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STUDIES ON THE BASIC STRUCTURE OF THE RED DRUM (*SCIAENOPS OCELLATUS*) GENOME

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ABSTRACT

Several basic parameters of the red drum (*Sciaenops ocellatus*) nuclear and mitochondrial genomes have been investigated as the initial phase of a long-term study of red drum genetics. (1) The red drum karyotype contains 2n = 48, differently-sized acrocentric chromosomes and a single pair of nucleolus organizer region (or NOR) bearing chromosomes. (2) The red drum genome size (DNA content) is estimated to be 1.65 ± 0.01 picograms of DNA per diploid nucleus. (3) The base composition of red drum nuclear DNA is 41.6 ± 0.1% GC (guanine-cytosine) base pairs; compositional heterogeneity and asymmetry values are 8.8 ± 0.2 and 2.3, respectively. The melting rate profile of red drum nuclear DNA indicates the presence of a highly repeated or satellite DNA family enriched in AT (adenine-thymine) base pairs. (4) Starch-gel electrophoresis of red drum tissue proteins has revealed five polymorphic or variable systems among the 27 putative gene loci now well resolved. (5) Highly purified red drum mitochondrial (mt) DNAs have been examined for digestion patterns using type-II restriction endonucleases. The size of the red drum mtDNA molecule is approximately 16.8 ± 0.2 kilobase pairs. An approximately 9.2 kilobase pair fragment of the red drum mtDNA has been cloned into a plasmid vector. The red drum mtDNA has been partially mapped.

INTRODUCTION

The red drum or redfish (*Sciaenops ocellatus*) is an important sport and commercial fish along the U.S. Gulf and Atlantic coasts. The apparent decline in the red drum fishery since the peak years between 1973-1978 (Matlock 1984) has caused widespread concern and accentuated the need for research on both wild and domesticated red drum populations, especially in the area of genetics. In this report, several basic parameters of the structure of the red drum nuclear and mitochondrial genomes are described as part of the initial phase of a long-term study of red drum genetics.

MATERIALS AND METHODS

Red drum samples for the research were obtained from four sources:

(i) Drs. Ed Robinson and Bill Neill of Texas A&M University and their projects on red drum nutrition and physiology;

(ii) Dr. Duncan MacKenzie of Texas A&M University and his project on red drum endocrinology;
(iii) Dr. Tom Linton of Texas A&M University and his Marine Advisory Service project with Dr. Jack Castle and Mr. Les Appelt of Silver Creek Farm near Palacios, Texas; and
(iv) a salt marsh near Grand Isle, Louisiana.

The samples from Drs. Robinson, Neill, and MacKenzie were obtained fresh. The samples from Silver Creek Farm were obtained by gill netting; those from Grand Isle were obtained by angling. Appropriate tissues were removed from both Silver Creek Farm and Grand Isle fish in the field and frozen in liquid nitrogen. All fish from Texas (i-iii) came from the Perry R. Bass Marine Fisheries Research Station near Palacios, Texas, and represent spawn from the John Wilson Marine Fish Hatchery at Flour Bluff, Texas. Both facilities are under the direction of the Texas Parks and Wildlife Department. The fish from Louisiana presumably represent spawn from a wild population.

Chromosome preparation and staining followed the methods of Gold (1984) and Amemiya and Gold (1987) employing live fish and gills as the tissue source. Photomicrography and computer-assisted measurement of chromosomes followed Gold and Amemiya (1986). Genome size (DNA content) measurements employed flow cytometric analysis of erythrocytes using live fish and following methods outlined in Bickham, Tucker and Legler (1985). Chicken erythrocytes were used as a DNA quantity standard. Isolation, purification, and thermal denaturation of nuclear DNA followed Karel and Gold (1987). Starch-gel electrophoresis of proteins followed procedures described in Morizot and Siciliano (1984) and employed muscle, liver, eye, and brain tissues which had been frozen in liquid nitrogen. Purified red drum mitochondrial (mt) DNAs were obtained from fresh liver and kidney tissue, digested with type-II restriction endonucleases (both singly and in pair-wise combinations), radioactively end-labeled, separated electrophoretically in agarose or polyacrylamide gels, baked onto filter papers, and autoradiographed. A few restriction sites were mapped by comparison of the single and double digestion patterns of specific enzymes. The methods used followed Carr and Griffith (1987) and Carr, Brothers and Wilson (1986). An approximately 9,200 base pair fragment of red drum mtDNA was inserted into a pTZ plasmid vector and cloned in *E. coli* strain DH5 after Maniatis, Fritsch and Sambrook (1982) and Hanahan (1984). Several restriction sites within the insert were mapped by removal of the insert from the vector, digestion with restriction endonucleases as before, separation of fragments using agarose electrophoresis, and UV-visualization of banding patterns after staining with ethidium bromide.

