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Temporal stability and spatial divergence of mitochondrial DNA haplotype frequencies in red drum (*Sciaenops ocellatus*) from coastal regions of the western Atlantic Ocean and Gulf of Mexico

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Abstract Restriction-site variation in mitochondrial (mt) DNA was assayed among 1675 red drum (*Sciaenops ocellatus* Linnaeus) sampled from 20 localities along the southeastern coast of the USA (western Atlantic) and the Gulf of Mexico (Gulf). Up to four consecutive year-classes (cohorts) were sampled at most localities. Nucleotide-sequence divergence among 170 mtDNA haplotypes identified ranged (in percentage) from 0.184 to 1.913, with a mean (±SD) of 0.887 ± 0.300. Comparisons of mtDNA haplotype frequencies across year-classes within localities were non-significant, indicating temporal stability of breeding components within localities. Significant heterogeneity in mtDNA haplotype frequencies was found across all localities, between (pooled) samples from the western Atlantic and the Gulf, and among geographically spaced, regional groupings in the Gulf. Genetic divergence between subpopulations of red drum in the western Atlantic and Gulf follows a pattern exhibited in other marine fishes, and probably stems from physical (historical environmental heterogeneity, absence of suitable habitat, and current patterns) and, perhaps, behavioral factors. Genetic differences among red drum in the Gulf appear to be due largely to an isolation-by-distance effect that is attributable to behavioral factors. The latter may include female philopatry to natal bays or estuaries, limited offshore (coastwise) movement of females relative to their natal bay or estuary, or both. Genetic divergence among red drum in the Gulf occurs despite high gene flow (estimated as the number of genetic effective migrants in an island mode). Conservation and management of red drum should be based on the premise that strategies for a given bay or estuary will impact geographically proximal bays or estuaries more than distal ones. Trajectories of correlograms in spatial autocorrelation analysis suggest a geographic neighborhood size, relative to genetic migration of red drum from a bay or estuary, of roughly 500 to 600 km.

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Introduction

The red drum (*Sciaenops ocellatus* Linnaeus) is a widely distributed, estuarine-dependent sciaenid fish found in the western Atlantic Ocean, primarily off the east coast of the USA (US) and in the Gulf of Mexico (Pattillo et al. 1997). Prior to closure of the commercial fishery in the Gulf of Mexico (Gulf) during the mid-to-late 1980s, red drum were among the most important of the sciaenid fishes in the commercial catch (Matlock 1984; Swingle 1987). The species still supports an important recreational fishery in US waters, with a total annual catch in the early 1990s of well over 700 metric tons (Van Voorhees et al. 1992). Because the recreational harvest of red drum in the US is primarily in bays and estuaries, fishing regulations are established by individual states and vary across the region (Gulf States Marine Fishery Commission 1993). A critical question to management of the red drum resource in US waters has been whether discrete subpopulations or stocks exist either within the Gulf or between the Gulf and the southeastern US coast (western Atlantic).

Several genetic studies utilizing nuclear-gene (allozyme) and mitochondrial (mt)DNA markers have been carried out to address the stock-structure question (Ramsey and Wakeman 1987; Bohlmeyer and Gold 1991; Gold and Richardson 1991, 1993; Gold et al. 1993a, 1994). Collectively, these studies have shown that red drum in the Gulf differ significantly in mtDNA haplotype frequency from those in the western Atlantic, suggesting that fish from the two regions comprise different genetic subpopulations. As discussed elsewhere

(Gold and Richardson 1998), limited sciaenid habitat and strong northerly currents along the southeastern coast of Florida may preclude significant gene exchange between red drum in the western Atlantic and Gulf. Within the northern Gulf, no consistent pattern of genetic heterogeneity has been observed. However, frequencies of mtDNA haplotypes are autocorrelated spatially (Gold et al. 1993a), indicating an isolation-by-distance effect where fish from neighboring bays or estuaries are more similar genetically to one another than to fish in more geographically distant bays or estuaries. These findings have implications for the conservation and management of red drum in the Gulf, in that ecological and other (e.g. overfishing) impacts on a given bay or estuary would probably affect adjacent bays and estuaries more than geographically distant ones.

Available life-history information on red drum, however, suggests that dispersal of individuals (or of genes) could be extensive. Adults spawn near the mouths of bays or estuaries (Matlock 1984, 1987), and oceanic currents could transport pelagic eggs or larvae to adjacent localities (Lyczkowski-Schultz et al. 1988). Moreover, even though juveniles appear to remain in nursery bays and estuaries until sexual maturity at Age 4 yr (Overstreet 1983; Wilson and Nieland 1994; Pattillo et al. 1997), sexually-mature adults can form large, migrating schools offshore (Overstreet 1983; Matlock 1984, 1987), and large fish in offshore waters of the Gulf are known to move considerable distances (Pattillo et al. 1997). Because relatively few effective genetic migrants are sufficient to maintain genetic continuity (Wright 1951; Allendorf and Phelps 1981), large-scale movement of adult red drum and possible gene exchange between individuals nursed in geographically distant bays or estuaries should minimize genetic divergence, at least within the Gulf.

