Abstract.— Red drum, *Sciaenops ocellatus*, from Mosquito Lagoon, east-central Florida, were examined for variation in products of nine polymorphic nuclear-gene (allozyme) loci and in mitochondrial (mt)DNA restriction sites. Genetic data from Mosquito Lagoon fish were compared to similar data from red drum sampled from the northeastern Gulf of Mexico (Gulf) and the Carolina coast of the southeastern United States. Significant heterogeneity among red drum from the three areas was found in the frequencies of inferred alleles at two to three allozyme loci and in the frequencies of six mtDNA haplotypes. Red drum from Mosquito Lagoon were as differentiated genetically from red drum in the northeastern Gulf and Carolina coast as the latter two were from each other. Genetic data are consistent with the hypothesis that red drum in Mosquito Lagoon are self-contained and at least partially isolated from red drum in other U.S. waters.

Manuscript accepted 17 August 1993
Fishery Bulletin: 92:58–66 (1994)

Genetic distinctness of red drum (*Sciaenops ocellatus*) from Mosquito Lagoon, east-central Florida*

John R. Gold
Department of Wildlife and Fisheries Science
Texas A&M University, College Station, Texas 77843

Linda R. Richardson
Department of Wildlife and Fisheries Science
Texas A&M University, College Station, Texas 77843

Over the past five years, our laboratory has carried out studies of spatial and temporal genetic variation among red drum (*Sciaenops ocellatus*) from the northern Gulf of Mexico (Gulf) and the Carolina coast of the southeastern United States (Bohlmeyer and Gold, 1991; Gold and Richardson, 1991; Gold et al., 1993, in press). Red drum currently support important recreational fisheries in both the northern Gulf and U.S. Atlantic (Matlock, 1984; Mercer, 1984), and both fisheries are now regulated to reduce growth and recruitment overfishing (Swingle et al., 1984[1]; Goodyear, 1989[2]). Collectively, our genetic data have indicated that red drum in U.S. waters are subdivided with weakly differentiated subpopulations in the northern Gulf and along the Carolina coast. No genetic heterogeneity has been found among red drum from different localities within either the northern Gulf or Carolina coast (Gold et al., 1993, in press). The genetic data are consistent with several aspects of red drum biology and life history that suggest red drum dispersal and gene flow among contiguous bays and estuaries could be extensive. These include 1) transport of eggs, larvae, or juveniles from spawning localities near the mouths of bays or estuaries to adjacent bays or estuaries by oceanic currents (Lyczkoski-Schultz et al., 1988[3]), 2) movement of sexually-mature adults from bay or estuarine juvenile nurseries into deeper, offshore waters prior to spawning (Matlock, 1984), and 3) formation of large, offshore schools that can migrate extensively (Overstreet, 1983; Matlock, 1984; Swingle et al., 1984[1]).

In this study, data on allozyme and mitochondrial (mt)DNA variation among red drum sampled from Mosquito Lagoon on the east coast of Florida are presented and compared to data from previous studies. The goal of the study was to

* Contribution No. 24 of the Center for Biosystematics and Biodiversity, Texas A&M University.

[1] Swingle, W., T. Leary, D. Davis, V. Blomo, W. Tatum, M. Murphy, R. Taylor, G. Adkins, T. McIlwain, and G. Matlock. 1984. Fishery profile of red drum. Gulf of Mexico Fish. Mngmt. Council and Gulf States Mar. Fish. Comm., Lincoln Cntr., Suite 331, 5401 West Kennedy Blvd., Tampa, FL.

[2] Goodyear, C. P. 1989. Status of red drum stocks of the Gulf of Mexico: report for 1989. Contrib. CRD 88/89–14, Southeast Fish. Cntr., Miami Lab., Coast. Res. Div., 75 Virginia Beach Drive, Miami, FL.

[3] Lyczkowski-Schultz, J., J. P. Steen Jr., and B. H. Comyns. 1988. Early life history of red drum (*Sciaenops ocellatus*) in the northcentral Gulf of Mexico. Mississippi-Alabama Sea Grant Consortium (Project No. R/LR-12). Gulf Coast Res. Lab., P.O. Box 7000, Ocean Springs, unpubl. ms.

test the hypothesis that red drum from Mosquito Lagoon and other U.S. waters are genetically homogeneous. Red drum in Mosquito Lagoon are of particular interest because they may represent a self-contained, at least partially isolated subpopulation. Evidence for the latter includes documentation within the system of both post-spawning females and red drum eggs (Murphy and Taylor, 1990; Johnson and Funicelli, 1991). In addition, physical access to the Atlantic from the lagoon is limited. In brief, Mosquito Lagoon (Fig. 1) is long and narrow (54 km × 4 km) and is separated from the Atlantic by a barrier beach. The lagoon represents the northern part of the Indian River lagoonal system and has two narrow outlets: one, Ponce de Leon Inlet, is a natural pass to the Atlantic located at the northern end of the lagoon; the other, Haulover Canal, is a man-made passageway at the southern end of the lagoon that leads into the Indian River. Access to or from the Atlantic through Ponce de Leon Inlet is restricted because of a series of islands and small passageways in the northern part of the lagoon. Access to or from the Atlantic through Haulover Canal (completed in 1929) would only be recent, and the nearest outlet to the Atlantic south from Haulover canal is roughly 90–100 km. We also were interested in studying red drum from Mosquito Lagoon because our earlier work (Gold et al., 1993, in press) did not include red drum from the east coast of Florida, an area of potential importance to tests of hypotheses regarding genetic subdivision between red drum from the northern Gulf and the U.S. Atlantic (Gold et al., in press). Finally, adult red drum from Mosquito Lagoon form a large part of the broodstock used by the Florida Department of Natural Resources (FDNR) to supplement and enhance the red drum fishery in Florida waters. The genetic composition of Mosquito Lagoon red drum is thus important to research in stocking hatchery-raised fish.

