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II. A protein electrophoretic analysis of population structure in the red drum *Sciaenops ocellatus* *

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Abstract. Nine polymorphic loci were found among 42 presumptive protein-coding gene loci surveyed among 474 red drum (*Sciaenops ocellatus*) sampled in 1987 from 13 nearshore and 1 offshore localities from the Atlantic coast of the southeastern USA and the northern Gulf of Mexico. The mean number of alleles over the polymorphic loci was 3.8, and the average heterozygosity over all loci examined ($\bar{H}$) was estimated as 0.047. These data indicate that red drum have "normal" levels of genetic variability. Wright's F_{ST} values (the standardized variance of allele frequencies between samples) over all polymorphic loci ranged from 0.009 to 0.027 (mean $F_{ST} = 0.019$), and estimates of the effective number of migrants ($N_e m$) per generation using Wright's island model ranged from 9.0 to 27.5. High levels of gene flow among the red drum samples were also indicated by Slatkin's qualitative analysis using conditional average allele frequencies. Nei's estimates of genetic distance between pairs of samples ranged from 0.000 to 0.009, indicating a high degree of nuclear gene similarity among all samples. Highly significant heterogeneity in allele frequencies at the locus for adenosine deaminase was detected between red drum sampled from the Atlantic and those sampled from the Gulf and among red drum sampled from the Gulf.

Introduction

The abundance of the red drum or redfish (*Sciaenops ocellatus*) has declined since 1973–1978 (Reagan 1985). This decline in red drum abundance has accentuated the need for studies on the population structure of the species. A major question is whether discrete breeding units or subpopulations occur within the overall population (McIlwain et al. 1986). Tagging studies on subadult red drum have indicated that juveniles generally remain in the vicinity of the tagging site and that movement of juveniles among nearshore localities is limited (Adkins et al. 1979, Matlock and Weaver 1979, Osburn et al. 1982). These and other findings have led to the suggestions that red drum from the south Atlantic and Gulf coasts comprise at least two, spatially-isolated subpopulations and that a third subpopulation may exist north of Cape Hatteras on the Atlantic coast (Matlock 1984, 1987 a). Ross et al. (1987), however, documented that yearling red drum in North Carolina waters can move over 150 km in less than 2 mo. In addition, several aspects of red drum biology and life-history could facilitate dispersal and, in theory, minimize spatial differentiation and subpopulation genetic divergence. Red drum spawn near the mouths of bays or estuaries (Matlock 1984, 1987 b, Reagan 1985), and their pelagic eggs (Holt et al. 1981) could be transported to adjacent spawning localities by oceanic currents. There also is evidence that some red drum in the northcentral Gulf of Mexico may spawn offshore and that the larvae or juveniles may enter various bays or estuaries at later dates (Lyczkowski-Schultz et al. 1988). Further, although larvae and juveniles remain in the bays and estuaries (Overstreet 1983, Matlock 1984, 1987 b), adults move into deeper, nearshore waters prior to sexual maturation and spawning (Matlock 1984, 1987 b). Finally, at least some adult red drum form large schools offshore and can migrate extensively, at least in the Gulf of Mexico (Overstreet 1983, Mercer 1984, Swingle et al. 1984). Taken together, these aspects of red drum life-history suggest that migration (gene flow) among Atlantic or Gulf red drum is considerable and that subpopulational divergence might be minimal.

The few genetic data on the issue of gene flow among Atlantic or Gulf red drum do not support the existence of separate subpopulations. Ramsey and Wakeman (1987) surveyed allelic variation at four polymorphic loci among red drum at two locations from the eastern (Atlantic) coast of Florida and twelve locations in the northwestern Gulf of Mexico and found a mean F_{ST} value of 0.019. Since F_{ST} is the standardized variance of allele frequencies between samples and measures the reduction in heterozygosity of a population due to nonrandom mating

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between subpopulations (Wright 1943, 1965), the value of 0.019 found by Ramsey and Wakeman suggested that red drum essentially comprise a single, randomly mating population. Significant heterogeneity in allele frequencies at a locus for glucose phosphate isomerase was detected by Ramsey and Wakeman, although no simple geographic pattern was evident. The level of locus polymorphism and average heterozygosity reported by Ramsey and Wakeman in red drum, however, were somewhat lower than those reported in other members of the family Sciaenidae (Beckwitt 1983) and in other marine fishes (Winans 1980, Smith and Fujio 1982). Genetic variation in an unspecified number of red drum from Texas also indicated no subpopulation differentiation (Wilder and Fisher 1986).

The purpose of the present study was to test the hypothesis of genetic homogeneity among red drum from the Atlantic coast of the southeastern USA and the Gulf of Mexico. Additional polymorphic loci were identified and used to provide more robust estimates of inbreeding within samples and of genetic variance among samples due to population subdivision (Allendorf and Phelps 1981).

Materials and methods

Specimens of *Sciaenops ocellatus* were collected during 1987 using gill nets, pound nets, haul seines, and hook and line. Four hundred and seventy-four fish were collected from 14 sampling localities in the Atlantic Ocean and Gulf of Mexico (Table 1, Fig. 1). Thirteen samples were from nearshore localities (bays or estuaries) and one sample was from offshore in the Gulf. The majority (>95%) of individuals taken from nearshore localities were yearlings from the 1986 year class, as judged by total length. A few 2 or 3 yr-old individuals were included in a few of the nearshore samples from the Gulf. All the offshore fish were mature adults. Tissue samples of white muscle, liver, brain, and eye were taken from each fish and stored in liquid nitrogen for transport to the laboratory, where they were stored at −80 °C in an ultracold freezer. Specimens from Sampling Sites 9 and 11 (Table 1) were frozen at −20 °C, transported to the laboratory on dry ice, and stored at −80 °C after tissue samples had been taken.