In this study, we address two issues. The first is temporal stability of mtDNA haplotype frequencies in red drum, primarily in the northern Gulf. Briefly, most studies (e.g. Bembo et al. 1995; Tringali and Bert 1995; Bentzen et al. 1996) of genetic stock structure in marine fishes represent a single "snapshot" in time relative to spatial patterns of genetic variation. Genetic variation between cohorts, or at least between samples from different years, has been examined in a few instances (Graves et al. 1992; Kinsey et al. 1994; Brown et al. 1996; Ruzzante et al. 1996, 1997), and in most cases genetic homogeneity between temporal samples has been observed. Exceptions include a study of orange roughy (Smolenski et al. 1993) and a study of Atlantic haddock (Purcell et al. 1996). In the latter study, differences in frequencies of four mtDNA haplotypes between the 1975 and 1985 cohorts sampled off the Georges Bank were interpreted to indicate that haddock spawning on Georges Bank did not represent a genetically discrete subpopulation (Purcell et al. 1996). The issue of temporal stability is important, as it implies that breeding components persist over time. Temporal stability combined with spatial heterogeneity supports the inference that spatially divergent subpopulations are exposed to different or independent population dynamics (Ruzzante et al. 1997). We examined the temporal issue previously in red drum (Gold et al. 1993b), but our samples sizes of year-classes spawned prior to 1986 were small and not partitioned spatially, i.e. by bay or estuary. The second issue addressed by this study is whether discrete subpopulations of red drum occur in the Gulf. Because we sampled at several localities over a 4 yr period, sample sizes available per locality are nearly double those we used previously (Gold et al. 1993a), thus decreasing the sampling variance of individual tests of genetic homogeneity. In addition, we incorporated use of the molecular analysis of variance developed by Excoffier et al. (1992). Software for this program was not available for use in our previous studies.

Materials and methods

A total of 1675 individuals of *Sciaenops ocellatus* Linnaeus, representing year-classes (cohorts) from 1986 through 1989, was obtained between 1987 and 1991 from 6 bays or estuaries along the southeastern coast of the USA (Atlantic) and 14 bays or estuaries in the northern Gulf of Mexico (Gulf). Collection localities and number of individuals taken at each locality by year-class are given in Fig. 1 and Table 1. White muscle, kidney, and heart tissues were removed from individual fish, placed in cryopreservation tubes, frozen in liquid nitrogen, and returned to the laboratory where they were stored at −80 °C. Fish were procured by a variety of methods, including gill nets, trammel nets, haul seines, and hook-and-line. Most fish were Age 0 (<300 mm total length) at the time of collection. Ages of individuals >300 mm total length (≃200 specimens) were determined from annuli on otoliths by procedures described in Bumguardner (1991). All fish included in the study could be assigned to one of four year-classes, i.e. 1986 to 1989.

Assay of mitochondrial (mt)DNA of individual fish followed methods outlined in Gold and Richardson (1991). We used 13 restriction enzymes (*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) to digest whole mtDNA molecules, followed by Southern transfer and hybridization to a red drum mtDNA probe. Autoradiography was used to identify individual mtDNA fragments. Lambda DNA digested with *Hin*dIII was used as a molecular weight (size) marker on individual gels. Homology of fragment patterns from single digestions was tested by multiple side-by-side comparisons or by double digestions as described in Gold and Richardson (1991). Restriction sites were either mapped (Schmidt and Gold 1992) or inferred from fragment patterns. A total of 104 mtDNA restriction sites was surveyed. Restriction sites surveyed per enzyme were: *Bam*HI (2), *Bcl*I (8), *Eco*RV (7), *Hin*dIII (6), *Nco*I (11), *Nsi*I (5), *Pst*I (6), *Pvu*II (7), *Sca*I (13), *Spe*I (11), *Stu*I (10), *Xba*I (9) and *Xmn*I (9). Individual mtDNA haplotypes (genotypes) were identified by differences in restriction fragment (site)-patterns. The data set included a total of 170 different mtDNA haplotypes. (A listing of all haplotypes, digestion patterns of each enzyme, and the distribution of haplotypes across sampling localities by year-class is available from the authors upon request).

Nucleotide-sequence divergence among mtDNA haplotypes was estimated after Nei and Li (1979), and intrapopulational (within-sample) nucleotide-sequence diversity (mtDNA diversity) was estimated after Nei and Tajima (1981). The latter is the average nucleotide-sequence difference between any two individuals drawn at random from a given sample. Because tests of temporal homogeneity between or among year-classes at individual localities were non-significant (see "Results"), we estimated intrapopulational mtDNA diversity within sampling localities (year-classes pooled). We then tested for homogeneity of intrapopulational mtDNA diversity among sampling localities using a Monte Carlo random-

