3 Population Structure of Spotted Seatrout (*Cynoscion nebulosus*) along the Texas Gulf Coast, as Revealed by Genetic Analysis

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CONTENTS

ABSTRACT

Allelic variation at (presumed) nuclear-encoded microsatellites was assayed among spotted seatrout sampled from localities along the Gulf Coast of Texas. Tests of Hardy-Weinberg equilibrium at each microsatellite within each sample and tests of genotypic equilibrium among pairs of microsatellites within and among samples were nonsignificant. Homogeneity (exact) tests of allele distributions at each microsatellite and estimates (the θ estimator of Wright's F_{ST}) of population structure were nonsignificant following correction for multiple tests executed simultaneously. The absence of a geographic pattern to microsatellite variation among spotted seatrout sampled from the Texas coast differs from that reported previously for the nuclear-encoded gene aspartate aminotransferase (*sAAT-2*) and for mitochondrial (mt)DNA: genetic divergence at *sAAT-2* and mtDNA was significant and related in part to increasing geographic distance between sample localities (isolation by distance).

Several possibilities to account for the difference in patterns of geographic variation among these three types of genetic markers are discussed. We suggest that population structure of spotted seatrout along the Texas coast is best modeled as a series of overlapping subpopulations or stocks distributed linearly along the coastline: individual subpopulations are centered in individual (natal) estuaries, but gene flow between geographically proximate estuaries is sufficient to prevent significant genetic divergence. This type of model also has been hypothesized for red drum (*Sciaenops ocellatus*), a related sciaenid, in the northern Gulf of Mexico. Microsatellite variation also was assayed in a sample of spotted seatrout from the Atlantic coast of Florida. Significant differences in allele frequencies between the sample from the east coast of Florida and four samples from the Texas Gulf coast were detected at all five microsatellites assayed. These findings parallel results from several studies on marine fishes (including other sciaenids) where regionally distinct populations reside in the northern Gulf of Mexico and along the southeast (U.S.) Atlantic coast.

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INTRODUCTION

Spotted seatrout, *Cynoscion nebulosus*, is an estuarine-dependent sciaenid fish distributed from coastal waters in Massachusetts to the Bay of Campeche on the Yucatan Peninsula in Mexico (Patillo et al., 1997), extending perhaps into the Mexican Caribbean (Aguilar-Salazar et al., 1993). The species is most abundant in the northern Gulf of Mexico (hereafter called Gulf) from Florida to Texas (Lassuy, 1983; Mercer, 1984) and at one time supported both recreational and commercial fisheries (Patillo et al., 1997). A small (~48,500 kg in 2000) commercial fishery industry still exists in western Florida, Mississippi, and Louisiana (http://www.st.nmfs.gov/st1/commercial/landings/annual_landings.html) but is dwarfed by the recreational fishery in the Gulf where more than 4 million kg were landed in 1998 and (exclusive of Texas waters) over 6,350,000 kg were landed in 2000 (http://www.st.nmfs.gov/st1/recreational/queries/catch/time_series.html; L. Green, Coastal Fisheries Division, Texas Parks and Wildlife, personal communication). Perceived declines in spotted seatrout abundance across the Gulf have led to decreases in or prohibition of commercial catches and increasing restrictions on recreational catches (references in Bortone et al., 1997). Both overfishing and loss of habitat are hypothesized (Shipp, 1986; Patillo et al., 1997) as instrumental in spotted seatrout declines.

Assessment and allocation of spotted seatrout recreational resources in the Gulf are the responsibility of individual states and vary from state to state (GSMFC, 1993). With the exception of Florida — where management of spotted seatrout is regionally based (Muller et al., 1997) — regulations in most Gulf Coast states are predicated on a single-stock model; i.e., allocation is the same across bays and estuaries within a state. Past studies asking whether a single-stock model is appropriate are equivocal. Nongenetic studies include those of Colura and King (1989), who found that shapes of scales and otoliths varied among spotted seatrout collected from several bays along the Texas coast, and Iverson and Tabb (1962), who reported different growth rates for spotted seatrout obtained from the Gulf Coast of Florida. The differences reported by Iverson and Tabb (1962), however, were hypothesized by Murphy and Taylor (1994) to stem from environmental or harvest factors, not from existence of discrete stocks. Tagging studies (Overstreet, 1983; Baker and Matlock, 1993), generally have indicated little "coastwise" movement of juveniles or adults, with most returns occurring less than 50 km from the release site. Movement of spotted seatrout from bays and estuaries does occur but appears to be primarily associated with spawning or in response to changes in salinity or temperature (Lorio and Perret, 1978; Helser et al., 1993).