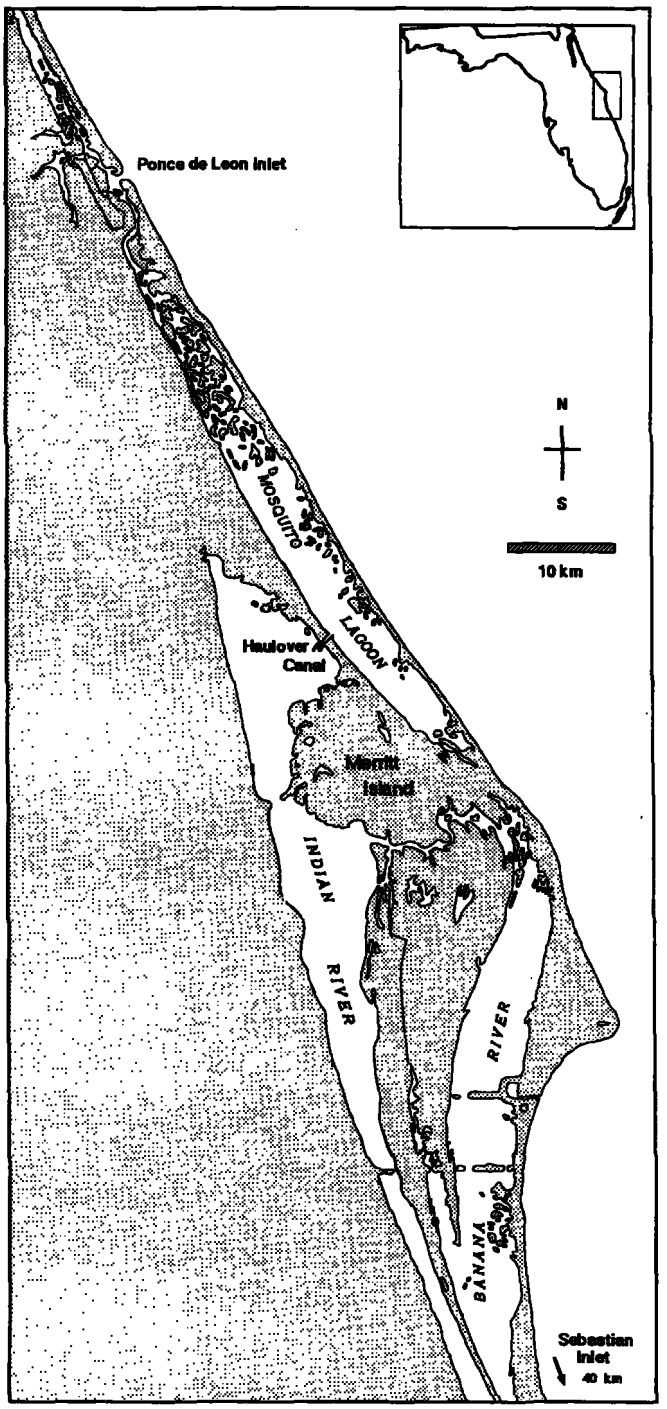

Figure 1
Mosquito Lagoon, east-central Florida, showing Ponce de Leon Inlet and Haulover Canal.

Materials and methods

Red drum were collected from Mosquito Lagoon during fall 1988, spring 1990, and spring 1991. Fish were captured with trammel nets. Tissues (heart, spleen, and muscle) were removed and placed in liquid nitrogen for transport to Texas A&M University where they were stored at –80°C. Ages of all but yearling (age zero) individuals (i.e., specimens less than 300 mm total length) were determined from annuli on otoliths by using methods described in Bumguardner (1991).

Individuals sampled in 1988 (41 total) were surveyed for variation at nine polymorphic allozyme

loci: *ACP-2** (acid phosphatase); *ADA** (adenosine deaminase); *ADH** (alcohol dehydrogenase); *sAAT-1** (aspartate aminotransferase); *EST-1** (esterase); *GPI-B** (glucose phosphate isomerase); and *PEPB**, *PEPD**, and *PEPS** (peptidases). Techniques for vertical starch gel electrophoresis, details of grinding and running buffers, starch composition of gels, protein staining, and interpretation of banding patterns may be found in Bohlmeyer (1989) and Bohlmeyer and Gold (1991). Designation of allelic variants was based on relative mobility to the most common allele (Allele **100*).

