A RESTRICTION ENZYME MAP OF THE MITOCHONDRIAL DNA OF RED DRUM, *Sciaenops ocellatus* (Osteichthys: Sciaenidae)

Over the last four years, we have been studying genetic variation and its geographic distribution in the red drum or redfish, *Sciaenops ocellatus*. The primary purpose of the study was to determine if separate stocks or breeding assemblages of red drum occur in the northern Gulf of Mexico or along the Atlantic coast of the southeastern United States. The primary genetic assay has been analysis of restriction fragment length polymorphisms (RFLPs) of red drum mitochondrial (mt) DNA (Gold and Richardson 1990, 1991,*unpubl.*).

A second purpose of the study was to uncover genetic markers that would be useful as tags in red drum stock enhancement or aquaculture programs. In Texas and other southeastern Gulf and Atlantic Coast 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 the stock enhancement programs will be genetic tags that can be used to discriminate among wild and hatchery-raised individuals.

To date, our studies of both red drum mtDNA and nuclear genes (the latter resolved using protein electrophoresis) have shown that red drum are weakly subdivided, with semi-isolated subpopulations or stocks occurring along the southeastern Atlantic Coast and in the northern Gulf of Mexico (Bohlmeyer and Gold 1991, Gold and Richardson 1991,*unpubl.*). MtDNA, in particular, has been found to vary considerably in red drum. One hundred and twenty-nine (129) different mtDNA genotypes or haplotypes have now been documented among >1,000 red drum surveyed (Gold and Richardson 1991, unpubl.). Of the 129 different mtDNA haplotypes, eleven were found in more than 30 individuals, seven were found in 11–30 individuals, 23 were found in 4–10 individuals, 19 were found in 2-3 individuals, and 69 were unique to individual fish. The high incidence of rare mtDNA genotypes suggests that mtDNA markers could prove highly effective as genetic tags in stocking programs. Reasons why mtDNA markers might be preferable to nuclear gene markers were given in Gold and Richardson (1991).

In this paper, we provide a restriction enzyme site map of red drum mtDNA. The map contains 123 different sites for 18 different, type II restriction endonucleases. Ninety-three (93) of the sites have thus far been found to be variable or "polymorphic" in red drum (Gold and Richardson 1991, *unpubl.*).

MATERIALS AND METHODS

The mtDNA restriction enzyme site map was generated using single and double digestions after Brown and Vinograd (1974). The 18 type II restriction endonucleases used were: *Bam*HI, *Bcl*I, *Bgl*II, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Nco* I, *Nsi*I, *Pst*I, *Pvu*II, *Sac*II, *Sca*I, *Spe*I, *Sph*I, *Stu*I, *Xba*I, and *Xmn*I. All digestions employed 1.0–1.5 μg of genomic DNA in 40 μl reactions following manufacturer's specifications. Procedures for isolating genomic DNA, separation of DNA fragments via agarose gels, Southern transfer to nylon membranes, hybridization to ^{32}P[dCTP] labelled mtDNA probes, and conditions for autoradiography may be found in Gold and Richardson (1991). A representative autoradiogram is shown in Figure 1. The probes used were a 9.00–9.25 kilobase (kb) fragment of the red drum mtDNA molecule inserted into a pTZ–18R plasmid (Gold *et al.* 1988) and an entire mtDNA molecule from the cyprinid fish *Cyprinella lutrensis* inserted into the bacteriophage

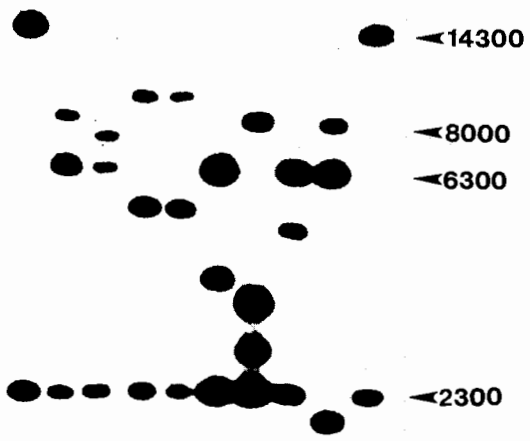

Figure 1. Autoradiogram of single digestions of red drum mtDNA with *Sca*I. Sizes are in base pairs.

