Restriction Analysis of the Nuclear DNA of Three Species of *Acipenser*

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The genomes of eukaryotes contain three classes of repeated DNA sequences: multigene families such as ribosomal RNA genes, interspersed short (SINES) and long (LINES) elements, and tandemly repeated satellite DNA sequences or stDNA (Singer, 1982). Multigene families plus SINES and LINES constitute a middle-repetitive DNA fraction, and stDNAs comprise a highly repetitive (HR) DNA fraction. HR DNA is localized in the heterochromatic regions of animal chromosomes and plays a significant role in genome and chromosome evolution (John, 1988). Originally stDNA was detected by isopycnic gradient centrifugation of total DNA (Miklos, 1985), but it was revealed by this method in only a few of many species of teleost fishes studied (Hudson et al., 1980; Bernardi and Bernardi, 1990). stDNA was not found by gradient centrifugation in the only *Acipenser* species investigated, *A. sturio* (Felix et al., 1956). Using another technique, restriction enzyme digestion of nuclear DNA followed by electrophoresis in agarose or acrylamide gels, several stDNA sequences were discovered in genomes of various salmonids (Goodier and Davidson, 1993, 1994; Hartley and Davidson, 1994), cyprinids (Datta et al., 1988; Moyer et al., 1988; Ekker et al., 1992; He et al., 1992), pollock (Denovan and Wright, 1990), pupfish (Elder and Turner, 1994), sparids (Garrido-Ramos et al., 1995), and cichlids (Wright, 1989; Frank et al., 1992, 1994).

The data on the genome structure and evolution of acipenseriforms are very scarce (review in Birstein et al., 1995). Only genome structure of the sterlet, *Acipenser ruthenus*, has been investigated so far (Vladychenskaya et al., 1980). Its genome consists of approximately 55% repetitive (including 24% of HR DNA) and 45% unique sequences. Repetitive sequences seem to be very similar in all acipenseriforms, at least within the subfamily Acipenserinae. A comparison of three species of *Acipenser* (*A. ruthenus*, *A. stellatus*, and *A. gueldenstaedti*) and *Huso huso* showed that the rate of divergence of repetitive sequences is very low in their genomes (Kedrova et al., 1980). The repetitive DNA contained only 0-2.6% of nucleotide change. In this paper we present the first results on several repeated sequences in *A. ruthenus*, *A. stellatus*, and *A. gueldenstaedti*, detected by restriction enzyme digestion of nuclear DNA.

Materials and Methods

Material. Three sturgeon species, the sterlet, *Acipenser ruthenus*, Russian sturgeon, *A. gueldenstaedti*, and stellate sturgeon, *A. stellatus*, were caught in the lower reaches of the Volga River near the city of Astrakhan. Samples of livers and muscles were collected and fixed in 70-80% alcohol in the spring of 1994.

DNA isolation. High molecular weight DNA was isolated from samples of liver and muscle tissues of each sturgeon species fixed in alcohol by a standard method (Sambrook et al., 1989) with some modifications. Tissue samples of about 0.5-1.0 g were frozen in liquid nitrogen and crushed into a powder. The powder was mixed with 10 ml of the buffer containing 10 mM EDTA (pH=8.0), 0.5% SDS, and 0.1 mg/ml of proteinase K or 1 mg/ml of pronase. The homogenate was incubated in a water bath at 55°C for 3 hours (proteinase K) or at 37°C for 2-3 hours (pronase). DNA was isolated using phenol/chloroform extraction and ethanol precipitation. DNA obtained was centrifuged in a CsCl-ethidium bromide gradient containing 0.5% sarcosyl at 35.000 rpm for 24 hours at room temperature. Ethidium bromide was removed by multiple isoamyl alcohol extraction. DNA was precipitated by ethanol, then was washed twice in ethanol, air-dried and redissolved in 1 ml of the buffer consisting of 10 mM Tris-HCl (pH=7.5) and 1 mM EDTA. DNA samples obtained were analysed by electrophoresis in 0.5% agarose gel with 0.5 mcg/ml of ethidium bromide using Tris-acetate buffer (40 mM Tris-acetate-1mM EDTA).