Techniques for vertical starch gel electrophoresis followed Siciliano and Shaw (1976) and Morizot and Siciliano (1984). Full details, including recipes for grinding and running buffers, may be found in Bohlmeyer (1989). Buffers used in an initial screening process were: tris-citrate, pH 7.0 (TC-1) and tris-borate-EDTA, pH 8.0 (TVB) (=tris-versene-borate) of Siciliano and Shaw (1976); Poulik, tris-citrate pH 6.3/6.7 (TC-2), and tris-maleate of Selander et al. (1971); LiOH (RW) (=Ridgway) of Ridgway et al. (1970); and APM citrate, pH 6.1/6.0 (APM=amino-propyl-morpholine) of Clayton and Tretiak (1972). The gels consisted of 11.7% starch except for TC-1 gels, which were 12.6% starch. The starch used was purchased from Connaught Laboratories, Swiftwater, Pennsylvania; specific lot numbers were 420-2 and 434-1. Protein staining generally followed the methods of Siciliano and Shaw (1976), Crowle and Kline (1977), and Harris and Hopkinson (1976).

The products of 42 presumptive gene loci were ultimately resolved. Banding patterns of the presumptive loci were interpreted according to the subunit composition of the same proteins in other fishes as described in the literature (Utter et al. 1974, Ferris and Whitt 1978, Buth 1984, Whitt 1987). The allozyme/isozyme nomenclature used generally follows that of Morizot and Siciliano (1984), and designation of allelic variants was based on relative mobilities to the most common allele, which was designated as Allele 100. Because of the large number of alleles initially detected at the locus for adenosine deaminase (Bohlmeyer and Gold 1990), allelic variation at this locus was scored first on the TVB buffer system followed by side-by-side comparisons of all variants on the TC-1 buffer system. This was done to insure accurate allele identification and to detect any cryptic variation. All gels were photographed using either Panatomic-X at ASA 32 or Technical Pan 2415 film at ASA 100. Fluorescent stains were photographed using Technical Pan 2415 film at ASA 100 and a #14 yellow filter.

Loci that could not be resolved easily on vertical starch gels were run on polyacrylamide (PAGE)-resolving gels using the meth-

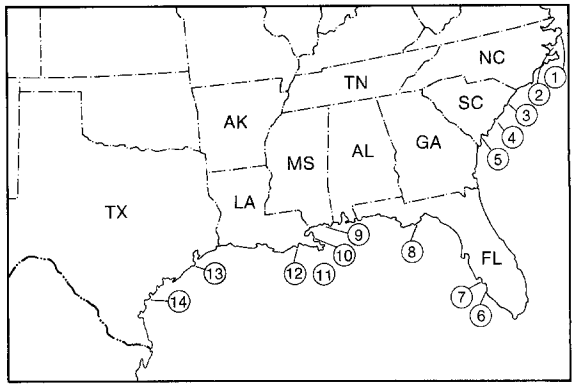

Fig. 1. Sample localities (1–14) of *Sciaenops ocellatus* examined in the study. Samples 1–5 are from southeastern Atlantic coast of USA, Samples 6–14 from northern Gulf of Mexico. Site descriptions are given in Table 1. NC: North Carolina; SC: South Carolina; GA: Georgia; FL: Florida; AL: Alabama; MS: Mississippi; LA: Louisiana; TX: Texas; AK: Arkansas; and TN: Tennessee

Table 1. *Sciaenops ocellatus*. Sampling localities (numbered as in Fig. 1) and numbers of specimens collected at each site. Location acronyms (in parentheses) are used in Fig. 3 and Tables 3 and 8

Location (Site No.)		No. of individuals
Atlantic		
(1)	Oregon Inlet, south of Nags Head, North Carolina (MNC)	15
(2)	Pamlico River, near Bath, North Carolina (BTH)	23
(3)	North Inlet, north of Georgetown, South Carolina (GTN)	18
(4)	Charleston Bay, South Carolina (CHS)	34
(5)	Calibogue Sound along Hilton Head Island, South Carolina (HIH)	50
Gulf of Mexico		
(6)	Sarasota Bay, near Long Key, Florida (SAR)	49
(7)	Riviera Bay, off Tampa Bay, Florida (RIV)	24
(8)	Apalachicola Bay, Florida (APP)	24
(9)	Biloxi Bay, Mississippi (OSP)	50
(10)	Black Bay, near Hopedale, Louisiana (HOD)	50
(11)	Offshore from Grand Isle, Louisiana (OLA)[a]	28
(12)	Salt marsh near Grand Isle, Louisiana (GIL)	50
(13)	West Bay, behind Galveston Island, Texas (GVB)	32
(14)	Redfish Bay, Port Aransas, Texas (PAR)	27
Total		474

[a] Samples obtained from National Marine Fisheries Service, Pascagoula, Mississippi

ods of Davis (1964) and Ornstein (1964). Details regarding exact procedures, stock solutions, stains, and recipes may be found in Bohlmeyer (1989). The loci examined using polyacrylamide included the polymorphic locus *Got-1* and six general protein loci in muscle.

Tests of Hardy-Weinberg equilibrium expectations, Wright's *F*-statistics, including F_{IS} and F_{ST}, the fixation index and standardized variance of allele frequencies between samples, respectively, and Nei's genetic identity (*I*) and distance (*D*) indices were computed using the BIOSYS-1 computer program of Swofford and Selander (1981). Deviations from Hardy-Weinberg equilibrium expectations were tested using pooled genotypes (as described in the Users Manual for BIOSYS-1) and the chi-square statistic with one degree of freedom. Mean F_{IS} and F_{ST} values were computed as the arithmetic averages of F_{IS} and F_{ST} values over all polymorphic loci. A UPGMA (unweighted pair-group method using arithmetic averages) cluster analysis of Nei's *D* values was carried out using a computer program written by Dr. N. Saitou from the University of Texas Health Science Center at Houston. This program also computes a standard error for each node in the phenogram according to equations in Nei et al. (1985). Estimates of gene flow ($N_e m$, i.e., the effective number of migrants per generation) were calculated using Wright's (1943) island model, where $F_{ST} \approx 1/4N_e m+1$. Significance testing of allele-frequency heterogeneity among localities was done using the *G*-statistic for goodness-of-fit (Sokal and Rohlf 1969) on contingency tables of allele counts, and the "*V*"statistic for sample heterogeneity (DeSalle et al. 1987) on arcsin square-root-transformed allele-frequency values. The *G*-tests were carried out using the BIOM-PC program package of Rohlf (1983), while the "*V*" tests were carried out using software kindly provided by T. Dowling. Slatkin's qualitative (1981) and quantitative (1985) methods to estimate average levels of gene flow were performed using a pocket calculator.