lambda using EMBL arms. The latter was used primarily to map regions of the red drum mtDNA molecule not covered by the red drum probe. After autoradiography, mtDNA fragments were sized by fitting migration distances to a least-squares regression line of lambda DNA-*Hin*dIII fragment migration distances. Procedures for amplification of a fragment of the 16S ribosomal RNA (rRNA) gene of the red drum mtDNA using polymerase chain reaction (PCR) followed the methods of Kocher *et al.* (1989).

RESULTS AND DISCUSSION

The map locations for 123 type II restriction endonuclease enzyme sites in red drum mtDNA are given in Table 1 and are based on an estimated genome size of red drum mtDNA of 16.75–16.80 kilobase (kb) pairs (Gold *et al.* 1988). Sites within 0.25 kb were placed at the same location, in large part because standard errors for mtDNA fragment sizes are typically in that range (Avise *et al.* 1984). Two restriction sites for *Spe*I were found to occur within 0.15 kb of one another and were mapped to position 15.75. The site closer to the *Bam*HI site at map position 11.75 is "polymorphic"; whereas the second site is fixed. Two "polymorphic" sites for *Sca*I were found to occur within 0.10 kb of one another and were mapped to position 8.00. The site closer to the *Bam*HI site at map position 11.75 has been found only in one red drum individual surveyed; whereas the second site has been found in all but one individual surveyed. Two "polymorphic" sites for *Stu*I were found to occur within 0.10 kb of one another and were mapped to position 12.50. Both sites are rare and have been found in only one individual each. Of the 123 restriction sites mapped, 93 have thus far been found to be "polymorphic" or variable; whereas 21 of the sites are conserved, *i.e.*, have been found in all red drum examined. The two *Hin*dIII sites at map positions 1.25 and 2.00, the two *Stu*I sites at map positions 4.50 and 15.50 and the *Xmn*I site at map position 5.50 occur outside of the region covered by the red drum probe and are flanked by other nearby sites for the same enzymes. As a consequence, these five sites were not surveyed among all red drum examined and it is not known if the five sites are "polymorphic" or conserved. The two *Sac*II sites at map positions 1.00 and 3.00, and the two *Hpa*I sites at map positions 6.00 and 15.00, were used in the mapping experiments in order to assist in the orientation of the red drum restriction site map relative to specific genes in the mitochondrial genome. Three of these sites (the two *Sac*II sites and the *Hpa*I site at map position 6.00) are known to be conserved in vertebrates (Carr *et al.* 1987).

In Figure 2, the red drum mtDNA restriction site map is oriented to the human mtDNA gene map in order to demonstrate approximate locations of red drum mtDNA restriction sites relative to specific mtDNA genes (Anderson *et al.* 1981). This orientation assumes that the gene order and content of red drum mtDNA is the same as in other vertebrates, which is likely as mtDNA gene order and content is conserved in all

known vertebrates (Moritz *et al.* 1987). The orientation of the red drum restriction site map to the human mtDNA gene map was carried out initially using the two *Sac*II sites and the *Hpa*I site at map position 6.00. In brief, one of the *Sac*II sites occurs in the 12S rRNA gene, whereas the other occurs in the 16S rRNA gene. The *Hpa*I site occurs in the asparagine tRNA gene, approximately 3.00 kb from the *Sac*II site in the 16S rRNA gene (Carr *et al.* 1987). This approach was confounded, however, by the presence of the second *Hpa*I site in the red drum mtDNA, which was nearly the same map distance from the *Sac*II site inferred to be in the 12S rRNA gene as was the first *Hpa*I site from the *Sac*II site inferred to be in the 16S rRNA gene (Table 1, Figure 2). To verify the orientation, an approximately 0.55 kb fragment of the red drum 16S rRNA gene was amplified via polymerase chain reaction (PCR) using primer sequences kindly provided by J. Derr of the Animal Sciences Department at Texas A&M University. The 0.55 kb fragment generated by these primers includes the conserved *Sac*II site in the 16S rRNA gene. The amplified fragments were isolated and digested singly with restriction enzymes known to have sites (Table 1, Figure 2) either in the vicinity of the *Sac*II site in the red drum 12S rRNA gene (*Hin*dIII, *Nsi*I, *Sph*I, and *Xba*I) or of the *Sac*II site in the 16S rRNA gene (*Eco*RI and *Sca*I). [Note: The individual used in these experiments did not possess the "polymorphic" *Sca*I site at map position 0.75.] Both *Eco*RI and *Sca*I cut the amplified fragment, whereas the remaining four restriction enzymes did not, verifying that the *Sac*II site at map position 3.00 is the one located in the 16S rRNA gene (Figure 3).

