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Population structure of red drum (*Sciaenops ocellatus*) in the northern Gulf of Mexico, as inferred from variation in nuclear-encoded microsatellites

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Abstract Allelic variation at eight nuclear-encoded microsatellites was assayed among 967 red drum (*Sciaenops ocellatus*) sampled from four consecutive cohorts at seven geographic localities (=28 samples total) in the northern Gulf of Mexico (Gulf). Number of alleles per microsatellite ranged from 6 to 21; average direct-count heterozygosity values per sample (±SE) ranged from 0.560 ± 0.018 to 0.903 ± 0.009. Tests of Hardy-Weinberg equilibrium revealed significant departures from expected genotype proportions at one microsatellite, which was omitted from further analysis. Tests of genotypic equilibrium indicated that genotypes between pairs of microsatellites were randomly associated. Homogeneity tests of allele distributions across cohorts within localities were non-significant following correction for multiple tests executed simultaneously, and results from molecular analysis of variance indicated that the genetic variance component attributable to variation among cohorts did not differ significantly from zero. Homogeneity tests of allele distributions among localities (cohorts pooled) revealed significant differences both before and after correction for multiple tests. Neighbor-joining clustering of a pairwise matrix of θ values (an unbiased estimator of F_{ST}), spatial autocorrelations, and regression analysis revealed a pattern of isolation by distance, where genetic divergence among geographic samples increases with geographic distance between sample localities. The pattern and degree of temporal and spatial divergence in the nuclear-encoded microsatellites paralleled almost exactly those of mitochondrial (mt) DNA, as determined in a prior study. Stability of both microsatellite and mtDNA allele distributions within localities indicates that the small but significant genetic divergence among geographic samples represents true signal and that overlapping populations of red drum in the northern Gulf may be influenced by independent population dynamics. The degree of genetic divergence in microsatellites and mtDNA is virtually identical, indicating that genetic effective size of microsatellites and mtDNA in red drum are the same. This, in turn, suggests either that gene flow in red drum in the northern Gulf could be biased sexually or that red drum populations may not be in equilibrium between genetic drift and migration. If a sexual bias exists, the observation that divergence in mtDNA is considerably less than 4 times that of microsatellites could suggest female-mediated dispersal and/or male philopatry. The observed isolation-by-distance effect indicates a practical limit to dispersal. Approximate estimates of geographic neighborhood size suggest the limit is in the range 700–900 km. Although the genetic studies of red drum indicate significant genetic divergence across the northern Gulf, the genetic differences do not delimit specific populations or stocks with fixed geographic boundaries.

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Introduction

Studies over the past decade of patterns of genetic variation and divergence in a variety of pelagic marine organisms have demonstrated that high dispersal potential at any of several life-history stages does not necessarily indicate high levels of actual gene flow and uniformity in population structure (Avise 1998). Examples among vertebrates include several fishes (Zwanenburg et al. 1992; Bentzen et al. 1996; O'Connell et al. 1998), turtles (Bowen et al. 1992; FitzSimmons et al. 1997), iguanas (Rassman et al. 1997), and whales (Palumbi and Baker 1994; Larsen et al. 1996; Brown

Gladden et al. 1999); examples among invertebrates include corals (Hellberg 1994), sea urchins (Palumbi et al. 1997), and squid (Shaw et al. 1999). The demonstration that gene flow has limits in these species is of considerable interest, in part because the physical and biological parameters that impact gene flow in pelagic marine organisms are poorly understood (Palumbi 1994, 1996), and in part because studies of population structure generally contribute to the understanding of how migration, genetic drift, and selection shape divergence among populations (Avise 1994). In addition, many pelagic marine species are of importance economically or in terms of conservation, and an understanding of population structure and gene flow in these species can enhance conservation and wise use of these resources (Avise 1998; Graves 1998). Mechanisms or factors that potentially could impede gene flow in pelagic marine species were discussed by Palumbi (1994, 1996) and Palumbi et al. (1997) and include physical processes (e.g., oceanic currents and/or circulation), natural selection, larval and/or adult behavior (e.g., natal philopatry), isolation by distance, stable migration routes, and recent history.

A significant component to the demonstration that gene flow is often limited in pelagic marine species has been advances in molecular technology and the increase in the number and type of genetic markers, particular at the DNA level, available for use (Ward 1989; Park and Moran 1994; Carvalho and Hauser 1995). Nuclear-encoded microsatellites (Weber 1990; Wright and Bentzen 1994) have proven perhaps the most informative, in that reduced gene flow and subtle population structure have been demonstrated with microsatellites when other genetic markers, e.g., mitochondrial DNA and/or protein polymorphisms, failed to detect genetic heterogeneity among geographic samples (Bentzen et al. 1996; Brunner et al. 1998; O'Connell et al. 1998; Shaw et al. 1999). The primary reason for the discriminating power of microsatellites in detecting population structure appears to be their generally high allelic diversity, which affords considerable statistical power to exact and other tests of allele-distribution homogeneity (Estoup et al. 1998; Ross et al. 1999). In addition, because microsatellites are diploid and biparentally inherited, they are less sensitive than haploid, uniparentally inherited mitochondrial (mt)DNA to reductions in effective population size (Birky et al. 1989). This means that microsatellites would be especially useful in detecting population structure when transitory bottlenecks (reductions in effective population size) occur prior to periods of genetic divergence. Examples of the latter are populations of charr in the salmonid genus *Salvelinus* (Angers and Bernatchez 1998; Brunner et al. 1998).

In situations where both microsatellites or other nuclear-encoded loci and mtDNA are sufficiently variable, the use of bi- and uniparentally inherited genetic markers has often revealed sex-biased patterns of genetic divergence that have been interpreted to indicate sex-biased differences in rates of gene flow. Generally, the degree of divergence in mtDNA has been greater than that in nuclear-encoded DNA, leading the authors to hypothesize that underlying mechanisms were behavioral and involved male-mediated dispersal, female philopatry, or both. Noteworthy examples among marine species include humpback (Palumbi and Baker 1994; Larsen et al. 1996) and beluga (Brown Gladden et al. 1999) whales, green turtles (Bowen et al. 1992; FitzSimmons et al. 1997), marine iguanas (Rassmann et al. 1997), blue marlin (Buonaccorsi et al. 1999), and sea trout (Ferguson et al. 1995). In theory, when populations are in equilibrium between genetic drift and migration, and migration rates of males and females are equal, the degree of divergence in mtDNA is expected to be 4 times that of divergence in analogous nuclear-encoded DNA (Birky et al. 1989). This suggests that for equilibrium populations, the magnitude of estimates of population divergence (e.g., F_{ST}) based on mtDNA should be significantly greater than 4 times the magnitude of estimates based on nuclear-encoded sequences when there is male-mediated dispersal and/or female philopatry. Conversely, the magnitude of divergence in mtDNA should be less than 4 times the magnitude of divergence in nuclear-encoded sequences if there is female-mediated dispersal and/or male philopatry (Birky et al. 1989). To our knowledge, the latter only has been documented in a study of two populations of broad whitefish (*Coregonus nasus*) from northern Alaska (Patton et al. 1997).

In this study, we employed microsatellites to assess population structure and gene flow among red drum (*Sciaenops ocellatus*) from the northern Gulf of Mexico (Gulf). Briefly, red drum is a widely distributed, economically important, sciaenid fish found in the western Atlantic Ocean, primarily in the northern Gulf and along the east coast of the United States (Pattillo et al. 1997). Juveniles are estuarine-dependent, spending the sexually immature part of their life cycle in bays and estuaries (Overstreet 1983; Wilson and Nieland 1994). At sexual maturity, individuals move offshore into the open ocean, where they often form large schools that can migrate extensively over time (Overstreet 1983; Matlock 1987; Pattillo et al. 1997). There also is indirect evidence that limited movement of individuals between adjacent bays or estuaries can occur at the egg, larval, and juvenile stages (Osborn et al. 1982; Lyczkowski-Schultz et al. 1988; C. Wenner, personal communication). The species once supported an important commercial fishery (Swingle 1987), which because of reduced landings stemming from overfishing and habitat decline (Heffernan and Kemp 1982; Swingle et al. 1984), was closed entirely in the northern Gulf by 1990 (GMFMC 1996). Red drum still support an important recreational fishery in bays and estuaries (Swingle 1987; Van Voorhees et al. 1992), and it is anticipated that offshore spawning stocks will be restored if escapement measures enacted to protect juveniles in bays and estuaries are effective (Swingle 1987; Pattillo et al. 1997). Because knowledge and geographic definition of discrete sub-

populations (stocks), if they exist, is an integral part of conservation and management of aquatic resources (Ryman 1991), a central issue addressed by our long-term research on red drum is whether population structure exists in the northern Gulf.