Results

Over 80 presumptive gene loci in *Sciaenops ocellatus* were initially screened. Forty-two of these were electrophoretically resolved, including 24 enzymes and 6 structural proteins (Table 2). Many other proteins exhibited activity but were not resolved satisfactorily. Some of the latter, e.g. aconitase, α-glucosidase, and *GPI-A*, appeared to be polymorphic.

The 42 presumptive loci resolved were screened in ten individuals from each of six collection localities (=60 individuals or 120 total gene products). The six red drum samples screened were: Pamlico River, North Carolina (BTH); Calibogue Sound, South Carolina (HIH); Sarasota Bay, Florida (SAR); Biloxi Bay, Mississippi (OSP); offshore Grand Isle, Louisiana (OLA); and Port Aransas, Texas (PAR). Nine of the presumptive loci were found to be polymorphic at the 0.01 level, giving a *P* value (percent of polymorphic loci) estimate of 21.4% at the 99% criterion. The remaining 33 presumptive loci were assumed to be monomorphic in red drum and were not examined further, although five of the monomorphic loci (*Acp-1, Est-2* and *3*, and *Sod-1* and *2*) were incidentally screened when examining the polymorphic loci. All five proved to be monomorphic in all red drum samples.

Allele frequencies for the nine polymorphic loci among all red drum samples are shown in Table 3. The number of alleles at the nine polymorphic loci ranged from 2 (at *Acp-2* and *Est-1*) to 11 (at *Ada*). The mean number of alleles over all polymorphic loci was 3.8. The observed heterozygosity (*h*) values at each polymorphic locus (averaged over all samples) ranged from 0.027 at *Pep-B* to 0.703 at *Ada* (Table 4). Assuming that the loci found to be monomorphic in the 60 individuals examined are monomorphic in all the individuals surveyed for the nine polymorphic loci, the average heterozygosity over all loci examined ($\bar{H}$) is estimated as 0.047.

Two significant deviations ($P<0.05$) from expected (Hardy-Weinberg equilibrium) genotypic frequencies were found: at *Adh* in the sample from North Inlet, South Carolina, and at *Got-1* in the sample from West Bay, Texas. However, when all genotypic classes at each locus were used to calculate chi-square values, the deviation from Hardy-Weinberg equilibrium at the *Adh* locus in the sample from North Inlet was not significant ($P=0.255$). The deviation from Hardy-Weinberg expectation at the *Got-1* locus in the sample from West Bay, Texas, was possibly due to two rare homozygotes and one rare heterozygote for Allele 90 (data not shown). All other loci in all samples were in Hardy-Weinberg equilibrium, even though the fish in a few of the samples were from different year classes.

Table 2. *Sciaenops ocellatus*. Proteins resolved electrophoretically from brain and eye (B, E), liver (L), and muscle (M). Buffers used were: amino-propyl-morpholine citrate (APM), tris-citrate (TC), tris-versene-borate (TVB), and LiOH of Ridgway (RW). One enzyme (GOT) and six muscle proteins were resolved using polyacrylamide gel electrophoresis (PAGE)

Protein	E.C. No.	No. of loci	Tissue	Buffer
Acid phosphatase (ACP)[a]	3.1.3.2	2	M	TVB
Adenosine deaminase (ADA)	3.5.4.4	1	M	TVB/TC
Adenylate kinase (AK)	2.7.4.3	1	M	TC
Alcohol dehydrogenase (ADH)	1.1.1.1	1	L	TVB
Creatine kinase (CK)	2.7.3.2	2	M	TC
Enolase (ENO)	4.2.1.11	1	B,E	TVB
Esterase (EST)[b]	3.1.1.1	3	M	TVB
Fumarase (FUM)	4.2.1.2	1	M	RW
Glucosephosphate isomerase (GPI)	5.3.1.9	1	M	TVB
β-glucuronidase (βGUS)	3.2.1.31	1	M	TVB
Glutamate-oxaloacetate transaminase (GOT)	2.6.1.1	2	L	PAGE/TVB
Glyoxalase I (GLO)	4.4.1.5	1	M	TVB
Lactate dehydrogenase (LDH)	1.1.1.27	3	B,E	TVB
Malate dehydrogenase (MDH)	1.1.1.37	2	M	APM
Malic enzyme (ME)	1.1.1.40	1	M	TVB
Mannosephosphate isomerase (MPI)	5.3.1.8	1	M	TC
α-mannosidase (αMAN)	3.2.1.24	1	M	TC
Muscle proteins (MP)	–	6	M	PAGE
Peptidase (PEP)	3.4.11	3	M	TVB
Phosphoglucomutase (PGM)	5.4.2.2	1	M	TC
Phosphoglycerate kinase (PGK)	2.7.2.3	1	M	TC
Pyruvate kinase (PK)	2.7.1.40	1	B,E	TVB
Superoxide dismutase (SOD)	1.15.1.1	2	M	TVB
Triosephosphate isomerase (TPI)	5.3.1.1	2	B,E	TVB
Uridine monophosphate kinase (UMPK)	2.7.4	1	B,E	TC

[a] 4-methylumbelliferal phosphate used as substrate
[b] 4-methylumbelliferal acetate used as substrate

Table 3. *Sciaenops ocellatus*. Allele frequencies of polymorphic loci. Locality abbreviations as in Table 1

