Continued 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 2007 and 2008 broodstock for parentage analysis
Deliverable 3): Evaluation of parentage analysis including AfuG 68

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Introduction

In 1994, the Kootenai River white sturgeon population was listed as a federally endangered distinct population segment due to its small size and continued recruitment failure (USFWS 1994, USFWS 1999). The Kootenai Tribe of Idaho (KTOI) initiated a conservation aquaculture program for Kootenai River white sturgeon to propagate the population until natural recruitment was restored. A genetic management plan was drafted for the KTOI conservation aquaculture program with the goal of preserving the remaining genetic diversity in the Kootenai River population. The genetic management plan has two objectives. First, continued genetic monitoring of the white sturgeon population will be conducted to detect declines in genetic diversity over time. Second, all broodstock will be genotyped so that genetic data can be used to determine familial relationships among future adults brought into hatchery for spawning. The Tribe will then design crosses that will reduce mating between relatives. This report continues the genetic monitoring of the Kootenai River population and describes the genetic diversity captured by the Tribe in the 2007 and 2008 broodstock. It also details results from parentage analysis tests incorporating the newly developed microsatellite, AfuG 68

Methods

Genetic Monitoring of Adult White Sturgeon

DNA was extracted from adult white sturgeon tissue samples collected by the Kootenai Tribe of Idaho in 2007 (N = 35) and 2008 (N = 38) using the PureGene^{TM} DNA Purification System Tissue Kit (GentraSystems). Of these adults, 25 and 28 were used by the Tribe as broodstock in 2007 and 2008 respectively. For an unknown reason, we were unable to obtain much data from one broodstock individual, KTF10-08, even after three extraction attempts. Therefore, the number of broodstock with full genotypes available for future parentage analysis is 52.

PCR was conducted with labeled primers for 15 microsatellite loci (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; Drauch and May 2007). Genotyping was conducted on an Applied Biosystems (ABI) 3130xl and allele scoring was completed in GeneMapper v 4.0 software (ABI). Due to the polyploid nature of the white sturgeon genome, each microsatellite allele is treated as a dominant locus and scored as present or absent in each individual (Rodzen and May 2002, Cordeiro et al. 2003, Drauch and May 2007).

Genetic diversity of the Kootenai River adult white sturgeon samples was evaluated in several ways. First, the total number of alleles from adults in 2007 and 2008 was counted for each year and the average number of alleles per individual was calculated as a proxy for heterozygosity. In a second analysis, the 2007 and 2008 broodstock were treated separately, and the number of alleles detected in both broodstock samples was calculated. This provided an estimate of the amount of genetic diversity in the total population that was captured by the broodstock in each year.

Parentage Analysis with AfuG 68

Parentage assignment was conducted with the suite of microsatellite markers described above. The log-likelihood method of Gerber (2000) was implemented in the

program Parent.exe (Rodzen and Famula, unpublished data) to identify the parents of 8 full sibling families from 19 broodstock adults of known sex. The statistic delta (δ) was used to determine how much more likely an assigned parent was to be the dam or sire of a particular individual than another adult in the population. More details about this analysis can be found in Drauch et al. (2006).

The parentage analysis was conducted in two ways. First, parentage analysis was conducted with a dataset excluding Atr 100 (see above) and AfuG 68 to determine if use of a higher resolution genotyping platform increased assignment accuracy. Second, parentage analysis was conducted while including the new marker, AfuG 68, to see if its inclusion increased assignment accuracy. The number of correct assignments was first assessed without the delta criterion, and then a delta criterion of 2.5 (Rodzen et al. 2004, Drauch et al. 2006) was applied.

Results

Genetic Monitoring of Adult White Sturgeon

The total number of alleles detected across the fifteen microsatellite loci was 103, ranging from 3 alleles (Atr 1101, Actm 53, Actm 177) to 14 (Actm 43). The number of alleles per individual ranged from 1.71 (Actm 53 in 2007 sample) to 4.88 (AfuG 68 in 2007 sample; Table 1, Table 2). The total number of alleles detected in the 2007 broodstock was 92, which is 89% of the total number of alleles observed in the population (Figure 1). In 2008, 90 alleles were detected, 87% of the total number (Figure 2).

