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Genetic studies in marine fishes. IV. An analysis of population structure in the red drum (*Sciaenops ocellatus*) using mitochondrial DNA

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ABSTRACT

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Variation in mitochondrial DNA (mtDNA) was examined among 397 individuals representing 13 geographic samples of red drum (*Sciaenops ocellatus*). Five of the samples were from the Atlantic coast of the southeastern United States, and eight were from the northern Gulf of Mexico. Seventy-six mtDNA haplotypes were found: eight haplotypes were found in 13 or more individuals, ten were found in four to 12 individuals, 22 were found in two to three individuals, and 36 were found in only one individual each. Mitochondrial DNA nucleon diversity values within samples ranged from 0.850 to 1.000; the value over all fish surveyed was 0.943. These data indicate that red drum have at least "normal" levels of genetic variability. Estimates of the percentage nucleotide sequence divergence among the 76 mtDNA haplotypes ranged from 0.137 to 1.962, with a mean ($\pm$SE) of 0.803 ± 0.006. Heterogeneity in the frequencies of four haplotypes was detected between pooled samples from the Atlantic vs. pooled samples from the Gulf. No heterogeneity was detected among samples from the Atlantic or among samples from the Gulf. F_{ST} values (a measure of the variance in mtDNA haplotype frequencies) over the four heterogeneous haplotypes ranged from 0.019 to 0.137 (mean $F_{ST}=0.057$), and the effective number of female migrants (N_em) per generation using Wright's island model ranged from 1.58 to 12.91 (mean $N_em=4.14$). The average percentage nucleotide sequence divergence within samples ranged from 0.494 to 0.734, with a mean ($\pm$SE) of 0.574 ± 0.021. The percentage nucleotide sequence divergence among pairwise comparisons of samples ranged from 0.498 to 0.713, with a mean ($\pm$SE) of 0.571 ± 0.005. Parsimony and phenetic analyses of individual mtDNA haplotypes and of sample localities revealed no strong evidence for geographic cohesion of haplotype or sample groupings. A matrix correlation (Mantel) test also revealed no significant association between matrices of mtDNA sequence divergence and geographic distance among sample localities. Collectively, these data indicate that the red drum population is weakly subdivided, with semi-isolated subpopulations occurring along the southeastern Atlantic coast and in the northern Gulf of Mexico. The large number of rare or unique mtDNA haplotypes found in the study may prove useful in evaluating the success of red drum stocking programs.

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INTRODUCTION

The red drum or redfish (*Sciaenops ocellatus*) is an important recreational species in the Gulf of Mexico and along the Atlantic coast of the southeastern United States. Recently, concern has been expressed over the apparent decline in the red drum fishery (G.C. Matlock, unpublished data, 1984; Reagan, 1985). A central question concerning the management of red drum is whether discrete breeding units or subpopulations (as defined by Hedgecock, 1986) occur within the species range (McIlwain et al., 1986).

Available information on the population structure of red drum appears contradictory. Tagging studies of subadult red drum have indicated that movement of juveniles among nearshore localities may be limited, although there is evidence that movement patterns of juveniles may differ among various bays and estuaries (Matlock and Weaver, 1979; Adkins et al.,1979; Osburn et al., 1982). These and other findings have led to the suggestions that at least two, possibly three spatially isolated subpopulations of red drum may exist along the Gulf and Atlantic coasts (G.C. Matlock, unpublished data, 1984; Matlock, 1987a). There are, however, several aspects of red drum biology and life history which could facilitate dispersal of individuals and thereby minimize spatial differentiation and divergence into discrete subpopulations. Adult red drum spawn near the mouths of bays or estuaries (G.C. Matlock, unpublished data, 1984; Reagan, 1985; Matlock, 1987b) and their highly pelagic eggs (Holt et al., 1981) could be transported to contiguous spawning localities by oceanic currents. In addition, there is evidence (Lyczkowski-Schultz et al., 1988) that some adult red drum in the north-central Gulf may spawn offshore and that larvae and juveniles could enter various bays or estuaries at a later time. Finally, although larvae and juveniles appear to remain in the bays and estuaries (Overstreet, 1983; G.C. Matlock, unpublished data, 1984; Matlock, 1987b), adults apparently move into deeper waters prior to sexual maturation and spawning (G.C. Matlock, unpublished data, 1984; Matlock, 1987b) and appear capable of extensive migration (Overstreet, 1983; Mercer, 1984; Swingle et al., 1984). These aspects of red drum life history suggest that migration or gene flow could be extensive and that subpopulational divergence might be minimal.

Previous genetic studies of red drum employed starch-gel electrophoresis of proteins encoded by nuclear genes. Ramsey and Wakeman (1987) surveyed allelic variation at four polymorphic loci among red drum sampled from 12 locations in the northwestern Gulf and two locations along the Atlantic coast. Significant heterogeneity in allele frequencies was found at a locus for glucose phosphate isomerase, although the differences in allele frequencies at this locus did not follow a simple geographic pattern. The mean F_{ST} value, a measure of the reduction in heterozygosity of a sample owing to non-random mating between samples (Wright, 1943, 1965), was 0.019, suggesting that the

samples essentially comprised a single, randomly mating population. Bohlmeyer and Gold (1991) surveyed allelic variation at nine polymorphic loci among red drum sampled from eight localities in the northern Gulf and five localities along the Atlantic coast. Highly significant heterogeneity in allele frequencies at the locus for adenosine deaminase was detected among red drum sampled from the Gulf and between red drum sampled from the Gulf and those sampled from the Atlantic. The mean F_{ST} value, however, was 0.019, suggesting high levels of gene flow among the localities sampled. The latter was also indicated by a qualitative analysis (after Slatkin, 1981) using conditional average allele frequencies.

In this paper, data on restriction fragment length polymorphisms (RFLPs) of the mitochondrial DNA (mtDNA) molecules of most of the individuals studied by Bohlmeyer and Gold (1991) are presented. Recent studies in a variety of organisms, including fishes, have shown that restriction fragment or site analysis of mtDNA is more powerful than protein electrophoresis for differentiating subpopulations within species (Brown, 1983; Avise and Lansman, 1983; Avise, 1986; Avise et al., 1987a; Moritz et al., 1987). In brief, mtDNA is a haploid, circular molecule which is uniclonally inherited through the maternal parent. This means that mtDNA sequence variants do not segregate and recombine during sexual reproduction. The joint effects of haploidy and maternal inheritance mean that the effective population sizes that measure the genetic impact of population substructuring and gene flow should be at least four times less for mtDNA than for nuclear encoded genes (Birky et al., 1983; Templeton, 1987). Finally, mtDNA appears to have a fairly rapid rate of nucleotide sequence evolution which means that mtDNA analysis should be useful in identifying discrete breeding subpopulations of relatively recent origin. The purpose of the present study was to test further the hypothesis that red drum are genetically subdivided.

MATERIALS AND METHODS

Red drum were collected during 1987 using gill nets, pound nets, haul seines, and hook and line. The 397 individuals surveyed in the study were collected from 13 nearshore localities in the Gulf of Mexico and Atlantic Ocean (Table 1, Fig. 1). The majority of individuals sampled were yearlings from the 1986 year class as judged by total length. Heart, kidney, and white muscle were taken from each fish and stored in liquid nitrogen for transport to College Station, TX. Tissue samples were stored at $-80°C$ in the laboratory. Individuals from sample site No. 9 (Biloxi Bay, MS) were provided by the Gulf Coast Research Laboratory in Ocean Springs, MS.

