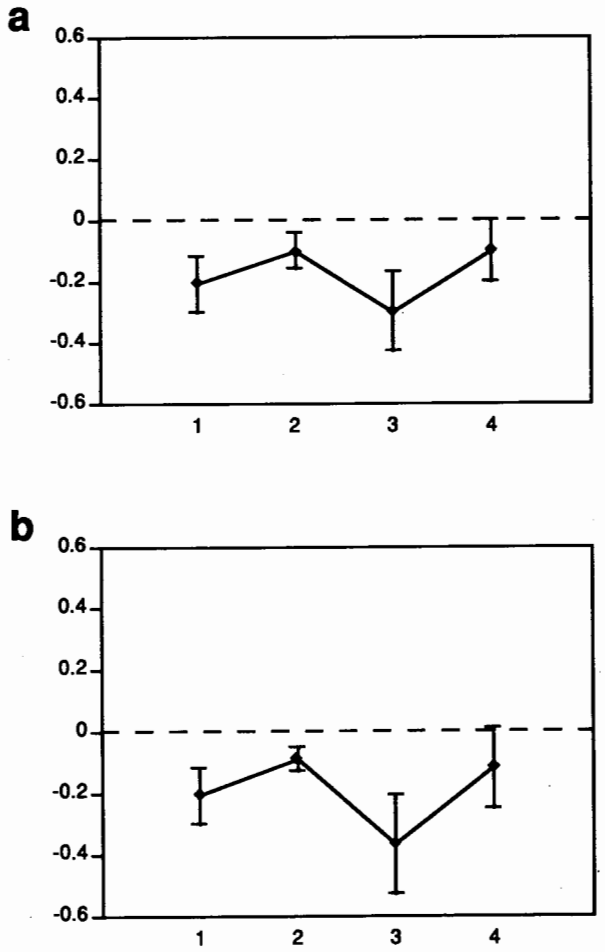

FIG. 9. Correlograms based on spatial autocorrelation analysis of mtDNA haplotype frequencies in red snapper. Details are the same as in Fig. 7.

classes, and negative in the last two distance classes in SAAP runs using equal frequencies or equal distances (Fig. 7). These results indicate autocorrelation among haplotype frequencies as a function of distance between pairs of localities, i.e., red drum in geographically proximate or neighboring localities are more similar genetically than are red drum in more geographically distant localities. In black drum, mean Moran's *I* values were effectively zero in the first two distance classes and negative in the last two distance classes in both SAAP runs (Fig. 8). These results also indicate autocorrelation among haplotype frequencies as a function of distance between pairs of localities, and that black drum in geographically proximate localities are more similar genetically than are black drum in more geographically distant localities. The lower autocorrelation coefficients in the first two distance classes in black drum, as compared with red drum, may indicate that black drum in neighboring localities are less similar genetically than are red drum in neighboring localities. In red snapper, mean Moran's *I* values were negative in all distance classes, with highest negative values occurring in penultimate distance classes in both SAAP runs (Fig. 9). We interpret this somewhat novel pattern as indicating that red snapper in geographically proximate localities are no more similar than are red snapper in geographically distant localities.

Intrapopulational mtDNA diversities appear to vary geographically in all three species (Fig. 10). MtDNA diversity values in both red drum and black drum appear higher in samples from the western Gulf, whereas the reverse appears to occur in red snapper (i.e., mtDNA diversity values appear higher in the eastern Gulf). Based on theoretical studies by Avise et al. (1988), and assuming that generation time and mtDNA mutation rate is the same across samples within each species, differences in intrapopulational mtDNA diversities may reflect differences in effective number of females that gave rise to different samples.

Discussion

Previous population-genetic studies in a variety of commercially and/or recreationally important marine fish species generally have demonstrated homogeneity in nuclear-gene or mtDNA allele frequencies among geographic samples of the same species. Species studied have included milkfish (*Chanos chanos*; Winans 1980), walleye pollock (*Theragra chalcogramma*; Grant and Utter 1980), skipjack tuna (*Katsuwonus pelamis*; Graves et al. 1984), Pacific (*Clupea pallasi*) and Atlantic herring (*Clupea harengus*; Grant and Utter 1984; Kornfield and Bogdanowicz 1987), halibut (*Hippoglossus* spp.; Grant et al. 1984), Atlantic cod (*Gadus morhua*; Mork et al. 1985), Pacific ocean perch (*Sebastes alutus*; Seeb and Gunderson 1988), and bluefish (*Pomatomus saltatrix*; Graves et al. 1992). Observed genetic homogeneity