All individuals collected (109 total) were assayed for 104 mtDNA restriction sites with 13 restriction enzymes: *Bam*HI, *Bcl*I, *Eco*RV, *Hin*dIII, *Nco*I, *Nsi*I, *Pst*I, *Pvu*II, *Sca*I, *Spe*I, *Stu*I, *Xba*I, and *Xmn*I. Methods used to assay mtDNAs of individual fish may be found in Gold and Richardson (1991). Homology of fragments from single digestions was tested by multiple, side-by-side comparisons. Variant patterns exhibiting only a single band of greater than 15 kb were tested for homology by using double digestions with *Bam*HI as described in Gold and Richardson (1991).

Red drum from Mosquito Lagoon were initially subdivided into year classes and tested for heterogeneity in both allozyme and mtDNA haplotype frequencies. Year classes (number of individuals) were 1985 (17), 1986 (25), 1987 (11), 1988 (7), and 1989 (49). No significant heterogeneity ($P>0.05$) in allozyme or mtDNA haplotype frequencies was found among year classes. Subsequent data analyses employed three test groups: 1) red drum from Mosquito Lagoon; 2) red drum from the northeastern Gulf; and 3) red drum from the Carolina coast. Data for the latter two were taken from Gold et al. (1993, 1994) and represent red drum from the following localities: northeastern Gulf — Apalachicola Bay, Riviera Bay, and Sarasota Bay (west coast of Florida); and Carolina coast — Calibogue Sound, Charleston Bay, and North Inlet (South Carolina), and the Pamlico River and Oregon Inlet (North Carolina). A map showing these localities may be found in Bohlmeyer and Gold (1991). A summary of allele frequencies at the nine polymorphic allozyme loci and the distribution of mtDNA haplotypes in each test group are given in Appendix Tables 1 and 2, respectively.

For allozyme data, tests of Hardy-Weinberg equilibrium expectations and generation of Nei's (1978) unbiased genetic distance were accomplished by using BIOSYS-1 (Swofford and Selander, 1981). Deviations from Hardy-Weinberg expectations were tested by using pooled genotypes and the chi-square statistic with one degree of freedom. Significance testing of allele-frequency differences among test groups was accomplished by using 1) the *G*-statistic (Sokal and Rohlf, 1969) on contingency tables of allele counts and the BIOM-PC program (Rohlf, 1983), and 2) the *V*-statistic (DeSalle et al., 1987) on arcsin, square-root transformed allele frequencies. For mtDNA data, significance testing of mtDNA-haplotype frequency differences was carried out by using the *G*- and *V*-statistics as described above and a Monte Carlo randomization procedure (Roff and Bentzen, 1989). Nucleon diversities and intra- and inter-populational nucleotide sequence diversities were estimated by using equations in Nei and Tajima (1981). Analysis of mtDNA data was facilitated by the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992). Significance levels for multiple tests performed simultaneously were adjusted after Cooper (1968).

Results

No significant deviations from Hardy Weinberg equilibrium expectations at any of the nine polymorphic allozyme loci were found following corrections for multiple tests. Two significant deviations were found in uncorrected tests: at *GPI-B** ($P=0.015$) and *PEPS** ($P=0.012$) in the northeastern Gulf. Both deviations appeared to be due to rare homozygotes for low frequency alleles. One new allele (Allele **110* at *EST-1**) was found among Mosquito Lagoon fish at a frequency of 1.2 percent (Appendix Table 1).

Estimates of allozyme variation (Table 1) indicate that red drum from Mosquito Lagoon have fewer

Table 1

Allozyme variation in red drum (*Sciaenops ocellatus*).

Test group	Mean sample size/locus	Mean number of alleles/locus (± SE)	Mean heterozygosity/locus[1] (±SE)
Northeastern Gulf of Mexico	246	3.9 ± 0.9	0.225 ± 0.076
Mosquito Lagoon, Florida	41	2.9 ± 0.6	0.206 ± 0.081
U.S. Carolina Coast	176	3.9 ± 0.9	0.213 ± 0.074

[1] Direct-count estimate.

alleles per locus or lower estimates of mean heterozygosity, or both, than do red drum from the northeastern Gulf and Carolina coast. The differences in genetic variation, however, are non-random across loci. Heterozygosity per locus values among Mosquito Lagoon fish at loci (e.g., *ACP-2**, *ADA**, *ADH**, *sAAT-1**, and *EST-1**) where alternate alleles occurred at frequencies of five percent or greater were equivalent to values among fish from the northeastern Gulf and Carolina coast (data not shown). Differences in heterozygosity per locus values were observed at loci (e.g., *GPI-B**, *PEPB**, and *PEPD**) where alleles occurring in a frequency of one to three percent in northeastern Gulf or Carolina coast fish, or both, were not found among Mosquito Lagoon fish (Appendix Table 1).