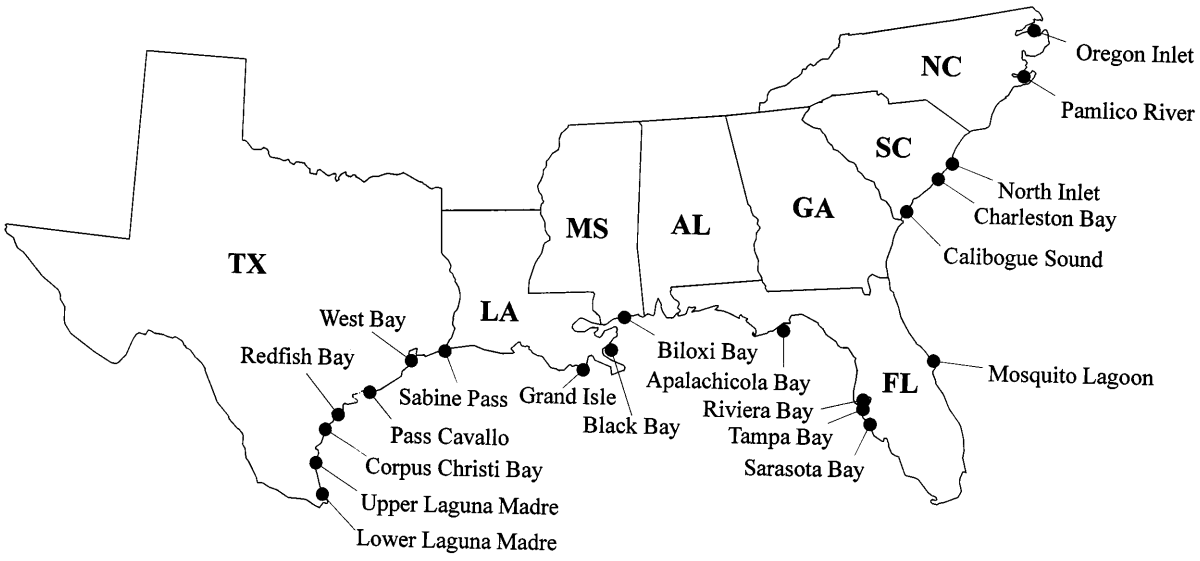

Fig. 1 Sampling localities for red drum, *Sciaenops ocellatus*, examined in present study (*TX* Texas; *LA* Louisiana; *MS* Mississippi; *AL* Alabama; *GA* Georgia; *FL* Florida; *SC* South Carolina; *NC* North Carolina)

ization procedure. This was necessary because nucleotide-sequence divergence values are pairwise (distance) values and violate assumptions of independence of traditional statistical tests. We constructed 100 randomized data sets by first pooling all haplotypes and then allocating *n* haplotypes at random (with replacement) to 20 groups, where *n* was equal to observed sample sizes in each group. Single classification (Sokal and Rohlf 1981) and Kruskal–Wallis (Siegel 1956) analyses of variance (ANOVA) were performed on the observed data and each randomized replicate. *F* and *X*-square statistics generated from the ANOVAs (based on observed data) were compared to distribution *F* and *X*-square from randomized data sets. Significant heterogeneity (at $\alpha = 0.05$) would be indicated if observed values exceeded 95% of randomized values.

Homogeneity of mtDNA haplotype frequencies was tested via a randomization (Monte Carlo) procedure developed by Roff and Bentzen (1989). We first tested temporal homogeneity of mtDNA haplotype frequencies among year-classes at localities (3 in the Atlantic, 13 in the Gulf) where multiple year-classes were sampled. Tests of spatial homogeneity were then carried out (i) among all localities (total of 20), (ii) among localities in the Atlantic (6) and localities in the Gulf (14), (iii) between localities (pooled) in the Atlantic and localities (pooled) in the Gulf, and (iv) within and among regions within the Gulf. For the last, localities sampled in the Gulf were subdivided into eastern Gulf (4 localities), central Gulf (3 localities), and western Gulf (7 localities). This a priori subdivision of localities within the Gulf was based in part on what appeared to be "logical" geographic subdivision (i.e. geographic

Table 1 *Sciaenops ocellatus*. Sampling localities, year class, and number of individuals assayed for variation in mtDNA in western Atlantic Ocean and Gulf of Mexico (*state abbreviations* as in legend to Fig. 1)

Locality	Number of individuals assayed in Year-Class:				
	1986	1987	1988	1989	Total
Western Atlantic	165	47	7	85	304
Oregon Inlet, NC	15	0	0	0	15
Pamlico River, NC	23	4	0	0	27
North Inlet, SC	18	0	0	0	18
Charleston Bay, SC	34	32	0	36	102
Calibogue Sound, SC	50	0	0	0	50
Mosquito Lagoon, FL	25	11	7	49	92
Gulf of Mexico	392	303	364	312	1371
Sarasota Bay, FL	87	24	0	0	111
Tampa Bay, FL	0	0	41	42	83
Riviera Bay, FL	24	45	0	0	69
Apalachicola Bay, FL	30	37	43	44	154
Biloxi Bay, MS	83	34	0	0	117
Black Bay, LA	20	0	0	0	20
Grand Isle, LA	43	47	31	0	121
Sabine Pass, TX	25	18	35	28	106
West Bay, TX	32	36	28	21	117
Pass Cavallo, TX	13	18	30	29	90
Redfish Bay, TX	17	21	0	0	38
Corpus Christi Bay, TX	0	0	50	54	104
Upper Laguna Madre, TX	0	2	48	44	94
Lower Laguna Madre, TX	18	21	58	50	147