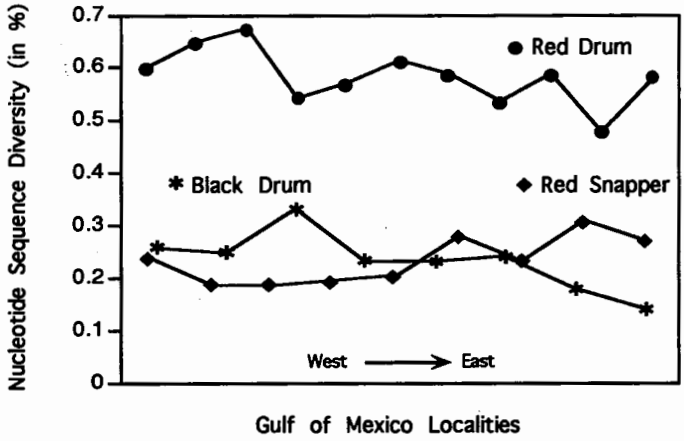

FIG. 10. Geographic variation in intrapopulational mtDNA diversity among samples of red drum, black drum, and red snapper from the Gulf of Mexico. The abscissa is the geographic samples oriented west to east (left to right) in the northern Gulf and the ordinate is the mean intrapopulational mtDNA diversities.

has been considered consistent with the hypothesis that gene flow (migration) among localities is sufficient to preclude significant genetic divergence.

As noted by Camper et al. (1993), there are caveats to the above hypothesis. First, genetic homogeneity does not indisputably establish the existence of a single, panmictic population. Proving a null hypothesis is impossible, and genetic homogeneity is simply consistent with the hypothesis that samples are drawn from a population with the same parametric allele frequencies. Moreover, small amounts of gene flow (e.g., 5%) will homogenize populations genetically, even though a species may be fragmented demographically (D. Campton, Department of Fisheries and Aquaculture, University of Florida, Gainesville, personal communication). A second caveat is that observed homogeneity may reflect historical rather than present-day events. Species could be isolated spatially in the present day, but have had sufficient genetic contact in the recent past to remain indistinguishable in allele frequencies. This possibility is also difficult to test.

The traditional approach of testing homogeneity in allele (mtDNA haplotype) frequencies revealed little evidence of significant genetic differentiation among samples of red drum, black drum, and red snapper from the Gulf. Clinal variation of two haplotypes in black drum, however, suggested the possibility of low-level divergence in this species between the eastern and western Gulf. This hypothesis could be tested further by sampling additional individuals.

In several recent publications, Avise (1989, 1992) and colleagues (Avise et al. 1987; Bowen and Avise 1990) have used a phylogenetic approach to ask whether genealogical lineages are nonrandomly distributed across geographic space. This "phylogeographic" approach has an advantage over traditional frequency-based approaches in that low frequency alleles (mtDNA haplotypes) can contribute to considerations of geographic subdivision. Phylogeographic analysis (including parsimony and phenetic methods) of mtDNA haplotypes and of samples in red drum, black drum, and red snapper, however, also revealed little evidence of geographic subdivision within the Gulf.

In our initial studies (Gold and Richardson 1991; Camper et al. 1993; C. Furman and J.R. Gold, unpublished data), genetic homogeneity and absence of phylogeographic structuring among geographic samples was taken as support for the hypothesis that each of the three species was comprised of a single genetic stock within the Gulf. The data also supported the hypothesis that gene flow throughout the Gulf was sufficient to homogenize haplotype frequencies. For red drum, these hypotheses were compatible with known life history. Both larval and juvenile red drum are largely estuarine dependent, but adults move offshore and form large schools that can migrate extensively (Matlock 1984, 1987, Swingle et al. 1984). For black drum and red snapper, however, life history considerations indicate that migration in the Gulf may be more limited than in red drum. Black drum also are estuarine dependent as larvae and juveniles, but are generally viewed as more sedentary as adults than are red drum (Osburn and Matlock 1984; Cody et al. 1985). For red snapper, the prevailing view is that both adults and juveniles are nonmigratory. This view is based largely on tagging studies and observations that juveniles and adults often exhibit substrate specificity (Bradley and Bryan 1974; Beaumariage and Bullock 1976; Fable 1980).