Parentage Analysis with AfuG 68

In general, it appears that both the higher resolution genotyping platform as well as the inclusion of AfuG 68 in the microsatellite marker suite increased the accuracy of parentage analysis (Tables 3, 4, 5). Without AfuG 68 or the delta criterion, the assignment accuracy of sires increased from 69% to 78% (Tables 3, 5). For dams, the accuracy decreased slightly from 84% to 83% (Tables 3, 5). When the delta criterion is applied, assignment accuracy increased from 79% to 89% for sires and decreased somewhat for dams (93%-89%; Tables 3, 5).

The inclusion of AfuG 68 in the dataset did appear to increase resolution of familial relationships. Without the delta criterion, assignment accuracy increased to 83% for sires and 87% for dams (Table 4). The use of the delta criterion increased accuracy to 88% for sires and 92% for dams, although fewer assignments could be made (Table 4).

Discussion

The number of alleles detected in the Kootenai River white sturgeon population has decreased slightly from what was reported previously (104; Drauch and May 2007). One reason for this change is that the locus Atr 100 has been removed from the marker set due to amplification difficulties. The other is related to the change in genotyping procedures since the initiation of the Kootenai River genetic monitoring program. Previously, genotype data was collected on a BaseStation acrylamide gel electrophoresis platform, but the Genomic Variation Lab (GVL) now utilizes a capillary electrophoresis platform. Capillary electrophoresis is more sensitive than acrylamide gel electrophoresis and we have detected several new alleles using the capillary instrument. To standardize

it with current and future data collection, all Kootenai River white sturgeon genetic data collected on the BaseStation platform now has been re-analyzed on the ABI 3130xl capillary instrument.

As reported in 2007, the KTOI white sturgeon conservation aquaculture program continues to represent most genetic diversity detected in the Kootenai River population. Many of the alleles not captured by the 2007 and 2008 broodstock were alleles found at low frequency, less than 0.05. Often, rare alleles not captured in the 2007 broodstock were captured in the 2008 broodstock, and vice versa. As noted in Drauch and May (2007) the difference of a few individuals between the 2007 and 2008 broodstock did not seem to affect the level of genetic diversity observed in each broodstock; in fact, the 2007 broodstock (N = 25) showed slightly higher genetic diversity than the 2008 broodstock (N = 27). The addition of more data from KTF10-08 may have increased the number of alleles detected in the 2008 broodstock.

In conducting these analyses, we noted that the number of alleles per locus described for the 2007 and 2008 samples differs from the number detected in individuals from a larger population sampled in the lower Columbia River below Bonneville Dam. For example, at Actm 35, a sample of 36 individuals from the lower Columbia River yields an average of 5.72 alleles per locus, compared to 4.74 and 4.76 alleles per locus in the 2007 and 2008 samples from the Kootenai River population (Drauch, unpublished data). This difference can likely be attributed to inbreeding in the Kootenai River population due to historical isolation and declining population size. In small populations, mating between relatives becomes more frequent due to chance. Individuals in inbreeding populations are more likely to possess two alleles that are identical by descent, thereby decreasing the number of unique alleles found in any one individual. It is not possible to quantify the degree of inbreeding in a polyploid population with current analytical techniques. Future work in the GVL involving relatedness analyses for white sturgeon may provide some insight into the degree of mating between relatives in the Kootenai River population. It is important to note, however, a measure of inbreeding alone cannot tell us if the Kootenai River population is experiencing inbreeding depression, a phenomenon of greater conservation importance (Hedrick 2005).

The increase in overall accuracy of parentage assignment with the higher resolution dataset (collected on ABI) appeared to be family specific, as some decreases in assignment accuracy are noted. There are two reasons that the higher resolution dataset may provide differing results from previous analyses. First, as mentioned above, the capillary electrophoresis platform has detected several new alleles in the Kootenai River population and has revealed a few previous errors in genotyping. One reason for a decrease in assignment accuracy, as observed in families 6453 and 6532, is due to missing data. Over time, the DNA extracted from the 2004 full-sibling families has degraded and we had some difficulty in obtaining a complete genotype dataset for these individuals when standardizing data on the ABI. Individuals missing genotypes at informative loci are more likely to be assigned to an incorrect parent. The additional power added by AfuG 68 increased overall accuracy and tended to increase accuracy for dams more than sires. There are some discrepancies between parents and offspring with this marker, however, and additional optimization with this locus may increase assignment accuracy.