Genetic studies have been less equivocal, as differences in general protein banding patterns (Weinstein and Yerger, 1976), allozymes (Ramsey and Wakeman, 1987; King and Pate, 1992), and mitochondrial (mt)DNA (Gold et al., 1999) have been reported among spotted seatrout sampled from various bays and estuaries of the Gulf. In three of these studies, i.e., Ramsey and Wakeman (1987), King and Pate (1992), and Gold et al. (1999), genetic divergence, although comparatively small, appeared to be, in part, a function of geographic distance between pairs of sample localities. In their study of 44 allozyme loci among 12 samples along the Gulf Coast of Texas and northern Mexico, King and Pate (1992) attributed this "isolation-by-distance" effect to westerly directed transport of eggs and larvae in nearshore waters. King and Zimmerman (1993) hypothesized that the difference in frequencies of alleles at the aspartate aminotransferase locus reported by King and Pate (1992) may reflect adaptation to temperature or salinity differences but that a nearshore dispersal mechanism might limit genetic divergence. Gold et al. (1999) assayed mtDNA variation across a broader geographic range, from the Lower Laguna Madre in southern Texas to Tampa Bay on the west coast of Florida, and hypothesized that the isolation-by-distance effect stemmed from factors (e.g., behavioral, physiological) that limited female dispersal from natal bays and estuaries.

In this chapter, we report results from ongoing, independent studies in our laboratories on variation in (presumed) nuclear-encoded microsatellites among geographic samples of spotted seatrout from the Gulf Coast of Texas. Briefly, microsatellites are abundant, short stretches of DNA composed of di-, tri-, or tetranucleotide arrays that are embedded in unique DNA, inherited in a Mendelian fashion, highly polymorphic, found in all eukaryotic species, and distributed evenly

throughout euchromatic regions of chromosomes (Weber, 1990; Wright, 1993; Weber and May, 1989). Microsatellites are considered superior to all other known genetic markers for population-genetic studies because of high levels of polymorphism and allele frequencies that are generally consistent with HW equilibrium expectations of diploid, Mendelian loci. In addition, because identification of each microsatellite is via amplification using specific polymerase-chain-reaction (PCR) primers, few problems are associated with homology of alleles (Weber, 1990; Wright and Bentzen, 1994). While our long-term interest is assessing population structure of spotted seatrout in the northern Gulf, our initial work (reported here) is restricted to spotted seatrout from the Texas Gulf Coast. Understanding the genetic structure of spotted seatrout in Texas waters has important management implications, especially for an ongoing stock enhancement program (King et al., 1995) conducted by Texas Parks and Wildlife.

MATERIALS AND METHODS

Our study was conducted in two different laboratories: one at Texas A&M University (TAMU) in College Station, Texas, and one at the Perry R. Bass Marine Fisheries Research Station of Texas Parks and Wildlife (TPW) in Palacios, Texas. Work at TAMU involved 162 spotted seatrout collected by angling and gill netting from three localities in Texas (Lower Laguna Madre, Tres Palacios Bay, and Sabine Pass) and one locality from the Atlantic coast of Florida (Mosquito Lagoon). Work at TPW involved 186 spotted seatrout sampled by gill netting from nine localities along the Texas coast (Lower Laguna Madre, Upper Laguna Madre, Corpus Christi Bay, Aransas Bay, San Antonio Bay, West Matagorda Bay, East Matagorda Bay, Galveston Bay, and Sabine Lake). Collection localities are shown in Figure 3.1; the number of individuals sampled at each locality is given in Tables 3.1A and 3.1B. For TAMU samples, heart and spleen tissues were removed, frozen in liquid nitrogen, and stored at –80°C until processed. For TPW samples, clippings of soft dorsal-fin tissue were removed, placed in 100% ethanol, and stored at room temperature. Genomic DNA was extracted for TAMU samples following Gold and Richardson (1991) and for TPW samples by the PureGene DNA isolation kit and protocols (Gentra Systems, Inc., Minneapolis, MN).

Polymerase-chain-reaction (PCR) primers for five microsatellites developed initially by Turner et al. (1998) for red drum were used for TAMU samples. The five microsatellite repeat motifs in red drum are *Soc*12 — $[GT]_7$, *Soc*50 — $[GT]_7$, *Soc*133 — $[TGC]_8$, *Soc*201 — $[CCT]_6$, and *Soc*243 — $[CCT]_9$. Details regarding primer sequences, length of the cloned allele, and annealing temperature may be found in Turner et al. (1998). The primers for *Soc*12 and *Soc*243 were used for TPW samples, along with primers for two additional microsatellites (*Cne*133 and *Cne*133′). Primers for the latter were amplified from primers designed by sequencing the product from amplifications of *Soc*133 and then identifying internal primers that gave more consistent results than did the original *Soc*133 primers. Primers (forward/reverse) for *Cne*133 and *Cne*133′ were 5′-CCGAGCTGAAA-CACATTCTTGC- 3′/5′-CTTGGCATTTCCAGACATCACTG-3′ and 5′-CATTTGGACCATCGC-TACTGCTG-3′/5′-TGTGTTTCAGCTCGGCTCG-3′, respectively.