Individual tissue samples were pulverized to a fine powder in liquid nitrogen using a pre-chilled mortar and pestle. Approximately 1 g of this powder was suspended in STE buffer (0.1 M NaCl, 50 mM Tris, 1 mM EDTA; pH

TABLE I

Sampling localities and number of red drum specimens examined from each site (acronyms given in parentheses)

Location	Number of individuals
Atlantic	
1. Oregon Inlet, south of Nags Head, NC (MNC)	15
2. Pamlico River, near Bath, NC (BTH)	22
3. North Inlet, north of Georgetown, SC (GTN)	18
4. Charleston Bay, SC (CHS)	34
5. Calibogue Sound along Hilton Head Island, SC (HIH)	50
Gulf of Mexico	
6. Sarasota Bay, near Long Key, FL (SAR)	49
7. Riviera Bay, off Tampa Bay, FL (RIV)	24
8. Apalachicola Bay, FL (APP)	24
9. Biloxi Bay, MS (OSP)	57
10. Black Bay, near Hopedale, LA (HOD)	20
11. Salt marsh near Grand Isle, LA (GIL)	43
12. West Bay, behind Galveston Island, TX (GVB)	32
13. Redfish Bay, Port Aransas, TX (PAR)	9
	Total=397

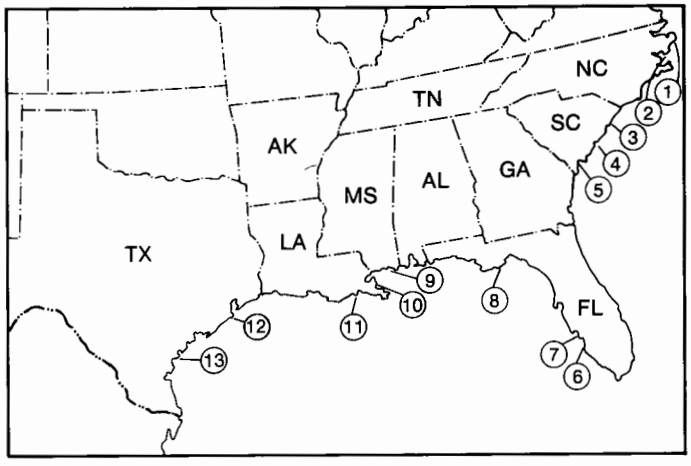

Fig. 1. Sample localities of red drum examined in the study. Site descriptions are in Table 1.

7.50). Cells were lysed by the addition of 20% sodium dodecyl sulfate (SDS) in water (0.05 g ml^{-1}). This mixture was shaken vigorously and immediately extracted twice with an equal volume of a 25:24:1 solution of phenol:chloroform:isoamyl alcohol. Samples were centrifuged in a high-speed microcentrifuge for 4 min at 14 000 rev min^{-1} during each extraction to fa-

cilitate separation of layers. The supernatant was then extracted twice with a 24:1 solution of chloroform:isoamyl alcohol and centrifuged as before. DNA was precipitated by adding a 1/10 volume of 2 M NaCl and two volumes of absolute ethanol and placing the solution at −20°C for 2 h. Precipitated DNA was recovered by centrifuging at 8000 rev min^{-1} for 10 min at 4°C. The DNA was then dissolved in 100 mM Tris, 0.1 mM EDTA at pH 7.50.

Sixteen restriction endonucleases were used to digest 1.0–1.5 μg of DNA in 40-μl reactions following the manufacturer's specifications. Enzymes used were : *Bam*HI, *Bgl*II, *Bcl*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Nco*I, *Nsi*I, *Pst*I, *Pvu*II, *Sca*I, *Spe*I, *Sph*I, *Stu*I, *Xba*I, and *Xmn*I. The samples of DNA were digested for 6–10 h, and the resultant fragments electrophoresed at approximately 5 V cm^{-1} for 12–15 h in 0.8% agarose gels (buffer system: 0.8 M Tris, 0.4 M sodium acetate, 0.36 M NaCl, 0.04 M EDTA; pH 8.00). Lambda DNA digested with *Hin*dIII was used as a molecular weight marker on each gel. After electrophoresis, the gels were stained with ethidium bromide, photographed under 300 nm UV light, and then denatured in 0.5 N NaOH for 1 h. DNA fragments were transferred to nylon filters (0.45 μm filter size; Micron Separations Inc., Westboro, MA) following the methods of Southern (1975). The filters were pre-hybridized at 65°C for 2 h in 10 ml (per filter) of a solution containing 0.5% SDS, 5×SSC, 5×Denhardt's solution, and 0.5 mg ml^{-1} denatured salmon sperm DNA, and then hybridized to a probe labeled with (^{32}P)dCTP (Amersham, sp. act. = 800 Ci mM^{-1}) by nick-translation following Rigby et al. (1977). The probe (pSOmt4) used was a 9.0–9.2 kilobase (kb) fragment of the red drum mtDNA molecule inserted into a pTZ-18R plasmid. Hybridized filters were incubated at 65°C for 12–18 h, washed twice with gentle shaking for 15 min at 37°C in a solution containing 2×SSC, 0.02% SDS, and 0.05% sodium pyrophosphate, followed by two additional washes of 1 h each at 37°C in a solution containing 1×SSC, 0.01% SDS, and 0.05% sodium pyrophosphate. The filters were then rinsed briefly in distilled H_2O, air dried, and exposed to X-ray film (XAR-5) with double intensifying screens for 2–4 days at −80°C. After autoradiography, mtDNA fragments were sized by fitting migration distances to a least-squares regression line of lambda DNA-*Hin*dIII fragment migration distances (excluding the 564 base pair (bp) fragment).

Genotypic ("nucleon") diversity values within geographic samples and among all red drum examined were calculated following Nei and Tajima (1981) and were based on the total number of mtDNA genotypes (haplotypes) identified by differences in restriction enzyme fragment patterns. Heterogeneity tests of haplotype frequencies among sample localities were carried out using the V statistic on arcsin square-root transformed haplotype frequency values (after DeSalle et al., 1987). F_{ST} values for each haplotype were calculated using the formulae in Weir and Cockerham (1984) and the computer programs described in Weir (1990). Estimates of gene flow ($N_e m$,

or the effective number of female migrants per generation) were calculated using Wright's (1943) island model, where $F_{ST} \approx 1/4N_e m + 1$.

Restriction fragment presence/absence (binary) matrices for estimation of nucleotide sequence divergence (**p**) values and for parsimony analyses were constructed using individual mtDNA haplotypes and individual sampling localities (referred to as samples, see Table 1 for acronyms used) as operational units. In the latter, a restriction fragment was scored as present (code 1) if it occurred within a sample, or absent (code 0) if it did not. Nucleotide sequence divergence values were estimated from these matrices using equations in Nei and Li (1979). The matrix of nucleotide sequence divergence values among haplotypes was simply the estimated **p** values between individual haplotypes. The sample matrix was generated using the frequency-based method shown in eqn. (1)

$$\mathbf{p}_{ab} = \sum_{i=1}^{i=m} \sum_{j=1}^{j=n} p_{ij}/mn \qquad (1)$$

where $\mathbf{p}_{ab}$ is the average nucleotide sequence divergence value between samples a and b, m is the number of individuals in sample a, n is the number of individuals in sample b, and p_{ij} is the nucleotide sequence divergence value between the ith individual of sample a and the jth individual of sample b. In a similar way, the average nucleotide sequence divergence values within samples were generated according to eq. (2)

$$\mathbf{p}_a = \sum_{i \gg 1}^{i=n_{a-1}} \sum_{j=i+1}^{j=n_a} p_{ij}/[n_a(n_{a-1})/2] \qquad (2)$$

where $\mathbf{p}_a$ is the average nucleotide sequence divergence value between all individuals in sample a, n_a is the total number of individuals in sample a, and p_{ij} is the nucleotide sequence divergence value between the ith and jth individuals in sample a. The $\mathbf{p}_{ab}$ values generated from eqn. (1) represent the average of all pairwise nucleotide sequence divergence estimates between all individuals of any two geographic samples. For example, the $\mathbf{p}_{ab}$ value between the MNC and BTH samples is the average of 330 separate nucleotide sequence divergence estimates as there were 15 and 22 individuals, respectively, in these two samples. The $\mathbf{p}_a$ values generated from eqn. (2) represent the average of all pairwise nucleotide sequence divergence estimates between all individuals of any one sample. For example, the $\mathbf{p}_a$ value within the MNC sample is the average of 105 separate nucleotide sequence divergence estimates as there were 15 individuals in that sample. These approaches to estimating nucleotide sequence divergence values within and among samples were used primarily to take into account similarities (or differences) in the frequencies of individual haplotypes both within and among samples.