As described in Drauch et al. (2006), the delta criterion greatly improves assignment accuracy, particularly when parentage analysis was conducted with AfuG68 (Table 4). Excluding families 6453 and 6532, there is a general increase in the number of assignments possible while implementing the delta criterion when AfuG 68 is included in the dataset. Families 6453 and 6532 do not show this increase likely due to the amount of missing data for individuals within these families. The high accuracy afforded by the delta criterion and an increased ability to maximize the number of assignments possible as shown here makes the implementation of this conservative measure seem more feasible in a hatchery setting. Still, the Tribe will have to decide whether to maximize either the number of assignments possible or the stringency of their assignments. This decision will likely be dependent on the number of broodstock available in a given year.

Future Directions

The GVL will continue to remain involved in Kootenai River white sturgeon genetic monitoring and broodstock genotyping. Storing samples in 95% ethanol as recommended by the GVL in 2007 has increased ease of DNA extraction, PCR, and genotyping. Additional white sturgeon research in the GVL may result in analytical advances that can be applied as appropriate to increase our understanding of the population genetics of the Kootenai River white sturgeon population. These will be reported to the Kootenai Tribe of Idaho as necessary.

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Table 1. Number of alleles per locus (N_T) and average number of alleles per individual (N_I) for the 2007 Kootenai River white sturgeon sample.

Locus	N_T	N_I
Atr 105	3	2.43
Atr 107	9	3.00
Atr 109	7	2.61
Atr 117	7	3.00
Atr 1101	3	1.97
Atr 1173	5	2.57
Actm 2	4	2.65
Actm 35	10	4.74
Actm 43	12	4.00
Actm 52	8	4.51
Actm 53	3	1.71
Actm 110	6	3.63
Actm 177	3	1.82
As015	4	3.09
AfuG 68	8	4.88
Total	92	

Table 1. Number of alleles per locus (N_T) and average number of alleles per individual (N_I) for the 2008 Kootenai River white sturgeon sample.

Locus	N_T	N_I
Atr 105	4	2.59
Atr 107	8	3.19
Atr 109	8	2.51
Atr 117	6	2.92
Atr 1101	3	2.03
Atr 1173	5	2.51
Actm 2	4	2.50
Actm 35	9	4.76
Actm 43	9	3.76
Actm 52	9	4.67
Actm 53	3	1.81
Actm 110	6	3.83
Actm 177	3	1.66
As015	5	3.05
AfuG 68	8	4.58
Total	90	

Table 3. Percentages of correct assignments with AfuG 68 excluded from the dataset. The value of the delta criterion is 2.5 (Drauch et al. 2006). All 2004 broodstock with sex information available was included in this analysis. In parentheses is the percentage of total assignments possible using the delta criterion.

	Without Delta		With Delta	
Family	% Correct sire assignment	% Correct dam assignment	% Correct sire assignment	% Correct dam assignment
KT261A	83	96	100 (54)	100 (87)
KT2972	100	100	100 (87)	100 (92)
KT270D	33[a]	96	60[a] (21)	100 (71)
KT3672	100	100	100 (83)	100 (96)
KT6453	75	33	100 (37)	42 (50)
KT627C	71	100	93 (62)	100 (92)
KT193C	58	96*	73 (46)	93* (58)
KT6532	62	67	90 (42)	75 (50)
Mean	78	84	89	89

*Assignment to 7F7D100A59

[a]True sire likely not sampled (Drauch et al. 2006)

Table 4. Percentages of correct assignments with AfuG 68 included in the dataset. The value of the delta criterion is 2.5 (Drauch et al. 2006). All 2004 broodstock with sex information available was included in this analysis. In parentheses is the percentage of the total assignments possible using the delta criterion.

