Genetic Analysis of the Sex Determination Mechanism of White Sturgeon (*Acipenser transmontanus* Richardson)

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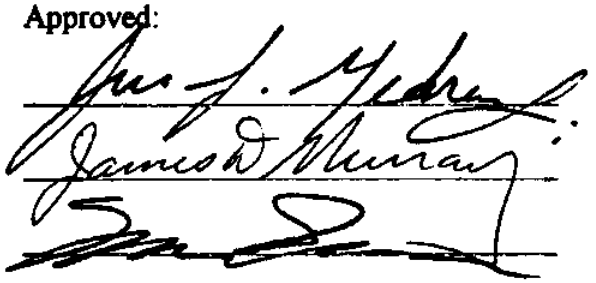

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PROJECT SUMMARY

Genetic Analysis of the Sex Determination Mechanism of White Sturgeon (*Acipenser transmontanus* Richardson)

The overall aim of this project was to understand the mode of sex determination in white sturgeon and to provide the first evidence as to what type of sex determination mechanism operates in chondrostean species. The following hypotheses were tested:

i) white sturgeon has a genetic sex determination system,

ii) one of the sexes in sturgeon is heterogametic, and

iii) sex-specific DNA polymorphisms exist between the sexes.

Several experimental approaches derived from the fields of transmission, molecular, and cytogenetics were used to examine the nature of the sex determination process.

In the first series of experiments (Chapter 2) ploidy manipulation techniques were used to produce gynogenetic and polyploid white sturgeon. A novel random amplified polymorphic DNA (RAPD)-based technique was developed to rapidly assess the overall success of treatments designed to induce gynogenesis, and measuring erythrocyte nuclei size with a Coulter Counter was found to be a rapid and accurate technique for ploidy analysis in sturgeon. Both sexes were observed in 23-24 month old gynogenetic progeny groups derived from four different females supporting the hypothesis that white sturgeon has a female heterogametic (ZW:ZZ) genetic sex determination system. The proportion of males in diploid (48%), gynogenetic (18%), and triploid (14%) progeny groups derived from the same female suggested that the sex-determining element on the W chromosome segregates independently of the centromere.

In the second series of experiments (Chapter 3) various molecular techniques were used in an attempt to isolate genetic markers for the identification of sex in white sturgeon. Subtractive hybridization, representational difference analysis and bulked segregant analysis were used, in conjunction with genetically unique groups of sturgeon, which were specifically developed to increase the probability of isolating sex-specific DNA sequences. DNA sequences associated with sex in white sturgeon were not identified. These results suggest that either 1) there are no sex-specific sequences in sturgeon, or 2) the sex-specific DNA is comprised of unusual sequences which were not complementary to the 1200 random decamer primers or the recognition sequence of the restriction endonucleases used in this set of experiments, or 3) the sex-specific sequences comprise a very small portion of the genome.

Chapter 4 details a synaptonemal complex (SC) analysis of white sturgeon spermatocytes. Synaptonemal complex analyses have not been previously reported for any sturgeon species and this study was initiated to determine if it was possible to directly identify heteromorphic sex chromosomes in meiotic prophase SC spreads. No bivalent consistently exhibited asynaptic behavior or had axes of unequal length suggesting that heteromorphic sex chromosomes are not present in white sturgeon spermatocytes which concurs with the findings of Chapter 2. Pachytene nuclei had varying numbers of univalents, self-paired foldback elements with no obvious centromeric region (1-7), and other SC peculiarities. No consistent evidence of alignment or pairing involving more than two lateral elements was found indicating that the process of diploidization is now complete in this ancient polyploid. The SC number was 139 (± 3.4) with both between and within animal variation.