distance between adjacent distal localities in each group), and in part on prior studies (Gold et al. 1993a) where significant heterogeneity of similar groupings had been suggested by "*V*" tests (DeSalle et al. 1987) of arcsine, square-root-transformed mtDNA haplotype frequencies. Significance levels for multiple tests were carried out using sequential Bonferroni corrections (Rice 1989). We also employed analysis of molecular variance, AMOVA (Excoffier et al. 1992) to generate estimates of (genetic) variance components and Φ statistics (a set of hierarchical *F*-statistic analogs that take into account the evolutionary distance among alleles). Significance of Φ statistics was tested by random permutation (1000 replicates). This approach avoids the parametric assumptions of normality and independence not typically met by molecular-distance measures (Excoffier et al. 1992). Sampling localities were nested into regional groupings for input into AMOVA. These groupings were the same as above and included Atlantic (6 localities), Gulf (14 localities), eastern Gulf (4 localities), central Gulf (3 localities), and western Gulf (7 localities). In this way, we tested spatial homogeneity among samples within regions. We also used AMOVA to test homogeneity (i) between samples from the Atlantic versus those from the Gulf, and (ii) among samples from the eastern Gulf vs central Gulf vs western Gulf (subdivided as above).

Spatial autocorrelation analysis of frequencies of mtDNA haplotypes was employed to examine whether haplotype frequencies at any given locality were independent of those in adjacent localities. Positive correlations between adjacent localities, with decreasing correlation as distance between localities increases, are generally interpreted to indicate an isolation-by-distance effect (Sokal and Oden 1978a). We used the SAAP (spatial autocorrelation analysis program) of Wartenberg (1989), and followed suggestions in Sokal and Oden (1978a, b). We also limited the analysis to the 14 localities in the Gulf, and minimized "noise" by including only haplotypes found in ≥7 individuals (total of 27 haplotypes). The first of two SAAP runs employed equal geographic distances between each of four distance-classes; the second employed equal numbers of pairwise comparisons in each distance-class. The number of pairwise comparisons in the former was 23, 22, 16, and 30; the number of pairwise comparisons in the latter was 22, 23, 23, and 23. Distance-classes in both runs were generated by SAAP from input longitude and latitude of each locality. We also searched for an isolation-by-distance effect by using the same haplotypes as employed in spatial autocorrelation analysis and converting pairwise genetic distances to $n/(1-n)$, where n represented pairwise Φ_{ST} values (the proportion of molecular genetic variation attributable to differences among populations). We then plotted genetic distance (pairwise Φ values) against physical distances (in kilometers), measured as the distance between sampling localities following the coastline. This approach approximates a linear stepping-stone model of migration (Rousset 1997). We used the regression extension of Mantel's test (Smouse et al. 1986) to test significance of the slope of the regression.

Results

Nucleotide-sequence divergence among the 170 mtDNA haplotypes identified among the 1675 *Sciaenops ocellatus* assayed ranged (in percentage) from 0.184 to 1.913, with a mean (± SD) of 0.887 ± 0.300. No evidence for size variation in the mtDNA of *S. ocellatus* was observed, although four individuals were found to be heteroplasmic for restriction-enzyme sites (Gold and Richardson 1990 unpublished data). These heteroplasmic individuals were excluded from further data analysis. Intrapopulational nucleotide-sequence (mtDNA) diversity (in percentage) among sampling localities (year-classes pooled) ranged from 0.452 ± 0.081 (mean ± SE) at Oregon Inlet, North Carolina to 0.712 ± 0.096 at North Inlet, South Carolina. Both single-classification ($P = 0.27$) and Kruskal–Wallis ($P = 0.39$) analysis of variance revealed homogeneity in mean values of mtDNA diversity among sampling localities.