Significant heterogeneity ($P<0.05$) in allele frequencies among test groups was found by using the *G*-test at *ADA** ($G=33.92$, df=22, $P\approx0.004$) and *sAAT-1** ($G=13.59$, df=6, $P\approx0.036$). Additional *G*-tests were carried out after pooling alleles whose frequency in any sample was less than 10%. Significant heterogeneity was again found at *ADA** ($G=9.62$, df=4, $P\approx0.048$) and also at *PEPB** ($G=6.86$, df=2, $P\approx0.034$). Examination of allele frequencies at *ADA**, *sAAT-1*, and *PEPB** did not reveal any striking differences among test groups, suggesting that heterogeneity was due to accumulation of small differences in frequencies of rare alleles. At *ADA**, for example, the frequency of Allele **115* was higher among Mosquito Lagoon fish and lower among Carolina coast fish; whereas the frequencies of Alleles **90* and **85* were higher among northeastern Gulf fish (Appendix Table 1). At *sAAT-1** and *PEPB**, slight frequency differences were apparent for Allele **110* (higher in Mosquito Lagoon fish) and Allele **115* (higher in northeastern Gulf fish and absent from Mosquito Lagoon fish), respectively (Appendix Table 1). The observation that *G*-test heterogeneity was due to small, cumulative frequency differences was corroborated by *V*-tests where no significant heterogeneity ($P>0.05$) in allele frequencies was found at any locus following corrections for multiple tests.

MtDNA fragment patterns from single digestions with 13 restriction enzymes generated 36 composite mtDNA haplotypes among fish from Mosquito Lagoon, eleven of which (numbers 114, 134–143) have been found only in Mosquito Lagoon red drum (Appendix Table 2). Estimates of mtDNA variation (Table 2) indicated that nucleon diversity (the probability of any two individuals differing in mtDNA haplotype) was highest in red drum from the northeastern Gulf and lowest in red drum from the Carolina coast; whereas intrapopulational nucleotide sequence diversity (the genetic difference between any two individuals) was greatest among Mosquito Lagoon fish. These estimates of mtDNA variation are among the highest reported to date for a non-clupeid, marine fish species (Richardson and Gold, 1993).

Highly significant heterogeneity in mtDNA-haplotype frequencies among test groups and between pairwise comparisons of test groups were found in both *G*-tests and Monte Carlo bootstrapping (Table 3). These results indicate that all three test groups differ significantly from each other. *V*-tests, carried out on haplotypes found in ten or more individuals (12 haplotypes total), identified six haplotypes (Table 4) that differed significantly among test groups. Genetic distances based on allozymes and mtDNAs (Table 5) indicate that red drum from Mosquito Lagoon are at least as divergent genetically from red drum in the northeastern Gulf and Carolina coast as the latter two are from each other.

Discussion

Tests of heterogeneity clearly indicate that red drum from Mosquito Lagoon differ genetically from red drum in the northeastern Gulf and along the Carolina coast and that at least three subpopulations of red drum occur in U.S. waters. That the genetic differences appear more pronounced in mtDNA than

Table 2

MtDNA variation in red drum (*Sciaenops ocellatus*).

Test group	Number of individuals	Number of haplotypes	Nucleon diversity	Nucleotide sequence diversity (± SD)[1]
Northeastern Gulf of Mexico	247	49	0.947	0.557 ± 0.298
Mosquito Lagoon, Florida	109	36	0.912	0.597 ± 0.321
U.S. Carolina Coast	174	43	0.904	0.560 ± 0.351

[1] Values are in percent. Standard deviations are used instead of standard errors because of the large number of pairwise comparisons used to generate mean values.

Table 3

Results of tests for heterogeneity in mtDNA haplotype frequencies among red drum (*Sciaenops ocellatus*) from the northeastern Gulf of Mexico, Mosquito Lagoon, Florida, and the U.S. Carolina coast.

Test group	Results of *G*-tests		*P*-value from Monte Carlo randomizations
	G-score	*P*-value	
Northeastern Gulf vs. Mosquito Lagoon vs. Carolina Coast	159.5	<0.001[1]	<0.001
Northeastern Gulf vs. Mosquito Lagoon	73.9	<0.001[2]	<0.001
Northeastern Gulf vs. Carolina Coast	76.2	<0.001[3]	<0.001
Mosquito Lagoon vs. Carolina Coast	66.2	<0.001[4]	0.006

Degrees of freedom in *G*-tests: 48[1], 18[2], 19[3], and 27[4].

Table 4

Frequency[1] of six significantly heterogeneous mtDNA haplotypes of red drum (*Sciaenops ocellatus*) in the northeastern Gulf of Mexico, Mosquito Lagoon, Florida, and the U.S. Carolina coast.

Haplotype	Northeastern Gulf (n=247)	Mosquito Lagoon (n=109)	Carolina Coast (n=174)	Probability value from *V*-test[2]
8	13.3	23.8	10.3	≈0.010
9	7.7	13.8	26.4	<0.001
11	9.3	1.8	7.5	≈0.019
12	0.0	7.3	3.4	<0.001
21	4.4	0.0	0.6	≈0.004
29	4.0	0.0	1.7	≈0.021

[1] Values are in percent.
[2] After DeSalle et al. (1987).

in (presumed) nuclear-coding genes is not surprising, given that mtDNA is expected to be at least four times more sensitive to population substructuring (Birky et al., 1983; Templeton, 1987). Because previous studies (Gold et al., 1993, in press) found no evidence of genetic heterogeneity among red drum from eleven estuaries or bays in the northern Gulf or among red drum from five estuaries or bays along the Carolina coast, red drum from Mosquito Lagoon are unusual in representing a genetically distinct red drum subpopulation existing within a single bay or estuary.