The final series of experiments (Chapter 5) involved mitotic analyses of metaphase chromosome spreads from four white sturgeon individuals of each sex. Conventional mitotic analyses provided no evidence for a heteromorphic sex chromosome pair or any sex-related chromosomal polymorphism. Likewise fluorescence *in situ* hybridization of labeled male and female sturgeon genomic DNA to metaphase chromosome spreads of each sex did not reveal a chromosome that was seen to be specifically hybridizing only to the genomic DNA of one sex. The average chromosome number was 271 (range 265-276) which concurred with the meiotic count found in Chapter 4. An improved C-banding technique using propidium iodide and epifluorescence microscopy revealed between animal variation (2-7) in the number of entirely heterochromatic metacentric chromosomes. These heterochromatic chromosomes and the self-pairing elements observed in Chapter 4 were approximately equal in number and relative size and they may represent accessory B chromosomes.

In combination these results suggest that white sturgeon has a female heterogametic ZW:ZZ genetic sex determination system, and that the sex-determining element segregates independently of the centromere. No evidence of a heteromorphic sex chromosome pair was found in meiotic analyses of white sturgeon spermatocytes supporting the hypothesis of male homogamety in this species. The inability to identify sex-specific DNA sequences despite evidence of a genetic sex determination system suggests that sex-specific DNA is rare, making up a very small portion of the genome. Mitotic analyses provided no evidence for a heteromorphic sex chromosome pair suggesting that the accumulation of sex chromosomal rearrangements is insufficient to be visible at the cytogenetic level. All of these results support the contention that white sturgeon sex chromosomes are at an early stage of differentiation.

CHAPTER 1. LITERATURE REVIEW: THE ELUCIDATION OF SEX DETERMINATION MECHANISMS - HISTORICAL PERSPECTIVE AND CURRENT APPROACHES.

Introduction

The fast-growing and highly prized white sturgeon, *Acipenser transmontanus* Richardson, is an increasingly important species to California aquaculture. Confined production of white sturgeon began in the United States in 1979 with the sturgeon program of the Aquaculture and Fisheries Program at the University of California, Davis (Logan et al. 1995). There are currently 51 private aquaculture operations registered to raise white sturgeon in California[1]. In addition to its value as a commercial food fish, this species has the potential to produce high quality domestic caviar for national and international markets. With the decline of the caviar fisheries in the former-Soviet Union and China (Birstein 1993), there is now a potential high value market for domestic caviar.

In analyzing the feasibility of sturgeon flesh/caviar production in the United States, Peykani (1993) found that maximum profitability was achieved when male fish (50% of the cohort) were sold very early in the production process and only females were kept in the growout system for caviar production. The current diagnostic technique for sex identification in this sexually-monomorphic species requires a surgical biopsy of sexually-differentiated gonads (Conte et al. 1988). This procedure requires the fish to be of 7-8 kg body weight and

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[1] State of California - Resources Agency, Department of Fish and Game, List of Registered Freshwater Aquaculturists as of December 31, 1996.

research and applications to aquaculture. One application would be to investigate the possibility that a proportion of female gynogens are WW "super"females which could prove to be of use as broodstock for the production of outbred monosex female populations. A novel technique to rapidly screen putative gynogenetic progeny for the presence of paternal inheritance using RAPD markers was also described. This simple technique could be easily adapted to verify gynogenesis or androgenesis in any progeny group with known parentage.

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CHAPTER 3. EXPERIMENTAL APPROACHES USED IN AN ATTEMPT TO ISOLATE MOLECULAR GENETIC MARKERS FOR THE IDENTIFICATION OF SEX IN WHITE STURGEON (*Acipenser transmontanus* RICHARDSON)

Alison L. Van Eenennaam and Juan F. Medrano

Introduction

The genetic sex of many species cannot be deduced by external morphology - a problem that is usually exacerbated when dealing with embryonic or juvenile forms. One effective solution to this problem is to use DNA markers to diagnose sex. Such markers will be present in species where one sex possesses an unique chromosome or DNA sequence (Griffiths and Tiwari 1993), and may not be present in species with XX:XO (ZO:ZZ), multilocus, or environmental sex determination systems. White sturgeon are sexually monomorphic and the current diagnostic technique for sex identification requires a surgical biopsy of sexually-differentiated gonads (Conte et al. 1988). This procedure requires the fish to be of 7-8 kg body weight and of at least three years of age. The availability of an age-independent, DNA-based sex identification procedure would significantly enhance the economic feasibility of domestic caviar production systems. Sexed males could be culled and/or used for flesh production at a young age while immature females could be maintained for the production of valuable caviar at 6-10 years of age (Peykani 1993, Logan et al. 1995).