Digestion and electrophoresis. Four restriction enzymes were used for digestion of the DNA samples: *Sau*3AI, *Acc*651, *Pst*I, and *Hae*III (Sibenzyme, Novosibirsk, Russia). The digestion followed the manufacturer's instruction. Fragments were separated by electrophoresis in 1.2% agarose or 3% polyacrylamide gels stained with ethidium bromide (0.5 mcg/ml). Fragments of λ DNA digested with *Bgl*I and *Pst*I were used as size markers.

Results and Discussion

Total nuclear DNA was extracted from ethanol-fixed tissues of *Acipenser gueldenstaedti*, *A. ruthenus*, and *A. stellatus* with a high yield: approximately 0.72 mg per 1 g of tissue. This yield is comparable with that of DNA extracted from fresh, non-fixed sturgeon muscles (4-6 mg/g; Vitvitskaya and Nikonorov, 1989). We assume that the method of DNA extraction used by us could be applied to the analysis of fish museum specimens fixed in alcohol as well.

The four restriction enzymes tested, *Acc*561, *Sau*3AI, *Pst*I, and *Hae*III, revealed repeated sequences in all three sturgeon species (Table 1). The sequences are seen as distinct bands in ethidium bromide stained gels and range in size approximately from 60 base pairs (bp) to 1,500 bp (or 1.5 kb). The characteristic position and distribution of bands are typical for HR DNA sequences. The sizes of the long and average short repetitive sequences were similar to those determined from the DNA reassociation data (1.35 kb and 600 bp, respectively; Vladychenskaya et al., 1980). There were no species specificity in the size or distribution of bands. After digestion with *Acc*651 only one band with the size of about 1.5 kb was revealed, whereas other enzymes revealed from two (*Sau*3AI) to 8 bands (*Hae*III). The size of sequences in the *Hae*III digest varied by 20 bp, and the distribution of bands showed a ladder pattern characteristic of the stDNA. The ladder-like pattern suggests that in this stDNA a restriction site may have appeared in some of the repeated units as a result of mutation.

Previously, two of the enzymes used by us, *Sau*3AI and *Hae*III, were used for the study of HR DNA in teleosts (Wright, 1989; Ekker et al., 1992; Garrido-Ramos et al., 1995). Digestion of the zebrafish, *Brachydanio rerio*, DNA with *Sau*3AI revealed two repeating sequences: stDNA-like monomer units of 186 bp, and dispersed SINES-like repeats of 165 bp (Ekker et al., 1992). The size of the repeated sequences revealed by the same enzyme in sturgeon DNA, is similar: 179 and 270 bp (Table 1). It is too early

Table 1. Size of Repeated DNA Fragments Revealed by Restriction Enzymes Digestion in Genomes of Three Species of Acipenser

Restriction Enzyme	Recognition Sequence	Fragment Size, base pairs
Acc561	GGTACC	1,500
*Sau3AI**	GATC	270
		170
PstI	CTGCAG	380
		230
		215
		170
		155
HaeIII	GGCC	559
		358
		165
		145
		120
		100
		80
		60

*The data for *A. ruthenus*.

to speculate on the possibile similarity of sequences in the genomes of sturgeons and teleosts without sequencing the sturgeon stDNA. But it is important to keep in mind that, according to the data on interspecies DNA hydridization, up to 20% of the repeated sequences in genomes of sturgeons and salmonids are common to both (Kedrova et al., 1980). Using the enzyme *HaeIII*, stDNA bands of 186 bp were obtained in the DNA of three sparid species, *Sparus aurata*, *Diplodus bellotti*, and *D. puntazzo*, whereas in the DNA of two species of *Pargus*, bands of 170 bp were obtained (Garrido-Ramos et al., 1995). The size of the repeats in the species of *Pargus* is similar to that in sturgeons, 165 bp (Table 1). In the DNA of tilapia, *Oreochromis mossambicus/hornorum*, an HR DNA sequence was produced by digestion with *HaeIII* (Wright, 1989). The size of monomers obtained, 237 bp, differed from the size of repeats revealed by the same enzyme in sturgeon DNA (Table 1).

