Genetic Monitoring of the Kootenai Tribe of Idaho White Sturgeon Conservation Aquaculture Program

Deliverable 1): Monitoring of Kootenai River white sturgeon genetic diversity
Deliverable 2): Genotyping of 2002, 2005, and 2006 broodstock for parentage analysis

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Introduction

The Kootenai River white sturgeon population was listed as endangered by the US Fish and Wildlife Service in 1994 primarily due to its small size and continued recruitment failure (USFWS 1994, USFWS 1999). The Kootenai Tribe of Idaho (KTOI) has developed a conservation aquaculture program with the goal of assisting reproduction of the Kootenai River white sturgeon until natural recruitment can be restored. A white sturgeon genetic monitoring program has been instituted by the tribe with two objectives. The first objective is to monitor the genetic diversity within the broodstock utilized by the program each year, which assists the program goal to maximize the amount of genetic diversity within each hatchery-reared cohort. The second objective is to use genetic data to identify familial relationships among potential broodstock in order to avoid crossing close relatives and introducing inbreeding depression into the already vulnerable population.

Last year, we reported the discovery and optimization of eight additional microsatellite markers to be used in the genetic monitoring of the Kootenai River white sturgeon population. This report details the development of one additional microsatellite marker for the monitoring project and presents genetic diversity data for 95 white sturgeon sampled by the tribe in 1998, 2002, 2005, and 2006.

Methods

Lake Sturgeon Microsatellite Screening

To increase the number of loci available for parentage analysis, we screened Kootenai River white sturgeon samples with a suite of lake sturgeon microsatellite loci previously developed by the Genomic Variation Laboratory. We performed PCR on a total of 120 lake sturgeon loci (Welsh et al. 2004) and found that seven loci (AfuG 1, AfuG 62, AfuG 68, AfuG 79, AfuG 96, AfuG 98, AfuG 108) amplified and were polymorphic in Kootenai River white sturgeon. Further optimization revealed that one these seven loci, AfuG 68, amplified consistently and exhibited a fair amount of polymorphism. Therefore it was incorporated as the sixteenth marker for genetic analyses. Another newly screened locus, AfuG 62 showed some promise as a good locus for genetic monitoring, but additional optimization is required before it can be used in analyses.

Genetic Monitoring of Adult White Sturgeon

DNA was extracted from 97 adult white sturgeon tissue samples using the PureGene™ DNA Purification System Tissue Kit (GentraSystems). Due to poor DNA yield after several extraction attempts, two samples were excluded from the study, leaving 95 samples for analysis. Sixty-four of these 95 samples were from broodstock used in the KTOI hatchery.

PCR was performed using fluorescently labeled primers for 16 microsatellite loci (Atr 100, Atr 105, Atr 107, Atr 109, Atr 117, Atr 1101, Atr 1173, Actm 2, Actm 35, Actm 43, Actm 52, Actm 53, Actm 110, Actm 177, As015, and AfuG 68). We used an ABI 3130xl capillary electrophoresis instrument to separate PCR products by size and we visualized them in ABI's GeneMapper v4.0 software. Due to the polyploid nature of the white sturgeon genome, microsatellite alleles were treated as dominant data; for each

locus, alleles were scored as either present or absent within individuals (Rodzen and May 2002; Rodzen et al. 2004; Drauch et al. 2006).

Genetic diversity of the white sturgeon samples was examined in two ways. First, all samples were pooled to evaluate the total amount of genetic diversity within all 95 fish. Second, we examined genetic diversity of broodstock used each year to estimate how well overall Kootenai River genetic diversity was represented in each hatchery-reared cohort. Of the four years of sampling data included in this report, only 2002 (N=25), 2005 (N=25), and 2006 (N=27) were subjected to this second analysis. The paucity of samples from 1998 (N=2) precluded this year from that analysis. We estimated genetic diversity in the pooled sample by quantifying the total number of microsatellite alleles per locus and the average number of alleles per individual. For each year, we quantified the number of alleles at each locus within broodstock fish and compared this to the total number for found in the pooled sample.

Results

A total of 104 alleles were found at sixteen microsatellite loci in the 95 Kootenai River white sturgeon. The number of alleles per locus ranged from 3 (Atr 1101, Actm 177, Actm 53) to 12 (Actm 43; Table 1). The average number of alleles per individual at each locus ranged from 1.74 (Actm 177) to 4.63 (Actm 35). Most Kootenai River genetic diversity was represented in broodstock each year, with 2005 broodstock possessing 89% of all alleles, 2006 with 88%, and 2002 with 84% (Figures 1, 2, 3). The newly developed AfuG 68 locus amplified in all 95 individuals and had 10 alleles, making it one of the more polymorphic loci available for white sturgeon.