Tests of temporal homogeneity in mtDNA haplotype frequencies across year-classes at localities where more than a single year-class was surveyed were non-significant (Table 2), as was a test of homogeneity of mtDNA haplotype frequencies across year-classes with localities combined ($P = 0.067$). Consequently, we pooled year-class samples at each locality for tests of spatial homogeneity in mtDNA haplotype frequencies. Results of homogeneity tests (Table 3) revealed considerable heterogeneity in mtDNA haplotype frequencies across the area sampled. In non-pooled comparisons, significant heterogeneity ($P < 0.05$, with sequential Bonferroni correction) was detected among all localities, among localities in the Gulf, and among localities in the western Gulf. Comparisons among Atlantic localities, and among eastern and central Gulf localities were non-significant ($P > 0.05$). We also tested a modified group of western Gulf localities, whereby the two samples from the Laguna Madre in southern Texas were removed from the analysis. We did so in part because the Laguna Madre differs ecologically, particularly in salinity, from other bays or estuaries in the Gulf (Hedgpeth 1967), and in part because previously, we had detected significant differences in mtDNA haplotype frequencies between samples of a related sciaenid species (the spotted seatrout *Cynoscion nebulosus*) from the Laguna Madre and elsewhere in the western Gulf (Gold and Richardson unpublished data). The Laguna Madre also is at the western extreme of the sampling area (Fig. 1). The homogeneity test among the modified western Gulf localities was non-significant ($P = 0.109$: Table 3). All pooled comparisons, including Atlantic vs Gulf and comparisons between and among the three regional groupings in the Gulf (using the modified western Gulf grouping) differed significantly in mtDNA haplotype frequencies (Table 3).

Analysis of molecular variation (AMOVA) revealed nearly identical results to the homogeneity tests. Significant or near-significant Φ values (Table 4) were found in the same non-pooled comparisons (among all localities and among localities from the Gulf) and pooled comparisons (between the Atlantic and Gulf and among the three regions within the Gulf). The P value of 0.014 in the comparison among Gulf localities was barely non-significant following Bonferroni correction (adjusted $\alpha = 0.010$), whereas the P values of 0.008 and 0.007 in the comparisons among regions in the Gulf were significant (but barely) following the same correction (adjusted α of 0.0083 and 0.0071, respectively). In the two hierarchical tests (i.e. Atlantic vs Gulf and among the three regions in the Gulf), Φ values for the proportion of the variance attributable to "among localities within regions" were non-significant ($P = 0.032$ and 0.135, respectively) following Bonferroni correction. We also used AMOVA to test the homogeneity of mtDNA

Table 2 *Sciaenops ocellatus*. Results of tests for temporal homogeneity in mtDNA haplotype frequencies among samples from western Atlantic Ocean and Gulf of Mexico

Locality	Year-classes	P^a
Western Atlantic		
Pamlico River, NC	1986, 1987	0.438
Charleston Bay, SC	1986, 1987, 1989	0.274
Mosquito Lagoon, FL	1986, 1987, 1988, 1989	0.061
Gulf of Mexico		
Sarasota Bay, FL	1986, 1987	0.060
Tampa Bay, FL	1988, 1989	0.059
Riviera Bay, FL	1986, 1987	0.391
Apalachicola Bay, FL	1986, 1987, 1988, 1989	0.751
Biloxi Bay, MS	1986, 1987	0.139
Grand Isle, LA	1986, 1987, 1988	0.649
Sabine Pass, TX	1986, 1987, 1988, 1989	0.267
West Bay, TX	1986, 1987, 1988, 1989	0.959
Pass Cavallo, TX	1986, 1987, 1988, 1989	0.724
Redfish Bay, TX	1986, 1987	0.568
Corpus Christi Bay, TX	1988, 1989	0.913
Upper Laguna Madre, TX	1987, 1988, 1989	0.740
Lower Laguna Madre, TX	1986, 1987, 1988, 1989	0.347

[a] Probability based on bootstrap analysis, 1000 replicates (after Roff and Bentzen 1989)

haplotype frequencies within the three regions in the Gulf (western Gulf with and without samples from the Laguna Madre). In each case, probability values were considerably greater than 0.05 (data not shown). Except for the (pooled) comparison between the Atlantic and Gulf, where 1.57% of the variance was attributable to between regions, >99% of the variance in mtDNA haplotype frequencies was distributed within sampling localities (Table 4).

The results of spatial autocorrelation analysis revealed a significant isolation-by-distance effect among samples of red drum from the Gulf. SAAP runs using mtDNA haplotypes found ≥7 individuals generated 108 Moran's *I*-values (27 haplotypes × 4 distance classes). When equal distances between distance-classes were used, 17 significant values ($P < 0.05$) were obtained. Of these, 6 (5 positive) occurred in the first two distance-classes, and 11 (9 negative) occurred in the last two distance-classes. Further, 4 of the positive values in the first distance-class were highly significant ($P < 0.01$), as were 4 of the negative values in the last distance-class. Virtually identical results were obtained in the SAAP runs using equal numbers of pairwise comparisons in each distance-class. Correlograms displaying geographic patterns of variation of haplotypes where significant Moran's *I*-values were found (Fig. 2) reveal a fairly regular decline from significant, positive autocorrelation at 300 km, to little or no autocorrelation at 750 km, to significant, negative autocorrelation at 1000 to 1500 km. These boundaries may represent estimates of geographic limits to gene flow from natal bays or estuaries. Additional evidence for a significant isolation-by-distance effect was revealed by the significant regression ($r = 0.22$, $P = 0.026$) of the plot between pairwise geographic distance and pairwise Φ_{ST} values between sampling localities (Fig. 3).