Parsimony analysis of the fragment presence/absence matrix of mtDNA

haplotypes was used to produce a "gene tree" (as used by Avise, 1989) and employed the MULPARS option in version 2.4.1 of the Phylogenetic Analysis Using Parsimony (PAUP) program of Swofford (1985). All autapomorphies (46 fragment characters) and symplesiomorphies (six fragment characters) were removed from the haplotype binary matrix, leaving 59 phylogenetically informative characters. Parsimony analysis of the sample fragment presence/absence matrix employed both PAUP (MULPARS and BANDB options) and the BOOT program in version 3.0 of the Phylogeny Inference Package (PHYLIP) of Felsenstein (1987). For bootstrapping, two different random number seeds were used and each was run with 50 replacements. Estimates of confidence for individual branches were summed from the two BOOT runs. All autapomorphies (26 fragment characters) and symplesiomorphies (45 fragment characters) were removed for parsimony analysis of the sample binary matrix, leaving 40 phylogenetically informative fragment characters.

Phenetic analysis of both mtDNA haplotype and sample nucleotide sequence divergence matrices was carried out using UPGMA clustering (Sneath and Sokal, 1973). A Fitch–Margoliash distance "tree" from the sample matrix was also generated using the FITCH option in PHYLIP. The Fitch–Margoliash procedure presumably is unrestrained by assumptions of rate homogeneity in DNA sequence evolution.

A matrix correlation (Mantel test) was carried out between the sample nucleotide sequence divergence matrix and a matrix of geographic distance (in miles) between all pairs of sample localities. Significance testing of the Mantel correlation employed a Student's t test as outlined in Douglas and Endler (1982).

RESULTS

Single digestions of mtDNA molecules from the 397 red drum surveyed using the 16 restriction enzymes produced a total of 110 presumed unique fragments. Homology of fragments was tested by multiple, side-by-side reruns of all variant patterns produced by each enzyme. Except for situations where only a single band of greater than 15 kb was observed, homology was assumed if fragments were the same size in rerun comparisons. This assumption is reasonable given that only 1–5 ($\bar{x}=2.77$) fragments were observed per single digest over all variant patterns for all enzymes. Variant patterns exhibiting only a single band of greater than 15 kb were tested for homology using double digestions with the enzyme *Bam*HI. With the exception of one individual which did not possess a *Bam*HI site, the remaining 396 individuals appeared to possess only a single *Bam*HI site in their mtDNA molecules. The latter was inferred from *Bam*HI/*Pst*I double digestions (see below) run on all 397 individuals.

All of the enzymes employed, except *Sph*I, produced variant digestion pat-

TABLE 2

Digestion patterns (fragment sizes in base pairs) of red drum mtDNA produced by 16 six-base restriction endonucleases. Fragments of the same size are assumed to be homologous except where indicated. Parentheses indicate fragments (or their sum) not seen with probe pSOmt4 (see text)

Bcl I

A	B	C	D	E	F	G	H
9800	7800	7000	9000	16800	9800	9800	9800
7000	7000	2600	7800		3650	5900	6600
	2000	(7200)			3350	(1100)	(400)

Nco I

A	B	C	D	E	F
11050	8400	8400	9700	3900	14950
3900	3900	3900	3900	2650	1850
1850	2650	1850	1850	2600	
	1850	1350	1350	1850	
		(1300)		(5800)	

Sca I

A	B	C	D	E	F	H
8200	14500	9250	6300	6300	7400	7400
6300	2300	5250	4800	3600	7100	6300
2300		2300	2300	2300	2300	2300
			(3400)	(4600)		(800)

Pvu II

A	B	C	D	E	F
8200	4500	7000	8200	12700	11200
4500	3000	4500	5600	3000	4500
3000	1850	3000	3000	1100	1100
1100	1100	1100			
	(6350)	(1200)			

Xba I

A	B	C	D	E	F	G
7150	7150	7150	7150	7150	10700	6100
6100	6100	6100	6100	3550	6100	6000
3550	2100	2350	3250	2150		3550
	1450	1200	(300)	(3950)		(1150)

Eco RV

A	B	C	D	E	F
13500	13500	13500	9650	15300	12600
1800	3300	3050	3850	(1500)	1800
(1500)		(250)	1800		900
			(1500)		(1500)

TABLE 2 (continued)

Spe I					*Stu* I					*Xmn* I			
A	B	C	D	E	A	B	C	D	E	A	B	C	D
8000	8000	5000	8000	8000	3900	3900	3900	2600	3900	8750	8750	5300	3900
5000	3600	3550	3450	5000	2600	2600	3550	2200	2600	4000	2600	4000	2600
3000	3000	3000	3000	2050	1700	1850	1700	1750	1700	2600	2100	2600	2100
(800)	1400	(5250)	1550	950	1300	1300	(7650)	1700	650	(1450)	1900	(4900)	1900
	(800)		(800)	(800)	(7300)	(7150)		1300	(7950)		(1450)		(6300)
								(7250)					

Pst I/*Bam* HI				*Pst* I				*Hind* III		
A	B	C	D	A	B	C	D	A	B	C
8400	9900	8400	15300	8400[1]	16800[2]	8400	16800[2]	5400	9500	4100
6900	6900	6400	1500			6400		4100	2900	2900
1500		1500				2000		2900	(4400)	2700
		(500)						(4400)		(7100)

Eco RI			*Nsi* I			*Bgl* II		*Bam* HI		*Sph* I
A	B	C	A	B	C	A	B	A	B	A
10950	16800	10950	9500	16800	8650	9050	7750	16800	uncut	11150
5850		4350	7300		7300	7750	5850			(5650)
		(1500)			(850)		3200			

[1]Doublet band revealed by *Pst* I/*Bam* HI double-digestions (see text).
[2]The 16,800 bp fragments observed in *Pst* I single digestions result from different *Pst* I restriction sites (see *Pst* I/*Bam* HI patterns and text).

terns (Table 2). All digestion profiles of variants were consistent with the hypothesis of single nucleotide substitutions (i.e. single gains or losses of restriction sites) among the variant patterns observed for each enzyme. Examples of variant single-digestion patterns for the enzymes *Nco*I and *Pvu*II are shown in Fig. 2. Note that not all digestion patterns produced fragments whose sizes summed to 16.8 kb, the inferred size of the red drum mtDNA molecule (see below). The reason for the latter is that the pSOmt4 probe does not cover the entire red drum mtDNA molecule, i.e. fragments in some digestion patterns are undetected. The mean genome size of all apparently complete digestion patterns was 16.8 ± 0.20 kb, in agreement with the previously estimated genome size of the red drum mtDNA molecule (Gold et al., 1988). No evidence for mtDNA size variation was observed among the individuals surveyed.

Several of the variant digestion patterns produced only a single band of greater than 15 kb in size (Table 2). These included patterns produced by *Bcl*I, *Eco*RV, *Eco*RI, *Nsi*I, and *Bam*HI (Table 2). The *Eco*RI B, and *Eco*RV E patterns were observed only in one individual each, whereas the *Bcl*I E and *Nsi*I B patterns were found in two and six individuals, respectively. Homol-

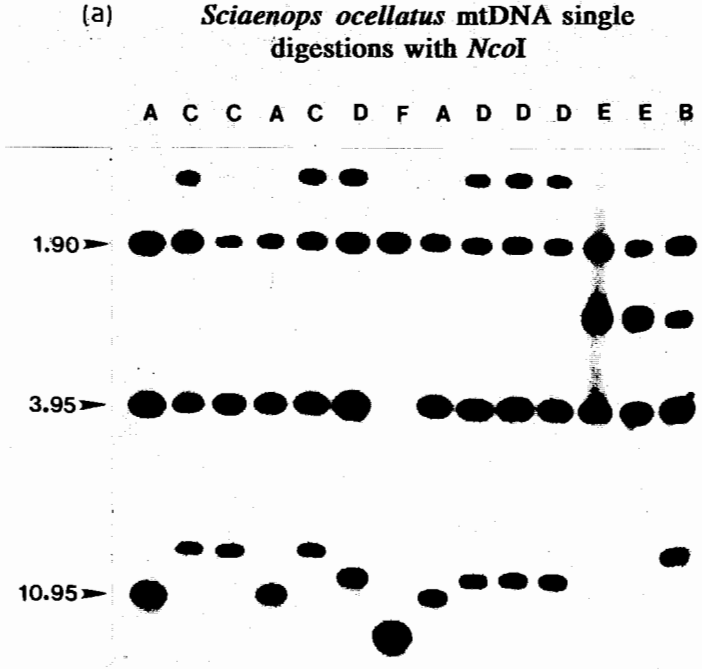

Fig. 2. (a) Autoradiogram of single digestions of red drum mtDNA with *Nco*I. Six different fragment patterns are shown. (b) Autoradiogram of single digestions with *Pvu*II. Six different fragment patterns are shown. Sizes in (a) and (b) are in kilobase (kb) pairs.