Discussion

Our results suggest that the KTOI conservation aquaculture program continues to capture most Kootenai River white sturgeon genetic diversity within and among hatchery cohorts. If all crosses result in surviving progeny, nearly 90% of all Kootenai River genetic diversity will be represented among hatchery-reared white sturgeon in each year. Of the three years examined, the 2002 broodstock showed somewhat lower genetic diversity. The number of broodstock utilized in 2002 was 13, which is considerably less than the number spawned in 2005 and 2006 (29 and 22, respectively). There is one male broodstock individual from 2002 that could not be considered in the study (PIT # 7F7D434C08) because the sample was destroyed upon shipping to the GVL. It is possible that we would have observed somewhat higher genetic diversity among 2002 broodstock if we had been able to examine that sample.

Although the number of broodstock used in 2005 and 2006 differs by 7 individuals, there is a very slight difference in the amount of overall genetic diversity represented in each year. With the inclusion of additional sampling years, we should eventually be able to determine a target number of broodstock to be used each year to maximize the amount of genetic diversity represented in hatchery-reared cohorts for minimum sampling effort. Although availability of broodstock will likely vary each year due to environmental fluctuations, we may be able to provide a target number to be used as a guideline in collection.

The addition of a highly variable microsatellite locus, AfuG 68, to the suite of loci to be used in parentage assignment will likely increase the power and accuracy of

assignment. This locus will replace Atr 113 which is being excluded from this study due to apparent sex-specific differences in inheritance (Rodzen and May 2002). If assignment accuracy is still not optimal, additional optimization of AfuG 62 will be conducted so it can be used to generate additional data for analysis.

Future Directions

We are still in the process of converting from the MJ Research BaseStation electrophoresis platform to the ABI 3130xl platform. The ABI 3130xl capillary electrophoresis instrument provides finer resolution in genotyping data; new alleles have been discovered in previously analyzed samples. Therefore, all samples from 2001, 2003, and 2004 must be reanalyzed on the new instrument. We will finish this process and complete genotyping of 2007 and 2008 broodstock samples for next year's report to the KTOI. Broodstock and progeny samples from 2004 must also be genotyped at AfuG 68 so we can test for improvement in parentage assignment accuracy. This also will be completed by the 2008 annual report.

It is notable that three samples could not be included in genetic analysis for this report because of either sample destruction or poor sample quality. We believe this may be due to the storage of samples in lysis buffer after collection. For future sample collection, it may be beneficial to consider storing samples in 95% ethanol to improve DNA quality and increase the number of samples we are able to include in the genetic monitoring program.

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Table 1. The total number of alleles per locus, N_T, and average number of alleles per individual per locus, N_I, for the all 95 adult white sturgeon samples.

Locus	N_T	N_I
Atr 100	5	2.23
Atr 105	4	2.56
Atr 107	9	3.11
Atr 109	8	2.67
Atr 117	8	2.79
Atr 1101	3	2.15
Atr 1173	5	2.77
Actm 2	5	2.98
Actm 35	10	4.63
Actm 43	12	3.81
Actm 52	10	4.55
Actm 53	3	1.79
Actm 110	5	3.13
Actm 177	3	1.75
As015	4	3.00
AfuG 68	10	3.87
Total	104	

Figure 1. The number of alleles found in the 2002 broodstock individuals (N = 12) compared to the total number of alleles exhibited by 95 white sturgeon samples described in this report.