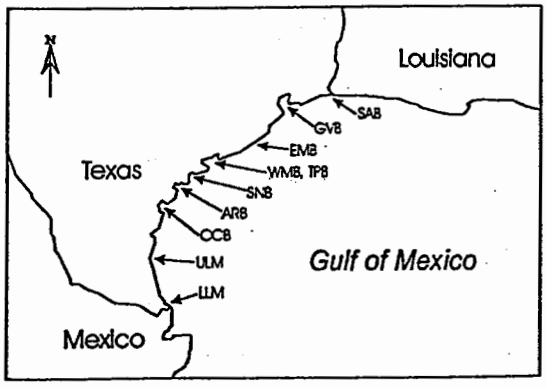

FIGURE 3.1 Sampling localities for spotted seatrout (*Cynoscion nebulosus*) examined from the Texas Gulf Coast. LLM – Lower Laguna Madre; ULM – Upper Laguna Madre; CCB – Corpus Christi Bay; ARB – Aransas Bay; SNB – San Antonio Bay; WMB – West Matagorda Bay; TPB – Tres Palacios Bay; EMB – East Matagorda Bay; GVB – Galveston Bay; and SAB – Sabine Pass and Sabine Lake.

TABLE 3.1A
Summary Statistics for Spotted Seatrout (*Cynoscion nebulosus*) Assayed in College Station

Microsatellite	Sample			
	LLM	TPB	SAB	EFL
*Soc*12				
N	55	35	31	41
n_a	6	3	4	2
P_{HW}	0.504	0.230	0.470	1.000
*Soc*50				
N	55	35	31	39
n_a	4	4	4	3
P_{HW}	0.503	0.781	0.116	0.664
*Soc*133				
N	55	35	31	41
n_a	4	4	4	3
P_{HW}	0.320	1.000	0.290	0.073
*Soc*201				
N	55	35	31	40
n_a	4	4	4	4
P_{HW}	0.425	0.378	1.000	0.010[a]
*Soc*243				
N	55	33	39	39
n_a	3	3	4	4
P_{HW}	0.793	0.870	1.000	1.000

Notes: Sample acronyms are as in Figure 3.1. N = number of individuals assayed; n_a = number of alleles; and P_{HW} = probability of conforming to Hardy-Weinberg (expected) proportions.

[a]Nonsignificant ($P > 0.05$) when corrected for multiple tests.

DNA amplifications (TAMU) were conducted in 10-µl reactions following conditions described in Turner et al. (1998). The forward primer of each pair was end-labeled with [γ^{32}P]-dATP and amplification reactions consisted of 25 cycles of denaturation (94°C, 30 sec), annealing (56°C, 30 sec), and extension (72°C, 30 sec), with an initial denaturation of 94°C for 2 min. Amplifications in Palacios were conducted in 25-µl reactions, utilizing a modified "touchdown" protocol where samples were denatured (95°C, 30 sec), annealed (55°C, 30 sec — decreasing 1°C per cycle), and extended (72°C, 1 min) for 10 cycles, followed by 20 additional cycles of denaturation (95°C, 30 sec), annealing (50°C, 30 sec), and extension (72°C, 1 min, increasing 3 sec per cycle). Amplifications concluded with a 7-min "hold" at 72°C. PCR products (TAMU) were separated on 6% polyacrylamide gels and visualized by autoradiography, whereas PCR products (TPW) were separated on 10% polyacrylamide gels and visualized by ethidium bromide (0.5 µg/ml in 1X TBE buffer for 20 min). Alleles in both laboratories were scored via comparisons with internal standards.