Our previous genetic studies utilized allozyme and mtDNA markers and demonstrated that red drum in the northern Gulf differ significantly from red drum along the east coast of the United States (Bohlmeyer and Gold 1991; Gold and Richardson 1991; Gold et al. 1993). A more recent study (Gold et al. 1999) that utilized mtDNA revealed that divergence followed a pattern of isolation-by-distance: mtDNA haplotype frequencies were positively autocorrelated among samples from proximate geographic localities and negatively autocorrelated among samples from distal geographic localities. The regression of pairwise Φ_{ST} values (a measure of population subdivision) with pairwise geographic distance among samples also was significant. We hypothesized that this pattern of genetic divergence likely was a function of (adult) female behavior, and could stem from philopatry to natal bays or estuaries, restricted coastwise movement relative to natal bays or estuaries, or both. We employed microsatellites in the present study to ask whether more subtle population structure exists than was revealed by mtDNA, given that microsatellites often reveal significant genetic divergence when mtDNA does not (Bentzen et al. 1996; Ruzzante et al. 1996a; Ball et al. 2000).

A second issue addressed in the research is temporal stability of allele diversity. Most population-genetic studies of pelagic marine organisms typically represent a single "snapshot" in time, in that individuals, often of mixed ages, are sampled only once from a given geographic locality. Targeted studies of genetic variation in different cohorts (age groups or year classes) or among samples taken in different years but at the same locality are few in number; some (Graves et al. 1992; Ruzzante et al. 1996b, 1997; Nielsen et al. 1999) have documented genetic stability over time, whereas others (Smolenski et al. 1993; Purcell et al. 1996) have not. Demonstrating temporal stability in patterns of genetic heterogeneity is important relative to inferring that different subpopulations are exposed to independent population dynamics and evolutionary forces (Ruzzante et al. 1997) and for discerning genetic signal from genetic noise (Waples 1998). The absence of temporal stability in patterns of genetic divergence, alternatively, may signal that subpopulations have experienced severe reductions in effective population size. Hedgecock (1994) described a "sweepstakes" hypothesis for marine organisms with high fecundity where chance events (e.g., oceanic currents, fortuitous absence of predators) could lead to a high variance in offspring survival that would result in a reduction in effective population size. Red drum fit well the model proposed by Hedgecock (1994), as they are highly fecund, broadcast spawners whose eggs and larvae are transported by oceanic currents into bays and estuaries (Matlock 1987; Lyczkowski-Schultz et al. 1988; Wilson and Nieland 1994). Based on variation at a single microsatellite, Chapman et al. (1999) felt the "sweepstakes" hypothesis was provisionally supported for subpopulations of red drum along the Atlantic coast of the southeastern United States. We found no statistically significant temporal variability of mtDNA variation in our studies of red drum in the northern Gulf (Gold et al. 1999). However, estimates of genetic effective size were three orders of magnitude lower than estimates of census size of red drum in the northern Gulf (Turner et al. 1999). A low effective size to census size ratio is expected under the "sweepstakes" hypothesis.

The last issue addressed in the research is whether genetic divergence and gene flow in red drum is biased sexually. Our hypothesis of philopatry and/or restricted coastwise movement of red drum females was predicated on geographic patterns of variation in maternally inherited mtDNA. The issue is of interest biologically, given the accumulating evidence of sex-biased genetic divergence and gene flow in marine species. It also is of concern relative to ongoing stock-enhancement programs, primarily in Texas, where the "wild" red drum fishery is augmented through the annual release of millions of hatchery-produced fingerlings (McEachron et al. 1995). The "genetic" issues of stock-enhancement programs are reviewed elsewhere (Blankenship and Leber 1995; Tringali and Bert 1998), and include the need for information regarding the population biology and spatial demography of an augmented species relative to selection of broodstock individuals and of localities where augmentation might occur.

Materials and methods

A total of 967 red drum, representing cohorts from 1986 to 1989, were sampled between 1987 and 1991 from seven bays or estuaries in the northern Gulf. Collection localities are shown in Fig. 1; the number of individuals by cohort sampled at each locality is given in Table 1. Details of tissue type, procurement, and storage are given in Gold et al. (1999). Almost all fish were age 0 (yearlings, < 300 mm in total length). Ages of individuals > 300 mm in total length were determined from annuli on otoliths, as described in Bumguardner (1991). All fish assayed were assigned to one of four cohorts (1986–1989).

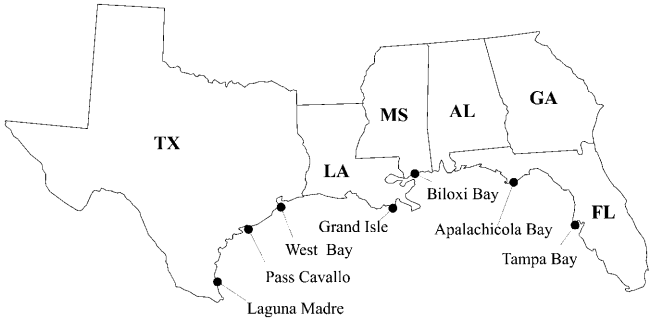

Fig. 1 Sampling localities for red drum (*Sciaenops ocellatus*) examined in the present study (*TX* Texas; *LA* Louisiana; *MS* Mississippi; *AL* Alabama; *FL* Florida)

Table 1 Localities (acronyms), cohorts, and number of individuals assayed for allelic variation at eight microsatellites among red drum (*Sciaenops ocellatus*) sampled from the northern Gulf of Mexico. State abbreviations are as in legend to Fig. 1

Locality	Number of individuals assayed				
	1986	1987	1988	1989	Total
Tampa Bay, FL (TBY)	24	42	44	39	149
Apalachicola Bay, FL (APA)	24	37	46	42	149
Biloxi Bay, MS (OSP)	94	36	19	27	176
Grand Isle, LA (GIL)	43	47	31	27	148
West Bay, TX (GVB)	32	29	29	20	110
Pass Cavallo, TX (PCV)	13	18	30	29	90
Laguna Madre, TX (LMA)	18	19	58	50	145
Totals	248	228	257	234	967

Polymerase chain reaction (PCR) primer sequences, length, and annealing temperature(s) for the eight microsatellites employed in the study may be found in Turner et al. (1998). For assay of individual fish, genomic DNA was isolated from frozen tissues as described in Gold and Richardson (1991); genotypes at each microsatellite were determined by PCR amplification and gel electrophoresis. Prior to amplification, one of the primers was kinase-labeled with γ^{32}P-ATP by T4 polynucleotide ligase (30 min, 37°C). PCR reactions contained approximately 5 ng of genomic DNA, 0.1 units of *Taq* DNA polymerase, 0.5 μM of each primer, 800 μM dNTPs, 1–2 mM $MgCl_2$, 1×*Taq* buffer at pH 9.0 (Promega), and sterile deionized water in a total volume of 10 μl. Thermal cycling was carried out in 96-well plates as follows: denaturation (45 s, 95°C), annealing (30 s), and polymerization (30 s, 72°C) for 30 cycles. Upon completion of thermal cycling, 5 μl of "stop" solution (Promega) was added to each sample. Aliquots (3 μl) of each PCR reaction were then electrophoresed in 6% denaturing polyacrylamide "sequencing" gels. Gels were dried and exposed to X-ray film. Alleles at individual microsatellites were scored as the size in base pairs of the fragment amplified by PCR. Genotypes at each microsatellite for each individual were scored and entered into a database.

Allele frequencies and direct-count heterozygosities were obtained with Biosys-1.7 (Swofford and Selander 1981). Significance testing of Hardy-Weinberg (HW) equilibrium proportions involved exact tests performed with Markov-chain randomization (Guo and Thompson 1992); probability (*P*) values for tests at each microsatellite within each sample were estimated via permutation (bootstrapping) with 1,000 resamplings (Manly 1991). Significance levels for simultaneous tests were adjusted with the sequential Bonferroni approach (Rice 1989). Tests of genotypic equilibrium at pairs of microsatellites were carried out as a surrogate to assess whether any microsatellites were genetically linked. Probability values for exact tests of genotypic equilibrium were generated by 1,000 resamplings, and significance levels for simultaneous tests were adjusted with the sequential Bonferroni approach. Tests of HW equilibrium and genotypic equilibrium employed the package Genepop (Raymond and Rousset 1995).