RESULTS AND DISCUSSION

The red drum karyotype consists of 2n = 48, differently-sized acrocentric chromosomes and a single pair of nucleolus organizer region (or NOR) bearing chromosomes (Fig. 1). The NORs are the chromosomal sites of the ribosomal RNA genes, and in some fishes vary considerably both within and between species (Gold and Amemiya 1986). No variation in chromosome number or type, or in the number of chromosomal NORs, was observed among 20 individuals examined. The 2n = 48 acrocentric karyotype and single pair of NOR-bearing chromosomes is fairly typical of a perciform fish (LeGrande 1987; Amemiya and Gold unpublished). The red drum chromosomal NOR, however, is situated sub-centromerically on the long arm of its chromosome (Fig. 1c). In most fishes, the (typically) single pair of NORs are situated terminally on the short arm of a submeta- or acrocentric chromosome (Gold and Amemiya 1986; and others).

The genome sizes (DNA contents) of eight individuals ranged from 1.57 - 1.69 picograms per diploid nucleus ($\bar{x}$ = 1.65 ± 0.01). The differences

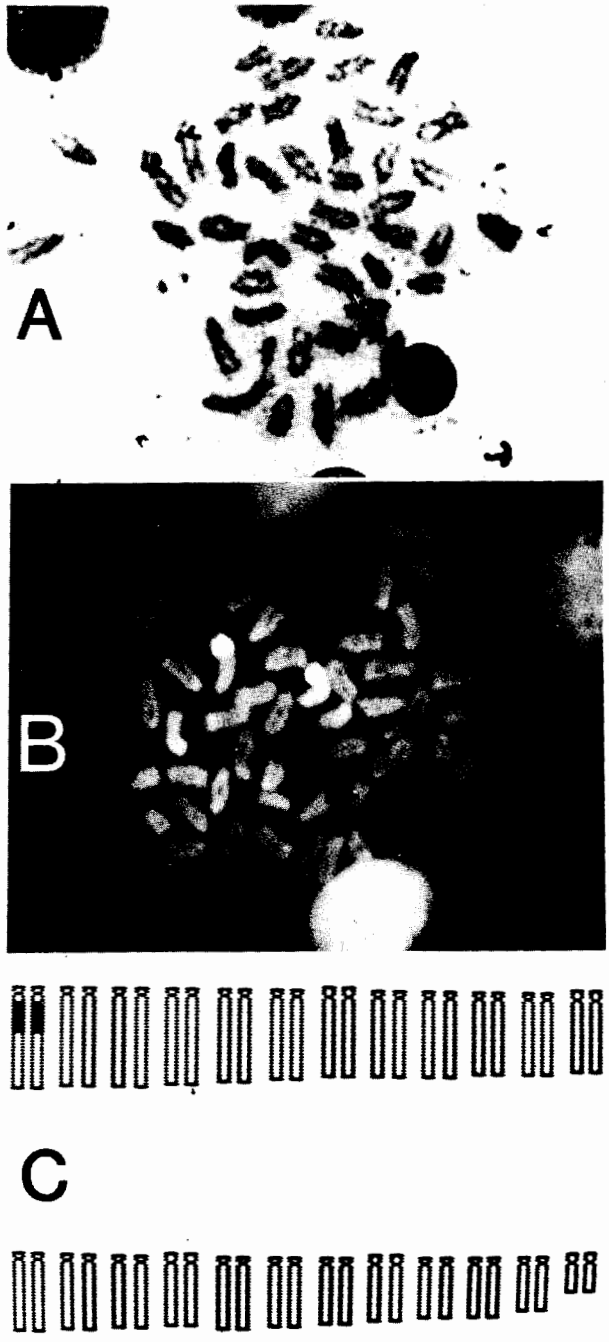

FIG. 1. (A) Silver- and (B) chromomycin A3-stained metaphase chromosomes from *Sciaenops ocellatus* showing the single pair of chromosomal nucleolus organizer regions or NORs. (C) Computer-assisted idiogram (from digitized measurements) of the *S. ocellatus* diploid karyotype; chromosomal NOR positions are indicated by darkened areas.

between individuals ranged from 0-6% and averaged around 3%. This level of genome size variation between individuals within populations is fairly typical of teleost fish (Gold and Amemiya 1987). The genome size of the red drum falls well within the range observed for other perciform fish (Hinegardner and Rosen 1972).