Locus/allele	Atlantic samples					Gulf samples								
	MNC	BTH	GTN	CHS	HIH	SAR	RIV	APP	OSP	HOD	OLA	GIL	GVB	PAR
Acp-2														
115	0.167	0.000	0.083	0.088	0.050	0.085	0.083	0.125	0.040	0.133	0.071	0.100	0.094	0.011
100	0.833	1.000	0.917	0.912	0.950	0.915	0.917	0.875	0.960	0.867	0.929	0.900	0.906	0.889
(*N*)	(15)	(23)	(18)	(34)	(50)	(47)	(24)	(24)	(50)	(45)	(28)	(50)	(32)	(27)
Ada														
150	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000
130	0.000	0.000	0.056	0.059	0.039	0.053	0.042	0.000	0.070	0.030	0.018	0.050	0.016	0.019
125	0.400	0.370	0.361	0.324	0.340	0.245	0.229	0.458	0.300	0.410	0.411	0.290	0.422	0.333
115	0.000	0.000	0.000	0.044	0.000	0.032	0.062	0.021	0.120	0.040	0.232	0.140	0.156	0.148
110	0.067	0.109	0.056	0.044	0.090	0.149	0.125	0.042	0.050	0.140	0.018	0.080	0.062	0.037
100	0.500	0.478	0.500	0.485	0.500	0.479	0.354	0.417	0.380	0.310	0.286	0.370	0.297	0.407
90	0.000	0.000	0.000	0.000	0.010	0.011	0.021	0.021	0.010	0.030	0.000	0.030	0.016	0.019
85	0.000	0.000	0.000	0.015	0.000	0.032	0.083	0.000	0.040	0.020	0.000	0.010	0.000	0.019
78	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000
75	0.033	0.022	0.028	0.029	0.030	0.000	0.083	0.021	0.020	0.020	0.036	0.010	0.000	0.019
65	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.010	0.000	0.000	0.000	0.031	0.000
(*N*)	(15)	(23)	(18)	(34)	(50)	(47)	(24)	(24)	(50)	(50)	(28)	(50)	(32)	(27)
Adh														
−100	0.600	0.630	0.667	0.529	0.550	0.534	0.354	0.562	0.550	0.500	0.661	0.530	0.453	0.500
−75	0.400	0.326	0.278	0.441	0.410	0.415	0.604	0.417	0.420	0.480	0.304	0.410	0.516	0.426
−50	0.000	0.043	0.000	0.015	0.020	0.032	0.042	0.021	0.030	0.020	0.018	0.060	0.031	0.074
−20	0.000	0.000	0.056	0.015	0.020	0.011	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000
(*N*)	(15)	(23)	(18)	(34)	(50)	(47)	(24)	(24)	(50)	(50)	(28)	(50)	(32)	(27)
Est-1														
100	0.900	0.957	0.917	0.838	0.920	0.862	0.937	0.917	0.940	0.950	0.893	0.950	0.906	0.963
95	0.100	0.043	0.083	0.162	0.080	0.138	0.062	0.083	0.060	0.050	0.107	0.050	0.094	0.037
(*N*)	(15)	(23)	(18)	(34)	(50)	(47)	(24)	(24)	(50)	(50)	(28)	(50)	(32)	(27)
Gpi-B														
−110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000
−100	0.967	0.978	1.000	0.971	0.960	0.957	0.958	1.000	0.960	0.967	0.982	0.980	0.937	0.963
−50	0.033	0.022	0.000	0.029	0.040	0.043	0.042	0.000	0.040	0.022	0.018	0.020	0.062	0.037
(*N*)	(15)	(23)	(18)	(34)	(50)	(47)	(24)	(24)	(50)	(45)	(28)	(50)	(32)	(27)
Got-1														
120	0.033	0.000	0.000	0.029	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000
110	0.033	0.065	0.139	0.162	0.150	0.156	0.091	0.187	0.122	0.110	0.089	0.060	0.100	0.148
100	0.933	0.935	0.833	0.794	0.840	0.844	0.909	0.771	0.878	0.890	0.911	0.930	0.883	0.852
90	0.000	0.000	0.028	0.015	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.017	0.000
(*N*)	(15)	(23)	(18)	(34)	(50)	(45)	(22)	(24)	(45)	(50)	(28)	(50)	(30)	(27)
Pep-B														
115	0.033	0.000	0.000	0.000	0.010	0.011	0.042	0.000	0.020	0.011	0.000	0.000	0.000	0.000
100	0.967	1.000	1.000	1.000	0.980	0.968	0.958	1.000	0.970	0.989	0.982	1.000	1.000	1.000
85	0.000	0.000	0.000	0.000	0.010	0.021	0.000	0.000	0.010	0.000	0.018	0.000	0.000	0.000
(*N*)	(15)	(23)	(18)	(34)	(50)	(47)	(24)	(24)	(50)	(45)	(28)	(50)	(32)	(27)
Pep-D														
115	0.000	0.022	0.000	0.015	0.010	0.011	0.000	0.000	0.000	0.011	0.036	0.010	0.000	0.019
100	0.967	0.957	1.000	0.956	0.980	0.979	0.958	0.958	0.990	0.922	0.893	0.970	0.937	0.926
85	0.033	0.022	0.000	0.029	0.010	0.011	0.042	0.042	0.010	0.067	0.071	0.020	0.062	0.056
(*N*)	(15)	(23)	(18)	(34)	(50)	(47)	(24)	(24)	(50)	(45)	(28)	(50)	(32)	(27)
Pep-S														
105	0.000	0.043	0.000	0.029	0.030	0.043	0.021	0.021	0.090	0.011	0.054	0.020	0.047	0.000
100	1.000	0.957	1.000	0.971	0.970	0.957	0.958	0.979	0.910	0.989	0.946	0.980	0.953	1.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(*N*)	(15)	(23)	(18)	(34)	(50)	(47)	(24)	(24)	(50)	(45)	(28)	(50)	(32)	(27)

Table 4. *Sciaenops ocellatus.* Observed heterozygosities (h) at each of nine polymorphic loci