Tests of genetic homogeneity of allele distributions included (1) the Monte Carlo procedure of Roff and Bentzen (1989), as implemented in the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992), (2) exact tests, as implemented in Genepop, and (3) the molecular analysis of variance (AMOVA) of Excoffier et al. (1992). AMOVA generates Φ statistics, a set of hierarchical *F*-statistic analogs that measure the proportion of molecular genetic variation attributable to different hierarchical levels and take into account the evolutionary distance among alleles. We employed all three approaches to test both temporal (among cohorts within localities) and spatial (among localities) genetic homogeneity at each microsatellite. Temporal comparisons with AMOVA assessed the proportion of the genetic variance within localities due to cohort and whether the associated Φ statistic (Φ_{SC}) differed significantly from zero. Spatial comparisons employed regional groupings of samples (eastern, central, and western Gulf) and assessed the proportion of the genetic variance among regions and among localities within regions and whether the associated Φ statistics (Φ_{CT} and Φ_{SC}, respectively) differed significantly from zero. Significance of tests of genetic homogeneity, and of whether Φ statistics differed significantly from zero, employed permutation with 1,000 resamplings per individual comparison; significance levels for simultaneous tests were adjusted by using the sequential Bonferroni approach. Finally, RST-CALC (Goodman 1997) was used to estimate *rho*, the proportion of the genetic variance among regions over all microsatellites.

In addition to homogeneity testing, clustering of genetic divergence matrices between pairs of samples and two approaches (spatial autocorrelation and regression) were employed to examine whether genetic divergence was correlated with geographic distance between sample localities. Genetic divergence metrics included the θ measure of F_{ST} (Weir and Cockerham 1984), obtained with Arlequin (Schneider et al. 1997), and the $\delta\mu^2$ distance statistic of Goldstein et al. (1995), obtained with RST-CALC. The former is based on the infinite alleles model (IAM) of microsatellite evolution, whereas the latter is based on the step-wise mutation model (SMM). Each genetic divergence matrix was clustered by neighbor joining (Saitou and Nei 1987) with the Neighbor program in Phylip (Felsenstein 1992). Spatial autocorrelation analysis was used to determine whether allele distributions at each microsatellite at a sample locality were independent of those at adjacent sample localities. Correlograms that plotted autocorrelation coefficients (Moran's I values) as a function of geographic distance between pairs of sample localities were employed to summarize patterns of geographic variation of allele distributions. We used the Spatial Autocorrelation Analysis Program (SAAP) of Wartenberg (1989) and followed procedures outlined in Sokal and Oden (1978a, b). "Noise" was minimized by including only alleles found in ≥20 individuals. Microsatellites (number of alleles used in SAAP runs) were Soc 11 (7), Soc 19 (15), Soc 35 (8), Soc 60 (5), Soc 156 (5), Soc 204 (8), and Soc 243 (6). The first of two SAAP runs employed equal geographic distances between geographic distance classes; the second employed (approximately) equal numbers of pairwise comparisons in each distance class. The number of pairwise comparisons in the former was 4, 6, 5, and 6; the number of pairwise comparisons in the latter was 5, 5, 5, and 6. Distance classes in both runs were generated by SAAP from input longitude and latitude of each sample locality. The correlation between genetic and geographic distance was evaluated by using a linear stepping-stone model of migration (Rousset 1997). Pairwise genetic distances were converted to $n/(1\text{-}n)$, where n represented pairwise θ values (Weir and Cockerham 1984), and plotted against pairwise geographic distances (in kilometers), measured as the distance between sample localities following the coastline. The regression extension of Mantel's test (Smouse et al. 1986) was employed to test significance of the slope of the regression, adjusted for non-independence.

Results

Summary statistics, including direct-count heterozygosity and results of tests of genotype conformance to expectations of HW equilibrium, for each of the eight microsatellites by cohort among the seven geographic

sample localities, are given in Appendix A. The distribution of alleles at each microsatellite by locality is given in Appendix B. Descriptive statistics are presented in Table 2 and include (1) the repeat sequence of the cloned allele, (2) the number of alleles detected, (3) the average (direct-count) heterozygosity (±SE) observed among sample localities, and (4) summarized results of tests of HW equilibrium. The eight microsatellites comprised a variety of di-, tri-, and tetranucleotide motifs, with the number of alleles per microsatellite ranging from six (Soc 60 and Soc 243) to 21 (Soc 19). Average direct-count heterozygosity ranged from 0.560 ± 0.018 (Soc 60) to 0.903 ± 0.009 (Soc 19), typical of heterozygosity values reported for microsatellites in other vertebrates, including fish (Turner et al. 1998; DeWoody and Avise 2000). Following Bonferroni correction (Rice 1989), genotype proportions at 5 of the microsatellites in all 28 samples (4 cohorts × 7 localities) were non-significant. Two significant HW tests (one involving the 1986 and one involving the 1987 cohorts at West Bay, Tex.) were found at Soc 35, and two significant HW tests (one involving the 1988 cohort at Grand Isle, La. and one involving the 1989 cohort at Pass Cavallo, Tex.) were found at Soc 204. The non-significance of the remaining 26 tests at both Soc 35 and Soc 204 suggests the significant departures from HW equilibrium are not meaningful biologically. Fifteen of 28 tests at Soc 252 were significant. In all 15 significant tests, there was a deficit of heterozygotes, suggesting the presence of null alleles. The possible presence of null alleles means that perceived allele distributions at Soc 252 may be erroneous, as some number of heterozygous genotypes may have been scored as homozygotes. For this reason, Soc 252 was omitted from all further analyses.

Initial tests of genotypic equilibrium between pairs of microsatellites were carried out with all samples pooled. Significant genotypic disequilibrium following Bonferroni correction was found only in the pairwise comparison of Soc 19 versus Soc 204 (Table 3). Tests of genotypic equilibrium within individual localities (cohorts pooled) were non-significant except for the comparison of Soc 19 versus Soc 204 ($P = 0.000$) in the pooled samples from Biloxi Bay, Miss. Probability values of tests of genotypic equilibrium between Soc 19 and Soc 204 at the remaining six localities ranged from 0.153 at Pass Cavallo, to 0.918 at West Bay, and averaged 0.464. The significant genotypic disequilibrium between Soc 19 and Soc 204 at Biloxi Bay appears to be due to a non-random distribution of genotypes only within the sample from the 1986 cohort ($P = 0.000$), as probability values for genotypic disequilibrium in the 1987, 1988, and 1989 cohorts at Biloxi Bay were 0.733, 1.000, and 1.000, respectively. Taken together, these results indicate that genotypes at pairs of the seven microsatellites are randomly associated and that all seven are inherited independently.

Significant heterogeneity in allele distributions among cohorts within sampling localities was detected prior to Bonferroni correction at six of the seven localities for one (five localities) or two (one locality) of the microsatellites (Table 4). None of the probability values were significant following Bonferroni correction. The Φ_{SC} statistic (an estimate of the proportion of the genetic variance due to among cohorts) and the probability that it differed significantly from zero for each of the seven microsatellites were: Soc 11 ($\Phi_{SC} = 0.001$, $P = 0.342$), Soc 19 ($\Phi_{SC} = -0.001$, $P = 0.601$), Soc 35 ($\Phi_{SC} = 0.004$, $P = 0.175$), Soc 60 ($\Phi_{SC} = 0.001$, $P = 0.323$), Soc 156 ($\Phi_{SC} = 0.008$, $P = 0.070$), Soc 204 ($\Phi_{SC} = 0.006$,

Table 2 Variation at eight microsatellites among red drum sampled from seven localities in the northern Gulf of Mexico

Microsatellite	Repeat sequence[a]	No. of alleles	Average heterozygosity ± SE	P_{HW}[b]
Soc 11	$[GA]_{12}$	14	0.651 ± 0.015	0/28
Soc 19	$[GATA]_{16}$	21	0.903 ± 0.009	0/28
Soc 35	$[CT]_5/[CA]_9$	19	0.632 ± 0.020	2/28
Soc 60	$[AGG]_8$	6	0.560 ± 0.018	0/28
Soc 156	$[CCT]_6/[TCC]_4$	12	0.580 ± 0.023	0/28
Soc 204	$[CTG]_{12}$	14	0.623 ± 0.014	2/28
Soc 243	$[CCT]_9$	6	0.708 ± 0.014	0/28
Soc 252	$[CA]_{10}$	19	0.606 ± 0.022	15/28

[a] Sequence of the cloned allele (Turner et al. 1998)
[b] Proportion of samples where $P < 0.05$ following Bonferroni correction for simultaneous tests

Table 3 Probability of genotypic equilibrium (pairwise comparisons) at seven microsatellites among red drum sampled from the northern Gulf of Mexico. Probability values are based on exact tests (1,000 permutations); corrected α (for initial test) = 0.002