The occurrence of an isolation-by distance effect suggests that the significant heterogeneity observed among red drum from eastern, central, and western regions in the Gulf may not necessarily identify discrete subpopulations where gene flow is limited between but not within regions. To test this possibility, we carried out homogeneity tests (after Roff and Bentzen 1989) between adjacent localities in different regions: Apalachicola Bay, Florida (westernmost locality in the eastern region) vs Biloxi Bay, Mississippi (easternmost locality in the central region) and Grand Isle, Louisiana (westernmost locality in the central region vs Sabine Pass (easternmost locality in the modified western region).

Table 3 *Sciaenops ocellatus*. Tests for spatial homogeneity in mtDNA haplotype frequencies among samples of red drum from western Atlantic and Gulf of Mexico

Test group	No. of samples	P^a
All localities	20	0.001
Atlantic localities	6	0.646
Gulf localities	14	<0.001
East Gulf localities[b]	4	0.554
Central Gulf localities[c]	3	0.289
West Gulf localities[d]	7	0.004
West Gulf localities[e]	5	0.109
Pooled comparisons		
Atlantic vs Gulf	2	<0.001
East vs Central vs West Gulf[e]	3	<0.001
East vs Central Gulf	2	<0.001
East vs West Gulf[e]	2	<0.001
Central vs West Gulf[e]	2	0.011

[a] Probability based on bootstrap analysis, 1000 replicates (after Roff and Bentzen 1989)
[b] East Gulf localities (Sarasota Bay, Tampa Bay, Riviera Bay, Apalachicola Bay)
[c] Central Gulf localities (Biloxi Bay, Black Bay, Grand Isle)
[d] West Gulf localities (Sabine Pass, West Bay, Pass Cavallo, Redfish Bay, Corpus Christi Bay, Upper Laguna Madre, Lower Laguna Madre)
[e] West Gulf localities (Sabine Pass, West Bay, Pass Cavallo, Redfish Bay, Corpus Christi Bay)

Table 4 *Sciaenops ocellatus*. Analysis of molecular variation (AMOVA) among mtDNA haplotypes of red drum from western Atlantic and Gulf of Mexico

Variance component	Observed partition		Φ values	P^a
	Variance	% total		
All localities				
Among localities	0.00318	0.67	0.007	<0.001
Within localities	0.47163	99.33		
Atlantic localities				
Among localities	−0.00014	−0.03	0.000	0.491
Within localities	0.45880	100.03		
Gulf localities				
Among localities	0.00100	0.21	0.002	0.014
Within localities	0.47443	99.79		
Atlantic vs Gulf				
Between regions	0.00752	1.57	0.016	<0.001
Among localities within regions	0.00080	0.17	0.002	0.032
Within localities	0.47163	98.26		
East[b] vs Central[c] vs West Gulf[d]				
Among regions	0.00072	0.15	0.002	0.008
Among localities within regions	0.00052	0.11	0.001	0.135
Within localities	0.47443	99.74		
East vs Central vs West Gulf[e]				
Among regions	0.00093	0.20	0.002	0.007
Among localities within regions	0.00007	0.01	0.000	0.429
Within localities	0.47429	99.79		

[a] Probability of finding a more extreme variance component by chance alone (1000 permutations)
[b] East Gulf localities (Sarasota Bay, Tampa Bay, Riviera Bay, Apalachicola Bay)
[c] Central Gulf localities (Biloxi Bay, Black Bay, Grand Isle)
[d] West Gulf localities (Sabine Pass, West Bay, Pass Cavallo, Redfish Bay, Corpus Christi Bay, Upper Laguna Madre, Lower Laguna Madre)
[e] West Gulf localities (Sabine Pass, West Bay, Pass Cavallo, Redfish Bay, Corpus Christi Bay)

Both tests were non-significant ($P = 0.608$ and 0.123, respectively), consistent with the notion that the observed heterogeneity among regions is a manifestation of the isolation-by-distance effect. Note, however, that the smaller sample sizes in between-locality tests may decrease robustness of the test.

Discussion

Our finding of temporal homogeneity of mtDNA haplotype frequencies at all sampling localities of *Sciaenops ocellatus* tested indicates that observed spatial differences (see following paragraphs) are temporally stable and not due to "chaotic temporal variation" (Bentzen et al. 1997). The degree to which chaotic temporal variation impacts genetic assessment of population structure in marine fishes is not known as most investigations (e.g. Bembo et al. 1995; Tringali and Bert 1995; Bentzen et al. 1996; Gold and Richardson 1998) have not provided a temporal perspective of genetic variation. However, genetic variation between cohorts, or at least between samples from different years, has been examined in a few instances (e.g. Graves et al. 1992; Brown et al. 1996; Ruzzante et al. 1996, 1997), and genetic homogeneity between temporal samples usually has been observed. Exceptions (e.g. Purcell et al. 1996) have been interpreted to indicate that samples represented genetically discrete subpopulations subjected to different or at least independent population dynamics. We also found no significant differences in intrapopulational (mtDNA) diversity among sampling localities. This parameter is the average nucleotide-sequence difference between any two individuals sampled randomly, and provides a relative index of evolutionary effective female population size (Avise et al. 1988; Ball et al. 1990). Our finding thus indicates that the evolutionary effective number of female parents is the same across localities.