Locus	h
Acp-2	0.162
Ada	0.703
Adh	0.495
Est-1	0.167
Gpi-B	0.062
Got-1	0.214
Pep-B	0.027
Pep-D	0.081
Pep-S	0.062

Table 5. *Sciaenops ocellatus.* G-statistics for goodness-of-fit from contingency-table analysis of allele counts. Degrees of freedom in parentheses

Locus	Atlantic	Gulf	Atlantic vs Gulf
Acp-2	10.937* (4)	6.938 (8)	1.501 (1)
Ada	26.335 (32)	120.790** (80)	51.835** (10)
Adh	11.693 (12)	22.181 (24)	9.518* (3)
Est-1	5.055 (4)	9.363 (8)	1.140 (1)
Gpi-B	2.615 (4)	10.539 (16)	0.798 (2)
Got-1	14.567 (12)	23.699 (24)	5.757 (3)
Pep-B	5.854 (8)	18.110 (16)	0.358 (2)
Pep-D	4.454 (8)	18.883 (16)	2.951 (2)
Pep-S	4.019 (4)	19.716 (16)	1.601 (2)

* $0.05 > P > 0.01$
** $P < 0.01$

Highly significant ($P < 0.01$) heterogeneity in allele frequencies using the G-test was found at *Ada* among samples from the Gulf of Mexico and between pooled samples from the Atlantic versus pooled samples from the Gulf (Table 5). Significant ($0.01 < P < 0.05$) heterogeneity using the G-test also was found at *Acp-2* among samples from the Atlantic and at *Adh* between pooled samples from the Atlantic versus those from the Gulf (Table 5). Additional G-tests were carried out at the following loci subsequent to pooling of alleles whose frequency in any sample was less than 10%: *Ada* (Alleles 150, 130, 90, 85, 78, 75, and 65 pooled); *Adh* (Alleles −50 and −20 pooled); *Gpi-B* (Alleles −110 and −50 pooled); *Got-1* (Alleles 120 and 90 pooled); *Pep-B* (Alleles 115 and 85 pooled); *Pep-D* (Alleles 115 and 85 pooled); and *Pep-S* (Alleles 105 and 95 pooled). Highly significant ($P < 0.01$) heterogeneity was again found at *Ada* among samples from the Gulf and between pooled samples from the Atlantic versus pooled samples from the Gulf. Significant heterogeneity was not detected at *Adh* between pooled samples from the Atlantic versus those from the Gulf. The heterogeneity at the *Acp-2* locus is possibly due to non-random sampling. The largest difference in allele frequencies at *Acp-2* among Atlantic samples was between the adjacent localities at Oregon Inlet (MNC) and the Pamlico River (BTH) in North Carolina (Table 3) and the sample size at these two localities (15 and 23 individuals, respectively) were among the lowest in the entire study. The heterogeneity at *Adh*, detected only in the initial G-tests, may be due to differing frequencies of the rare −50 and −20 *Adh* alleles between Atlantic and Gulf samples (Table 3), or may be artifactual, given that at least one significant result from the 27 G-tests would be expected by chance alone at an alpha level of 0.05.

Table 6. *Sciaenops ocellatus.* "V"-statistics for tests of heterogeneity based on arcsin square-root transformations of allele frequencies

Locus/allele	Atlantic	Gulf	Atlantic vs Gulf
Acp-2			
100	11.428*	6.969	1.399
Ada			
125	0.694	16.378*	0.146
115	5.524	29.534**	36.475**
110	2.028	16.912*	0.190
100	0.085	10.062	12.359**
Adh			
−100	2.597	11.922	2.609
−75	3.510	12.287	2.462
Est-1			
100	4.511	8.532	1.378
Gpi-B			
−100	1.966	5.650	0.106
Got-1			
110	5.567	8.172	0.201
100	6.195	9.053	1.097
Pep-B			
100	3.284	10.012	0.185
Pep-D			
100	2.823	12.520	1.684
Pep-S			
100	2.786	12.678	0.938

* $0.05 > P > 0.01$
** $P < 0.01$

Heterogeneity in the frequencies of all alleles at each locus whose frequency in any sample was greater than 10% was tested using the "V" test recently developed by DeSalle et al. (1987). Significant ($0.01 < P < 0.05$) heterogeneity was detected at *Acp-2* 100 among samples from the Atlantic, and at *Ada* 125 and *Ada* 110 among samples from the Gulf; highly significant ($P < 0.01$) heterogeneity was detected at *Ada* 115 among samples from the Gulf, and at *Ada* 115 and *Ada* 100 between pooled samples from the Atlantic versus pooled samples from the Gulf (Table 6). The heterogeneity at *Acp-2* may stem from non-random sampling. The heterogeneity at *Ada*, however, is real and appears to result from frequency differ-

Table 7. *Sciaenops ocellatus.* Estimates of F_{IS} (fixation index), F_{ST} (standardized variance of allele frequencies between samples), and N_em (effective number of migrants per generation) based on nine polymorphic loci. Mean N_em value calculated from mean F_{ST} value using Wright's (1943) island model; arithmetic mean of N_em values is 14.15

Locus	F_{IS}	F_{ST}	N_em
Acp-2	−0.118	0.020	12.25
Ada	−0.002	0.027	9.01
Adh	0.032	0.025	9.75
Est-1	−0.043	0.016	15.37
Gpi-B	−0.040	0.009	27.53
Got-1	−0.005	0.020	12.25
Pep-B	−0.028	0.017	14.46
Pep-D	−0.024	0.017	14.46
Pep-S	0.025	0.021	12.25
Mean	−0.022	0.019	12.91

ences in up to four *Ada* alleles. Visual inspection of allele frequencies at *Ada* (Table 3) among samples from the Gulf indicates that the significant heterogeneity detected at *Ada* Alleles 125 and 110 may be due to a lower frequency of *Ada* 125 and a higher frequency of *Ada* 110 in the two samples from central Florida (viz. SAR and RIV) compared to most other samples from the Gulf. This possibility is supported by additional "*V*" tests involving these two alleles and excluding the SAR and RIV samples where no significant heterogeneity was detected. Alternatively, since 42 different "*V*" tests were initially performed (Table 6), at least two of these would be expected by chance alone to be significant at an alpha level of 0.05. The highly significant heterogeneity at *Ada* 115 among the Gulf samples does indicate genetic differentiation among red drum within the Gulf. Inspection of allele frequencies in Table 3 suggests that this difference may be due to higher frequencies of *Ada* 115 among samples from the northwestern Gulf. The highly significant differences between Atlantic and Gulf samples at *Ada* 115 and *Ada* 100 (Table 6) are due to higher frequencies of *Ada* 115 among Gulf samples and higher frequencies of *Ada* 100 among Atlantic samples (Table 3).