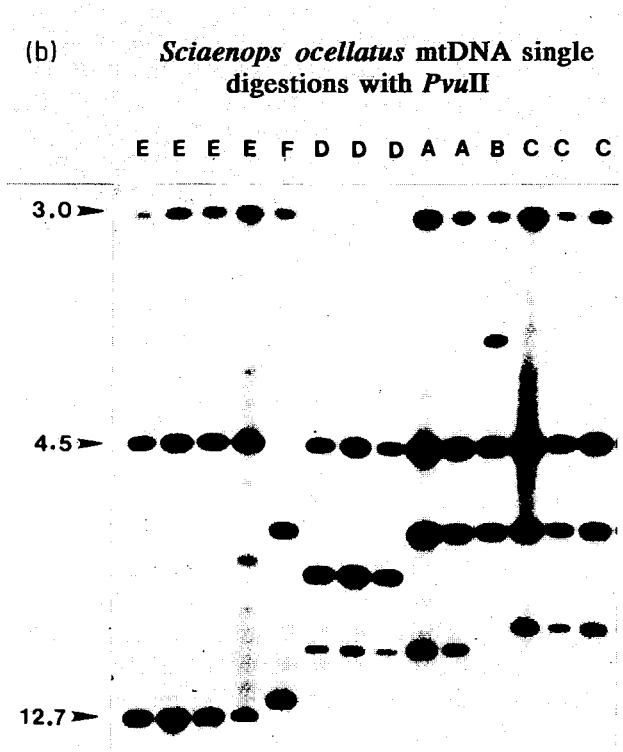

Fig. 2. Continued.

ogy of the single bands in the *Bcl*I E and *Nsi*I B patterns were confirmed by double digestions with *Bam*HI where identical double-digestion patterns for each enzyme were observed. Homology of the single band produced by *Bam*HI digestions was confirmed by *Bam*HI/*Pst*I double digestions. The *Bam*HI/*Pst*I double digestions were necessitated in large part by the single-digestion patterns with *Pst*I which typically revealed only a single band of approximately 8.4 kb. Double digestions with *Bam*HI/*Pst*I revealed that the common *Pst*I (A) pattern was actually a doublet, and that the two individuals possessing only a single *Pst*I band of 16.8 kb did not possess the same single *Pst*I restriction site (see Table 2). Subsequent mapping experiments of *Bam*HI and *Pst*I restriction sites (data not shown) have shown the latter to be the case.

Digestion patterns of all 16 restriction enzymes were used to generate composite digestion patterns. Seventy-six unique composite patterns or haplotypes were found among the 397 red drum individuals examined. The distribution of the 76 haplotypes with respect to sample locality is given in Appendix Table A1. Of the 76 different haplotypes, eight were found in 13 or more individuals, ten were found in four to twelve individuals, 22 were found in two to three individuals, and 36 were found in only one individual each. The

most common haplotypes were numbers 1, 8, 9 and 11 which were found in 30, 51, 56, and 30 individuals, respectively. Of all haplotypes found in four or more individuals, only one (haplotype 14) was found exclusively in the Atlantic, and only one (haplotype 24) was found exclusively in the Gulf.

Mitochondrial DNA nucleon diversity values within each of the 13 geographic samples of red drum are shown in Table 3. These values represent an index of mtDNA polymorphism (Avise et al., 1989) and range from zero (where all individuals exhibit the same mtDNA haplotype) to one (where each individual has a unique mtDNA haplotype). Observed values within the red drum samples ranged from 0.850 in the CHS sample to 1.00 in the PAR sample. The mean ($\pm$SE) value within samples was 0.945 ± 0.010, which is nearly identical to the overall value (among all fish surveyed) of 0.943. These nucleon diversity values are at the high end relative to other fish species thus far surveyed (Table 4) and demonstrate that red drum are no less variable in mtDNA than other fishes, and that the polymorphism in red drum mtDNA occurs throughout the geographic area surveyed.

Estimates of the percentage nucleotide sequence divergence (after Nei and Li, 1979) among the 76 red drum haplotypes ranged from 0.137 to 1.962. The mean ($\pm$SE) of 0.803 ± 0.006 is slightly higher than those reported by Avise et al. (1987a) for several species of marine fishes, and about half of the mean value reported by Kornfield and Bogdanowicz (1987) among 26 haplotypes of the Atlantic herring, *Clupea harengus*.

TABLE 3

Mitochondrial DNA nucleon diversity values within 13 geographic samples of red drum, *Sciaenops ocellatus*

Sample locality	Individuals	Different mtDNA haplotypes	MtDNA nucleon diversity
Atlantic			
Oregon Inlet, NC	15	10	0.943
Pamlico River, NC	22	13	0.931
North Inlet, SC	18	13	0.961
Charleston Bay, SC	34	17	0.850
Calibogue Sound, SC	50	23	0.907
Gulf of Mexico			
Sarasota Bay, FL	49	20	0.947
Riviera Bay, FL	24	15	0.953
Apalachicola Bay, FL	24	17	0.960
Biloxi Bay, MS	57	21	0.934
Black Bay, LA	20	17	0.984
Grand Isle, LA	43	24	0.966
West Bay, TX	32	19	0.948
Redfish Bay, TX	9	9	1.000

TABLE 4

Mitochondrial DNA nucleon diversity values documented in various fish species

Taxon (common name)	Individuals	Different mtDNA genotypes	MtDNA genotypic diversity
Sciaenops ocellatus[1] (red drum)	397	76	0.943
Brevoortia tyrannus[2] (Atlantic menhaden)	33	32	0.998
Clupea harengus[3] (Atlantic herring)	69	26	0.909
Amia calva[2] (bowfin)	75	13	0.806
Lepomis punctatus[2] (spotted sunfish)	79	17	0.788
Stizostedion vitreum[4] (walleye)	221	10	0.624
Opsanus tau[2] (oyster toadfish)	43	5	0.578
Anquilla rostrata[2] (American eel)	109	23	0.538
Arius felis[2] (hardhead catfish)	60	11	0.473

[1]Includes both Atlantic and Gulf of Mexico samples of red drum.
[2]Data are from Avise et al. (1989).
[3]Data are from Kornfield and Bogdanowicz (1987).
[4]Data are from Ward et al. (1989).

Heterogeneity in mtDNA haplotype frequencies among the five samples from the Atlantic, among the eight samples from the Gulf, and between pooled samples from the Atlantic vs. pooled samples from the Gulf was tested for each mtDNA haplotype that was found in four or more individuals in each of the comparison groups. All tests among samples from the Atlantic and among samples from the Gulf were non-significant (Table 5). Heterogeneity between pooled samples from the Atlantic vs. pooled samples from the Gulf was detected for four haplotypes (Table 5). In two of the tests (for haplotypes 12 and 24), the heterogeneity was significant only at the 5% level. This is important to the extent that 43 different V tests were performed and one might expect at least two tests to be significant by chance alone at an alpha level of 0.05.