Genotype frequencies at each microsatellite for both TAMU and TPW samples were tested for deviation from Hardy-Weinberg (HW) equilibrium frequencies by using exact tests performed with Markov-chain randomization (Guo and Thompson, 1992). Probability (P) values for HW tests at each microsatellite within each sample were estimated via permutation with 1000 resamplings (Manly,

TABLE 3.1B
Summary Statistics for Spotted Seatrout (*Cynoscion nebulosus*) Assayed in Palacios

	Sample								
Microsatellite	LLM	ULM	CCB	ARB	SNB	WMB	EMB	GVB	SAB
*Soc*12									
N	40	40	41	40	40	40	40	39	30
n_a	3	4	4	3	2	3	2	3	3
P_{HW}	0.322	0.376	0.230	0.581	1.000	0.564	0.216	0.381	0.346
*Soc*243									
N	40	40	41	40	40	40	40	39	30
n_a	3	3	3	3	4	3	3	3	3
P_{HW}	0.844	0.306	0.186	0.007[a]	0.035[a]	0.347	0.102	0.105	1.000
*Cne*133									
N	40	40	41	40	40	40	40	39	30
n_a	3	4	3	4	3	4	4	3	3
P_{HW}	0.748	1.000	1.000	0.201	1.000	0.310	0.315	0.358	1.000
*Cne*133′									
N	40	40	41	40	40	40	40	39	30
n_a	2	3	3	3	2	2	2	4	2
P_{HW}	0.398	1.000	0.172	1.000	1.000	1.000	0.247	0.016	1.000

Notes: Sample acronyms are as in Figure 3.1. N, n_a, and P_{HW} are as in Table 3.1A.
[a]Non-significant ($P > 0.05$) when corrected for multiple tests.

1991); significance levels for simultaneous tests were adjusted using the sequential Bonferroni approach (Rice, 1989). Tests of genotypic equilibrium at pairs of microsatellites were used to assess whether any microsatellites might be linked. Significance of probability values obtained from exact tests of genotypic equilibrium was generated by 1000 resamplings. Tests of HW and genotypic equilibrium were executed using the statistical program GENEPOP (Raymond and Rousset, 1995).

Homogeneity of allele distributions at each microsatellite was assessed for both TAMU and TPW samples via exact tests as implemented in GENEPOP. Significance of tests of genetic homogeneity employed permutation with 1000 resamplings per individual comparison. Estimates of population subdivision among TAMU samples employed Weir and Cockerham's (1984) θ generated via GENEPOP. Statistical significance of θ was assessed using the analysis of molecular variance (AMOVA) of Excoffier et al. (1992), employing 1000 random permutations. For TPW samples, θ values and determination of whether individual θ values differed significantly from zero (1000 random permutations) were obtained using the statistical program ARLEQUIN (Schneider et al., 1999).

RESULTS

Summary statistics, including sample sizes, number of alleles detected at each microsatellite, and results of tests of genotype conformance to expectations of HW equilibrium for each microsatellite at each sample locality are given in Tables 3.1A and 3.1B. The distribution of alleles and allele frequencies at each microsatellite for each locality may be obtained from JRG (TAMU samples) or RW (TPW samples). The number of alleles over all samples ranged between two and four for most microsatellites at most localities; six alleles were detected at *Soc*12 in the TAMU sample from Lower Laguna Madre (LLM), and six alleles (total) at *Soc*201 were found among all TAMU samples. Following Bonferroni correction, genotype proportions for all microsatellites in all samples did not differ significantly from expected HW equilibrium proportions. Tests of genotypic equilibrium within sample localities also were nonsignificant following Bonferroni correction, as were tests carried out with all sample localities pooled (Table 3.2).

Exact tests of allele distribution homogeneity and the θ measure (after Weir and Cockerham, 1984) of population structure among TAMU and TPW samples were nonsignificant following Bonferroni correction (Table 3.3). A probability value of 0.035 before Bonferroni correction was obtained for allele distributions at *Soc*201 among TAMU samples. Frequency plots (Figure 3.2) of the four common alleles at *Soc*201 indicate an elevated frequency of *Soc*201–9 in the sample from Sabine Pass (SAB) and an elevated frequency of *Soc*201–10 in the sample from Lower Laguna Madre (LLM). It is also interesting that frequencies of three alleles (*Soc*201–9, *Soc*201–10, and *Soc*201–11) appear to display a pattern of east-to-west clinal variation. A probability value of 0.013 before Bonferroni correction was obtained for allele distributions at *Soc*12 among TPW samples; this value is near the Bonferroni adjusted alpha of 0.0125. Frequency plots (Figure 3.2) indicate that the heterogeneity likely is due to varying frequencies of alleles *Soc*12–82, *Soc*12–84, and *Soc*12–94, none of which appears to display a pattern of clinal variation. Estimates (θ) of population structure (Table 3.3) ranged from zero (including negative values) to 0.015 for TAMU samples; however, none of the θ values differed significantly from zero. For TPW samples (Table 3.3), θ estimates ranged from 0.004 to 0.013. The θ estimate of 0.013 (for *Soc*12) differed significantly from zero before but not after Bonferroni correction (adjusted alpha of 0.0125).