Locus	Soc 11	Soc 19	Soc 35	Soc 60	Soc 156	Soc 204	Soc 243
Soc 11	–	0.161	0.681	0.294	0.015	0.608	0.512
Soc 19		–	0.373	0.694	0.799	0.000*	0.082
Soc 35			–	0.929	0.141	0.931	0.331
Soc 60				–	0.155	0.782	0.735
Soc 156					–	0.324	0.284
Soc 204						–	0.363
Soc 243							–

* Significant values after Bonferroni correction for simultaneous tests

Table 4 Probability values of tests for homogeneity in allele distributions at seven microsatellites among four consecutive cohorts of red drum sampled at each of seven geographic localities in the northern Gulf of Mexico. Upper value is probability based on 1,000 bootstrap pseudoreplicates (Roff and Bentzen 1989); lower value is probability based on exact test, with 1,000 permutations. Corrected α for initial test = 0.001

Locus	Soc 11	Soc 19	Soc 35	Soc 60	Soc 156	Soc 204	Soc 243
Locality							
Tampa Bay, FL	0.424	0.830	0.487	0.098	0.755	0.069	0.441
	0.334	0.909	0.325	0.303	0.873	0.035*	0.507
Apalachicola Bay, FL	0.487	0.751	0.192	0.803	0.758	0.010*	0.567
	0.563	0.807	0.345	0.744	0.514	0.010*	0.460
Biloxi Bay, MS	0.402	0.201	0.853	0.056	0.403	0.263	0.773
	0.476	0.144	0.784	0.029*	0.566	0.061	0.865
Grand Isle, LA	0.231	0.413	0.554	0.212	0.892	0.747	0.603
	0.266	0.513	0.504	0.229	0.755	0.877	0.497
West Bay, TX	0.333	0.488	0.208	0.256	0.109	0.036*	0.864
	0.406	0.584	0.305	0.222	0.093	0.088	0.710
Pass Cavallo, TX	0.179	0.740	0.082	0.346	0.083	0.138	0.413
	0.065	0.608	0.042*	0.314	0.044*	0.099	0.437
Laguna Madre, TX	0.972	0.039*	0.789	0.661	0.053	0.865	0.974
	0.983	0.118	0.671	0.470	0.097	0.832	0.976

* Significant values before Bonferroni correction for simultaneous tests; none of the probability values were significant after correction

Table 5 Results of tests for (spatial) homogeneity in allele distributions at seven microsatellites among geographic samples of red drum from the northern Gulf of Mexico. P_{rb} Probability of allele-frequency homogeneity based on 1000 bootstrapped pseudoreplicates (after Roff and Bentzen 1989). P_{exact} Probability of allele-frequency homogeneity based on exact test, with 1000 permutations. Φ_{ST} Hierarchical F_{ST} analogue derived from AMOVA (Excoffier et al. 1992); P is the probability of finding a more extreme variance component by chance alone (5,000 permutations)

Microsatellite	P_{rb}	P_{exact}	Φ_{ST}	P
Soc 11	0.087	0.020	0.005	0.001*
Soc 19	0.000*	0.000*	0.003	0.000*
Soc 35	0.000*	0.000*	0.003	0.016
Soc 60	0.180	0.160	0.003	0.025
Soc 156	0.015	0.004*	0.005	0.009*
Soc 204	0.000*	0.000*	0.003	0.011*
Soc 243	0.644	0.659	0.000	0.540

*Significant probability values ($P < 0.05$) following Bonferroni correction for simultaneous tests

$P = 0.053$), and Soc 243 ($\Phi_{SC} = -0.004$, $P = 0.845$). These results indicate that from zero to roughly 0.80% of the genetic variance over all localities is due to variation among cohorts but that the variance component attributable to variation among cohorts within localities does not differ significantly from zero. Because of this, cohort samples at each locality were pooled for subsequent investigation of spatial variation (among localities) at each microsatellite.

Results of homogeneity tests (Table 5) revealed significant differences in allele distributions among localities at five of the seven microsatellites, both before and after Bonferroni correction. At two of the microsatellites (Soc 19 and Soc 204), significant heterogeneity following Bonferroni correction was found in all three approaches employed (viz., the Roff-Bentzen procedure, exact test, and AMOVA), while two of the three approaches yielded significant heterogeneity at Soc 35 and Soc 156. Φ_{ST} values (the proportion of the genetic variance among localities) derived from AMOVA ranged from 0.000 to 0.005 (Table 5), and averaged 0.003 over all seven microsatellites. The latter conformed closely to an estimate of 0.003 for all seven microsatellites combined.

We examined further the spatial distribution of the genetic heterogeneity by subdividing the samples into regional groups. The subgroup localities were: eastern Gulf (Tampa Bay and Apalachicola Bay), central Gulf (Biloxi Bay and Grand Isle), and western Gulf (West Bay, Pass Cavallo, and Laguna Madre). Results of homogeneity tests among the three regional subgroups (not shown) paralleled the tests carried out among localities (Table 5), in that significant heterogeneity was detected at the same five microsatellites. Alternatively, the hierarchical AMOVA revealed that the proportion of the variance attributable to "between/among localities within regional subgroups" (reflected as Φ_{SC} statistics) was non-significant at all seven microsatellites. Probability values for the test of the null hypothesis that $\Phi_{SC} = 0$ for each microsatellite were Soc 11 ($P = 0.342$), Soc 19 ($P = 0.601$), Soc 35 ($P = 0.175$), *Soc* 60 ($P = 0.323$), Soc 156 ($P = 0.070$), Soc 204 ($P = 0.053$), and Soc 243 ($P = 0.845$). This pattern indicates that divergence in microsatellite allele frequency is at least partially a function of geographic distance, as significant heterogeneity occurs among subregional groupings but not between spatially contiguous localities within groupings. This was confirmed by pairwise exact tests, where samples from geographically adjacent localities in different subregional groupings (i.e., Apalachicola Bay, Fla. vs Biloxi Bay, and Grand Isle vs West Bay) generally did not differ significantly from one another. A similar pattern where genetic divergence was attributable at least in part to geographic distance was indicated by estimates of genetic distance between pairs of sample localities. Both the θ measure of F_{ST} and the $\delta\mu^2$ distance statistic increased with increasing geographic distance between localities (data not shown), and the two were correlated significantly ($r = 0.59$, $t_{[19]} = 3.193$, $P < 0.01$). The neighbor-joining topology (Fig. 2) derived from the pairwise matrix of θ values clearly

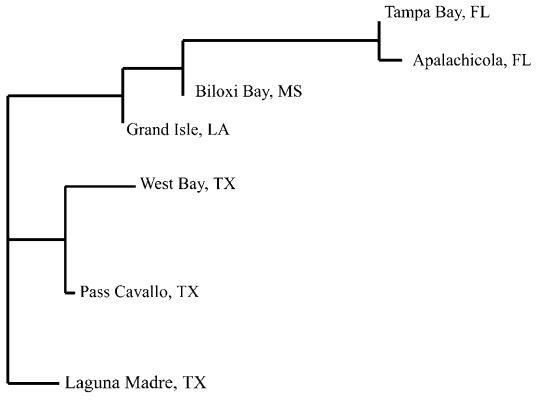

Fig. 2 Neighbor-joining topology generated from a matrix of pairwise genetic distances (the θ measure of F_{ST}) between samples of red drum

demonstrated a relationship between genetic and geographic distance, as geographically proximate sample localities clustered successively.

Results of spatial autocorrelation analysis also indicated a distance effect relative to genetic divergence among geographic samples. SAAP runs that employed alleles found in ≥20 individuals generated 216 Moran's I values (54 alleles over 7 microsatellites × 4 distance classes). When equal distance between distances classes were used, 23 significant ($P < 0.05$) and eight highly significant ($P < 0.01$) Moran's I values were obtained. Of the 31 significant or highly significant values, 14 (12 positive) occurred in the first distance class, three (all positive) occurred in the second distance class, six (four negative) occurred in the third distance class, and eight (seven negative) occurred in the last distance class. Five of the positive values occurring in the first distance class were highly significant, as were three negative values occurring in the last distance class. Nearly identical results were obtained in SAAP runs that used equal numbers of pairwise comparisons in each distance-class. There were 24 significant and 5 highly significant Moran's I values, with the distribution of positive and negative scores virtually the same as SAAP runs with equal distances between distance-classes. Graphical representation of these results is shown in Fig. 3, where the mean (±2SE) Moran's I values for SAAP runs that employed equal numbers of pairwise comparisons are plotted by distance class for each microsatellite. For comparison, a plot of mean Moran's I values (±2SE), based on mtDNA haplotypes where significant Moran's I values were found in our prior study (Gold et al. 1999), is also shown in Fig. 3. For nearly all of the microsatellites, and for mtDNA, autocorrelations (mean Moran's I values) are generally positive in the first distance class, near zero in the second distance class, near zero or negative in the third distance class, and negative in the fourth distance class. The autocorrelation profiles are consistent with the hypothesis that genetic divergence among red drum in the northern Gulf is at least in part a function of geographic distance between the bays and estuaries from which individuals were sampled and follows a classical isolation-by-distance model (Sokal and Oden 1972a, b). Of interest are the similarities in autocorrelation profiles among microsatellites and mtDNA and that the geographic distance between localities at which spatial autocorrelations become effectively zero is in the proximity of 700–900 km.