The incidence of the four haplotypes which differed significantly in frequency between Atlantic and Gulf red drum is shown in Table 6. Three of the haplotypes (9, 12, and 14) occur primarily in the Atlantic, whereas haplotype 24 was found only in the Gulf. Two approaches were adopted to examine the hypothesis that the three haplotypes occurring primarily in the Atlantic were closely related to one another. The minimum number of mutational steps (i.e.

TABLE 5

V-values based on arcsin square-root transformations of mtDNA haplotype frequencies[1]

Haplotype No.	Atlantic	Gulf	Atlantic vs. Gulf
1	6.131	5.312	1.900
2	–	8.560	2.955
3	4.650	5.820	0.441
6	–	5.045	0.696
8	1.606	3.211	0.753
9	4.716	1.896	25.574***
10	2.416	12.907	0.186
11	1.474	3.899	0.058
12	2.762	–	7.279*
13	1.244	–	3.976
14	1.244	–	8.011**
16	–	3.823	0.000
18	–	5.347	2.305
21	–	7.487	3.647
22	–	5.147	0.858
23	3.026	8.463	0.162
24	–	5.068	7.060*
29	–	6.482	2.339

[1]Degrees of freedom: Atlantic, 4; Gulf, 7; Gulf vs. Atlantic, 2.
*$P<0.05$; **$P<0.025$; ***$P<0.001$.

TABLE 6

Incidence of four significantly heterogeneous haplotypes among Gulf and Atlantic red drum

	Haplotype				N[1]
	9	12	14	24	
Atlantic	37	6	4	0	139
Gulf	19	1	0	8	258

[1]Number of individuals surveyed.

gains or losses of a restriction site) among all four haplotypes was estimated by comparing the digestion profiles (see Table 2 and Appendix Table A1). The mean ($\pm$SE) number of mutational steps separating haplotypes 9, 12, and 14 was 4.0 ± 0.58, whereas that separating these three haplotypes from haplotype 24 was 4.33 ± 1.20. The percentage nucleotide sequence divergence among the four haplotypes was also estimated. The mean ($\pm$SE) percentage nucleotide sequence divergence among haplotypes 9, 12, and 14 was 0.849 ± 0.143, a value which is essentially the same as the average percentage nucleotide sequence divergence between any two of the 76 haplotypes (see

above). The mean (±SE) percentage nucleotide sequence divergence between haplotypes 9, 12, and 14 and haplotype 24 was 0.791±0.238. Collectively, these data suggest that haplotypes 9, 12, and 14 are no more closely related to one another than any is to haplotype 24.

Estimated F_{ST} values for these four haplotypes are shown in Table 7. F_{ST} values are shown only for these four haplotypes as the null hypothesis of the V test is equivalent to the null hypothesis of $F_{ST}=0$ (DeSalle et al., 1987) and it was assumed that all other F_{ST} values were not significantly different from zero. The estimated F_{ST} values for the four haplotypes ranged from 0.019 for haplotype 24 to 0.137 for haplotype 9. Estimates of the gene flow parameter N_em using these F_{ST} values ranged from 1.58 to 12.91 (Table 7). The average estimate of N_em (calculated from the arithmetic mean F_{ST} value of 0.057) was 4.14. As these estimates are based on mtDNA molecules, the effective number of migrants per generation represents the effective number of female migrants.

Estimates of the average percentage nucleotide sequence divergence within and among the 13 red drum samples are shown in Table 8. The estimates were generated using eqns. (1) and (2) described in the section Materials and Methods. The average percentage nucleotide sequence divergence within samples ranged from 0.494 in the MNC sample to 0.734 in the GTN sample. The overall mean (±SE) within samples was 0.574±0.021. The percentage nucleotide sequence divergence among pairwise comparisons of samples ranged from 0.498 (MNC vs. OSP) to 0.713 (GTN vs. PAR), with a mean (±SE) of 0.571±0.005. The near identity of the percentage nucleotide sequence divergence values within and among the red drum samples indicates that, on average, any two individuals drawn at random from any given sample will be about as different in mtDNA genotypes as any two individuals drawn at random from two different samples. There also is no evidence for marked genetic differentiation between Atlantic and Gulf red drum as the mean (±SE) percentage nucleotide sequence divergence between samples from the Atlantic vs. those from the Gulf was 0.572±0.008.

Parsimony analysis (using PAUP) and phenetic clustering (using UPGMA)

TABLE 7

F_{ST} values and gene flow estimates between pooled samples from the Atlantic and pooled samples from the Gulf based on four mtDNA haplotypes

Haplotype	F_{ST}	N_em
9	0.137	1.58
12	0.038	6.33
14	0.035	6.89
24	0.019	12.91

TABLE 8

Estimated average percentage mtDNA sequence divergence within and among 13 samples of red drum

	Atlantic samples					Gulf samples							
	MNC	BTH	GTN	CHS	HIH	SAR	RIV	APP	OSP	HOD	GIL	GVB	PAR
MNC	**0.494**	0.528	0.630	0.535	0.519	0.519	0.506	0.525	0.498	0.546	0.545	0.510	0.578
BTH		**0.562**	0.628	0.555	0.524	0.560	0.527	0.500	0.539	0.580	0.567	0.558	0.615
GTN			**0.734**	0.645	0.621	0.653	0.625	0.633	0.637	0.672	0.660	0.654	0.713
CHS				**0.540**	0.519	0.570	0.537	0.557	0.544	0.573	0.572	0.574	0.628
HIH					**0.507**	0.552	0.509	0.531	0.530	0.565	0.555	0.555	0.601
SAR						**0.567**	0.544	0.555	0.543	0.586	0.581	0.554	0.627
RIV							**0.527**	0.525	0.524	0.568	0.554	0.541	0.593
APP								**0.550**	0.532	0.563	0.565	0.545	0.608
OSP									**0.515**	0.560	0.559	0.522	0.596
HOD										**0.612**	0.597	0.585	0.641
GIL											**0.596**	0.578	0.630
GVB												**0.544**	0.611
PAR													**0.719**

Values on the diagonal (and in boldface) are estimates of average percentage mtDNA nucleotide sequence divergence within samples.

of the fragment presence/absence and percentage nucleotide sequence divergence matrices, respectively, of the 76 different mtDNA haplotypes were carried out to determine if there was a geographic component to the distribution of the haplotypes. The gene trees (as used by Avise, 1989) produced may be obtained, upon request, from the first author. Visual inspection of both gene trees, i.e. the unrooted parsimony network and the phenogram, revealed no evidence for geographic cohesion of haplotype groupings. Putatively monophyletic lineages and phenetic clusters of haplotypes contained haplotypes from both the Atlantic and Gulf, and haplotypes from the same locality did not necessarily occur in the same phyletic group or phenetic cluster.

Parsimony analysis of the sample fragment presence/absence matrix yielded three minimum length trees of 73 steps, with a consistency index of 0.479. The unrooted, strict consensus tree produced from the CONTREE option in PAUP is shown in Fig. 3. Superimposed on the consensus tree are the results from bootstrapping where the numbers shown represent the percentage of times (from 100 replicates) that a putatively monophyletic clade in the consensus tree is supported. Only two putative clades, one comprised of the OSP and GVB samples (85 occurrences in 100 replicates) and one comprised of the BTH, CHS, HIH, and GTN samples (72 occurrences in 100 replicates), were supported at greater than the 50% level. Given that the BTH, CHS, HIH, and GTN samples are from the Atlantic coast, and considering the results