Campton (1992)[4] examined red drum from Mosquito Lagoon for allelic variation at several allozyme loci and found genetic homogeneity among red drum from Mosquito Lagoon, the northern Gulf, and the Carolina coast. He suggested that our initial study (Bohlmeyer and Gold, 1991) of allozyme variation among northern Gulf and Carolina coast red drum did not account for temporal variation among samples within localities. Our subsequent studies (and this one), however, have included temporal sampling of variation in *both* allozymes and mtDNA and have demonstrated that weak (but significant) genetic heterogeneity exists (Gold et al., 1993, in press). Sampling error associated with specimen procurement in varying time and space may account for the different results obtained in Campton's (1992)[4] study and this one. However, in Campton's (1992)[4] study, the total *G*-statistic, obtained by summing individual *G*-values and their associated degrees of freedom, was significant at the 0.01 level. This suggests the existence of spatial or temporal genetic heterogeneity, or both, among the localities sampled.

Genetic differentiation of red drum in Mosquito Lagoon is consistent with the hypothesis that red drum in Mosquito Lagoon represent a self-contained, at least partially isolated subpopulation. Three lines of evidence support this hypothesis. First, genetic differences between red drum from Mosquito Lagoon and red drum sampled elsewhere involve frequencies of alleles at two or three putative nuclear-gene loci and frequencies of at least six mtDNA haplotypes. Differentiation of several, presumably independent and selectively-neutral, genetic markers suggests a genome-wide effect related to at least partial isolation and reduced gene flow (Wright, 1978; Hartl and Clark, 1989). Second, inferred nuclear-gene alleles present in low frequency in red drum sampled outside of Mosquito

[4] Campton, D. E. 1992. Gene flow estimation and population structure of red drum (*Sciaenops ocellatus*) in Florida. Final Rep. Coop. Agrmt. No. 14-16-009-1522, U.S. Fish & Wildl. Serv., Natl. Fish. Res. Cntr., 7920 N.W. 71st St., Gainesville, FL.

Table 5

Matrix of Nei's (1978) unbiased genetic distance based on allozymes (upper diagonal) and Nei and Tajima's (1981) corrected interpopulational nucleotide sequence divergence based on mtDNAs (lower diagonal) among red drum (*Sciaenops ocellatus*) from the northeastern Gulf of Mexico, Mosquito Lagoon, Florida, and the U.S. Carolina coast. Interpopulational nucleotide sequence divergence values are in percent.

	Northeastern Gulf	Mosquito Lagoon	Carolina Coast
Northeastern Gulf	—	0.000	0.001
Mosquito Lagoon	0.006	—	0.002
Carolina Coast	0.006	0.009	—

Lagoon were not found in red drum from Mosquito Lagoon; whereas one inferred allele and eleven mtDNA haplotypes were unique to red drum from Mosquito Lagoon. The distribution of low frequency nuclear-gene alleles and mtDNA haplotypes is consistent with reduced gene flow concomitant with allele-frequency drift expected in isolated subpopulations. Finally, both females with ovaries containing postovulatory follicles and spawned red drum eggs have been documented in Mosquito Lagoon (Murphy and Taylor, 1990; Johnson and Funicelli, 1991), clearly indicating that red drum spawn within the system.

Assuming red drum in Mosquito Lagoon represent a partially isolated, self-contained subpopulation, one question of interest is how long the subpopulation has been semi-isolated. Geological evidence (Mehta and Brooks, 1973, cited from Johnson and Funicelli, 1991) indicates that several tidal inlets once connected Mosquito Lagoon to the Atlantic, the last of which is estimated to have closed about 1,500 years ago. Assuming some variation in the geological estimate, this date does not differ substantially from an estimate of 2,900 ± 1,550 (SD) years based on 1) a corrected interpopulational nucleotide sequence divergence (between red drum in Mosquito Lagoon and red drum elsewhere) of 0.0058 ± 0.0031 (SD) percent, and 2) an evolutionary rate for vertebrate mtDNA of 0.01 substitutions/bp/lineage/Myr (Brown et al., 1979; Wilson et al., 1985). Given ongoing debates about molecular clocks, the correspondence between the two temporal estimates is noteworthy.

Because the genetic distinctness of Mosquito Lagoon red drum appears to stem largely from physical isolation, the biological reasons for subdivision between red drum in the northern Gulf and those along the Carolina coast remain unknown. Possible reasons for this subdivision could include 1) current patterns between the Gulf and U.S. Atlantic, 2) absence of suitable near-shore habitats along the southeastern coast of Florida, or 3) differences in biogeographic provinces (Gold et al., 1993, in press). Similar genetic discontinuities between U.S. Atlantic and Gulf coast fauna have been described by Avise and co-workers (reviewed in Avise, 1992). Their hypothesis is that the concordant phylogeographic patterns provide evidence of similar vicariant histories that are tentatively related to episodic changes in environmental conditions during the Pleistocene (Avise, 1992). The relative inaccessibility of Mosquito Lagoon suggests that sampling red drum from north or south of Mosquito Lagoon may be more informative for testing hypotheses regarding phylogeographic subdivision between the northern Gulf and the U.S. Atlantic.

