

INHERITANCE OF MICROSATELLITE LOCI IN LAKE STURGEON

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Introduction

Lake sturgeon (*Acipenser fulvescens*) were at one time abundant throughout the Great Lakes drainage but overharvesting, pollution, and the damming of rivers have pushed the sturgeon's abundance to low levels. For example, in Lake Erie, the initial commercial harvest of sturgeon was 2,250,000 kg in 1860 but by 1895 this level had dropped by 80% (Scott and Crossman 1973). Populations that still remain today generally exist at low levels and exploitation is restricted to a few populations in Wisconsin and Canada.

The genetics of sturgeon are poorly understood for several reasons. The first is that the life history of sturgeon make genetic investigations difficult. Sexual maturation in sturgeon requires 10-30 years and then spawning is intermittent (1-6 years). Another problem is the difficulty in capturing an organism that lives on the bottom of large lakes and rivers. Current techniques to study fish genetics generally rely on allozymes which require the sacrifice of the fish in order to sample their internal tissues. Thus, allozymes require the mortality of individuals from already threatened populations. Genetic studies are further complicated by their octoploid nature (Blacklidge and Bidwell 1993).

Development of microsatellites as genetic markers for lake sturgeon would facilitate genetic studies. Microsatellites can be used as a genetic marker that requires non-lethal tissue sampling. Microsatellites often provide greater discriminatory power than allozymes, especially if there are low levels of gene flow. These markers have already been shown to be useful in genetic studies of brown trout (Estoup et al. 1993). If the markers are shown to work for lake sturgeon, they will provide a useful tool for examining the genetics of lake sturgeon at a population level. Population genetics data are particularly needed for the development and implementation of species restoration projects.

A restoration effort involving lake sturgeon is being undertaken by the Menominee Indian Tribe of Wisconsin. Lake sturgeon were historically used by the Menominee people and are important culturally and spiritually to the Menominee (Beck 1993). The fish are blocked by dams from returning to traditional spawning sites, particularly Keshena Falls on the Wolf River, and so the tribe currently has no access to lake sturgeon. As a result, a multi-agency Menominee Reservation Sturgeon Management Planning Committee was formed and includes members from the Menominee Tribe, the Wisconsin Department of Natural Resources, the Bureau of Indian Affairs, and the U.S. Fish and Wildlife Service. The committee adopted the management goal of establishing a self-sustaining population of lake sturgeon within waters of the reservation. In 1994, the Planning Committee drafted the "Menominee Reservation Lake Sturgeon Management Plan" and initiated efforts to re-establish lake sturgeon. The plan identifies genetics as being important to the success and health of establishing a population. This research should provide a tool to address this issues.

In a previous study, a genomic library of lake sturgeon DNA was screened to identify tri- and tetrameric repeat motif microsatellite loci. A screen of 60,000 clones identified twelve primer pairs that amplified microsatellite loci that produced products in most North American sturgeon species (May et al., in review). This paper describes the segregation of these loci in single pair matings (families) of lake sturgeon to describe their inheritance applicability for population studies.

Methods

Sample collection: Fish from the Menominee River in Wisconsin were collected below the White Rapids dam using a Wisconsin Department of Natural Resources (WIDNR) electroshocking boat. A portion of fin was sampled from each fish. Half of the fin clip was stored in 100% alcohol and the other half placed in lysis buffer (150 mM EDTA, 50 mM Tris pH 8.0, 2% n-lauroylsarcosine) for later extraction of DNA. Adults in spawning condition when captured were held in 1.8 meter diameter tanks to let them spawn on their own. This technique was used successfully in spring 1994 (Thuemler, personal communication). Tanks were examined every day to determine whether the fish were ready to spawn. Milt was collected from three males using a syringe and stored on ice. Eggs were then stripped from a single female into separate containers to create three separate families when fertilized with milt from the different males. The fertilized eggs were held in the Wild Rose, Wisconsin hatchery for several days before being transported to the hatchery at Cornell University. All fry from the Menominee River families (N=14,20,30) were sampled at hatch because the hatch was poor due to a fungus problem. Similarly, seven families were made from two females and four males captured in the St. Lawrence river at Montreal, Quebec. One hundred fry from each St. Lawrence River family were sampled with 50 being frozen and stored in an ultra-cold freezer and 50 stored in lysis buffer.