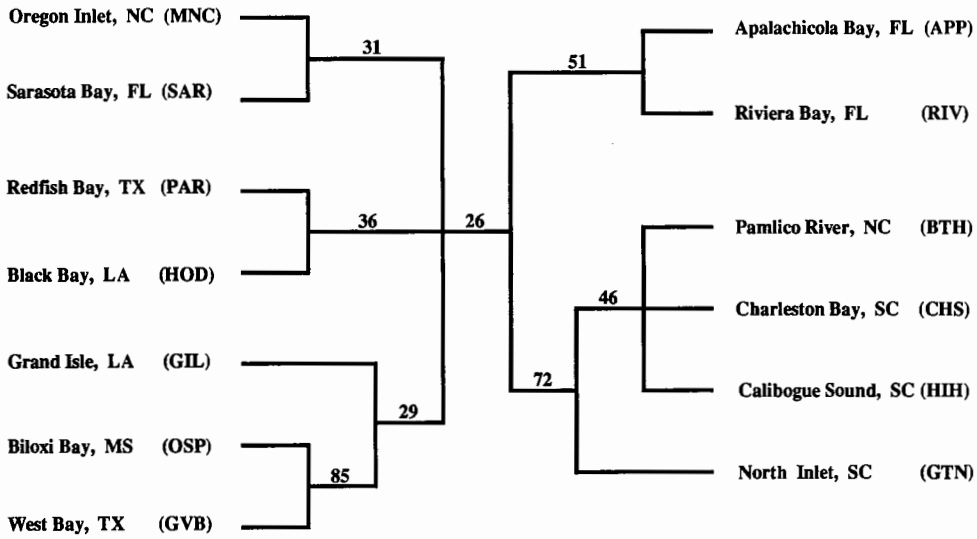

Fig. 3. Strict (unrooted) consensus tree produced by parsimony analysis (PAUP) of the binary coded (presence/absence) sample fragment matrix. Numbers along branches indicate the proportion of times (from 100 replicates) that a group was distinguished in bootstrap analysis. Branch lengths are not accurate representations of the number of character state changes between operational units.

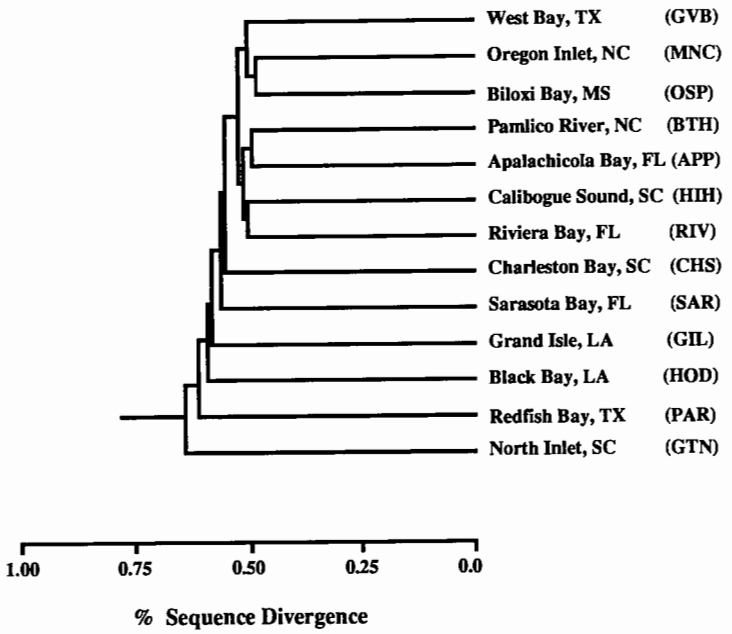

Fig. 4. UPGMA phenogram derived from mtDNA sequence divergence values among geographic samples of red drum.

from heterogeneity testing of mtDNA haplotype frequencies (see above), geographic cohesion of samples from the Atlantic may be weakly supported. The bootstrap values, however, are very likely inflated, as the data are from restriction fragments (as opposed to restriction sites) and there is a redundancy of information concerning single gains or losses of restriction sites (Felsenstein, 1985; Bermingham and Avise, 1986). As a consequence, support for geographic cohesion of mtDNA haplotype assemblages should be considered minimal at best.

The UPGMA-generated phenogram of similarity among samples is shown in Fig. 4 and was based on the estimates of percentage nucleotide sequence divergence among samples presented in Table 8. Some of the groupings suggested by phenetic analysis are similar to those inferred from parsimony analysis, while others are not. However, the relatively small difference between the initial cluster value of 0.498%, where MNC joins OSP, and the terminal cluster value of 0.647%, where GTN joins the remaining samples (Fig. 4), suggests that most, if not all, of the cluster nodes are probably not significantly different from one another. Nearly identical results were also found in the distance tree using the Fitch–Margoliash procedure (data not shown).

As a final test of the hypothesis that red drum are geographically substructured, a matrix correlation between the sample nucleotide sequence divergence matrix and a matrix of geographic distance (in miles) between all pairs

of sample localities was generated using the Mantel test (Douglas and Endler, 1982). The matrix correlation value was 0.108 and was not significantly different from zero (Student's t test, $t=0.860$, $P>0.05$).

DISCUSSION

The large number of mtDNA haplotypes and the comparatively high values of mtDNA nucleon diversity observed within and among the red drum samples surveyed in this study indicate that red drum have at least "normal" levels of genetic variability. A similar conclusion was reached by Bohlmeyer and Gold (1991) based on average heterozygosity values of red drum nuclear coding genes. These findings of appreciable levels of genetic variability in red drum suggest that the perceived decline in red drum abundance (G.C. Matlock, unpublished data, 1984; Reagan, 1985) has not affected the genetic diversity base of the species.

Interestingly, the red drum nucleon diversity value of 0.943 (over all individuals surveyed) is amongst the highest reported for a lower vertebrate species. Comparable data are not extensive, but equivalent values (i.e. more than 0.900) are known only for the Atlantic herring, *Clupea harengus*, the Atlantic menhaden, *Brevoortia tyrannus*, and the warmouth sunfish, *Lepomis gulosus* (Kornfield and Bogdanowicz, 1987; Avise et al., 1989). Two of these species, *Clupea harengus* and *B. tyrannus*, like the red drum, are marine species that school in large numbers and have the capability (geographic and otherwise) for extensive dispersal. Therefore, one might expect to find a high degree of genetic variation in species with these life history attributes. However, there are species, e.g. the American eel, *Anguilla rostrata*, the hardhead catfish, *Arius felis*, the spotted seatrout, *Cynoscion nebulosus*, and the black drum, *Pogonias cromis*, which share these life history attributes, but which have mtDNA nucleon diversity values of less than 0.600 (Avise et al., 1989; C. Furman and J.R. Gold, unpublished data, 1990). The latter, coincident with the high mtDNA nucleon diversity value of 0.962 reported in *L. gulosus*, a non-schooling, freshwater fish species largely confined to impoundments and sluggish streams (Cross and Collins, 1975) indicates that life history characters alone are insufficient to predict levels of mtDNA variability.

The distribution and relative frequencies of the 76 red drum haplotypes supports the general conclusion that the red drum population as a whole is very weakly subdivided, with semi-isolated subpopulations occurring along the southeastern Atlantic coast and in the Gulf of Mexico. This conclusion is based on the following observations. First, nearly all of the "common" haplotypes (i.e. those found in four or more individuals) occur in both the Atlantic and Gulf. While commonality by itself need not necessarily imply current gene exchange (Avise et al., 1984; Neigel and Avise, 1986), it seems unlikely that all of the common haplotypes represent ancestral mtDNA haplotypes

which were widespread prior to any period of genetic isolation. Secondly, significant heterogeneity between Atlantic and Gulf red drum was detected in the frequencies of four haplotypes; whereas no heterogeneity in haplotype frequency was detected among the Atlantic samples or among the Gulf samples. Thirdly, genetic differentiation, as estimated by percentage nucleotide sequence divergence, within geographic samples of red drum was essentially identical to that among all samples and between samples from the Atlantic vs. those from the Gulf. On average, any two individuals drawn at random from any given sample were about as divergent in mtDNA haplotypes as any two individuals drawn at random from different samples. The limited mtDNA sequence divergence (i.e. $\mathbf{p} \approx 0.005$) observed within and among the red drum samples is likely a function of the stochastic mtDNA lineage extinction expected to occur in species with historically high levels of gene flow (Neigel and Avise, 1986; Avise et al., 1987a). Finally, there was little evidence from parsimony or phenetic analysis (or from matrix correlation analysis using the Mantel test) of a geographic component to the distribution of the red drum mtDNA haplotypes. Presumed sister haplotypes were broadly distributed within and among both Atlantic and Gulf samples, and there was no statistical support for phyletic integrity of either Atlantic or Gulf red drum or of contiguous geographic samples.