An isolation-by-distance effect also was indicated by a significant regression ($r = 0.772$, adjusted $r^2 = 0.575$, $P = 0.001$, $df = 1,7$) in the plot of linear pairwise geographic distances between sample localities and pairwise estimates of genetic divergence (the θ measure of F_{ST}) among microsatellites (Fig. 4). As indicated, genetic divergence increases in an approximately linear fashion with increasing geographic distance, with the slope of the regression line (3.15×10^{-6}) also differing significantly from zero (95% CI = 1.90×10^{-6} and 4.39×10^{-6}). Interestingly, the Φ_{ST} value (0.003) for the three microsatellites (Soc 19, Soc 35, and Soc 204), where significant heterogeneity in allele distributions was revealed by both homogeneity tests (Table 5), corresponds to approximately 900 km, roughly the same geographic distance where spatial autocorrelations become effectively zero. For comparison, we also regressed pairwise genetic distances between the same sample localities and pairwise estimates of genetic divergence (Φ_{ST}) based on mtDNA haplotypes (data from Gold et al. 1999). The regression for mtDNA also was significant ($r = 0.548$, adjusted $r^2 = 0.263$ $P = 0.024$), as was the slope of the regression line (4.10×10^{-6}, 95% CI = 1.09×10^{-6} and 7.10×10^{-6}). The slopes of the two regression lines fell well within the 95% confidence intervals of one another.

Discussion

As part of a continuing analysis of genetic population structure in red drum, we addressed three issues in this study. The first was whether microsatellites would reveal more subtle population structure than had been detected previously with mtDNA. Studies in other marine species such as Atlantic cod (Bentzen et al. 1996; Ruzzante et al. 1996a), Pacific herring (O'Connell et al. 1998), European hake (Lundy et al. 1999), squid (Shaw et al. 1999), and wreckfish (Ball et al. 2000) have shown that microsatellites often reveal population structure undetected by other genetic markers. The latter includes mtDNA, even in situations where there was sufficient variation such that significant heterogeneity could have been detected should it have existed. This distinction, that there be sufficient variation in a genetic marker, is necessary as several studies have demonstrated significant heterogeneity in nuclear markers (including microsatellites) in situations where variation in mtDNA was minimal to nil, presumably as a consequence of a severe, recent bottleneck in female effective size (Ovenden and White 1990; Angers et al. 1995; Jones et al. 1996; Brunner et al. 1998). In general, microsatellites are expected to have better resolving power than many genetic markers be-

cause of higher mutation rates leading to higher allelic diversity (Angers and Bernatchez 1998; Estoup et al. 1998; Ross et al. 1999).

In this study, we detected significant genetic heterogeneity among geographic samples of red drum at four to five of the seven microsatellites assayed. The proportion of genetic variance due to among samples, measured as the average of Φ_{ST} values for each microsatellite, was approximately 0.003, as was an overall F_{ST} (*rho*). The genetic heterogeneity followed a pattern of isolation-by-distance, where genetic divergence increased with increasing geographic distance between sample localities. Interestingly, both spatial autocorrelation analysis and regression of genetic distance on geographic distance between pairs of geographic samples suggest that significant genetic divergence becomes apparent when sample localities are separated by 700–900 km; we refer to this as the geographic neighborhood size as a means to define geographic boundaries between overlapping subpopulations. Virtually identical results were obtained in our previous studies of mtDNA (Gold et al. 1999): homogeneity testing and AMOVA detected significant heterogeneity among localities, and the Φ_{ST} value for mtDNA was 0.002. The spatial pattern of mtDNA divergence also followed a pattern of isolation-by-distance and the geographic neighborhood size was estimated to be 500–700 km. Microsatellites in red drum thus paralleled and strongly reinforced earlier findings based on analysis of mtDNA, but did not reveal more subtle population structure.

The second issue addressed by this study was whether spatially divergent genetic patterns were stable temporally. Previous studies in other marine fishes have not necessarily addressed this issue fully, as most (e.g., Bembo et al. 1995; Tringali and Bert 1995; Gold and Richardson 1998) are essentially single "snapshots" in time. However, although there are exceptions (Smolenski et al. 1993; Purcell et al. 1996), temporal stability gen-

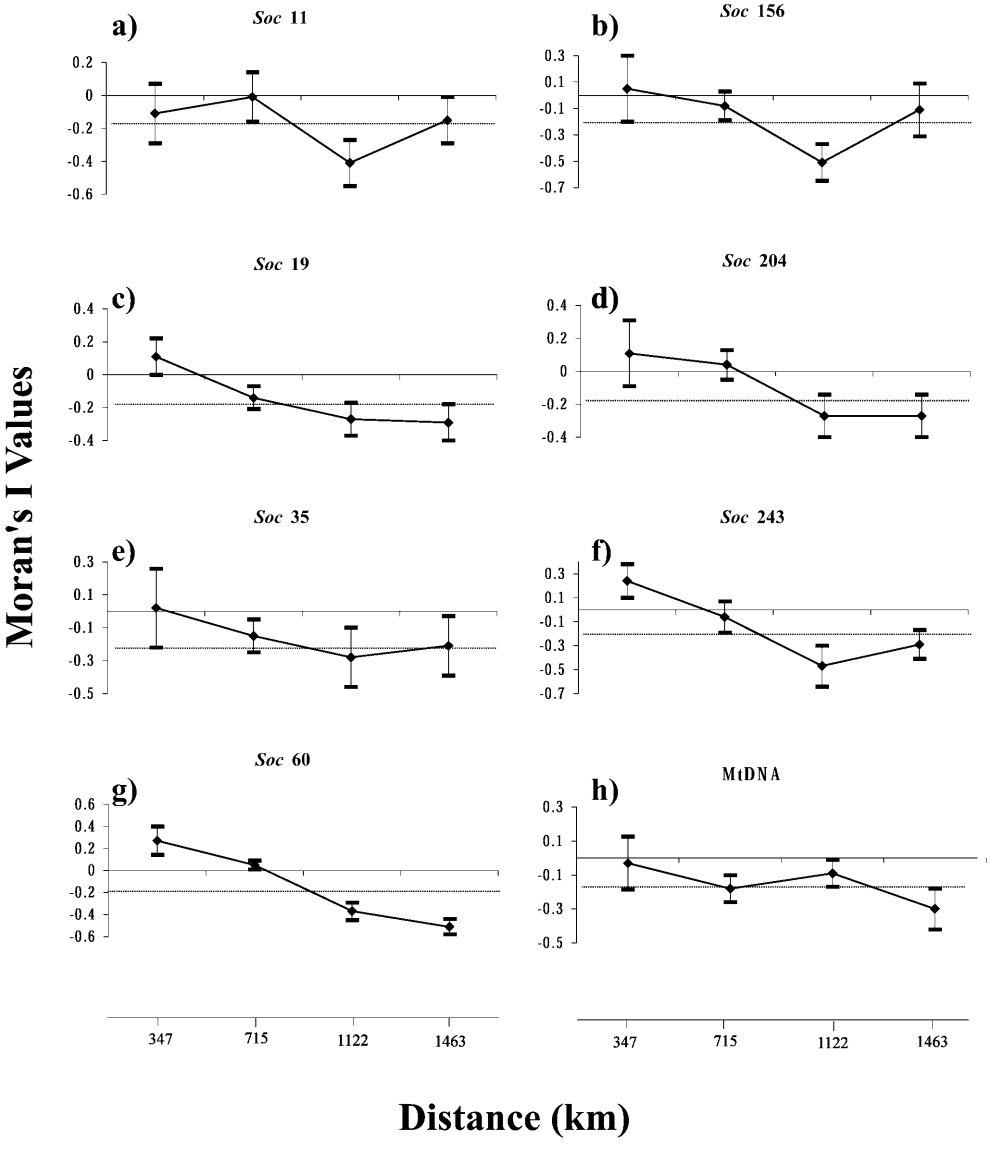

Fig. 3 Correlograms based on frequencies of alleles found in ≥20 individuals at each of seven microsatellites (*a–g*) and on frequencies of mtDNA haplotypes (*h*) found in ≥10 individuals. *Abscissas* Distance classes (left to right) based on equal frequencies per distance class; ordinates: mean autocorrelation coefficients (Moran's I values) for each distance class. *Bars* about each mean represent two standard errors on either side of a mean; *dashed lines* are expected Moran's I values when no correlation exists