Sturgeon are bony fish (Class Osteichthyes) which belong to the monophyletic ancestral group of ray-finned fish, Order Acipenseriformes, Infraclass Chondrostei. No

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CHAPTER 4. SYNAPTONEMAL COMPLEX ANALYSIS IN SPERMATOCYTES OF WHITE STURGEON (*Acipenser transmontanus* RICHARDSON).

Alison L. Van Eenennaam, James D. Murray, and Juan F. Medrano

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Introduction

White sturgeon (Class Osteichthyes) belong to the phylogenetically unique ancestral group of ray-finned fish, Order Acipenseriformes. Karyotypes of extant Acipenseriformes can be divided into two groups, both of which are characterized by a very large number of chromosomes, about half of which are microchromosomes. The first group has a chromosome number of approximately 120 and a mean genome size (1C) of 1.6-2.5 pg, and the second group has a chromosome number of 240-250 and a genome size of 4.0-4.8 pg (Birstein et al. 1993, Blacklidge and Bidwell 1993). It is considered that the contemporary Acipenseriformes have a polyploid origin with the $2n \approx 120$ group being of a tetraploid (4N) origin, and the $2n \approx 240$-250 group being of an octoploid (8N) origin (Ohno et al. 1969, Dingerkus and Howell 1976, Birstein and Vasiliev 1987, Birstein et al. 1993, Blacklidge and Bidwell 1993); although on the basis of nucleolus organizer region (NOR) location Fontana (1994) contends that species with 120 chromosomes should in fact be considered diploid, and that species with 240-250 chromosomes are of a tetraploid origin.

Synaptonemal complex (SC) analyses have been used in cytogenetic studies of plants and animals to investigate the process of meiotic chromosome synapsis because of the high-resolution permitted by the electron microscope. Two techniques for the preparation of

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CHAPTER 5. MITOTIC ANALYSES OF THE NORTH AMERICAN WHITE STURGEON (*Acipenser transmontanus* RICHARDSON).

Alison L. Van Eenennaam, James D. Murray, and Juan F. Medrano

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Introduction

Some of the most ancient living ray-finned fish, sturgeon and paddlefish, belong to the order Acipenseriformes. Karyotypes of the sturgeon and paddlefish are characterized by a very large number of chromosomes, about half of which are microchromosomes (Table 3, Chapter 1). The order can be divided into two groups; the first group has a chromosome number of approximately 120 and a mean genome size (1C) of 1.6-2.5 pg and the second group has a chromosome number of 240-250 with a genome size of 4.0-4.8 pg (Birstein et al. 1993, Blacklidge and Bidwell 1993). Various authors have claimed that the first group is of a tetraploid origin and the second group is of an octoploid origin (Ohno et al. 1969, Burtzev et al. 1976, Dingerkus and Howell 1976, Birstein and Vasiliev 1987). No extant Acipenseriformes species has been found to have a diploid chromosome number of 60. Early Russian papers suggested that certain sturgeon species have a chromosome number of 60 (Serebryakova 1969, Burtzev et al. 1976), but it seems that the microchromosomes were not included in these chromosome counts. Conventional mitotic studies have not revealed heteromorphic sex chromosomes in any sturgeon species (Fontana and Colombo 1974, Holčík 1986).

Conclusions

The average chromosome number of 8 California white sturgeon was found to be 271 (range 265-276). This number is significantly higher than previous estimates for this species. A representative karyotype was found to consist of 132 meta- and submeta-centric chromosomes, 44 acrocentric chromosomes, and 98 microchromosomes. An improved C-banding technique revealed variation between animals in the number (2-7) of entirely heterochromatic metacentric chromosomes. These heterochromatic chromosomes may represent accessory B chromosomes. There was no evidence of a heteromorphic sex chromosome pair or any sex-related chromosomal polymorphism in this species.

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