DNA extraction: DNA extraction was done using a CTAB extraction described by Grewe et al. (1993), with the following modifications: 30 ug

of proteinase K was used in each sample and the samples were incubated overnight at 55⁰ C

PCR conditions: The 50 ul reaction contained 1ul of genomic DNA (20 ng), 5 ul 10X PCR buffer (Gibco BRL), 1.5-2.5 ul 50mM MgCl₂, 1 ul each of 20uM forward and reverse primer, 2.0-3.5 (ul 2.5mM dNTPs), 0.2 ul (5 U/ul) GibcoTaq polymerase, and 35.8-38.3 ul H₂O. PCR was performed in a Perkins-Elmer DNA Thermocycler. Thirty-five cycles were run as a 3 step function. The first step was a denaturing step at 94⁰ C for 1 minute. The second step was an annealing step at 57⁰ C for 30 seconds. The final step was an extension step at 72⁰ C for 30 seconds. The initial denaturing step was run for 3 minutes and the final extension step was run for 5 minutes.

Gel conditions: Samples were run on a 4% Metaphor^R gel at 460V with the 0.5 TBE gel buffer run through a Neslab RTE 100 cooling unit to keep the temperature at 20⁰ C. In a pre-run step, the gel was run for 5 minutes at 460V in 12-13⁰ C buffer. The voltage was then turned off until the buffer was brought up 20⁰ C and then restored and the gel run for 1-1.5 hours (run time was determined by the clone size based on 15 minutes for every 25 base pairs). Gels were stained with ethidium bromide and examined under UV light. A polaroid picture was taken and used for scoring individuals.

Results and Discussion

Polymorphisms were found in five of the twelve loci examined. The loci that were polymorphic are LS-19, 34, 39, 54, and 68. LS-19, 34, and 54 exhibit two alleles, LS-39 three alleles, and LS-68 four alleles. The three monomorphic loci include LS-58, 62 and 73. Loci LS-22, 23, 57, and 69 either did not amplify well or did not resolve well enough to be used further. Table 1 outlines some of the specifications for the loci used.

Table 1. Locus designation with size, repeat motif and current status.

Locus	Clone Size	Repeat Motif	Status
LS-19	133	(TTG)9	Polymorphic
LS-22	218	(AAAT)6 (AAG)30	Not resolved
LS-23	162	(GTTT)8	Not resolved
LS-34	137	(GTT)10	Polymorphic
LS-39	129	(GTT)10	Polymorphic
LS-54	177	(GATA)6 (GACA)7	Polymorphic
LS-57	206	(GAA)29	Not resolved
LS-58	198	(GATA)20	Monomorphic
LS-62	85	(GATA)7	Monomorphic
LS-68	120	(GATA)13	Polymorphic
LS-69	206	(TATC)13	Not resolved
LS-73	214	(ACTC)12	Monomorphic

Polymorphic loci are currently being examined to determine the mode of inheritance. Preliminary results suggest the polymorphic loci exhibit tetrasomic inheritance. Evidence suggesting tetrasomic inheritance includes gene dosage in loci LS-19,34,39 and 54; see Figs. 1 and 2 and note assymmetric phenotypes in LS-19 and LS-54 and three alleles present in progeny of a LS-68 cross from parents that exhibit three alleles (Fig. 3).

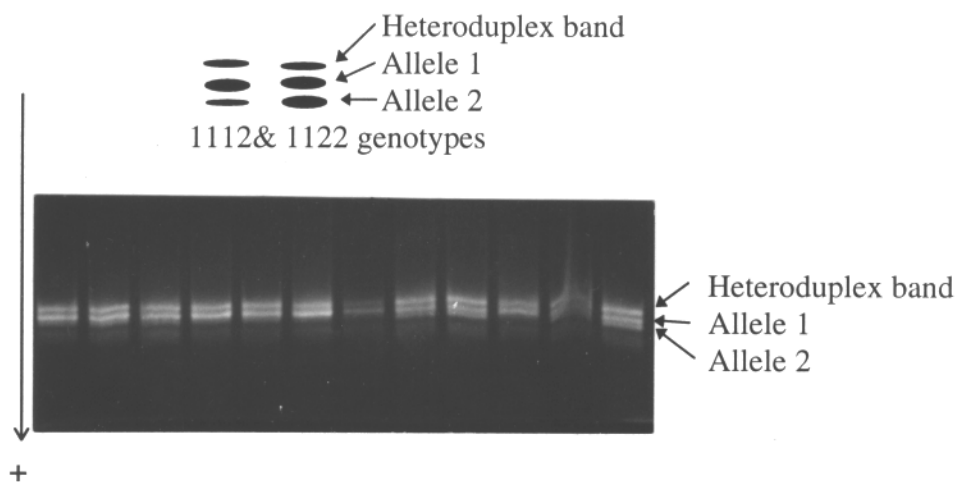


Figure 1. LS-19 cross of a 1222 male X 1111 female with progeny showing genotypes 1112 (lanes 1-7 and 10) and the 1122 (lanes 8, 9, 11 and 12). All individuals exhibit a heteroduplex band as the upper band. The homoduplex bands are under the heteroduplex band with the upper band being designated the 1 allele.