Our results showed that: 1) families of highly repeated stDNA sequences are present in the genomes of sturgeons; 2) the structure of these stDNAs is complex; 3) there is no species specificity in the size and patterns of the main families of stDNA. A lack of species specificity in the size and patterns of HR DNA sequences revealed by restriction digestion supports the hypothesis of a high degree of genetic conservation within the acipenseriforms (Birstein et al., 1995). All extant acipenseriforms are polyploids, the sterlet and stellate being tetraploids, 4n=120, and the Russian sturgeon being an octoploid, 8n=240 (Birstein et al., 1993, 1995). Genome doubling seems to have been one of the main genetic mechanisms of speciation within the genus *Acipenser* (Birstein et al., 1995). These peculiar genetic and evolutionary characteristics of acipenseriforms point to the need for investigation of stDNA in these fishes in more detail.

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References

Bernardi, G., and G. Bernardi. 1990. Compositional patterns in the nuclear genome of cold-blooded vertebrates. J. Mol. Evol. 31 :265-281.

Birstein, V. J., R. Hanner, and R. DeSalle. 1995. Phylogeny of the Acipenseriformes: Cytogenetic and molecular approaches. Environ. Fish Biol. (submitted).

Birstein, V. J., A. I. Poletaev, and B. F. Goncharov. 1993. The DNA content in Eurasian sturgeon species determined by flow cytometry. Cytometry 14 :377-383.

Datta, U., P. Dutta, and R. K. Mandal. 1988. Cloning and characterization of highly repetitive fish nucleotide sequence. Gene 62 :331-336.

Denovan, E. M., and J. M. Wright. 1990. A satellite family from pollock (*Pollachius virens*). Gene 87 :279-283.

Ekker, M., A. Fritz, and M. Westerfield. 1992. Identification of two families of satellite-like repetitive DNA sequences from the zebrafish (*Brachydanio rerio*). Genomics 13 :1169-1173.

Elder, J. E., Jr., and B. J. Turner. 1994. Concerted evolution at the population level: Pupfish *HindIII* satellite DNA sequences. Proc. Natl. Acad. Sci. USA 91 :994-998.

Felix, K., I. Jilke, and R. K. Zahn. 1956. Die Deoxyribonucleinsaure Einiger Fischspermien. Hoppe Seyler's Z. Physiol. Chem. 303 :140-152.

Franck, J. P. C., J. M. Wright, and B. J. Mc Andrew. 1992. Genetic variability in a family of satellite DNAs from tilapia (Pisces: Cichlidae). Genome 35 :719-725.

Franck, J. P. C., I. Kornfield, and J. M. Wright. 1994. The utility of SATA satellite DNA sequences for inferring phylogenetic relationships among the three major genera of tilapine cichlid fishes. Mol. Phylogenet. Evol. 3 :10-16.

Garrido-Ramos, M. A., M. Jamilena, R. Lozano, S. Cardenas, C. Ruiz Rejon, and M. Ruiz Rejon. 1995. Phylogenetic relationships of the Sparidae family (Pisces, Perciformes) inferred from satellite-DNA. Hereditas 122 :1-6.

Goodier, J. L., and W. S. Davidson. 1993. A repetitive element in the genome of Atlantic salmon, *Salmo salar*. Gene 131 :237-242.

Goodier, J. L., and W. S. Davidson. 1994. Characterization of a repetitive element detected by *Nhe*I in the genomes of *Salmo* species. Genome 37 :639-645.

Hartley, S., and W. S. Davidson. 1994. Distribution of satellite DNA sequences isolated from Arctic char, *Salvelinus alpinus*, in the genus *Salvelinus*. Can. J. Fish Aquat. Sci. 51(Suppl.) :277-283.

He, L., Z. Zhu, A. J. Faras, K. S. Guise, P. B. Hackett, and A. R. Kapuscinski. 1992. Characterization of *AluI* repeats of zebrafish (*Brachydanio rerio*). Mol. Mar. Biol. Biotechnol. 1 :125-135.

Hudson, A. P., G. Cuny, J. Cortadas, A. E. V. Haschemeyer, and G. Bernardi. 1980. An analysis of fish genomes by density gradient centrifugation. Eur. J. Biochem. 112 :203-210.