The above results (and conclusions) are supported, in part, by previous studies of red drum nuclear gene variation. Ramsey and Wakeman (1987) found significant heterogeneity at a locus for glucose phosphate isomerase among red drum sampled from the Gulf. The maximum difference in allele frequencies at this locus, however, were small and occurred between geographically adjacent localities in the north-central Gulf. Bohlmeyer and Gold (1991) found significant heterogeneity at an acid phosphatase locus among samples from the Atlantic, and at a locus for adenosine deaminase among samples from the Gulf and between pooled samples from the Atlantic vs. pooled samples from the Gulf. However, as discussed by Bohlmeyer and Gold (1991), their findings of heterogeneity within both Atlantic and Gulf red drum were constrained by several factors including: (i) the possibility of non-random sampling of two localities in the Atlantic; (ii) several problems relating to the heterogeneity tests carried out on samples from the Gulf. As a consequence, they concluded that the only heterogeneity observed which was real was that between red drum in the Atlantic vs. those in the Gulf. This conclusion is also supported by the mtDNA data.

The heterogeneity in both mtDNA and nuclear gene frequencies between Atlantic and Gulf red drum appears, initially, to be inconsistent with the estimates of population subdivision as inferred from F_{ST} values. In the two studies of nuclear gene variation, F_{ST} values of polymorphic loci ranged from 0.009 to 0.030 and averaged 0.019 (Ramsey and Wakeman, 1987; Bohlmeyer and Gold, 1991). In this study, F_{ST} values of the four heterogeneous mtDNA

haplotypes ranged from 0.019 to 0.137 and averaged 0.057. Using the island model of Wright (1943), the F_{ST} value of 0.019 (from the nuclear gene studies) gives an estimate of the effective number of migrants (N_em) per generation of 12.91; whereas the F_{ST} value of 0.057 (from the mtDNA data) gives an N_em estimate of 4.14. The finding that estimated F_{ST} values are higher (and N_em values lower) from the mtDNA data set is not surprising given the increased sensitivity of mtDNA (relative to nuclear genes) in detecting population subdivision and gene flow (DeSalle et al., 1987). The apparent inconsistency with regard to Atlantic and Gulf red drum stems from the concept that subpopulations are not expected to diverge genetically from one another if the effective number of migrants per generation is greater than one (Spieth, 1974). However, as pointed out by Allendorf and Phelps (1981), statistically significant genetic divergence can occur even with substantial gene exchange, and a small value of F can coincide with appreciable amounts of genetic differentiation among subpopulations (Wright, 1969). This suggests that the genetic heterogeneity between Atlantic and Gulf red drum is real, despite significant levels of gene flow.

As noted by Bohlmeyer and Gold (1991), the genetic heterogeneity among Atlantic and Gulf red drum is curious given the several aspects of red drum biology and life history which should, in theory, facilitate dispersal and minimize geographic subdivision. Red drum are relatively strong swimmers, and at least some adult red drum form large schools offshore and are capable of extensive migration (Overstreet, 1983; Mercer, 1984; Swingle et al., 1984). Red drum are also fairly long-lived (M.D. Murphy and Taylor, 1990), meaning that individuals could spawn at multiple localities throughout their lifetimes. In other marine fishes with similar life histories and/or the capability for long-distance dispersal, genetic divergence is typically small (if it occurs at all), and most of the genetic variation occurs within localities (Winans, 1980; Gyllensten, 1985; Kornfield and Bogdanowicz, 1987; Avise et al., 1987a, b). The extreme case is the skipjack tuna, *Katsuwonus pelamis*, where samples from the Atlantic and Pacific Oceans hardly differed at all in mtDNA nucleotide sequence divergence (Graves et al., 1984).

The concordance of both mtDNA and nuclear gene data sets suggests that the genetic discontinuity is real and not an artifact or accident of sampling. In a general way, the genetic difference between Atlantic and Gulf red drum likely relates to a historical or recent interaction between red drum dispersal and biogeographic or ecological impediments to gene flow. The exact nature of these impediments is unknown. Bohlmeyer and Gold (1991) noted that there are several recognizable biogeographic provinces which separate Atlantic from Gulf marine fauna, one of which is found near Cape Canaveral, FL, and demarks a boundary between northern and southern subpopulations of the coastal horseshoe crab, *Limulus polyphemus* (Saunders et al., 1986; Avise et al., 1987a). Another possibility is that the offshore currents utilized by red

drum are somehow not conducive to movement between the Atlantic and Gulf. Finally, there also is the possibility that the present-day genetic heterogeneity represents a remnant of a historical separation among formerly existing red drum subpopulations. As discussed by Larson et al. (1984), genetic similarities or dissimilarities in present-day populations may often reflect historical rather than current conditions.

Two final points regarding mtDNA variation in red drum merit brief mention. The first concerns the use of mtDNA molecules (as opposed to nuclear genes) as genetic markers to evaluate the success of red drum stocking programs. In Texas and other southeastern Gulf and Atlantic coastal states, several management units have initiated red drum stocking programs as a means to revitalize the historic red drum fishery (Dailey and Matlock, 1987). Vital to evaluating the success of these stocking programs will be genetic tags that can be used to discriminate between wild and hatchery-reared fish. Past efforts in other (primarily freshwater) fishes to utilize genetic tags in stocking programs have exclusively employed nuclear gene markers (B.R. Murphy et al., 1983; Chilcote et al., 1986). Although successful in some cases (e.g. Taggart and Ferguson, 1984), the use of nuclear genes as tags is limited by: (i) the associated reduction in genetic variation which stems from the inbreeding required to generate adults homozygous for rare allelic variants (Allendorf and Utter, 1979); (ii) the difficulties in finding heterozygotes which carry the rare alleles needed to initiate the tagging program. By utilizing red drum mtDNA molecules, both of these limitations can be at least partially alleviated. First, of the 76 mtDNA haplotypes thus far identified in red drum, 36 are unique to individual fish. This means that identifying one or more reproductively active females carrying a rare mtDNA molecule should be relatively straightforward. Secondly, as mtDNA molecules are haploid, it is not necessary to propagate the fish to produce homozygous (diploid) genotypes. Finally, as mtDNA is maternally inherited, targeted females can be crossed with multiple males to produce genetically tagged fish, thereby decreasing considerably the reduction in nuclear gene variation associated with producing individuals homozygous for a nuclear gene marker.

The second point concerns the pattern of mtDNA divergence and geographic distribution in red drum compared with other organisms. Avise et al. (1987a) outlined five major categories of phylogenetic/geographic mtDNA distribution patterns. Categories I and II defined situations were mtDNA is discontinuously distributed phylogenetically, whereas categories III–V defined situations where mtDNA parsimony networks were more or less continuous. The differences among categories III–V related to whether spatial separation of contiguous, usually phyletically related mtDNA haplotypes was evident (category III), non-existent (category IV), or partial (category V). Red drum would appear to be best placed in category IV as none of the mtDNA haplotypes (or their phyletic relatives) appeared to be confined to a subset of

geographic localities (category III), nor does there appear to be geographic localization of a nested series of phyletically allied haplotypes (category V). Red drum thus appear to be an exception to other category III species in that geographic differentiation of mtDNA haplotypes does exist. As a consequence, the categories of Avise et al. (1987a) may need to be more broadly defined.