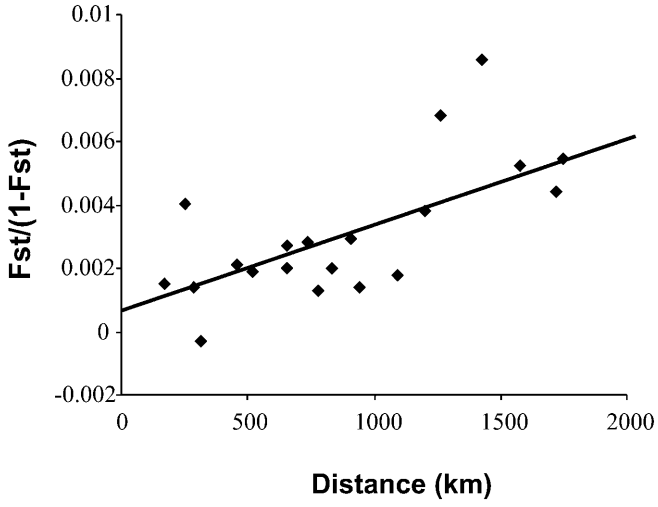

Fig. 4 Relationship between genetic distance ($F_{ST}/(1-F_{ST})$, where F_{ST} is the θ measure of Weir and Cockerham (1984) and geographic distance (km) for samples of red drum. Geographic distance between sample localities followed the coastline of the northern Gulf of Mexico

erally has been observed in those studies where sampling occurred over time (Graves et al. 1992; Brown et al. 1996; Ruzzante et al. 1997; Beacham and Dempson 1998), in one instance (Nielsen et al. 1999) over a time span of several decades. It is worth noting that "temporal sampling" in these studies did not necessarily mean sampling consecutive cohorts.

The issue of temporal stability is of interest for at least two reasons. First, temporal sampling permits discrimination of genetic signal from genetic noise that potentially could arise from non-random sampling or some form of lineage sorting (Waples 1998). This is particularly critical in marine species where degree of genetic divergence among samples (generally measured by some analog of Wright's F_{ST} statistic) is substantially less than that typically observed among freshwater or terrestrial species (Ward 2000). The importance of temporal stability relates to inferences that spatially divergent subpopulations actually exist and potentially exhibit different or independent population dynamics (Ruzzante et al. 1997). The second reason is that temporal instability could signal a "sweepstakes" type situation (*sensu* Hedgecock 1994), where temporal genetic change occurs because of small effective population size (N_e) and random genetic drift. This is of considerable interest in recruitment and other issues related to conservation and management and has been implicated as an explanation for patterns of microsatellite variation in red drum along the Atlantic coast of the southeastern United States (Chapman et al. 1999). Briefly, Chapman et al. (1999) surveyed variation in a single microsatellite among both temporal and spatial samples of red drum from the coasts of Virginia, South Carolina, and Georgia and found that genotypes at 6 of 11 sample localities exhibited heterozygote deficiencies and were not in HW equilibrium. Although no differences in allele frequencies were found among cohorts, there were significant differences in allele frequencies among samples from different localities. Based on comparisons with microsatellite variation/divergence in the related species *Cynoscion nebulosus*, Chapman et al. (1999) hypothesized that the differences in allele frequencies in red drum likely stemmed from reductions in effective population size.

In our study of microsatellite variation among red drum from the Gulf, genotype proportions in all samples were in HW equilibrium and genotypes at different microsatellites were independent of one another indicating the absence of linkage disequilibrium. In addition, allele frequencies among cohorts at each sample locality were homogeneous and the variance component (derived from AMOVA) attributable to variation among cohorts within localities did not differ significantly from zero. The temporal stability observed over the four cohorts of red drum reinforces inferences drawn from patterns of spatial genetic heterogeneity (i.e., genetic divergence in red drum in the Gulf follows an isolation-by-distance effect), and indicates that (1) the small but significant genetic divergence among spatial localities represents true signal, and (2) overlapping subpopulations may be influenced by different or independent population dynamics. The microsatellite data also indicate that red drum in the Gulf may not be experiencing a "sweepstakes effect" (Hedgecock 1994) that is of sufficient magnitude to cause significant changes in allele frequencies across cohorts or to lower genetic diversity of cohorts with respect to breeding adults. Populations undergoing a "sweepstakes effect" are predicted to exhibit (1) significant and random gene-frequency shifts between cohorts, (2) less genetic diversity in new recruits than in the adult population from which they came, and (3) linkage disequilibrium (non-independence) at multiple loci (Hedgecock 1994; Chapman et al. 1999). We observed none of these effects in either mtDNA (Gold et al. 1999) or microsatellites (this study). Turner et al. (1999), however, showed that the effective number (N_{ef}) of red drum females (14,308) in the northern Gulf was much lower than the estimated adult female census size (N_f) of approximately 3.5 million, suggesting that red drum could experience a "sweepstakes" effect.

The third issue addressed was whether genetic divergence and gene flow in red drum were biased sexually. In theory, the degree of divergence in mtDNA, under equilibrium conditions, is expected to be 4 times that of divergence in analogous nuclear-encoded DNA (Birky et al. 1989). In some marine species, observed divergence of mtDNA relative to nuclear genes or sequences exceeded that expected under equilibrium conditions, leading the authors to hypothesize the existence of male-mediated dispersal, female philopatry, or both (Palumbi and Baker 1994; FitzSimmons et al. 1997; Rassman et al. 1997; Brown Gladden et al. 1999). The same was hypothesized in studies of sea trout (Ferguson et al. 1995) and blue marlin (Buonaccorsi et al. 1999), although in both cases the degree of divergence in mtDNA was only

3–5 times that of divergence in nuclear-encoded DNA. The converse situation, where the magnitude of divergence in mtDNA is significantly less than 4 times the magnitude of divergence in nuclear-encoded sequences, would be predicted under female-mediated dispersal and/or male philopatry. To our knowledge, this has been documented only in broad whitefish (*Coregonus nasus*) sampled from two populations in northern Alaska (Patton et al. 1997).

Based on Φ_{ST} values derived from AMOVA, the degree of genetic divergence in red drum mtDNA ($\Phi_{ST} = 0.002$; Gold et al. 1999) appears to be approximately the same (or less) than that of microsatellites, where Φ_{ST} values for six of the seven microsatellites assayed were 0.003 or greater. It is important to note that Φ_{ST} values for mtDNA and for most of the microsatellites differed significantly from zero, i.e., the genetic divergence for both genetic markers is real. The near identity of microsatellite and mtDNA divergence also can be inferred from the observation that the slopes of the regression of divergence in each marker on geographic distance did not differ significantly from one another. We interpret this finding to indicate that the genetic effective size of both microsatellites and mtDNA in red drum are the same, as the inverse of this slope is hypothesized (Rousset 1997), under equilibrium conditions, to represent an estimate of effective size. Given that allele frequencies at microsatellites and mtDNA differ significantly across the northern Gulf, the identity in degree and pattern of genetic divergence at the two classes of genetic markers suggests either that gene flow in red drum could be biased sexually or that red drum populations may not be in equilibrium between genetic drift and migration. If a sexual bias exists, the fact that mtDNA divergence is considerably less than 4 times that of microsatellites would suggest female-mediated dispersal and/or male philopatry.

Our studies over the last several years, along with those of Seyoum et al. (2000), have generally demonstrated genetic divergence among geographic, but not temporal, samples of red drum. Collectively, these findings are in contrast with expectations based primarily on adult life history, where sexually mature red drum are known to form large offshore schools that can migrate considerable distances (Overstreet 1983; Matlock 1987; Patillo et al. 1997). Red drum thus provide another example of the general pattern (Avise 1998) that pelagic marine organisms with high dispersal potential may not necessarily exhibit high levels of actual gene flow and uniformity in population structure.

Based on the studies of red drum mtDNA, Gold et al. (1999) offered the suggestion that older females might not spawn as frequently as younger females that had not yet joined large offshore schools. However, Wilson and Nieland (1994) examined ovaries in large, mature red drum females sampled offshore during the spawning season and found no evidence of mid-spawning-season atresia, sterility, or females that were not in spawning condition. Ross et al. (1995) did find two senescent females (aged as 49 and 51 years) that had atrophied ovaries, but five other females (aged as 40–52 years) were spent or resting. Because these observations indicated that older, presumably migratory females do spawn annually, Gold et al. (1999) suggested that the isolation-by-distance effect observed with mtDNA could stem from natal-site philopatry (i.e., homing), limited coastwise movement relative to natal bays or estuaries, or both.