The rapid rate of evolution of mtDNA, its maternal mode of inheritance, and the ease with which mtDNA can be experimentally utilized, suggest that mtDNA variants could be extremely useful as genetic markers for identifying hatchery-raised from wild individuals. In this sense, analysis of red drum mtDNA could prove useful in ultimately evaluating the success of red drum stock enhancement programs. In addition, the presence of conserved restriction sites in the red drum mitochondrial genome should

Table 1. Restriction enzyme site locations in the mitochondrial DNA of *Sciaenops ocellatus*. Map locations are in kilobase (kb) pairs. Conserved (non-polymorphic) restriction sites are indicated by asterisks (*). Sites not routinely screened for polymorphisms are indicated by daggers (†).

Restriction enzyme	Map location	Restriction enzyme	Map location	Restriction enzyme	Map location
*Eco*RV	0.00*	*Hin*dIII	6.50	*Stu*I	12.00
*Pst*I	0.00	*Pvu*II	7.25	*Pst*I	12.25
*Xba*I	0.00	*Xba*I	7.25*	*Eco*RV	12.50
*Bcl*I	0.25	*Bcl*I	7.50	*Stu*I	12.50
*Hin*dIII	0.25*	*Hin*dIII	7.50*	*Stu*I	12.50
*Pvu*II	0.25	*Sca*I	7.75*	*Xmn*I	12.50*
*Bgl*II	0.50*	*Spe*I	7.75*	*Nsi*I	12.75
*Nsi*I	0.75	*Nsi*I	8.00	*Nco*I	13.00
*Sca*I	0.75	*Sca*I	8.00	*Nco*I	13.25
*Sph*I	0.75	*Sca*I	8.00	*Pst*I	13.25
*Nco*I	1.00	*Xmn*I	8.00	*Sca*I	13.25
*Sac*II	1.00†	*Bgl*II	8.25*	*Eco*RV	13.50
*Xba*I	1.00*	*Nco*I	8.25*	*Eco*RV	13.75
*Xmn*I	1.00	*Xba*I	8.25	*Sca*I	13.75
*Hin*dIII	1.25†	*Bcl*I	8.50	*Stu*I	13.75
*Xmn*I	1.50	*Sca*I	8.50	*Bcl*I	14.00
*Hin*dIII	2.00†	*Stu*I	8.50	*Nco*I	14.00
*Nco*I	2.75	*Xmn*I	8.50	*Spe*I	14.00
*Eco*RI	3.00	*Bgl*II	8.75	*Hin*dIII	14.25
*Sac*II	3.00†	*Eco*RI	8.75*	*Bcl*I	14.50
*Sca*I	3.00	*Spe*I	9.00	*Spe*I	14.50
*Eco*RV	3.50	*Bcl*I	9.25	*Hpa*I	15.00†
*Sca*I	4.25	*Stu*I	9.25	*Nco*I	15.00
*Spe*I	4.25	*Xba*I	9.25	*Pst*I	15.00
*Eco*RI	4.50	*Eco*RV	9.50	*Spe*I	15.00
*Stu*I	4.50†	*Stu*I	9.75*	*Eco*RV	15.25
*Xmn*I	4.50	*Nco*I	10.00*	*Sca*I	15.25
*Hin*dIII	4.75*	*Pvu*II	10.25*	*Stu*I	15.25
*Nsi*I	4.75	*Sca*I	10.25*	*Stu*I	15.50†
*Pst*I	5.00	*Xba*I	10.50	*Pvu*II	15.75
*Xba*I	5.00	*Pvu*II	10.75	*Spe*I	15.75
*Pvu*II	5.25	*Spe*I	10.75	*Spe*I	15.75*
*Xmn*I	5.50†	*Xba*I	10.75	EcoRI	16.00
*Nco*I	5.75	*Xmn*I	10.75	*Xba*I	16.25
*Xmn*I	5.75	*Bcl*I	11.00	*Xmn*I	16.25
*Hpa*I	6.00†	*Pst*I	11.25	*Bcl*I	16.50
*Spe*I	6.00	*Pvu*II	11.25	*Nco*I	16.50
*Stu*I	6.00*	*Bgl*II	11.50	*Nsi*I	16.50
*Nco*I	6.25	*Bam*HI	11.75	*Sca*I	16.50
*Sph*I	6.25*	*Hin*dIII	11.75	*Spe*I	16.50*
*Stu*I	6.25	*Sca*I	12.00	*Bam*HI	16.75