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APPENDIX

TABLE A1

Distribution of 76 mtDNA composite digestion patterns (haplotypes) among 13 geographic samples of red drum

Composite designation (haplotype)	Composite mtDNA digestion pattern[1]	Atlantic samples					Gulf samples							
		MNC	BTH	GTN	CHS	HIH	SAR	RIV	APP	OSP	HOD	GIL	GVB	PAR
1	ABAAAAAAAAAAAAAA	1	2	–	–	4	2	2	3	8	1	2	4	1
2	ABCCAAAAAAAAAAAA	–	–	–	–	1	4	1	2	–	–	1	1	–
3	ABBACAAAAAAAAAAA	1	1	2	–	2	3	–	1	3	–	–	1	–
4	EAAAAABAAAAAAAAA	–	–	–	–	–	–	–	1	1	–	1	–	–
5	BAAAACBAAAAAAAAA	–	–	–	–	–	–	–	1	–	–	–	–	–
6	CBAAAAAAAAAAAAAA	–	–	–	–	1	–	–	1	2	–	2	–	–
7	AAABAAAAAAAAAAAA	–	–	–	–	–	1	–	1	–	–	–	–	1
8	AAAAAABAAAAAAAAA	2	3	3	3	4	7	4	4	8	2	5	6	–
9	BAAAAAAAAAAAAAAA	3	5	2	13	14	5	2	2	3	1	4	2	–
10	BBAAAAAAAAAAAAAA	–	–	–	1	3	2	3	–	–	1	3	–	1
11	AAAAAAAAAAAAAAAA	2	2	2	2	3	3	2	1	8	1	2	2	–
12	CBAAAABAAAAAAAAA	–	2	–	2	2	–	–	–	1	–	–	–	–
13	ABCAAACAAAAAAAAA	–	1	1	1	1	–	–	–	–	–	1	–	–
14	BBFAAAAAAAABAABA	–	1	1	1	1	–	–	–	–	–	–	–	–
15	AAAAAABACAAAAAAA	–	–	1	–	1	–	–	–	–	–	–	–	–
16	ACAAAAAAAAAAAAAA	1	–	1	–	1	2	–	–	2	–	2	–	–
17	ABBACAAAAABAAAAA	–	–	–	1	–	–	–	–	–	–	–	–	–
18	ABAACAAAAAAAAAAA	–	–	–	1	–	–	1	1	2	–	2	2	–
19	BBAAADAAAAAAAAAA	–	1	–	1	1	–	–	–	–	–	–	–	–
20	ABBAAAAAAAACAAAA	–	1	–	1	–	–	–	–	–	–	–	–	–
21	BABAAAAAAAAAAAAA	–	–	–	1	–	3	–	–	2	2	3	–	–
22	BAAAAABAAAAAAAAA	–	–	–	1	1	1	–	1	3	1	1	–	1
23	AAAABAAAAAAAAAAA	2	1	–	1	3	5	2	1	1	1	–	1	–
24	AAAAAAAAAAAAAACA	–	–	–	–	–	2	1	–	2	1	–	2	–
25	ADCCAAAAAAAAAAAA	–	–	–	–	–	–	1	–	–	–	2	–	–

TABLE A1 (continued)

Composite designation (haplotype)	Composite mtDNA digestion pattern[1]	Atlantic samples					Gulf samples							
		MNC	BTH	GTN	CHS	HIH	SAR	RIV	APP	OSP	HOD	GIL	GVB	PAR
26	BABABAAAAAAAAAAA	–	–	–	–	1	–	1	–	–	–	–	–	–
27	AACCAAAAAAAAAAAA	–	1	–	–	–	–	–	–	–	–	–	–	–
28	ABAADAAAAAAAAAAA	1	–	–	–	1	–	–	–	–	–	–	–	–
29	AAAAABABAAAAAAAA	1	–	–	2	–	2	–	–	4	2	3	2	1
30	EAAAAAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	–	1	–	–
31	DBCAAAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	1	1	–	1
32	BBBACAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	1	–	–	–
33	AAAAABABBAAAAAAA	–	–	–	–	–	–	–	–	–	1	–	–	–
34	AABAAAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	1	–	1	–
35	ABBAAAAAAAAAAAAA	–	–	–	1	1	–	–	–	–	1	–	–	–
36	ABADAAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	–	–	1	–
37	EAAAAABAAAADAAAA	–	–	–	–	–	–	–	–	1	–	–	1	–
38	AAAAAAAAAAAACAAB	–	–	–	–	–	–	–	–	–	–	1	–	–
39	ABEAAEAAAAAAAAAA	–	–	–	–	–	–	–	–	–	–	–	–	1
40	AAAAAAAAAAAABAAA	–	–	–	–	–	–	–	–	–	–	–	–	1
41	AAAABAAADAAAAAAA	–	–	–	–	–	–	–	–	1	–	–	1	1
42	BAAACAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	1	–	–	–
43	AEAAABABAAAAAAAA	–	–	–	–	–	–	–	–	2	–	–	–	–
44	BADAAAAAAAAAAAAA	–	–	–	–	–	–	–	–	1	–	–	–	–
45	BABAAABAAAAAAAAA	–	–	–	–	–	1	–	–	–	–	–	–	–
46	ABEAAAAAAAAAAAAA	–	–	–	–	–	–	–	1	–	1	–	–	–
47	BBAAAFAAEAAAAAAA	–	–	–	–	–	–	–	1	–	–	–	–	–
48	AAEAAAAAAAAAAAAA	–	–	–	–	–	–	–	1	–	–	–	–	–
49	CBBAAAAAAAAAAAAA	–	–	–	–	–	–	1	1	–	–	–	–	–
50	BBHAAAAAAAABAABA	–	–	1	–	–	–	–	–	–	–	–	–	–
51	ABCAAAAAAAAAAAAA	–	–	1	–	–	–	–	–	–	–	1	–	–
52	BBAAAAACAAABAABA	–	–	1	–	–	–	–	–	–	–	–	–	–
53	ABBAAAAAAAAFAAAA	–	–	1	–	–	1	–	–	–	–	–	–	–
54	BAAEAAAAAAAAAAAA	–	–	1	–	–	–	–	–	–	–	–	–	–
55	AHCCAAAAAAAAAAAA	1	–	–	–	–	–	–	–	–	–	–	–	–

56	AGAAAAAAAAAAAAAA	–	1	–	–	–	–	–	–	–	–	–	–	–
57	AAAAAABAAACEAAAA	–	–	–	1	–	–	–	–	–	–	–	–	–
58	BBAAAFAAAAAAAAAA	–	–	–	–	–	–	1	–	–	–	–	1	–
59	ABAAAGAAAAAAAAAA	–	–	–	–	–	–	1	–	–	–	–	–	–
60	FBBAAAAAAAACAAAA	–	–	–	–	1	–	–	–	–	–	–	–	–
61	AAAAAAAADAAAAAAA	–	–	–	–	1	–	–	–	–	–	–	–	–
62	BBBAAAAAAAAAAAAA	–	–	–	–	1	–	–	–	–	–	–	–	–
63	BBADAAAAAACAAAAA	–	–	–	–	1	–	–	–	–	–	–	–	–
64	AAAEAABAAAAAAAAA	–	–	–	–	–	2	–	–	–	–	–	1	–
65	AAAAAABBAAAAAAAA	–	–	–	–	–	–	–	–	–	–	–	1	–
66	BBADAAAAAAAAAAAA	–	–	–	–	–	–	–	–	1	–	–	1	–
67	AADAAAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	–	1	1	–
68	BBAEAAAAAAAAAAAA	–	–	–	–	–	1	–	–	–	–	–	–	–
69	AFAAAABAAAAAAAAA	–	–	–	–	–	1	–	–	–	–	–	–	–
70	ACAAAAAACAAAAAAA	–	–	–	–	–	1	–	–	–	–	–	–	–
71	AAAFAAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	–	1	–	–
72	AAAAAADAAAAAAAAA	–	–	–	–	–	–	–	–	–	–	1	–	–
73	BBAAAAAAAAAADAAA	–	–	–	–	–	–	–	–	–	–	1	–	–
74	ABCAEAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	–	1	–	–
75	DBBACAAAAAAAAAAA	–	–	–	–	–	–	–	–	1	–	–	–	–
76	BAAAAAAAABAAAAAA	–	–	–	–	–	–	1	–	–	–	–	–	–

[1]Letters (from left to right) are digestion patterns (Table 2) for *Nco*I, *Bcl*I, *Sca*I, *Pvu*II, *Spe*I, *Xba*I, *Xmn*I, *Hin*dIII, *Stu*I, *Bam*HI, *Eco*RI, *Eco*RV, *Pst*I, *Sph*I, *Nsi*I, and *Bgl*II.