Exact tests for TAMU samples, including the sample from the Atlantic coast of Florida, were significant before and after Bonferroni correction: *Soc*12 (P = 0.002), *Soc*50 (P = 0.011), *Soc*133 (P = 0.004), *Soc*201 (P = 0.017), and *Soc*243 (P = 0.000). Except for *Soc*201 (P = 0.128), θ values in comparisons that included the sample from the Atlantic coast of Florida differed significantly from zero (data not shown). Genetic distances, estimated as the $(\delta\mu)^2$ metric of Goldstein et al. (1995), revealed that the average difference between the sample from the Florida east coast and the three TAMU samples from Texas waters was considerably greater than the average difference among the three Texas samples.

DISCUSSION AND CONCLUSIONS

The primary purpose of our independent studies was to assess population structure of spotted seatrout in coastal waters of Texas. Divergence (population structure) among spotted seatrout in coastal waters might be expected (Chapman et al., 1999) based on observed life history patterns and results from tag-and-release studies. Briefly, spotted seatrout are generally perceived to be resident to individual estuaries and to spend their entire life cycle in inshore waters (Patillo et al., 1997). Spawning locations tend to be inside estuaries, and larvae and juveniles are found primarily in grass beds, although they can be abundant in areas without seagrass (McMichael and Peters, 1989). Sexual maturity generally occurs after age one (Saucier and Baltz, 1993; Saucier et al., 1992). Tagging studies are consistent with the notion of estuarine residency, as movement of juveniles and adults between estuaries appears to be limited (Overstreet, 1983; Baker and Matlock, 1993). In one study (Moffett, 1961), a re-

TABLE 3.2
Probability of Genotypic Equilibrium (pairwise comparisons) at Microsatellites among Spotted Seatrout (*Cynoscion nebulosus*) Assayed in College Station (above diagonal) and Palacios (below diagonal)

Microsatellite	*Soc*12	*Soc*50	*Soc*133	*Soc*201	*Soc*243	*Cne*133	*Cne*133′
*Soc*12	— -	0.546	0.682	0.893	0.090	— -	— -
*Soc*50	— -	— -	0.445	0.197	0.181	— -	— -
*Soc*133	— -	— -	— -	0.201	0.516	— -	— -
Soc201	— -	— -	— -	— -	0.810	— -	— -
*Soc*243	0.362	— -	— -	— -	— -	— -	— -
*Cne*133	0.323	— -	— -	— -	0.026[a]	— -	— -
*Cne*133′	0.177	— -	— -	— -	0.802	0.469	— -

[a] Non-significant (P > 0.05) when corrected for multiple tests.

TABLE 3.3
Results of Tests for Homogeneity in Allele Distributions and Estimates of Population Structure among Geographic Samples of Spotted Seatrout (*Cynoscion nebulosus*) Sampled from Coastal Waters of Texas

	College Station Samples[a]			Palacios Samples		
Microsatellite	P_{EXACT}	θ	P	P_{EXACT}	θ	P
*Soc*12	0.305	0.015	0.103	0.013[b]	0.013	0.043[b]
*Soc*50	0.878	–0.011	0.981	—	—	
*Soc*133	0.362	0.000	0.331	—	—	
*Soc*201	0.035[b]	0.005	0.180	—	—	
*Soc*243	0.285	–0.012	0.936	0.211	0.004	0.233
*Cne*133	—	—		0.250	0.006	0.095
*Cne*133′	—	—		0.054	0.006	0.196

Notes: P_{EXACT}: Probability of allele distribution homogeneity based on exact test. θ: Estimate of population subdivision (after Weir and Cockerham, 1984). P: probability that θ = 0.

[a] Results are for samples from the Texas coast (i.e., the sample from the east coast of Florida was excluded).

[b] Nonsignificant (P > 0.05) when corrected for multiple tests.

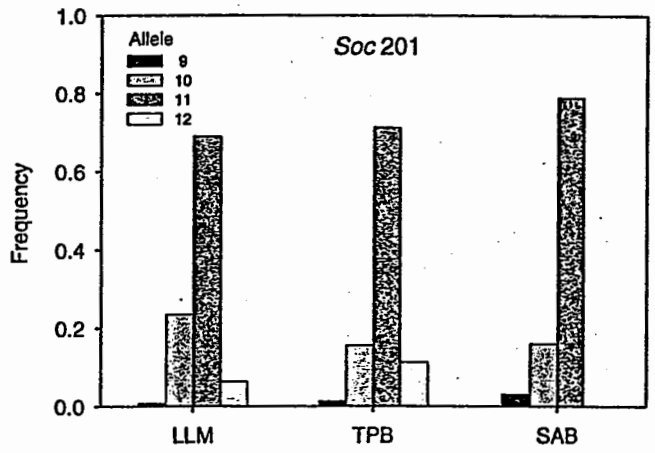

FIGURE 3.2 Allele-frequency histograms for microsatellite *Soc*201 among College Station samples of spotted seatrout (*Cynoscion nebulosus*). Abscissa: sample localities (LLM – Lower Laguna Madre, TPB – Tres Palacios Bay; and SAB – Sabine Pass); ordinate: frequencies of alleles *Soc*201-9, -10, -11, and -12.