The base composition of red drum nuclear DNA is 41.6 ± 0.1% GC (guanine-cytosine) base pairs as estimated by thermal denaturation; the compositional heterogeneity and asymmetry values of red drum DNA are 8.8 ± 0.2 and 2.3, respectively. These values (including base composition) are well within the range found in other teleost fishes (Hudson, Cuny, Cortadas, Haschemeyer and Bernardi 1980; Karel and Gold 1987). The melting rate profile of red drum nuclear DNA is shown in Figure 2. The prominent, minor

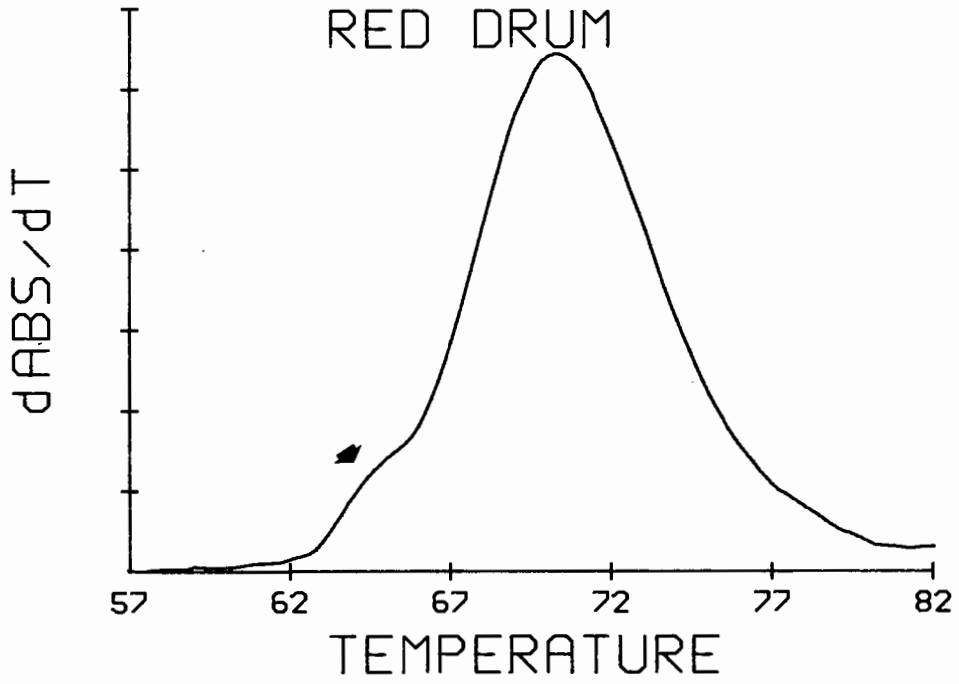

FIG. 2. Differential melting rate profile of red drum nuclear DNA. Abscissa: denaturation temperature; ordinate: increase in absorbance for each step increase (0.2°C) in temperature. The minor peak, possibly representing a family of repeated DNA sequences, is indicated by an arrow.

peak indicates the presence in the red drum genome of a highly repeated or satellite DNA family enriched in AT (adenine-thymine) base pairs. The estimated base composition of this putative satellite DNA family is 26.4 - 26.6% GC.

Systems for 27 presumptive red drum protein-coding gene loci are now well resolved. The enzymes, loci, tissues employed, and buffers used are shown in Table 1. Systems for another 15-20 loci show varying degrees of staining

TABLE 1

Protein Coding Loci Resolved

Enzyme	Enzyme commission number	Tissue source*	Buffer system**
Aconitase (ACON)	4.2.1.3	L	1
Adenosine deaminase (ADA)	3.5.4.4	M	2
Adenylate kinase (AK)	2.7.4.3	M	2
Alcohol dehydrogenase (ADH)	1.1.1.1	L	1
Creatine kinase (CK)	2.7.3.2	M	2
Enolase (ENO)	4.2.1.11	B,E	1
Esterase (ES-1)	3.1.1.1	M	1
Esterase (ES-2)	3.1.1.1	M	1
Esterase (ES-3)	3.1.1.1	M	1
Fumarase (FUM)	4.2.1.2	M	3
α Glycerophosphate dehydrogenase (αGPD)	1.1.1.8	M	2
Glyoxylase (GLO)	4.4.1.5	M	1
Isocitrate dehydrogenase (IDH)	1.1.1.42	L	1
Lactate dehydrogenase (LDH-1)	1.1.1.27	B,E	2
Lactate dehydrogenase (LDH-2)	1.1.1.27	B,E	2
Lactate dehydrogenase (LDH-3)	1.1.1.27	B,E	2
Malic enzyme (ME)	1.1.1.40	M	1
Mannose phosphate isomerase (MPI)	5.3.1.8	M	2
Peptidase (PEP B)	3.4.11	M	1
Peptidase (PEP D)	3.4.11	M	2
Peptidase (PEP S)	3.4.11	M	1
Phosphoglucomutase (PGM)	2.7.5.1	M	2
Phosphoglycerate kinase (PGK)	2.7.2.3	M	2
Pyruvate kinase (PK)	2.7.1.40	B,E	1
Superoxide dismutase (SOD)	1.15.1.1	B,E	2
Triosephosphate isomerase (TPI)	5.3.1.1	B,E	1
Uridine monophosphate (UMPK)	2.7.4	B,E	2