A last point to consider is the use of Mosquito Lagoon red drum as broodstock for stock enhancement programs. It could be argued that red drum from Mosquito Lagoon differ genetically from red drum sampled elsewhere (e.g., the northeastern Gulf) and should be used only for stock enhancement at localities where no genetic differences exist. Alternatively, it could be argued that the genetic distinctiveness of red drum in Mosquito Lagoon is relatively small and possibly inconsequential. This follows from the observation that the documented genetic difference between red drum in Mosquito Lagoon and red drum sampled elsewhere is considerably less than that, on average, among races of man (Cann et al., 1987). One other consideration might be to cross red drum from Mosquito Lagoon with red drum from elsewhere (e.g., the northeastern Gulf) in order to increase performance from potential heterotic effects.

Acknowledgments

Assistance in procuring red drum specimens from Mosquito Lagoon was provided by J. Burch, J. Camper, B. Denis, C. Furman, M. Murphy, G. Ramos, and D. Roberts. Their assistance is gratefully acknowledged. Special thanks are extended to C. Amemiya and D. Roberts for providing no-cost lodging during field trips. We also thank B. Colura and B. Bumguardner for carrying out age determinations from otoliths, D. Bohlmeyer and C. Furman for assistance in the laboratory, R. Taylor for providing historical information on the construction of Haulover Canal, and M. Murphy for providing criti-

cal comments on a draft of the manuscript. Work was supported by the Texas A&M University Sea Grant College Program (grants NA85AA-D-SG128 and NA89AA-D-SG139), by the MARFIN Program of the U.S. Department of Commerce (grants NA89–WC-H-MF025 and NA90AA-H-MF107), and by the Texas Agricultural Experiment Station (Project H-6703). This paper represents number XI in the series "Genetic Studies in Marine Fishes."

Literature cited

Avise, J. C.
1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63:62–76.

Birky Jr., C. W., T. Maruyama, and P. Fuerst.
1983. Mitochondrial DNAs and phylogenetic relationships. *In* S. K. Dutta (ed.), DNA systematics, p. 107–137. CRC Press, Boca Raton, FL.

Bohlmeyer, D. A.
1989. A protein electrophoretic analysis of population structure in the red drum (*Sciaenops ocellatus*). M.S. thesis, Texas A&M University, College Station, TX.

Bohlmeyer, D. A., and J. R. Gold.
1991. Genetic studies in marine fishes. II: A protein electrophoretic analysis of population structure in the red drum *Sciaenops ocellatus*. Mar. Biol. 108:197–206.

Brown, W. M., M. George Jr., and A. C. Wilson.
1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Academy Sci. (USA) 76:1967–1971.

Bumguardner, B. W.
1991. Marking subadult red drums with oxytetracycline. Trans. Am. Fish. Soc. 120:537–540.

Cann, R. L., M. Stoneking, and A. C. Wilson.
1987. Mitochondrial DNA and human evolution. Nature 325:31–36.

Cooper, D. W.
1968. The significance level in multiple tests made simultaneously. Heredity 23:614–617.

DeSalle, R., A. Templeton, I. Mori, S. Pletscher, and J. S. Johnston.
1987. Temporal and spatial heterogeneity of mtDNA polymorphisms in natural populations of *Drosophila mercatorum*. Genetics 116:215–233.

Gold, J. R., and L. R. Richardson.
1991. Genetic studies in marine fishes. IV: An analysis of population structure in the red drum (*Sciaenops ocellatus*) using mitochondrial DNA. Fish. Res. 12:213–241.

Gold, J. R., L. R. Richardson, C. Furman, and T. L. King.
1993. Mitochondrial DNA differentiation and population structure in red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. Mar. Biol. (In press.)

Gold, J. R., T. L. King, L. R. Richardson, D. A. Bohlmeyer, and G. C. Matlock.
In press. Genetic studies in marine fishes. VII: Allozyme differentiation within and between red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. J. Fish Biol. 116:175–185.

Hartl, D. L., and A. G. Clark.
1989. Principles of population genetics, 2nd ed. Sinauer Assoc., Inc., Sunderland, MA.

Johnson, D. R., and N. A. Funicelli.
1991. Spawning of the red drum in Mosquito Lagoon, east-central Florida. Estuaries 14:74–79.

Matlock, G. C.
1984. A basis for the development of a management plan for red drum in Texas. Ph.D. diss., Texas A&M University, College Station, TX.

McElroy, D., P. Moran, E. Bermingham, and I. Kornfield.
1992. REAP-The Restriction Enzyme Analysis Package. J. Hered. 83:157–158.

Mehta, A. J., and H. K. Brooks.
1973. Mosquito Lagoon barrier beach study. Shore and Beach 41:27–34.