	Without Delta		With Delta	
Family	% Correct sire assignment	% Correct dam assignment	% Correct sire assignment	% Correct dam assignment
KT261A	83	100	100 (42)	100 (92)
KT2972	100	100	100 (100)	100 (100)
KT270D	29[a]	96	44[a] (17)	100 (71)
KT3672	100	100	100 (100)	100 (96)
KT6453	79	42	100 (54)	58 (50)
KT627C	87	100	94 (75)	100 (96)
KT193C	58	96*	80 (42)	100* (92)
KT6532	71	71	90 (42)	78 (58)
Mean	83	87	88	92

*Assignment to 7F7D100A59

[a]True sire likely not sampled (Drauch et al. 2006)

Table 5. Results of parentage assignment reported in Drauch et al. (2006), before conversion to the ABI platform and before inclusion of AfuG 68. The value of the delta criterion is 2.5 (Drauch et al. 2006). All 2004 broodstock with sex information available (N = 19) were included in this analysis. In parentheses is the percentage of total assignments possible using the delta criterion.

	Without Delta		With Delta	
Family	% Correct sire assignment	% Correct dam assignment	% Correct sire assignment	% Correct dam assignment
KT261A	87.5	92	91 (46)	100 (79)
KT2972	62	92	83 (50)	95 (87)
KT270D	8[a]	83	12.5 [a] (33)	94 (71)
KT3672	100	96	100 (96)	100 (79)
KT6453	92	50	100 (54)	53 (62)
KT627C	92	92	94 (71)	100 (79)
KT193C	75	83*	67 (50)	100* (67)
KT6532	33	92	83 (50)	100 (71)
Mean	69	85	79	93

Figure 1. Number of alleles captured by the KTOI 2007 broodstock relative to the total detected in the Kootenai River white sturgeon population.

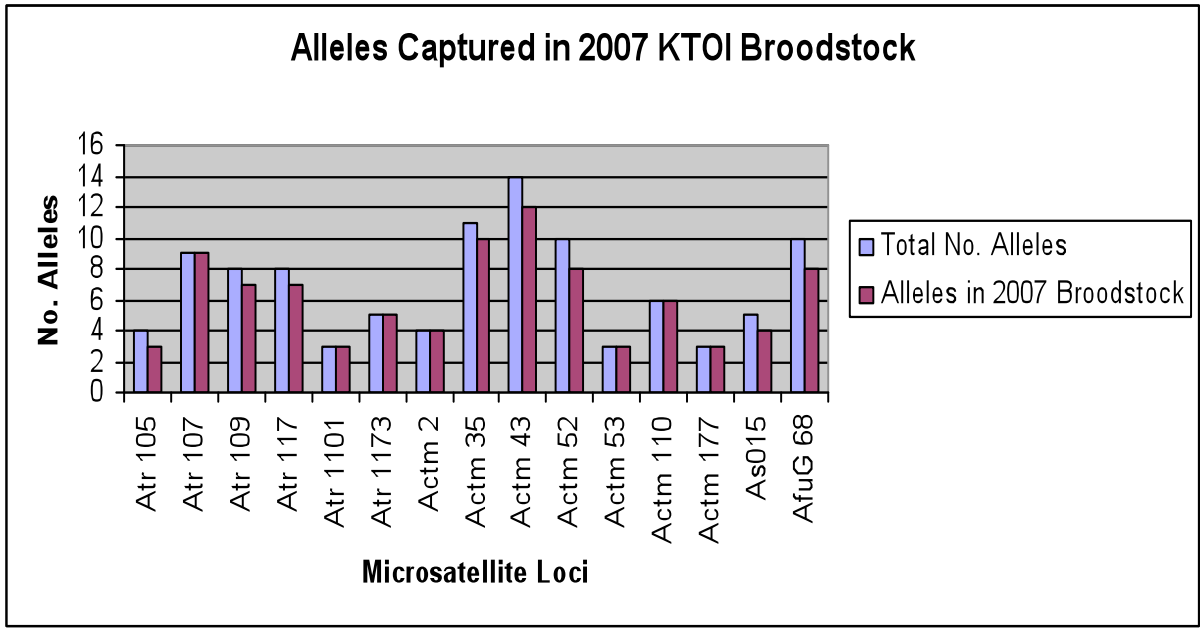

Figure 2. Number of alleles captured by the KTOI 2008 broodstock relative to the total detected in the Kootenai River white sturgeon population.