Our previous studies of spatial genetic variation in red drum had revealed significant heterogeneity between samples from the western Atlantic and the Gulf (Gold et al. 1993a, 1994). The data in those studies were from the 1986 and 1987 year-classes, and included both mtDNA and nuclear-encoded proteins (allozymes). Data in the present study reinforce the previous findings by demonstrating temporal stability of mtDNA haplotype differences. Genetic divergence between subpopulations in the western Atlantic and Gulf has been documented for a number of marine species (Avise 1992; Gold and Richardson 1998), and has been attributed to: (i) episodic changes in environments during glacial times: (ii) absence of suitable habitat at the spatial junction between subpopulations; and/or (iii) stronger currents into the western Atlantic from the Gulf than the reverse. Existence of an isolation-by-distance effect in red drum (see following paragraphs) suggests that be-

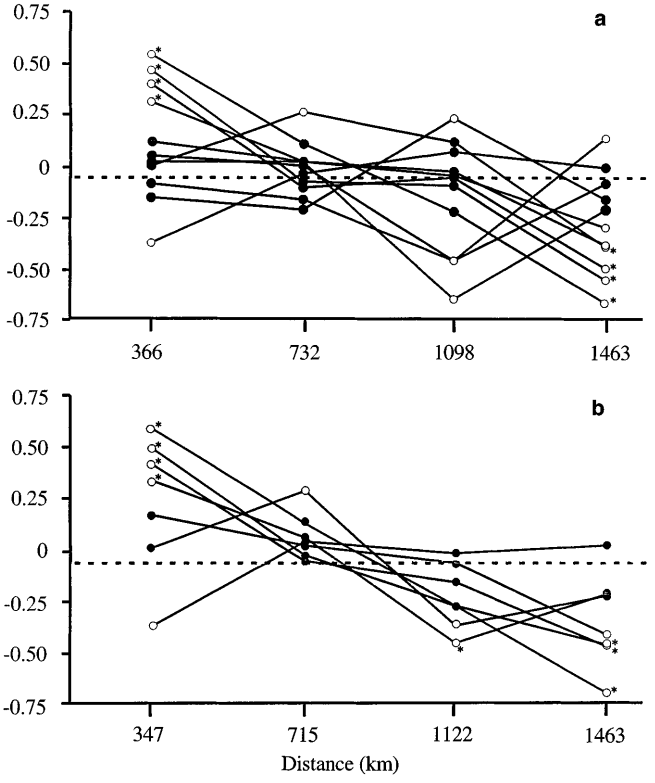

Fig. 2 *Sciaenops ocellatus*. Correlograms of mtDNA haplotypes with significant Moran's *I*-value in one or more distance classes. **a** equal distance between distance classes; **b** equal frequencies per distance class [○ significant *P* values (<0.05); * highly significant *P* values (<0.01); *abscissas* distance classes; *ordinates* Moran's *I*-value, *dashed lines* expected Moran's *I*-value when no correlation exists]

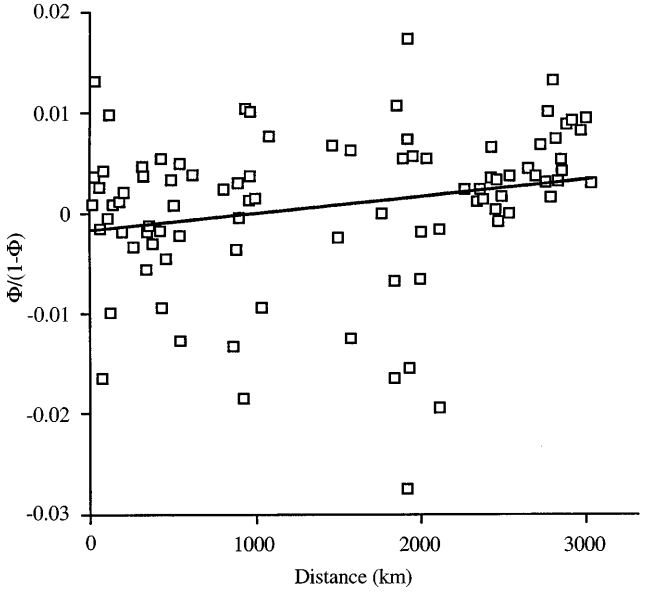

Fig. 3 *Sciaenops ocellatus*. Relationship between genetic distance ($\Phi_{ST}/1-\Phi_{ST}$) and geographic distance (km) for samples of red drum. Comparisons use distances along coastline of northern Gulf of Mexico

havior may play a role as well. We also reported previously (Gold and Richardson 1993) that red drum in the Mosquito Lagoon system on the east coast of Florida (western Atlantic) differed from red drum elsewhere in the western Atlantic. No such difference was found in a recent comparison of sequences from the red drum mtDNA-control region (T. Bert personal communication), a finding corroborated by the expanded data set in the present study. The issue of whether red drum from Mosquito Lagoon are unique is of interest, as broodstock for large-scale supplementation of red drum along the Florida east coast have been obtained from Mosquito Lagoon (T. Bert personal communication).