Mercer, L.
1984. A biological and fisheries profile of red drum, *Sciaenops ocellatus*. Spec. Sci. Rep. 41, North Carolina Dep. Nat. Resour. Community Dev., Div. Mar. Fish., Raleigh, NC.

Murphy, M. D., and R. G. Taylor.
1990. Reproduction, growth, and mortality of red drum, *Sciaenops ocellatus*, in Florida. Fish. Bull. 88:531–542.

Nei, M.
1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Nei, M., and F. Tajima.
1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145–163.

Overstreet, R. M.
1983. Aspects of the biology of the red drum, *Sciaenops ocellatus*, in Mississippi. Gulf Res. Rep. (Suppl.) 1:45–68

Richardson, L. R., and J. R. Gold.
1993. Mitochondrial DNA variation in red grouper (*Epinephelus morio*) and greater amberjack (*Seriola dumerili*) from the Gulf of Mexico. ICES J. Mar. Sci. 50:53–62.

Roff, D. A., and P. Bentzen.
1989. The statistical analysis of mitochondrial polymorphisms: chi-square and the problem of small samples. Mol. Biol. Evol. 6:539–545.

Rohlf, F. J.
1983. BIOM-PC: a package of statistical programs to accompany the text BIOMETRY. W. H. Freeman & Co., San Francisco, CA.

Sokal, R. R., and F. J. Rohlf.
1969. Biometry. The principles and practice of sta-

tistics in biological research. W. H. Freeman & Co., San Francisco, CA.

Swofford, D. L., and R. B. Selander.
1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281–283.

Templeton, A. R.
1987. Genetic systems and evolutionary rates. *In* K. F. S. Campbell and M. F. Day (eds.), Rates of evolution, p. 218–234. Australian Acad. Sci., Canberra.

Wilson, A. C., R. L. Cann, S. M. Carr, M. George Jr., U. B. Gyllensten, K. M. Helm-Bychowski, R. G. Higuchi, S. R. Palumbi, E. M. Prager, R. D. Sage, and M. Stoneking.
1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linnaean Soc. 26:375–400.

Wright, S.
1978. Evolution and the genetics of populations. Univ. Chicago Press, Chicago, IL.

Appendix Table 1

Allele frequencies at nine polymorphic loci among red drum (*Sciaenops ocellatus*) from the northeastern Gulf of Mexico, Mosquito Lagoon, Florida, and the U.S. Carolina coast.

Locus allele	Northeastern Gulf of Mexico[1]	Mosquito Lagoon, Florida	U.S. Carolina coast[1]
*ACP-2**			
**125*	0.002	0.012	0.000
**115*	0.087	0.073	0.063
**100*	0.911	0.915	0.937
(*n*)	(246)	(41)	(175)
*ADA**			
**150*	0.000	0.012	0.003
**130*	0.036	0.024	0.028
**125*	0.315	0.354	0.372
**118*	0.006	0.000	0.003
**115*	0.081	0.122	0.028
**113*	0.002	0.000	0.003
**110*	0.061	0.012	0.060
**100*	0.443	0.452	0.469
**90*	0.010	0.000	0.003
**85*	0.024	0.000	0.003
**78*	0.000	0.000	0.000
**75*	0.018	0.024	0.028
**65*	0.004	0.000	0.000
(*n*)	(247)	(41)	(176)
*ADH**			
**–100*	0.508	0.451	0.566
**–75*	0.458	0.525	0.391
**–50*	0.028	0.012	0.020
**–20*	0.006	0.012	0.023
(*n*)	(246)	(41)	(175)
*sAAT-1**			
**120*	0.000	0.012	0.017
**110*	0.134	0.171	0.120
**100*	0.856	0.817	0.854
**90*	0.010	0.000	0.009
(*n*)	(242)	(41)	(175)

Locus allele	Northeastern Gulf of Mexico[1]	Mosquito Lagoon, Florida	U.S. Carolina coast[1]
*EST-1**			
**110*	0.000	0.012	0.000
**100*	0.911	0.915	0.898
**95*	0.089	0.073	0.102
(*n*)	(246)	(41)	(176)
*GPI-B**			
**–110*	0.004	0.000	0.003
**–100*	0.976	1.000	0.971
**–50*	0.020	0.000	0.026
(*n*)	(247)	(41)	(176)
*PEPB**			
**115*	0.022	0.000	0.006
**100*	0.974	1.000	0.991
**85*	0.004	0.000	0.003
(*n*)	(247)	(41)	(176)
*PEPD**			
**115*	0.002	0.012	0.009
**100*	0.968	0.988	0.968
**85*	0.030	0.000	0.020
**75*	0.000	0.000	0.003
(*n*)	(247)	(41)	(176)
*PEPS**			
**105*	0.040	0.024	0.023
**100*	0.958	0.976	0.977
**95*	0.002	0.000	0.000
(*n*)	(247)	(41)	(176)

[1] Data are from Gold et al. (in press).

Appendix Table 2

Distribution of mtDNA haplotypes among red drum (*Sciaenops ocellatus*) from the northeastern Gulf of Mexico, Mosquito Lagoon, Florida, and the U.S. Carolina coast.