John, B. 1988. The biology of heterochromatin. *In*: Verma, R. S. (ed.) Heterochromatin. Molecular and Structural Aspects. Cambridge: Cambridge Univ. Press. Pp. 1-149.

Kedrova, O. S., N. S. Vladychenskaya, and A. S. Antonov. 1980. Divergence of unique and repetitious sequences in fish genomes. Molek. Biol. 14 :1001-1012 (in Russian; English translation: Mol. Biol. 14 :787-797).

Miklos, G. L. 1985. Localized highly repetitive DNA sequences in vertebrate and invertebrate genomes. *In*: Molecular Evolutionary Genetics. Plenum Press: New York. Pp. 241-321.

Moyer, S. P., D. P. Ma, T. L. Thomas, and J. R. Gold. 1988. Characterization of highly repeated satellite DNA from the cyprinid fish *Notropis lutensis*. Comp. Biochem. Physiol. 91B :639-646.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning. A Laboratory Manual. 2nd ed. New York: Cold Spring Harbor Laboratory.

Singer, M. F. 1982. Highly repeated sequences in mammalian genome. Int. Rev. Cytol. 76 :67-112.

Vitvitskaya, L. V., and S. I. Nikonorov. 1989. Nucleic acids content in muscles of the russian sturgeon with muscle myopathy. *In*: Sturgeon Farming in the USSR. Astrakhan. Pp. 51-52 (in Russian).

Vladychenskaya, N. S., O. S. Kedrova, and N. B. Petrov. 1980. Organization of nucleotide sequences in the genome of the sterlet, *Acipenser acipenser*. Molek. Biol. 14 :986-1000 (in Russian; English translation: Mol. Biol. 14 :774-786).

Wright, J. M. 1989. Nucleotide sequence, genomic organization and evolution of a major repetitive family in tilapia (*Oreochromis mossambicus/hornorum*). Nucl. Acids Res. 17 :5071-5079.

Roskin, G. I., and L. B. Levinson. 1957. Microscopic Technique. Sovetskaya Nauka, Moscow. (in Russian).

Ruban, G. I. 1997. Species structure, contemporary distribution and status of populations of the Siberian sturgeon, *Acipenser baerii*. Env. Biol. Fish. (in press).

Ruban, G. I., and N. V. Akimova. 1993. Ecological charactetristics of Siberian sturgeon, *Acipenser baeri*, from the Kolyma River. Voprosy Ikhtiologii 33 :84-92 (in Russian, English translation: J. Ichthyol. 33 :66-80).

Sedel'nikov, A. K. 1910. Lake Zaisan. Zapiski Zapadno-Sibirskogo Otdela Imperatorskogo Russkogo Geograficheskogo Obshchestva 35 :1-253 (in Russian).

Shagaeva, V. G., M. P. Nikol'skaya, N. V. Akimova, K. P. Markov, and N. G. Nikol'skaya. 1993. A study of the early ontogeny of Volga River sturgeons (Acipenseridae) subjected to human activity. Voprosy Ikhtiologii 33 :230-240 (in Russian; English translation: J. Ichthyol. 33 :23-41).

Votinov, N. P. 1963. Biological foundations of artificial reproduction of the Ob River sturgeon. Trudy Ob-Tazovskogo Otdeleniya Gosudarstvennogo Nauchno-Issledovatelskogo Instituta Ozernogo i Rechnogo Rybnogo Khozyaistva. Novaya Seria 3 :5-102 (in Russian).

Votinov, N. P., V. N. Zlokazov, V. P. Kas'yanov and R. I. Setsko. 1975. Status of Sturgeon Reserves in the Rivers of Siberia and Measures Aimed to Increase These Reserves. Sverdlovsk. 94 pp. (in Russian).

Sturgeons in the Lower Danube: A Trip to Romania

In January, 1996, I visited Romania as Chairman of the Sturgeon Specialist Group (SSG), IUCN. I am very glad to have an opportunity to visit this area since I was anxious to understand the status of sturgeons in the Danube and Black Sea. Moreover, the Danube Delta Reserve is a very interesting area. Historically there were five sturgeon species which migrated from the Black Sea into the Danube River: the beluga sturgeon, *Huso huso*, the Russian sturgeon, *Acipenser gueldenstaedti*, the stellate sturgeon, *A. stellatus*, the ship sturgeon, *A. nudiventris*, and, perhaps, the European Atlantic sturgeon, *A. sturio*. The freshwater sterlet, *A. ruthenus*, also thrived in the Danube and its tributaries. In addition, it provides a home for 320 species of birds!