Different patterns of spatial autocorrelation of mtDNA haplotype frequencies were found in the three species. In red drum, positive autocorrelations were found in geographically proximate or neighboring sample localities, whereas increasingly negative autocorrelations were found with increasing geographic distance between sample localities. This pattern is consistent with an "isolation-by-distance" model where migration of individuals is inversely related to geographic distance (Sokal and Oden 1978). In red drum, migration would be hypothesized to be inversely proportional to distance from the estuary or bay of natal origin. This hypothesis suggests that red drum may be semiisolated spatially, but that overall gene flow within the Gulf is sufficient to maintain similar haplotype frequencies and neutralize potential phylogeographic structuring.

In black drum, the overall pattern of spatial autocorrelation was similar to that in red drum: increasingly negative autocorrelation was found with increasing geographic distance between sample localities. Unlike red drum, however, autocorrelation between geographically proximate sample localities was essentially zero in black drum. We interpret this pattern to indicate that migration of black drum also is inversely related to geographic distance from the estuary or bay of natal origin, but that the frequency of migration between neighboring estuaries or bays in considerably less than that in red drum. This interpretation is consistent with the prevailing view that black drum are less migratory than are red drum.

In red snapper, spatial autocorrelations were either negative or effectively zero, and a pattern of increasingly negative autocorrelation with increasing geographic distance between sample localities was not observed. The negative autocorrelation found between geographically proximate sample localities indicates either (1) little or no effective migration between localities, or (2) environmental heterogeneity and strong "patch" selection where patch size is less than interlocality distances (Sokal and Oden 1978). We favor the first alternative, in part because we assume the mtDNA variants under study are selectively neutral, and in part because little to no migration between contiguous localities also would account for the absence of increasingly negative autocorrelation as distance between localities increases.

The foregoing indicates that spatial subdivision and reduced gene flow among Gulf localities occurs in all three species despite the absence of significant frequency differences and phylogeographic structuring of mtDNA haplotypes. Similar results were found recently among haddock from the western North Atlantic (Zwanenburg et al. 1992), where the existence of reduced gene flow and discrete stocks was inferred from correlations between genetic dissimilarity and geographic distance. Relative to the Gulf species studied here, we hypothesize that gene flow is highest in red drum and lowest in red snapper. This hypothesis is consistent with known life history information in all three species (Matlock 1984, 1987; Bradley and Bryan, 1974; Beaumariage and Bullock, 1976; Osburn and Matlock 1984; Murphy and Taylor 1989; C. Wilson, personal communication).

The variation in intrapopulational mtDNA diversity among samples of all three species is also suggestive of geographic differences within the Gulf. Avise et al. (1988) demonstrated that estimates of times to shared mtDNA haplotype ancestry were essentially a function of effective size of female populations, and that estimates of effective female population size ($N_{f(e)}$ value) for any sample or population could be derived from intrapopulational mtDNA diversities. The major assumptions were that mtDNA molecules are matriclinously inherited and that mtDNA variants are selectively neutral. Estimates of absolute $N_{f(e)}$ values require conversion to sidereal time using a presumed rate of mtDNA sequence evolution and an estimate of the generation time of a species (Avise 1992). Assuming that mtDNA sequence evolution and generation time are constant among geographic samples within species, the variation in intrapopulational mtDNA diversities among samples of red drum, black drum, and red snapper suggests differences in effective number of female parents or ancestors associated with individuals sampled among localities.

The differences in intrapopulational mtDNA diversities appear to vary geographically among the three species: in red and black drum, values are higher in the western Gulf and lower in the eastern Gulf, whereas the reverse was found in red snapper. These observed patterns may be important for two reasons. First, assuming spatial subdivision within the Gulf occurs in all three species, differences in intrapopulational mtDNA diversities may be useful in stock assessment. Secondly, the observation that differences in intrapopulational mtDNA diversities exist among samples within species, and that reverse geographic patterns occur among species, suggest that intrapopulational mtDNA diversities (and estimates of $N_{f(e)}$) may reflect contemporary as well as historical events.

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