The similarity in pattern and degree of genetic divergence in both microsatellites and mtDNA of red drum suggests that the same general factors that impact genetic divergence/gene flow in red drum are operative in both sexes and, moreover, lead to an isolation-by-distance effect where genetic divergence and gene flow are related directly and inversely, respectively, to geographic distance from a natal bay or estuary. In general, the very limited data from zooplankton sampling, mark-and-recapture of juveniles, and sonic tracking of adults are consistent with this hypothesis. Briefly, Lyczkowski-Schultz et al. (1998), based on extensive ichthyoplankton sampling in the north-central Gulf, hypothesized that oceanic currents could transport larval red drum during their 12- to 16-day planktonic existence to adjacent localities. In addition, although mark-and-recapture studies in both the Gulf (Osborn et al. 1982) and along the southeastern Atlantic coast (Pafford et al. 1990 and Pugliese 1990, cited from Nicholson and Jordan 1994), have shown that juvenile red drum generally exhibit strong fidelity to a local bay or estuary, limited movement to adjacent bays or estuaries does occur and in a distribution consistent with an isolation-by-distance effect. Osborn et al. (1982), for example, found that 78–97% of fish tagged along the Texas coast were recaptured in the same bay or estuary in which they were tagged initially and that the majority of inter-bay movement was to an adjacent bay. Similarly, in a recent study by C. Wenner and colleagues, 4,215 red drum were recovered from a release of slightly over 17,000 yearlings tagged in Charleston harbor: of 4,215 recaptures, 4,107 (97.4%) were recovered within 10 nautical miles (18 km) of the release site, 78 (1.9%) were recovered in the range 10–70 nautical miles (18–126 km), and 30 (0.7%) were recovered in the range 70–170 nautical miles (126–306 km) (C. Wenner, personal communication). Finally, Nicholson and Jordan (1994) were able to follow the movements of 50 of 75 adult red drum implanted with ultrasonic transmitters and found that fish tagged in the fall along shoals and beaches in Georgia overwintered offshore then relocated the following spring and fall to the same shoal where they were tagged initially. Although how strong or widespread such site fidelity of adults may be is not known, it is noteworthy that two-thirds (50 of 75) of the fish implanted exhibited site-fidelity. Taken together, these data at various life-history stages are fully consistent with the hypothesis that gene flow in red drum is inversely related to geographic distance from a natal bay or estuary.

The possibility that gene flow in red drum might be biased sexually would seem to argue against the notion of extensive gene flow at the larval and possibly juvenile stages, as coastwise currents (implicated in larval transport) would not be expected to affect sexes differently and sex-related behavioral patterns might not be fully developed in juveniles. In addition, our estimates of geographic neighborhood size (700–900 km) are greater than the average distance between adjacent bays or estuaries, suggesting that gene flow in the Gulf occurs beyond adjacent estuaries, further implicating migratory adults. However, even assuming the majority of gene flow in red drum stems from migrating adults, the observed isolation-by-distance effect relative to genetic divergence indicates a practical limit to dispersal that is less than the range of the species (Slatkin 1993; Chambers 1995; Palumbi et al. 1997) and that long-distance dispersal in red drum is insufficient to preclude genetic divergence.

In closing, we note that conservation and management of red drum in the Gulf relative to assessment and allocation currently occurs on a state-by-state basis. Although our genetic studies indicate significant genetic divergence across the northern Gulf, the genetic differences per se do not delimit specific stocks with fixed geographic boundaries. The estimates of genetic neighborhood size represent an attempt to generate a spatial framework that might be useful in management planning, but it should be realized that the spatial framework is overlapping and ostensibly moves in both directions from any specific geographic locality. This may indicate that management planning should be carried out on a regional basis involving cooperation of independent state management agencies. The region in this context, however, is likely not the entire northern Gulf of Mexico, and the isolation-by-distance effect (and assuming dispersal distances are distributed normally) means that the effective neighborhood size (*sensu* Wright 1946) will be smaller than the range of the population (Chambers 1995).

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Appendix A

Summary statistics for each of eight microsatellites in seven geographic samples of red drum (*Sciaenops ocellatus*) from the northern Gulf of Mexico. Acronyms refer to collection localities (Table 1); individual cohorts (year classes) are designated by year, i.e., 86–89 represents 1986–1989. *n* Number of individuals scored;

Sample	Microsatellite locus							
	Soc 11	Soc 19	Soc 35	Soc 60	Soc 156	Soc 204	Soc 243	Soc 252
TBY-86								
n	24	24	24	24	24	24	24	24
H_{DC}	0.625	0.917	0.750	0.708	0.708	0.625	0.792	0.833
P_{HW}	0.211	0.091	0.431	0.145	0.472	0.143	0.010	0.190
TBY-87								
n	42	42	42	42	42	42	42	42
H_{DC}	0.690	0.857	0.714	0.476	0.714	0.619	0.714	0.690
P_{HW}	0.951	0.682	0.328	0.237	0.107	0.009	0.312	0.050
TBY-88								
n	44	44	44	44	44	44	44	43
H_{DC}	0.523	0.841	0.750	0.636	0.705	0.614	0.773	0.791
P_{HW}	0.381	0.622	0.074	0.897	0.134	0.021	0.175	0.399
TBY-89								
n	39	39	38	38	39	38	39	39
H_{DC}	0.641	0.872	0.816	0.500	0.641	0.711	0.744	0.846
P_{HW}	0.346	0.348	0.760	0.239	0.270	0.502	0.992	0.205
APA-86								
n	24	22	24	24	22	24	24	24
H_{DC}	0.625	1.000	0.625	0.583	0.500	0.583	0.667	0.417
P_{HW}	0.733	0.863	0.047	0.962	0.493	0.058	0.375	0.000*
APA-87								
n	37	37	37	37	37	37	37	37
H_{DC}	0.541	0.865	0.676	0.595	0.514	0.703	0.676	0.622
P_{HW}	0.424	0.085	0.922	0.220	0.754	0.394	0.704	0.001*

Sample	Microsatellite locus							
	Soc 11	Soc 19	Soc 35	Soc 60	Soc 156	Soc 204	Soc 243	Soc 252
APA-88								
n	46	45	46	46	45	46	46	45
H_{DC}	0.587	0.889	0.565	0.609	0.556	0.609	0.739	0.578
P_{HW}	0.631	0.438	0.700	0.106	0.923	0.131	0.042	0.000*
APA-89								
n	42	42	42	42	42	42	42	42
H_{DC}	0.595	0.929	0.667	0.595	0.595	0.643	0.595	0.524
P_{HW}	0.870	0.047	0.903	0.673	0.009	0.215	0.171	0.000*
OSP-86								
n	94	94	94	94	94	94	94	94
H_{DC}	0.691	0.851	0.745	0.479	0.574	0.670	0.681	0.649
P_{HW}	0.333	0.148	0.026	0.004	0.094	0.213	0.402	0.000*
OSP-87								
n	36	35	36	36	36	36	36	36
H_{DC}	0.806	0.886	0.722	0.556	0.528	0.611	0.639	0.694
P_{HW}	0.203	0.109	0.730	0.076	0.942	0.141	0.413	0.001*
OSP-88								
n	19	18	19	19	19	19	19	19
H_{DC}	0.526	0.889	0.474	0.737	0.684	0.632	0.737	0.421
P_{HW}	0.369	0.533	0.487	0.156	0.828	0.184	0.537	0.000*
OSP-89								
n	27	27	27	27	27	26	27	27
H_{DC}	0.630	0.852	0.519	0.556	0.630	0.692	0.519	0.481
P_{HW}	0.200	0.228	0.009	0.675	0.346	0.801	0.128	0.000*
GIL-86								
n	43	43	43	43	43	43	43	43
H_{DC}	0.628	0.884	0.674	0.605	0.535	0.605	0.674	0.465
P_{HW}	0.482	0.570	0.925	0.837	0.579	0.058	0.128	0.000*
GIL-87								
n	47	47	47	47	47	47	47	47
H_{DC}	0.574	0.915	0.660	0.766	0.426	0.681	0.702	0.489
P_{HW}	0.121	0.666	0.008	0.135	0.174	0.381	0.860	0.000*
GIL-88								
n	31	31	31	31	31	31	31	31
H_{DC}	0.645	0.935	0.613	0.613	0.645	0.484	0.710	0.484
P_{HW}	0.733	0.877	0.676	0.591	0.739	0.000*	0.261	0.000*
GIL-89								
n	27	26	27	27	26	26	27	26
H_{DC}	0.667	1.000	0.704	0.519	0.654	0.577	0.741	0.500
P_{HW}	0.261	0.942	0.725	0.587	0.896	0.077	0.103	0.000*
GVB-86								
n	32	32	32	31	31	32	32	32
H_{DC}	0.750	0.969	0.469	0.484	0.742	0.656	0.719	0.656
P_{HW}	0.140	0.845	0.000*	0.455	0.292	0.178	0.864	0.018
GVB-87								
n	29	29	29	29	29	29	29	29
H_{DC}	0.690	0.828	0.448	0.655	0.655	0.690	0.793	0.621
P_{HW}	0.790	0.117	0.000*	0.233	0.284	0.258	0.029	0.009
GVB-88								
n	29	27	29	29	28	29	29	29
H_{DC}	0.724	0.852	0.655	0.586	0.679	0.483	0.655	0.621
P_{HW}	0.031	0.161	0.568	0.806	0.523	0.021	0.824	0.008
GBV-89								
n	20	20	20	20	20	19	20	20
H_{DC}	0.700	0.850	0.600	0.550	0.700	0.632	0.650	0.750
P_{HW}	0.073	0.550	0.375	0.738	0.339	0.009	0.166	0.053
PCV-86								
n	13	10	13	13	12	13	13	13
H_{DC}	0.538	0.900	0.769	0.538	0.417	0.615	0.538	0.692
P_{HW}	0.486	0.338	0.602	0.186	0.132	0.309	0.083	0.051
PCV-87								
n	18	17	18	18	15	18	18	18
H_{DC}	0.778	0.941	0.500	0.444	0.267	0.444	0.833	0.556
P_{HW}	0.192	0.575	0.608	0.074	0.083	0.006	0.887	0.002*
PCV-88								
n	30	29	30	30	29	30	30	30
H_{DC}	0.633	0.931	0.600	0.633	0.655	0.733	0.700	0.567
P_{HW}	0.116	0.912	0.156	0.636	0.830	0.046	0.927	0.000*