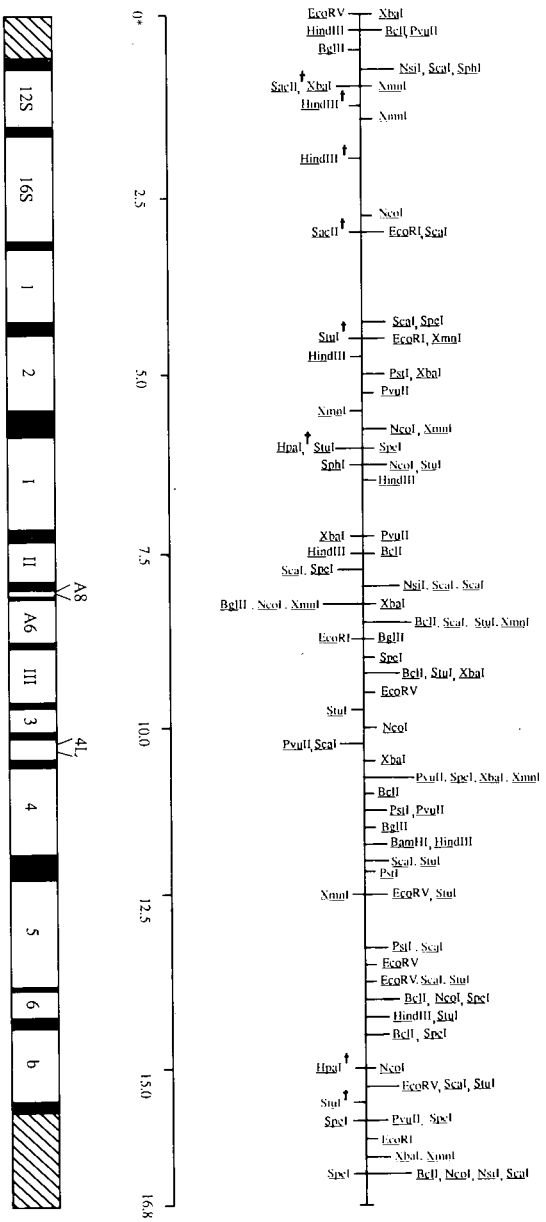

Figure 2. Restriction enzyme map for mitochondrial DNA of red drum, *Sciaenops ocellatus*. The red drum map is oriented to the human mtDNA gene map. In the latter, the clear boxes refer to the small and large rRNA genes (12S and 16S), NADH dehydrogenase subunits (1–6, 4L), cytochrome oxidase subunits (I–III), ATPase subunits (A6, A8), and cytochrome b (b); transfer RNA genes and spacers appear as black areas and the D-loop or control region is shaded. Red drum restriction sites above the line are "polymorphic", those below the line are conserved. Restriction sites marked with asterisks (*) are not routinely surveyed in our laboratory. On the scale in the middle, one map unit corresponds to 1000 nucleotides.

assist in the cloning and analysis of specific regions of the mtDNA molecule (*e.g.*, the D-loop or control region) which are known to be rapidly evolving. The information presented here should be useful in the on-going efforts to evaluate red drum stock enhancement programs.