In contrast, in our prior studies we were unable to discern significant genetic differences among spatial samples of red drum from the Gulf (Gold et al. 1993a, 1994), although existence of a weak isolation-by-distance effect had been suggested by spatial autocorrelation analysis of mtDNA haplotype frequencies (Gold et al. 1993a). In the present study, we detected significant heterogeneity both among all samples (total of 14) from the Gulf and in a test of geographically-spaced regional groupings. However, samples from geographically-adjacent localities in different regional groupings did not differ in haplotype frequency. This suggests that the heterogeneity observed among regional groupings may reflect the nearly two-fold increase in sample size (from 695 individuals in the previous study to 1371 in the present study) rather than discrete subpopulations (stocks) of red drum in the northern Gulf. Briefly, the significant isolation-by-distance effect indicates that migration of adult, reproductively active red drum within the Gulf is inversely related to geographic distance from a bay or estuary of natal origin. Thus, one might expect that geographically-separate groupings would differ significantly in haplotype frequency when the isolation-by-distance effect is amplified by increased sample sizes. The distinction here is important to conservation and management, as we are not hypothesizing the existence of discrete genetic stocks, even though significant genetic differences occur across the region. It also is important to note that our sampling of estuarine-dependent juveniles was critical to detecting the isolation-by-distance effect, as sampling from the offshore, adult schools would probably be random with respect to natal origin.

The above results appear somewhat at odds with mark–recapture and other studies which suggest sexually-mature red drum form large, offshore schools that can migrate considerable distances in the northern Gulf (Overstreet 1983; Matlock 1984, 1987; Pattillo et al. 1997). Assuming that mature, older fish in the large, offshore schools spawn and leave their offspring near bays or estuaries proximal to offshore localities where they may have migrated, one might expect both homogeneity of haplotype frequencies across localities and absence of an isolation-by-distance effect. One possibility is that older (and presumably migratory) females may not spawn frequently, with recruitment

into estuaries stemming primarily from younger females that have not joined large, offshore schools. However, studies of ovaries in large red drum females sampled offshore from several consecutive cohorts have revealed no evidence of mid-spawning-season atresia (egg absorption) or sterility, nor have females of mature size and age been found that were not in spawning condition during the spawning season (D. Nieland and C. Wilson personal communication). This indicates that older (and presumably migratory) red drum females do spawn on an annual basis. Consequently, the isolation-by-distance effect appears more likely to be a function of female behavior, and could stem from either natal-site philopatry (i.e. homing), from limited, offshore (coastwise) movement of females relative to their natal bay or estuary, or both. Homing, at least over long distances, is well known in several fish species (e.g. salmon) but has not, to our knowledge, been reported for a sciaenid fish. Alternatively, there is evidence that movement of adult red drum in the Gulf is primarily inshore–offshore, with relatively little coastwise migration (Simmons and Breuer 1962; Adkins et al. 1979; Osburn et al. 1982). A final point is that the Φ value of 0.002, obtained from molecular analysis of variance of the spatial distribution of red drum haplotypes in the Gulf, differed significantly from zero but is consistent with a relatively high level of genetic effective migration in the Gulf. Briefly, substituting the Φ in place of F_{ST} [proportion of genetic variance attributable to allele (mtDNA haplotype) frequency differences among samples], and employing the simple island model of Wright (1943), where $F_{ST} \simeq 1/(2N_e m_f)$, the effective number of female migrants per generation ($N_e m_f$) is $\simeq 250$. This underscores first, that genetic divergence can occur in the face of high gene flow (Wright 1969), and second, that some proportion of reproductively active red drum must leave offspring in bays or estuaries other than their natal bays or estuaries.

Current management of red drum in the Gulf in terms of assessment and allocation occurs on a state-by-state basis, whereby fishing regulations and conservation measures are established by individual states. The results of this study indicate that although genetic differences occur among red drum across the region, genetic migration, at least between geographically proximal bays or estuaries, can be considerable. Conservation and management of red drum in the Gulf should thus be based on the premise that management action for a given bay or estuary may impact geographically adjacent bays or estuaries, even if state lines are thereby crossed. The trajectories of the correlograms in spatial autocorrelation analysis revealed fairly regular declines, from significant, positive autocorrelation at 300 km to little or no autocorrelation at 750 km. This may suggest a geographic neighborhood size, relative to genetic migration from an individual bay or estuary, of 500 to 600 km. This distance is roughly the mid-point between strong, positive autocorrelation of mtDNA haplotype frequencies and little to no autocorrelation, suggesting that females are most likely to breed within 500 to 600 km of their natal bay or estuary. The concept of a geographic neighborhood size relative to management of species such as red drum merits further consideration and study, perhaps by a combination of mark–recapture and molecular genetics.

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