Estimates of genetic differentiation within and among the red drum samples are shown in Table 7. Fixation index (F_{IS}) values ranged from −0.118 at *Acp-2* to +0.032 at *Adh*. The overall mean F_{IS} value was −0.022. The negative F_{IS} values observed at seven of the nine loci indicate a slight excess of heterozygotes over what might be expected from a randomly mating population. The largest negative value was −0.118 at *Acp-2* and is due primarily to an observed excess of heterozygotes in the sample from Oregon Inlet, North Carolina (data not shown). Levels of population subdivision, as reflected by F_{ST} values, are also shown in Table 7. The estimated F_{ST} values ranged from 0.009 at *Gpi-B* to 0.027 at *Ada*. The overall mean F_{ST} value was 0.019. Estimates of the gene flow parameter N_em, using single-locus F_{ST} values, ranged from 9.01 to 27.53 (Table 7). The average estimate of N_em (calculated from the mean F_{ST} value) was 12.91.

Slatkin's (1981) qualitative method to estimate gene flow produced a graph typical of species with high gene

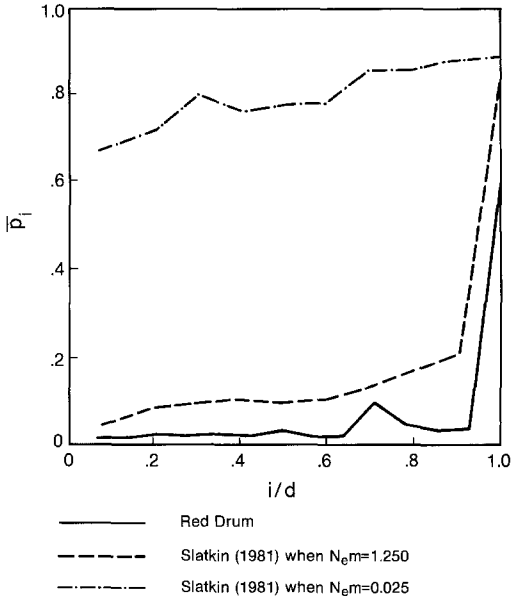

Fig. 2. *Sciaenops ocellatus.* Qualitative profile of gene flow. Values were obtained by plotting conditional-average allele frequencies ($\bar{p}_i$) against occupancy rate (i/d); see Slatkin (1981) for further details. Also shown are theoretical profiles (from Slatkin 1981) when N_em (effective number of migrants per generation) equals 1.250 and 0.025

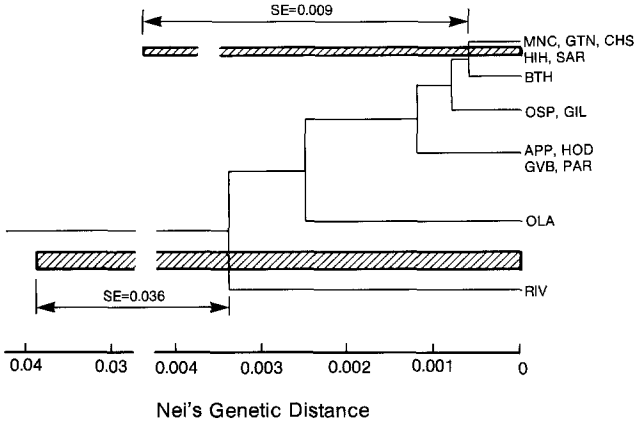

Fig. 3. *Sciaenops ocellatus.* UPGMA (unweighted pair-group method using arithmetic averages) cluster analysis of Nei's (1978) unbiased genetic distances. Hatched bars are standard errrors for first and last nodes. Acronyms for sample localities are given in Table 1

flow (Fig. 2). Slatkin's (1985) quantitative method gave an N_em estimate of 37.38. Note that this latter estimate is based on only three private alleles: Allele 78 at *Ada*, Allele −110 at *Gpi-B*, and Allele 95 at *Pep-S*. Presumably, more rare alleles would have surfaced if all loci had been scored for all individuals. Both of Slatkin's methods, however, yielded relatively high estimates of gene flow.

Estimates of Nei's (1978) identity and distance indices generated from the nine polymorphic and five monomorphic loci examined in all individuals are shown in Table 8. Estimates of Nei's identity ranged from 0.990 to 1.0, while estimates of Nei's distance ranged from 0.000 to 0.009. These results demonstrate that the red drum samples are almost identical genetically, particularly since the

Table 8. *Sciaenops ocellatus*. Matrix of Nei's (1978) unbiased genetic identity (upper diagonal) and unbiased genetic distance (lower diagonal) among 14 samples, based on nine polymorphic and five monomorphic loci. Locality acronyms are as in Table 1