ACKNOWLEDGMENTS

We thank M. Birkner and L. Richardson for providing technical assistance, J. Derr for providing the primer sequences which allowed PCR amplification of the 16S mtDNA rRNA gene, and L. Richardson for providing critical comments on an early draft of the manuscript. Work was supported in part by grants from the Texas A&M Sea Grant College Program and the MARFIN Program (U.S. Department of Commerce), and in part by the Texas Agricultural Experiment Station under Project H-6703.

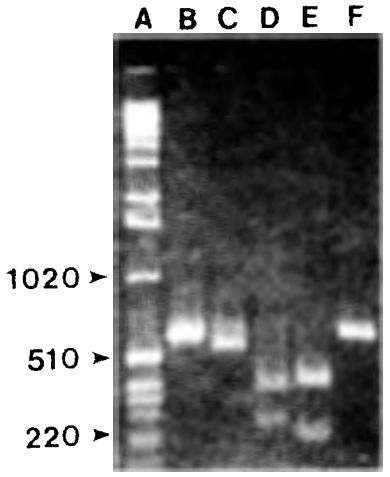

Figure 3. Restriction enzyme digestions of a PCR-amplified, 550 base pair fragment of the 16S rRNA gene from red drum. The gel was 2% agarose and fragments were visualized over UV light after staining with ethidium bromide. Lane A — BRL (Bethesda Research Laboratories) 1 kb ladder; lane B — uncut; lane C — *Sac*II digestion; lane D — *Sca*I digestion; lane E — *Eco*RI digestion; and lane F — *Hin*dIII digestion. Sizes are in base pairs.

LITERATURE CITED

Anderson, S., A.T. Bankier, B.G. Barrell, M.H.L. De Bruijn, A.R. Coulson, A.J. Drouin, I.C. Eperon, D.P. Nierlich, B.A. Roe, F. Sanger, P.H. Schreier, A.J.H. Smith, R. Staden, and I.G. Young. 1981. Sequence and organization of the human mitochondrial genome. Nature 290: 457-465.

Avise, J.C., E. Bermingham, L.G. Kessler, and N.C. Saunders. 1984. Characterization of mitochondrial DNA variability in a hybrid swarm between subspecies of bluegill sunfish (*Lepomis macrochirus*). Evolution 38: 931-941.

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. Mar. Biol. 108: 197-206.

Brown, W.M., and J. Vinograd. 1974. Restriction endonuclease cleavage maps of animal mitochondrial DNAs. Proc. Nat. Acad. Sci. (USA) 71: 4617-4621.

Carr, S.M., A.J. Brothers, and A.C. Wilson. 1987. Evolutionary inferences from restriction maps of mitochondrial DNA from nine taxa of *Xenopus* frogs. Evolution. 41: 176-188.

Dailey, J., and G.C. Matlock. 1987. Fish stocking in Texas bays: 1975-1986. Tex. Pks. Wildl. Dept., Cstl. Fish. Br., Data Mngmt. Ser. 16: 1-8.

Gold, J.R., and L.R. Richardson. 1990. Restriction site heteroplasmy in the mitochondrial DNA of the marine fish *Sciaenops ocellatus*. Animal Genet. 21: 313-316.

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. Fisheries Res. 12:213-241.

Gold, J.R., K.M. Kedzie, D.A. Bohlmeyer, J.D. Jenkin, W.J. Karel, N. Iida, and S.M. Carr. 1988. Studies on the basic structure of the red drum (*Sciaenops ocellatus*) genome. Contrib. Mar. Sci. 30 (Supplement): 57-64.

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Paabo, F.X. Villablanca, and A.C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Nat. Acad. Sci. (USA) 86: 6196-6200.

Moritz, C., T.E. Dowling, and W.M. Brown. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. An. Rev. Ecol. Syst. 18: 269-292.

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