My trip was organized by Dr. Radu Suciu from the Danube Delta Institute (DDI) and Prof. Nicolae Bacalbasa-Dobrovici of the University in Galati. Dr. Suciu is a head of the Sturgeon Research Group (SRG) at the DDI which is located in the town of Tulcea, and Prof. Bacalbasa is a member of the SSG. They brought me by car from Bucharest to Tulcea, the regional capital of the Danube Delta. In Tulcea I spent three days making acquaintance with the Institute and the Danube (a boat trip around the Delta was especially informative). There is an aquarium at the local Museum of Natural History with live Russian and stellate sturgeons from the Danube. After Tulcea, I went to Galati with Prof. Bacalbasa. In Galati I had a meeting with another group of "sturgeon people" working at the Center of Research for Fish Aquaculture, Fishing and Fishprocessing. I spent my last day in Bucharest.

1. The Danube Delta Institute (Tulcea)

The DDI is a research center of the Danube Delta Biosphere Reserve (DDBR) which was organized in 1990. The DDBR covers a huge area with 18 strictly protected regions. There are a few villages and small towns in the area. This creates many problems as there are state, private, and cooperatively owned pieces of land within it. Also, there are areas formerly used unsuccessfully for industry and agriculture. Now these places are mostly abandoned and in a process of natural recovery. The DDBR belongs to the Ministry of Waters, Forests and Environmental Protection of Romania.

Originally, the DDI was the Tulcea Hydrobiological Station established in 1932, becoming the institute in 1970. In 1991, the DDI was divided into two parts, the Research Department and the Design Department. The Research Department "has the responsibility of providing the scientific basis for the formation of governmental policy and activities pertaining to the conservation and management of natural resources of the fauna and flora of the DDBR" (from the brochure about the DDI). The main goals of the Institute's studies are: constant environmental monitoring of the main aquatic and terrestrial ecosystems of DDBR, assessment of the resources (fish, pasture land, reed, forests), and elaboration of regulations for their sustenance.

There are a few research groups at the DDI working in different fields. I was most impressed with the group which is working on an atlas of the area. This is a joint project with the Forestry and Wildlife Management Department of the University of Massachusetts, USA (Prof. Curtice Griffin). The group is well-equipped with computers, color printers, etc. The draft of the atlas looks very impressive. There are also small groups of ornithologists, botanists and other specialists.

The SRG consists of Dr. Suciu and six collaborators. They worl mainly as ichthyologists, but they also use biochemical (protein electrophoresis) and karyological methods. Dr. Suciu plans to organize studies on mtDNA. In 1994, the SRG started a project entitled "Research for protection and recovery of marine sturgeons which migrate into the Danube for spawning" which is financed by the Ministry of Research and Technology of Romania and the World Bank. The preliminary field studies in 1994-1995 showed that *H. huso* and *A. gueldenstaedti* are extremely threatened now. Their depletion was caused mainly by the construction of the Iron Gates II Dam twenty years ago which cut off these species from their natural spawning grounds in the Danube. *A. ruthenus* and *A. nudiventris* practically disappeared from the lower reaches of the Danube (as in all European countries, *A. sturio* disappeared many years ago). *A. stellatus* is the only sturgeon species which is still relatively numerous in the lower Danube.

The future plans of the SRG include monitoring and assessment of sturgeon populations (mainly of *A. stellatus*), a search for their spawning grounds in the Delta branches, the influence of pollution on the development and reproduction of sturgeons in the Delta, etc. Some of the studies (radio- and ultrasonic tagging and telemetry) will be conducted as a joint project with an American scientist, Dr. Boyd Kynard of the Conte Anadromous Fish Research Center, National Biological Survey, Turners Falls, Massachusetts. Dr. Kynard is a member of SSG. After a period of research work, a restocking program for at least *A. stellatus* will be organized (following the technology of sturgeon breeding and raising elaborated by the German company Fisheries Consultancy Service of