	MNC	BTH	GTN	CHS	HIH	SAR	RIV	APP	OSP	HOD	OLA	GIL	GVB	PAR
MNC		1.000	1.000	1.000	1.000	1.000	0.996	1.000	0.999	1.000	0.998	1.000	0.999	1.000
BTH	0.000		1.000	0.998	1.000	0.999	0.994	0.998	0.999	0.998	0.998	0.999	0.996	0.999
GTN	0.000	0.000		1.000	1.000	1.000	0.992	1.000	0.999	0.997	0.998	0.998	0.995	0.999
CHS	0.000	0.002	0.000		1.000	1.000	0.997	1.000	0.999	0.998	0.996	0.999	0.998	1.000
HIH	0.000	0.000	0.000	0.000		1.000	0.997	1.000	1.000	1.000	0.996	0.999	0.998	1.000
SAR	0.000	0.001	0.000	0.000	0.000		0.998	0.999	1.000	0.998	0.995	0.999	0.997	0.999
RIV	0.004	0.006	0.008	0.003	0.003	0.002		0.996	0.998	0.999	0.991	0.999	0.999	0.999
APP	0.000	0.002	0.000	0.000	0.000	0.001	0.004		0.999	1.000	0.997	0.998	1.000	1.000
OSP	0.001	0.001	0.001	0.001	0.000	0.000	0.002	0.001		0.998	0.998	1.000	0.999	1.000
HOD	0.000	0.002	0.003	0.002	0.002	0.002	0.001	0.000	0.002		0.997	1.000	1.000	1.000
OLA	0.002	0.002	0.002	0.004	0.004	0.005	0.009	0.003	0.002	0.003		0.999	0.998	0.990
GIL	0.000	0.001	0.002	0.001	0.001	0.001	0.001	0.002	0.000	0.000	0.001		0.999	1.000
GVB	0.001	0.004	0.005	0.002	0.002	0.003	0.001	0.000	0.001	0.000	0.002	0.001		1.000
PAR	0.000	0.001	0.001	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.001	0.000	0.000	

remaining 28 loci which were assumed to be monomorphic in all 14 samples were not included in the calculations of identity and distance.

A cluster analysis using the unweighted pair group method (UPGMA) on Nei's D values clustered all samples below the 0.0013 level (Fig. 3). The samples from the Atlantic tended to cluster together, followed by random clustering together with the samples from the Gulf of Mexico. However, the standard errors on the first and last nodes of the phenogram were 0.009 and 0.036, respectively, collapsing all of the nodes of the phenogram and suggesting that all 14 red drum samples are not strongly differentiated genetically.

Discussion

Two studies of nuclear gene variation using protein electrophoresis have been carried out on 29 geographic samples of *Sciaenops ocellatus*: 7 from nearshore localities along the Atlantic coast of the southeastern USA, 21 from nearshore localities throughout the northern Gulf of Mexico, and 1 from an offshore Gulf locality near Grand Isle, Louisiana, (Ramsey and Wakeman 1987, and present study).

The percent of polymorphic loci (99% criterion) reported in the two studies ranged from 15.0% (Ramsey and Wakeman 1987) to 21.4% (present study). Of the total of 54 presumptive loci adequately resolved in both studies, 10 were polymorphic by the 99% criteria, giving an overall estimate of 18.5% polymorphic loci. This estimate does not include loci for an aconitase protein, an α-glucosidase protein, a transferrin protein, and a glucose phosphate isomerase protein, all of which have been reported to be polymorphic (but not surveyed) in red drum (Ramsey and Wakeman 1987, and present study). Observed average heterozygosity values (averaged over all loci) in the two studies ranged from 0.029 (Ramsey and Wakeman 1987) to 0.047 (present study). The two values, however, are not significantly different (Student's t test $=0.66$, $P>0.05$). These estimates of genetic variability in red drum agree closely with those reported in other marine fishes (Winans 1980, Smith and Fujio 1982, Waples 1987), and were not unexpected since estimates of genetic variability are similar across a broad spectrum of vertebrate taxa (Nevo 1978).

That red drum have appreciable ("normal") levels of genetic variability is of interest for two reasons. First, the perceived decline in red drum abundance, at least in the Gulf of Mexico, has not affected the genetic variability base of the species. This assertion is based on the premise that historical levels of genetic variability in red drum were not significantly higher than those found at present. The absence of a reduction in genetic variability in red drum is not surprising given recent estimates (Nichols 1988) of adult red drum densities in the northern Gulf of Mexico. Nelson and Soulé (1987) cited studies which indicated that a minimum effective population size of 500 individuals would be needed for a population to maintain its historical level of genetic variability and to retain its long-term adaptive potential. Nichols' estimate of roughly 5×10^6 adult red drum in the northern Gulf of Mexico is several orders of magnitude greater than 500, suggesting that even if only 1% of adult Gulf red drum were reproductively active, there should be no detectable loss of genetic variability.

Secondly, coincident with (and largely because of) the decline in wild red drum abundance, red drum stocking programs have been initiated. In Texas, 6 to 8 million red drum eggs, fry, or fingerlings have been stocked annually since 1975 into various bays and estuaries (Dailey and Matlock 1987). Effective genetic monitoring (*sensu* Allendorf and Ryman 1987) of these stocking programs in terms of detecting potential erosion of the genetic variability base requires reasonable levels of variability such as now documented in red drum.

Both studies of red drum nuclear gene variation have detected significant heterogeneity in allele frequencies. Ramsey and Wakeman (1987) found significant heterogeneity among red drum from the Gulf at a locus for glucose phosphate isomerase. The maximum differences in allele frequencies at this locus were not large and occurred between adjacent sample localities in the northern Gulf. In our study, significant heterogeneity using the

G-test was found at a locus for acid phosphatase among samples from the Atlantic, at the locus for alcohol dehydrogenase between pooled samples from the Atlantic and the Gulf, and at the locus for adenosine deaminase among samples from the Gulf and between pooled samples from the Atlantic and the Gulf. Using the "*V*" test on arcsin square-root transformed allele frequencies, significant heterogeneity was found at the acid phosphatase locus among samples from the Atlantic and at the adenosine deaminase locus both among samples from the Gulf and for the pooled comparison of Atlantic vs Gulf samples.