Sample	Microsatellite locus							
	Soc 11	Soc 19	Soc 35	Soc 60	Soc 156	Soc 204	Soc 243	Soc 252
PCV-89								
n	29	29	29	29	29	29	29	29
H_{DC}	0.793	0.966	0.483	0.448	0.414	0.552	0.724	0.690
P_{HW}	0.757	0.839	0.035	0.060	0.725	0.001*	0.413	0.234
LMA-86								
n	18	18	18	18	18	17	18	18
H_{DC}	0.611	0.889	0.667	0.500	0.611	0.529	0.833	0.500
P_{HW}	1.000	0.298	0.128	0.808	0.476	0.084	0.977	0.021
LMA-87								
n	19	19	19	19	19	19	19	19
H_{DC}	0.632	0.895	0.789	0.316	0.316	0.737	0.789	0.632
P_{HW}	0.209	0.309	0.573	0.004	0.054	0.709	0.795	0.036
LMA-88								
n	58	57	58	57	55	57	58	58
H_{DC}	0.724	0.930	0.552	0.456	0.527	0.702	0.707	0.603
P_{HW}	0.727	0.932	0.000*	0.013	0.921	0.159	0.196	0.000*
LMA-89								
n	50	50	50	50	50	50	50	50
H_{DC}	0.660	0.940	0.500	0.540	0.640	0.600	0.780	0.600
P_{HW}	0.591	0.506	0.128	0.471	0.698	0.103	0.782	0.000*

H_{DC} observed heterozygosity (direct counts); P_{HW} probability of conforming to expected Hardy-Weinberg proportions, based on exact tests (1,000 permutations). * Significant P_{HW} values after correction for multiple tests.

Appendix B

Distribution of alleles at eight microsatellite loci in seven geographic samples of red drum from the northern Gulf

Locus (allele)*	Sample locality						
	TBY	APA	OSP	GIL	GVB	PCV	LMA
Soc 11							
213	0	0	0	0	1	0	2
217	0	0	0	0	0	0	1
219	174	190	194	173	99	82	156
221	46	36	47	33	33	32	38
223	0	0	2	1	2	0	2
225	0	0	1	2	3	0	2
226	37	41	59	43	38	32	49
229	17	12	19	24	19	12	15
231	17	9	18	11	12	9	11
233	3	2	6	4	1	5	6
235	2	1	2	1	3	1	1
237	1	7	3	4	8	6	6
239	1	0	0	0	1	1	1
241	0	0	1	0	0	0	0
Soc 19							
193	0	0	0	1	0	0	1
197	0	0	1	0	0	0	0
201	22	21	9	8	5	1	8
205	0	3	2	1	0	1	1
209	0	1	0	0	0	0	0
213	0	4	6	7	1	0	7
217	4	2	8	1	6	5	1
221	13	17	34	30	22	19	30
225	30	15	30	19	20	16	19
229	41	28	43	44	21	24	44
233	30	33	56	29	37	24	29
237	41	44	44	40	19	18	40
241	37	34	39	36	28	16	36
245	23	25	20	18	20	19	18
249	14	24	16	17	12	10	17
253	18	16	14	22	11	5	22

Locus (allele)*	Sample locality						
	TBY	APA	OSP	GIL	GVB	PCV	LMA
257	12	11	11	5	6	5	5
261	2	11	7	6	6	5	6
265	10	2	3	6	0	2	6
269	0	1	3	3	2	0	3
273	1	0	2	1	0	0	1
Soc 35							
244	103	113	143	96	69	55	106
246	0	1	0	0	2	0	0
248	0	0	1	0	1	3	2
250	0	3	0	0	1	0	0
252	112	116	157	140	105	94	125
254	12	17	7	7	3	2	3
256	7	10	14	11	7	4	17
258	4	1	0	2	0	4	1
260	15	12	8	7	8	5	10
262	18	12	7	16	6	6	9
264	6	1	4	5	4	1	5
266	7	4	5	5	7	3	7
268	1	2	1	1	0	0	1
270	0	3	2	1	2	0	2
272	7	2	2	1	2	2	2
274	0	0	1	0	2	0	0
276	1	0	0	3	0	1	0
278	3	0	0	0	0	0	0
280	0	1	0	1	1	0	0
Soc 60							
147	0	0	0	0	0	0	1
153	11	15	13	12	9	6	4
156	13	14	14	17	8	10	7
159	136	138	154	142	121	84	164
162	126	121	165	116	79	76	105
165	10	10	6	9	3	4	7
Soc 156							
167	0	1	0	0	0	0	0
170	27	28	26	27	26	13	28
176	2	1	4	4	5	1	3
182	0	0	0	1	0	0	0
185	4	2	3	0	4	4	4
188	149	156	197	175	109	108	178
194	108	98	120	86	71	44	71
200	8	2	2	1	1	0	0
248	0	1	0	0	0	0	0
260	0	1	0	0	0	0	0
266	0	1	0	0	0	0	0
314	0	1	0	0	0	0	0
Soc 204							
172	4	0	1	2	3	0	1
175	0	1	4	2	0	0	1
178	0	0	1	0	0	0	0
181	15	13	17	8	6	13	14
184	78	94	85	79	46	35	54
187	81	84	108	94	82	56	85
190	94	92	97	81	53	61	92
193	5	4	13	16	11	12	13
196	12	1	8	4	2	0	5
199	2	3	0	1	5	2	8
202	2	1	1	0	0	0	0
205	2	3	13	7	9	1	10
208	0	0	1	0	0	0	0
211	1	2	1	0	1	0	3
Soc 243							
88	4	5	10	3	3	8	6
91	86	98	114	96	61	49	95
94	55	62	64	60	43	29	56
97	93	80	118	89	77	67	84
100	55	50	42	46	34	25	46
106	5	3	4	2	2	2	3

Locus (allele)*	Sample locality						
	TBY	APA	OSP	GIL	GVB	PCV	LMA
Soc 252							
110	2	1	0	0	0	0	0
112	5	4	6	9	4	1	3
114	57	51	39	39	38	41	57
116	9	3	7	4	5	5	2
118	34	32	42	31	30	20	29
120	7	10	14	10	6	6	11
122	31	42	58	39	31	21	37
124	28	24	26	14	10	8	11
126	72	53	77	81	41	47	66
128	10	19	29	27	22	9	35
130	31	42	28	29	15	15	29
132	5	10	10	6	9	3	5
134	3	3	3	1	6	1	1
136	1	0	5	4	0	0	3
138	0	2	0	0	0	2	0
140	1	0	3	0	0	1	1
142	0	0	2	0	0	0	0
144	0	0	3	0	1	0	0
148	0	0	0	0	2	0	0

of Mexico. Acronyms refer to collection localities (Table 1). * Allele number represents size in base pairs of the fragment amplified.

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