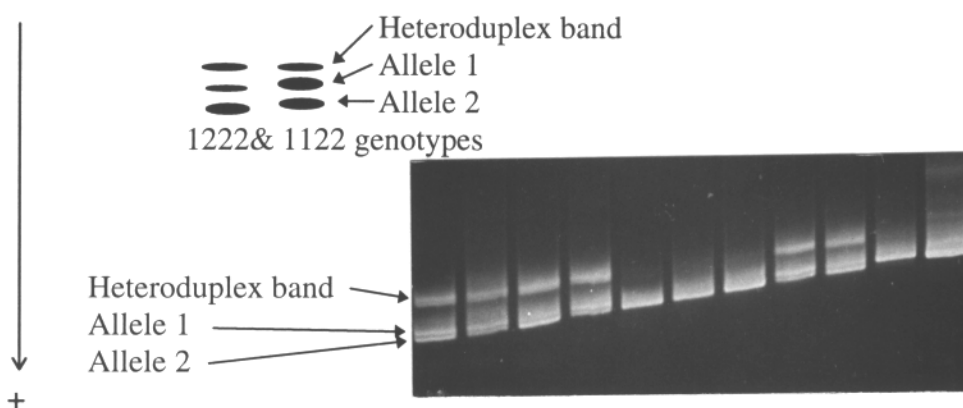


Figure 2. LS 54 cross of a 1122 male X 1122 female with progeny expressing genotypes of 2222(lanes 5, 6, and 7), 1122(lanes 1, 2, 4, and 8), 1222(lanes 3 and 9), and 1111(lane 10). Lane 11 is the LS-54 clone.

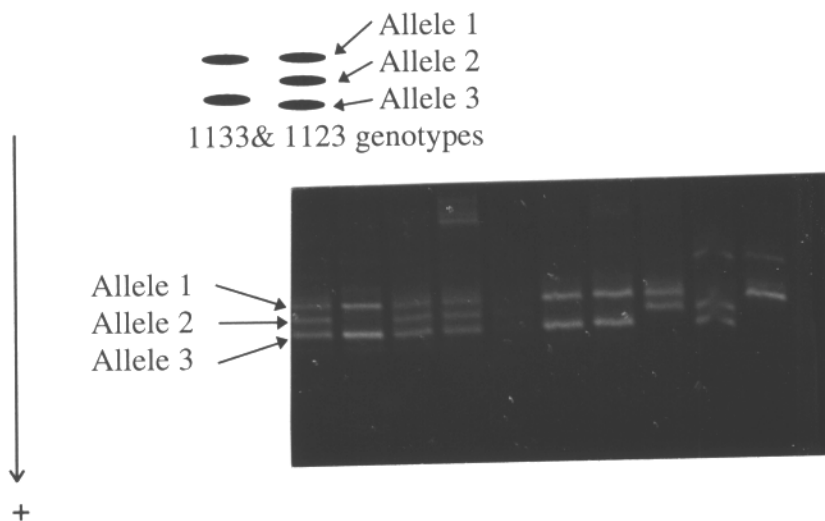


Figure 3. LS 68 cross of a 1111 male X 2233 female with individual progeny exhibiting all three alleles. Lanes 1, 3, and 5, are individuals exhibiting all three alleles. Lanes 2, 6, and 7 are individuals exhibiting alleles 1 and 3 and lane 8 is an individual with alleles 1 and 2. Lane 9 is the female's genotype and lane 10 is the male's genotype. Lane 5 was unresolved for this individual.

Additional progeny are being run to determine if Mendelian ratios shown in these crosses are consistent with tetrasomic inheritance. These results will determine if these genetic markers can be used in determining the population structure of remaining wild sturgeon stocks and as a tool to monitor the effects of transplanting and stocking of hatchery reared fish in the wild.

References

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