Haplotype	Composite mtDNA digestion pattern[1]	Northeastern Gulf of Mexico[2]	Mosquito Lagoon, Florida	U.S. Carolina coast[2]
1	ABAAAAAAAAAAA	19	4	10
2	ABCCAAAAAAAAA	10	6	3
3	ABBACAAAAAAAA	11	1	10
4	EAAAAABAAAAAA	1	—	—
5	BAAAACBAAAAAA	1	—	—
6	CBAAAAAAAAAAA	2	1	1
7	AAABAAAAAAAAA	7	1	—
8	AAAAAABAAAAAA	33	26	18
9	BAAAAAAAAAAAA	19	15	46
10	BBAAAAAAAAAAA	9	2	4
11	AAAAAAAAAAAAA	23	2	13
12	CBAAAABAAAAAA	—	8	6
13	ABCAAACAAAAAA	1	—	4
14	BBFAAAAAAABAB	—	—	4
15	AAAAAABACAAAA	—	1	2
16	ACAAAAAAAAAAA	6	2	4
18	ABAACAAAAAAAA	5	2	1
19	BBAAADAAAAAAA	—	2	5
20	ABBAAAAAAACAA	—	3	2
21	BABAAAAAAAAAA	11	—	1
22	BAAAAABAAAAAA	4	1	2
23	AAAABAAAAAAAA	17	6	8
24	AAAAAAAAAAAAC	5	2	1
25	ADCCAAAAAAAAA	2	—	1
26	BABABAAAAAAAA	3	1	1
27	AACCAAAAAAAAA	—	5	1
28	ABAADAAAAAAAA	—	2	2
29	AAAAABABAAAAA	10	—	3
31	DBCAAAAAAAAAA	1	—	—
35	ABBAAAAAAAAAA	—	2	4
36	ABADAAAAAAAAA	1	—	1
45	BABAAABAAAAAA	2	—	—
46	ABEAAAAAAAAAA	1	1	—
47	BBAAAFAAEAAAA	2	—	—
48	AAEAAAAAAAAAA	1	—	—
49	CBBAAAAAAAAAA	3	—	—
50	BBHAAAAAAABAB	—	—	1
51	ABCAAAAAAAAAA	—	1	1
52	BBAAAAACAABAB	—	—	1
53	ABBAAAAAAAFAA	2	—	1
54	BAAEAAAAAAAAA	—	—	1
55	AHCCAAAAAAAAA	—	—	1
56	AGAAAAAAAAAAA	—	—	1
57	AAAAAABAAAEAA	—	—	1
58	BBAAAFAAAAAAA	3	—	—
60	FBBAAAAAAACAA	—	—	1
61	AAAAAAAADAAAA	—	—	1
62	BBBAAAAAAAAAA	—	—	1
64	AAAEAABAAAAAA	5	—	—
66	BBADAAAAAAAAA	—	—	1
68	BBAEAAAAAAAAA	1	—	—
69	AFAAAABAAAAAA	4	—	—
70	ACAAAAAACAAAA	1	—	—
76	BAAAAAAAABAAA	2	—	—
77	ABAAAGFAAAAAA	1	—	—
82	ABAAAAFAAAAAA	4	—	—
89	BIAAAAAAAAAAA	—	—	1
90	BAAAAAGAEAAAA	—	1	1
91	AAAAAAABAAAAA	—	—	1
92	ABBAFAAAAACAA	—	—	1
93	AAAFGAAAEAAAA	1	—	—
94	AAAAAABAAAADA	1	—	—
95	BAAAAHAAAAAAC	2	—	—
96	BCAAAAAAAAAAA	1	—	—
97	HBAAAAAAAAAAA	1	—	—
98	BAAABAAAAAAAA	1	—	—
99	BBBAAAAAAAFAA	1	—	—
100	AAIAAABAAAAAA	1	—	—
101	ABCCAAFAAAAAA	1	—	—
106	AAAAIAAAAAAAC	1	—	—
107	BAAAABABAAAAA	2	—	—
114	ACBAAAAAAAAAA	—	1	—
121	ABADAAAADAAAA	1	—	—
134	AAAAGABAAAAAA	—	1	—
135	BBJAADAAAAAAA	—	1	—
136	BBADAAABAAAAA	—	1	—
137	BBAAAAACAABAA	—	1	—
138	BBAAAAAAAABAB	—	1	—
139	AAACAAAAAAAAA	—	1	—
140	ABACAAAAAAAAA	—	1	—
141	AACABAAAAAAAA	—	1	—
142	AACAAABAAAAAA	—	1	—
143	ABAAGAAAAAAAA	—	1	—

[1] Letters (from left to right) are digestion patterns for: *Nco*I, *Bcl*I, *Sca*I, *Pvu*II, *Spe*I, *Xba*I, *Xmn*I, *Hin*dIII, *Stu*I, *Bam*HI, *Eco*RV, *Pst*I, and *Nsi*I. Details regarding fragment sizes of individual digestion patterns are available upon request.

[2] Data are from Gold et al. (1993).