* L = liver; M = muscle; B,E = brain, eye.
** 1 = Tris-versene-borate, pH 8.0; 2 = Tris-citrate, pH 7.0; 3 = Ridgeway.

activity, and require further work. Of the resolved systems, five loci have been found to be polymorphic (Table 2); additional polymorphisms are apparently present among the unresolved systems. Allele frequencies at the polymorphic loci have not been calculated since the primary focus has been on locus resolution.

Highly purified mitochondrial (mt) DNAs were isolated from several individuals and examined for digestion patterns using 25-30 type-II restriction endonucleases. Fifteen enzymes cut the red drum mtDNA and 35-40 restriction sites have been identified by end-labeling. The latter method was used to map a few sites, including the two Sst II sites (Fig. 3) which apparently

TABLE 2

Polymorphic Loci Resolved

Locus	# Alleles
Aconitase	2*
Adenosine deaminase	7
Alcohol dehydrogenase	3
Esterase - 1	2
Isocitrate dehydrogenase	2*

* Rare (< 0.05) allele.

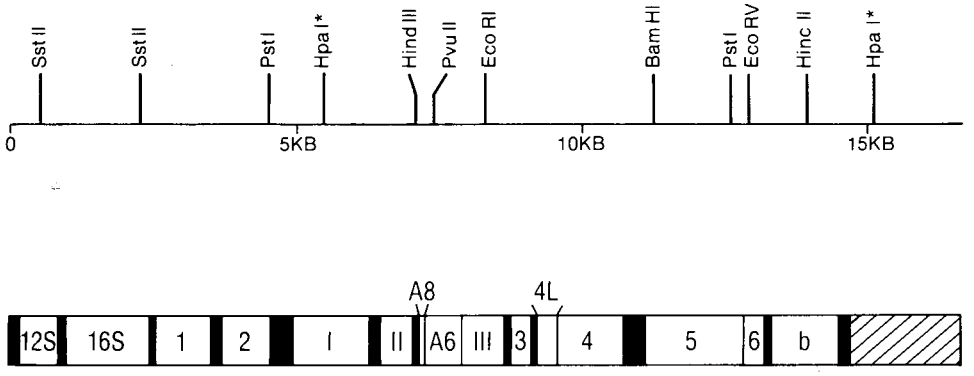

FIG. 3. Current restriction enzyme map (above) of the red drum mitochondrial (mt) DNA molecule. The red drum map is oriented to the human mtDNA gene map (below) by the two Sst II sites (see text). Asterisks (*) indicate the approximately 9,200 base pair fragment cloned into the pTZ plasmid.

are conserved throughout vertebrates and can be used to orient most vertebrate mtDNA maps to the mtDNA gene map in humans (Carr *et al.* 1986). Based on these experiments, the red drum mtDNA was estimated to be 16,800 ± 200 base pairs in length. Additional sites shown in Figure 3 were generated from mapping experiments using the approximately 9,200 base pair red drum mtDNA insert in the pTZ plasmid. No polymorphisms in size or restriction sites have yet been identified.

Data on genetic variability in red drum are few. Ramsey and Wakeman (1983) examined red drum from Louisiana and Texas for allelic variation at 41 presumptive gene loci; only three loci (including glucose phosphate isomerase or GPI) were demonstrably polymorphic. Attempts to resolve red drum GPI in our laboratory have been only partially successful. Satellite or artifactual bands often appear in GPI gels, regardless of the buffer employed, and no simple genetic models can yet explain the patterns of GPI variation observed. Wilder and Fisher (1986) surveyed the protein products of 30-40 loci from an unspecified number of red drum from Texas. They noted that

genetic variability was low, but did not give details as to the loci examined. The preliminary inference from the above is that red drum may not possess high levels of variability in nuclear protein-coding genes and hence "...illustrate the case where significant heterogeneity accompanies low population divergence" (Ramsey and Wakeman 1983). Whether or not protein electrophoresis will prove useful in discriminating red drum populations remains to be thoroughly tested. Mitochondrial DNA analysis, alternatively, holds great promise (Avise and Lansman 1983) for discriminating discrete red drum populations (should they exist), and current work in our laboratory is focused in that direction.

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