The interpretation of the heterogeneity tests is problematic. Using the "*V*" test, significant heterogeneity ($0.05 < P < 0.01$) was detected at *Acp-2* 100 among samples from the Atlantic and at *Ada* 125 and *Ada* 110 among samples from the Gulf. Since 42 different "*V*" tests were initially performed, approximately two tests are expected to be significant by chance alone at an alpha level of 0.05. As noted previously, the heterogeneity at *Acp-2* may be due to non-random sampling at the Oregon Inlet and Pamlico River localities. Sample sizes at the two localities were small in comparison to other localities, and the largest observed differences in allele frequencies at *Acp-2* occurred between these two geographically contiguous localities. The evidence for heterogeneity at *Ada* 125 and *Ada* 110 is more compelling in that observed allele frequencies do appear to follow a geographic pattern; i.e., there are apparent differences in the frequencies of *Ada* 125 and *Ada* 110 between samples from central Florida versus most of those taken elsewhere from the Gulf. This suggested division of red drum samples from the Gulf is further supported by the highly significant heterogeneity ($P < 0.01$) detected at *Ada* 115: samples from the northwestern Gulf have higher frequencies of this allele compared to samples from the northeastern Gulf. However, both *Ada* 115 and *Ada* 110 are relatively rare alleles and the sampling distributions given by the null hypothesis do not necessarily approximate a chi-square distribution when alleles are rare (Chakraborty and Leimar 1987). Moreover, the separate tests carried out on allelic variants at *Ada* are not independent, since frequency differences at any one allele affect frequencies at alternative alleles. As a consequence, conclusions that significant genetic differentiation at *Ada* occurs among Gulf samples are tenuous. The highly significant ($P < 0.01$) heterogeneity detected between Atlantic and Gulf red drum at both *Ada* 115 and *Ada* 100, however, is real, particularly since *Ada* 100 is the most common allele at this locus.

We favor the conservative interpretation that the only unequivocal heterogeneity observed is that between red drum in the Atlantic and those in the Gulf. Patterns of allele-frequency heterogeneity among red drum samples within the Atlantic or within the Gulf are variable in terms of loci and/or localities and appear to stem in large part from differences in the frequencies of rare alleles (Ramsey and Wakeman 1987, and present study). Allele 115 at *Ada* may be an exception and indicate the existence of genetic differentiation within the Gulf between red drum from northwestern and northeastern Gulf localities.

The apparent heterogeneity in allele frequencies observed among the red drum samples would appear to be at odds with the estimates of population subdivision and gene flow as inferred from the *F*-statistics. Fixation index (F_{IS}) values in this study ranged from -0.118 at a locus for acid phosphatase to $+0.032$ for the alcohol dehydrogenase locus; the overall mean F_{IS} value was -0.022. The mean F_{IS} value reported by Ramsey and Wakeman was -0.030. In both studies, there was an excess of loci with negative F_{IS} values, suggesting a slight excess of heterozygotes over that expected in a randomly mating population. However, nearly all samples in both studies were in Hardy-Weinberg equilibrium for most or all loci, indicating the absence of subdivision *within* samples. F_{ST} values, averaged over all polymorphic loci, were estimated to be 0.019 in both studies. This estimate of gene flow is similar to those reported for other marine fishes inferred to have the potential for high gene flow (Gyllensten 1985, Waples 1987). Using the island model of Wright (1943), the F_{ST} value of 0.019 gives an estimate of the effective number of migrants ($N_e m$) per generation of approximately 13. Since, in theory, populations will not tend toward fixation of alternate alleles as a function of genetic drift if the effective number of migrants is greater than 1 (Spieth 1974), these estimates of F_{ST} and $N_e m$ indicate the absence of subdivision *among* the red drum samples. Additional evidence in this study for high gene flow and a lack of genetic differentiation among all red drum sampled were provided by the conditional average allele-frequency analysis (after Slatkin 1981) and the high levels of genetic similarity (after Nei 1978). As pointed out by Allendorf and Phelps (1981), however, the principle that populations will not diverge at selectively neutral genes provided there is one migrant individual per generation has been misinterpreted or inappropriately applied in several instances. Although one migrant individual per generation is sufficient to insure that the same alleles will be shared over time, it is not sufficient to maintain identical allele frequencies, particularly over short periods of time. Statistically significant allelic divergence can occur even if substantial gene exchange occurs, and a small value of *F* can be associated with a considerable amount of differentiation among subpopulations (Wright 1969). This suggests that the allele frequency heterogeneity observed in this study could be real, despite significant levels of gene flow.

The apparent heterogeneity in allele frequencies among red drum is curious given the several aspects of red drum biology and life history which should facilitate dispersal and minimize spatial differentiation. In other marine fishes with similar life histories or with similar capability for long-distance dispersal, genetic divergence, whether determined by protein electrophoresis of nuclear gene products or restriction enzyme analysis of mitochondrial genes, is usually minimal and nonsignificant, and most of the total gene diversity is allocated within localities (Winans 1980, Gyllensten 1985, Avise et al. 1987). It is possible that the observed heterogeneity in red drum is artifactual and an accident of sampling.

The observed genetic heterogeneity between Atlantic and Gulf red drum suggests that a biological or geo-

graphical barrier separates, or perhaps historically separated, red drum in the Atlantic from those in the Gulf. We emphasize the latter, since Larson et al. (1984) noted that genetic differentiation, particularly when coupled with evidence of present-day high gene flow, may reflect historical isolation (or differentiation) rather than present-day equilibrium conditions. Recognizable biogeographic provinces separate Atlantic from Gulf marine fauna. One occurs near Cape Canaveral, Florida, and appears to separate northern and southern populations of the horseshoe crab (Avise et al. 1987). However, at least three species of nearshore marine fishes occur along the Atlantic and Gulf coasts and exhibit little or no evidence of genetic discontinuity (Avise et al. 1987). Another possibility could be that the offshore currents utilized by red drum are not conducive to movement between the Atlantic and Gulf.

Regardless of the reason for the observed heterogeneity, the genetic data indicate that red drum from the Atlantic coast and the northern Gulf are weakly subdivided despite considerable levels of gene flow. Red drum within the Gulf may also be weakly subdivided

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