turn of more than 450 km from a release site was reported, but in general, nonrandom movements of spotted seatrout are thought to be largely in response to changes in temperature or salinity (Lorio and Perret, 1978; Helser et al., 1993). Nongenetic studies based on growth rates (Iverson and Tabb, 1962, but see Murphy and Taylor, 1994) and shapes of scales and otoliths (Colura and King, 1989) also have been consistent with the notion of resident stocks within estuaries.

Prior genetic studies on spotted seatrout from the northern Gulf have documented varying degrees of divergence among samples from different estuaries. Weinstein and Yerger (1976) assayed general proteins and reported significant differences among spotted seatrout from various estuaries on the west (Gulf) coast of Florida. Ramsey and Wakeman (1987) found significant heterogeneity at three of 40 putative allozyme loci among spotted seatrout sampled from Texas to Florida. However, homogeneous subsets of samples were generated upon removal of specific alleles at one or a few loci in one or a few samples; overall, the only evident spatial patterning was a weak isolation-by-distance effect indicated by a significant Moran's I value at a locus for glucose-6-phosphate isomerase (*Gpi-B*). King and Pate

(1992), alternatively, surveyed 44 putative allozyme loci among spotted seatrout sampled from 11 localities along the Texas coast and one locality from northern Mexico. They found significant heterogeneity at a locus for aspartate aminotransferase (*sAAT-2*) and significant spatial autocorrelations (indicative of isolation by distance) for alleles at both *sAAT-2* and a locus for tripeptide aminopeptidase (*PEP-B*). Finally, Gold et al. (1999) examined mtDNA restriction-site variation among spotted seatrout sampled from eight localities in the northern Gulf (five from Texas) and found significant heterogeneity among all samples from the northern Gulf and among samples from the western Gulf. Heterogeneity among samples from the western Gulf appeared to stem primarily from mtDNA haplotype frequency differences in a sample from the Lower Laguna Madre. Spatial autocorrelation analysis of common mtDNA haplotypes revealed an isolation-by-distance effect, where significant, positive Moran's I values were found between proximal sample localities and significant, negative values were found between geographically distant ones. Collectively, the studies of Ramsey and Wakeman (1987), King and Pate (1992), and Gold et al. (1999) have not demonstrated significant genetic differences among spotted seatrout in individual estuaries but, rather, a pattern in which genetic divergence increases with geographic distance and significant differences arise ostensibly from comparisons between samples near the extremes of geographic sampling. Thus, while there may be different subpopulations or stocks of spotted seatrout in the northern Gulf, sufficient gene flow appears to occur between geographically proximate subpopulations such that significant genetic divergence is observed only between geographically distal bays and estuaries. King and Pate (1992) suggested that the intracoastal waterway linking most bays and estuaries of the northern Gulf may provide the mechanism whereby gene flow could occur in a species such as spotted seatrout that spends virtually all of its life cycle in nearshore waters.

Results from our independent studies of microsatellite variation among spotted seatrout from the Texas coast are not directly comparable with the studies of Weinstein and Yerger (1976) and Ramsey and Wakeman (1987), because their samples came from Florida and from across the northern Gulf, respectively. The study by Ramsey and Wakemen (1987) also included only a single sample from Texas waters. However, results from this study are comparable with those of King and Pate (1992) and Gold et al. (1999), as all three involved spotted seatrout sampled along the Texas Gulf Coast from Sabine Lake (or Sabine Pass) near the Louisiana border to the Lower Laguna Madre near the border with Mexico.

Patterns of geographic variation for different types of genetic markers used in the three studies differ. King and Pate (1992) and Gold et al. (1999) found significant divergence among samples in allele frequencies of allozymes and mtDNA, respectively, whereas in this study allele frequencies at microsatellites essentially were homogeneous across the same geographic sampling surface. King and Pate (1992) and Gold et al. (1999) also found that genetic divergence at *sAAT-2* and mtDNA, respectively, was related in part to increasing geographic distance between sample localities (isolation by distance), giving rise to an east-west clinal pattern along the Texas coast; little evidence of such an effect was detected for any of the microsatellites.