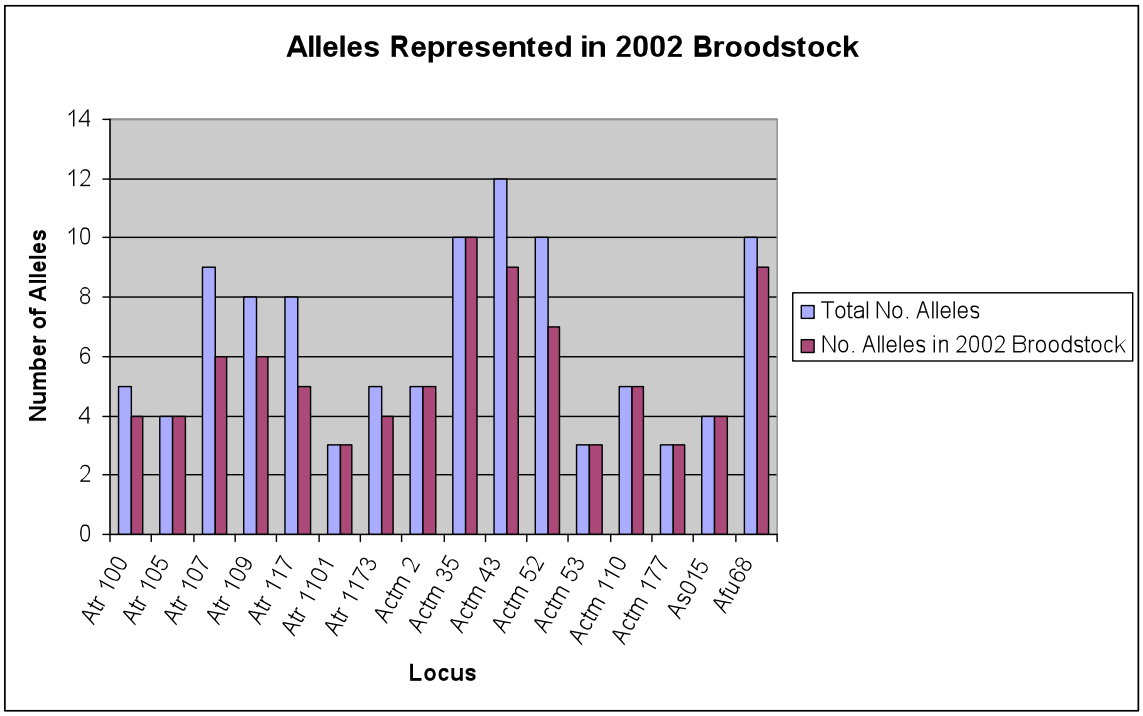

Figure 2. The number of alleles found in the 2005 broodstock (N=29) individuals in comparison to the total number of alleles exhibited by 95 white sturgeon samples described in this report.

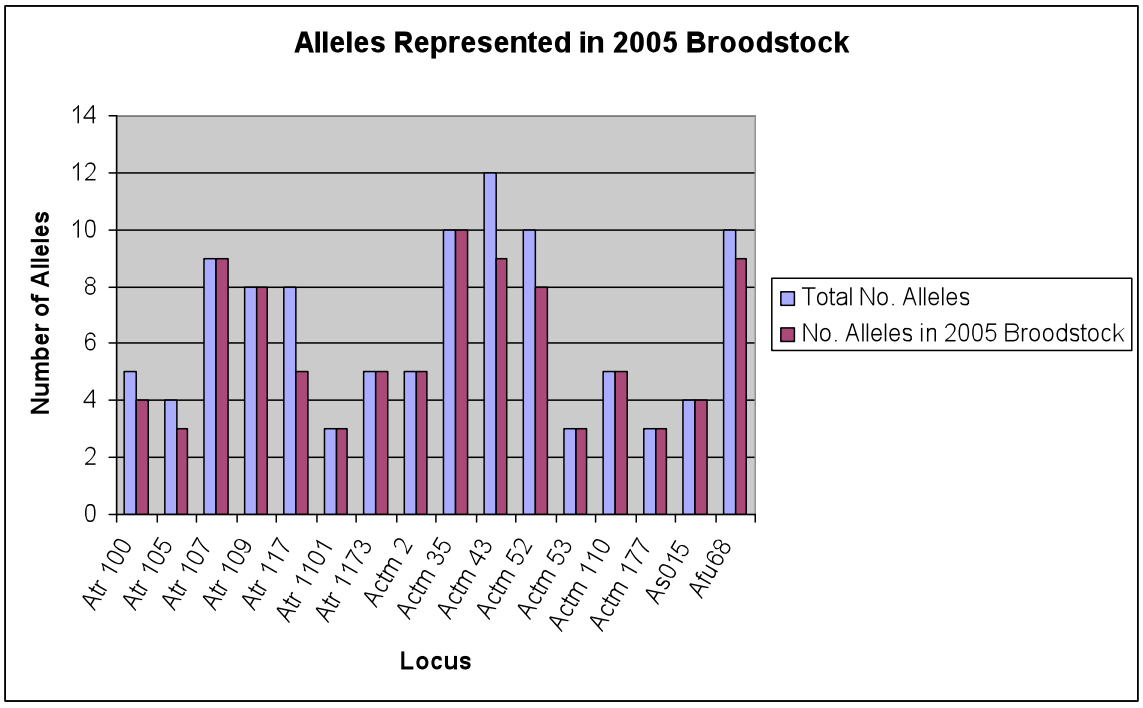

Figure 3. The number of alleles found in the 2006 broodstock (N=22) individuals in comparison to the total number of alleles exhibited by 95 white sturgeon samples described in this report.