Differences in patterns of geographic variation between or among various genetic markers have been reported previously in other marine species, including yellowfin tuna, *Thunnus albacares* (Ward et al., 1994), Atlantic cod, *Gadus morhua* (Pogson et al., 1995), and American oyster, *Crassostrea virginica*. The last study is perhaps the best known. Buroker (1983) assayed 14 polymorphic allozyme loci among *C. virginica* sampled from Texas to Massachusetts and found little detectable geographic heterogeneity; however, Reeb and Avise (1990) found large differences in mtDNA restriction fragment length polymorphisms (RFLPs) between samples from the northern Gulf and the southeastern U.S. Atlantic coast. Subsequently, Karl and Avise (1992) identified restriction site polymorphisms at four anonymous nuclear-encoded sequences that also exhibited large differences in allele frequency between the northern Gulf and the southeastern U.S. Atlantic coast. In general, when different types of polymorphic genetic markers exhibit different patterns of geographic variation, the usual inference is that at least one type of polymorphism may be affected by natural selection in addition to genetic drift (McDonald, 1994).

Assuming that anonymous (and presumably noncoding) nuclear DNAs and mtDNA RFLPs are selectively neutral, Karl and Avise (1992) interpreted the different patterns in *C. virginica* as indicating that balancing selection was acting to maintain allele frequencies at the allozyme loci. Under this hypothesis, the geographic divergence in mtDNA and anonymous nuclear DNAs would be hypothesized to stem from small effective population size (mtDNA) and from a combination of reduced gene flow, effective population size, and genetic drift.

Pogson et al. (1995) employed similar logic in their study of genetic variation in Atlantic cod, where the average F_{ST} value of several anonymous nuclear DNA polymorphisms was significantly greater than the average of several allozyme polymorphisms. The findings in *C. virginica*, however, were questioned subsequently by the discovery that six additional anonymous nuclear DNA polymorphisms, sampled over essentially the same geographic surface, displayed geographic variation that was not significantly greater than that exhibited by the allozymes (McDonald et al., 1996).

The patterns of variation exhibited by the three types of genetic markers, i.e., allozymes, mtDNA, and nuclear DNA, in spotted seatrout from the Texas coast differ from the patterns in American oysters and Atlantic cod, in that significant divergence among samples of spotted seatrout was detected in mtDNA and allozymes but not in noncoding nuclear DNAs (microsatellites in this case). The differences in patterns of geographic variation in the three types of genetic markers among spotted seatrout may stem from one of several possibilities. The first and simplest possibility is that the pattern of variation in one or more of the genetic markers may not be representative of "true" geographic variation in the type of marker. In the study by King and Pate (1992), for example, only a single (putative) allozyme locus (*sAAT-2*) varied significantly among localities, representing effectively a sample size of one. However, a second allozyme locus (*PEP-B*) also varied clinally, supporting the inferred pattern of isolation by distance. The same is true for the mtDNA study by Gold et al. (1999), in that two mtDNA haplotypes differed significantly in frequency across localities and both showed strong east-west clines across the northern Gulf.

Relative to the microsatellites, the studies of anonymous nuclear DNA loci in American oyster have shown that different patterns may be obtained in different studies carried out in different laboratories (Karl and Avise, 1992; McDonald et al., 1996). Our studies of microsatellites in spotted seatrout, however, have revealed essentially the same pattern of geographic variation even though the studies were conducted in different laboratories and involved different samples. It may be that too few microsatellites have been assayed relative to a random sampling of variation at microsatellites in spotted seatrout. This can be tested easily in the future.

A second possibility is that observed patterns in the three types of markers are real and reflect different evolutionary, ecological, or genetic processes. King and Zimmerman (1993) hypothesized that the clinal variation in *sAAT-2* observed by King and Pate (1992) could reflect adaptation to temperature or salinity gradients and, moreover, that divergence along such gradients might be minimized by gene flow, given that the effective number of migrants (N_em) estimated by King and Pate (1992) ranged from 9.75 (at *sAAT-2*) to 49.75 (at *PEP-B*). The hypothesis that nonrandom patterns of allozyme variation can stem from natural selection is not without precedent (Christiansen and Frydenberg, 1974), and the observation of clinal variation is certainly consistent with expectations based on directional selection where one genotype is favored at one end of an environmental gradient and disfavored at the other end (Hartl, 1980).

Alternatively, clines theoretically can arise from migration (gene flow) coupled with founder effects at geographic extremes and hence are not necessarily *prima facie* evidence of selection (Hartl, 1980). The latter (i.e., gene flow coupled with founder effects) would seem the more likely explanation for the cline observed at *sAAT-2*, given that the same clinal pattern is observed for mtDNA haplotypes. That is, a directional selective force acting jointly on both *sAAT-2* and mtDNA and along the same geographic gradient would seem unlikely, in part because the two are inherited independently, and in part because variation in mtDNA RFLPs typically stems from synonymous base substitutions in third codon positions of protein-coding genes, which generally are assumed to be selectively neutral.

The absence of (spatial) heterogeneity or a marked cline in allele frequencies at any of the spotted seatrout microsatellites assayed, however, is puzzling because polymorphisms at microsatellites are typically assumed to be selectively neutral. This means that one would expect geographic patterns at spotted seatrout microsatellites to parallel patterns observed at *sAAT-2* and mtDNA if, in fact, observed clinal variation at *sAAT-2* and mtDNA is primarily a function of gene flow, effective population size, and genetic drift. One explanation might be that too few microsatellites were assayed relative to detecting clinal variation. King and Pate (1992), for example, assayed seven polymorphic loci, yet significant divergence was found only at *sAAT-2*. Another explanation might be allele-size homoplasy (Estoup et al., 1995), where co-migrating alleles at microsatellites are assumed to be homologous yet represent different sequence motifs and hence are not identical by descent. This phenomenon has been documented in a number of animals, including fishes (Angers and Bernatchez, 1997), and clearly could confound population diversity assessment (Culver et al., 2001).

A last possibility is that variation in *sAAT-2* is a consequence of directional selection, as suggested by King and Zimmerman (1993), and that variation in mtDNA and microsatellites reflects the interactions among gene flow, effective population size, and genetic drift expected of selectively neutral genetic markers. If so, the finding that divergence in spotted seatrout mtDNA is greater than that at any of the spotted seatrout microsatellites may suggest a sex-biased difference in gene flow. Because mtDNA provides information only on female gene flow, greater divergence in mtDNA relative to nuclear-encoded DNA has been inferred in other marine species (Ferguson et al., 1995; Rassmann et al., 1997; Buonaccorsi et al., 1999) to indicate male-mediated dispersal, female philopatry, or both. Interestingly, the opposite (i.e., female-mediated dispersal, male philopatry, or both) has been suggested as an explanation to account for patterns of mtDNA and microsatellite divergence in the red drum (Gold and Turner, 2001). Clearly, this issue bears further investigation in spotted seatrout.

Although we cannot distinguish satisfactorily between the latter two possibilities, both are consistent with the hypothesis that genetic divergence in spotted seatrout is in part a function of the interaction among gene flow, effective population size, and genetic drift, and that gene flow occurs primarily (but not exclusively) between adjacent or neighboring estuaries. Population structure of spotted seatrout along the Texas coast may thus be modeled as a series of overlapping subpopulations or stocks distributed linearly along the coastline, where individual subpopulations are centered in individual (natal) estuaries but where gene flow between geographically proximate estuaries is sufficient to prevent significant genetic divergence. This type of model also has been hypothesized (Gold et al., 2001) for red drum (*Sciaenops ocellatus*) in the northern Gulf. The genetic data in red drum, however, were far more extensive, both spatially and temporally, permitting an estimate of the geographic neighborhood size of individual populations. We suggest that future genetic studies of spotted seatrout should be directed along similar lines, as migration and its extent from individual estuaries may be an important component to recruitment in adjacent estuaries. This could mean that conservation and management planning for spotted seatrout should perhaps include a wider geographic context and that adults employed in hatchery-based supplementation programs should not necessarily be procured from the same bay or estuary.

Our finding that the sample of spotted seatrout from the Atlantic coast of Florida differed significantly in allele frequencies from the samples in the northern Gulf paralleled those findings of Ramsey and Wakeman (1987) and Gold et al. (1999), in which significant divergence at two allozyme loci and in mtDNA haplotype frequencies, respectively, were documented between spotted seatrout from the two regions. Wiley and Chapman (2001) also found significant differences in allele frequencies at two microsatellites between a sample from the northern Florida Gulf coast and several samples from the southeast (U.S.) Atlantic coast. These findings parallel results from several marine fishes (including other sciaenids) for which regionally distinct populations reside in the northern Gulf and along the southeast (U.S.) Atlantic coast (Avise, 1992; Gold and Richardson, 1998a, b). As noted by Gold et al. (1999) and Wiley and Chapman (Chapter 4, this volume), these shared patterns of regional population structure likely stem from similar vicariant histories possibly related to climatic changes occurring during glaciation, absence of suitable habitat at spatial junctures between populations, or oceanographic currents that minimize movement from the Atlantic into the Gulf.

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