1520

Interim Report

MICROSATELLITE TOOLS FOR GENETIC IDENTIFICATION OF SCAPHIRHYNCHUS

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ranges from 0.034 to 0.82 with the number of alleles ranging from 4 to 33. An estimate of F_{ST}, an index of genetic differentiation between samples, calculated for each locus independently showed the majority of markers detecting relatively high levels of differentiation for microsatellite markers with several markers showing statistical significant deviations from a null hypothesis of panmixia detected by non-parametric tests of genetic homogeneity (Table 2). F_{ST} calculations combined across all loci had a value of $F_{ST} = 0.0745$. This value corresponds to a highly significant deviation detected by the non-parametric test of homogeneity ($P < 0.001$). The microsatellite markers show significant differences in allele frequency being detected between samples identified as either pallid sturgeon or shovelnose sturgeon by the CI of Willis et al. Therefore, there is at least some level of historical reproductive isolation between pallid sturgeon and shovelnose sturgeon.

Assignment testing has found evidence for two groups of sturgeon in the Middle Mississippi River, and these genetically identified groups largely correspond to morphological and mersitically defined species. A model-based clustering program found strong evidence supporting two clusters in the Middle Mississippi River. This analysis uses genetic data as its sole criterion to search the microsatellite genotype data. These genetic determined clusters largely matched the initial CI species identification (Table 3). However, some CI-identified pallid sturgeon and CI-identified shovelnose sturgeon clustered with the opposite group.

Likelihood based assignment has shown similar results to the model-based assignment (Table 3). Here, 5 shovelnose sturgeon and 1 pallid sturgeon were found that had disagreement between morphological and genetic identification. Removing the 5

shovelnose sturgeon and the 1 pallid sturgeon whose genetic and morphologically identifications are not in agreement results in a baseline data set of 52 pallid sturgeon and 64 shovelnose sturgeon that have perfect agreement between morphological and meristic identification. Further, setting a stringency that the fish have a 0.01 chance of error in assignment or less has 46 of the 52 pallid sturgeon being assigned. This baseline data set could be very useful in future efforts to investigate hybridization and to refine the CI index of Wills et al (2002)

The results to date are very encouraging. We have genetic data which finds strong evidence for two groups in the Middle Mississippi River and these two genetically identified groups largely agree with the initial CI identification. We also have likelihood-based assignment baseline data that identifies 46 of the initial CI identified pallid sturgeon with 0.01 chance of error in assignment or less. However, there are at least 5 shovelnose sturgeon and at least 1 pallid sturgeon that are not in agreement, morphological and genetic identifications do not match. It is possible that the fish which have disagreement are actually hybrid fish, which we should be able to address objectively with data generated in phase II of this project.

Data collection is progressing. We have optimized a total of 20 microsatellite loci and have received pallid and shovelnose samples from cooperating researchers from the Missouri and Middle Mississippi Rivers, with additional samples of shovelnose sturgeon being collected from the Platte and Wabash Rivers. We have CI identified hybrid fish, these hybrids have been culled from the initial data analysis, however they will be used in the next phase of analysis to provide information about the occurrence and prevalence of hybridization between pallid sturgeon and shovelnose sturgeon.

We will also compare pallid sturgeon and shovelnose sturgeon within the Missouri River, then compare the Missouri River fish to Middle Mississippi River, then range wide for the shovelnose sturgeon, to generate information about population structure which could affect the amount of information required in baseline sample for correct assignment. We are confident that these techniques will result in a more clearly defined species boundary between the pallid and shovelnose sturgeons.

Literature Cited

McQuown, E.C., B.L. Sloss, R.J. Sheehan, J. Rodzen, G.J. Tranah & B. May. 2001. Microsatellite analysis of genetic variation in sturgeon (Acipenseridae): new primer sequences for *Scaphirhynchus* and *Acipenser*.

Wills, P.S., R.J. Sheehan, R.C. Heidinger, and B.L. Sloss. 2002. Differentiation of pallid sturgeon *Scaphirhynchus albus* and shovelnose sturgeon *Scaphirhynchus platorynchus* using an index based on meristics and morphometrics. *In* Biology, Management and Protection of Sturgeon a Symposium. Special Publication of the American Fisheries Society, Bethesda, MD.

Table 1. Summary statistics for twelve microsatellite loci currently being used to screen variation in pallid and shovelnose sturgeon. Number of alleles per locus, observed heterozygosity, and expected heterozygosity are reported for all loci. Eight additional loci have been optimized and are currently being used to screen variation. Asterisk denotes a locus that was found to deviate from Hardy-Weinberg equilibrium after sequential Bonferroni adjustment.

Locus	Number of alleles	Observed Heterozygosity	Expected Heterozygosity
Spl-07	4	0.34	.038
Spl-35 *	33	0.77	0.93
Spl-19	12	0.80	0.80
Spl-53	11	0.82	.081
Spl-15	25	0.81	0.85
Spl-30	17	0.57	0.62
Spl-18 *	7	0.47	0.66
Spl-23	13	0.46	0.70
Spl-40	18	0.77	0.87
Spl-56 *	16	0.68	0.89
Spl-12	10	0.55	0.61
Spl-60 *	9	0.77	0.72

Table 2. Estimation of genetic differentiation levels between pallid sturgeon and shovelnose sturgeon identified by morphological and meristic based index. FST estimated for each locus independently and an overall total combining all loci. Significance values calculated by a non-parametric test of genetic homogeneity. Asterisk denotes statistical significance after sequential Bonferroni adjustment indicating a significant amount of genetic differentiation occurs among samples compared.

Locus	F_{ST}
Spl-07	- 0.0079
Spl-35	0.0309*
Spl-19	0.0028
Spl-53	0.0271
Spl-15	0.0272*
Spl-30	0.0612*
Spl-18	0.1946*
Spl-23	0.3384*
Spl-40	0.0072
Spl-56	0.1060*
Spl-12	0.1093*
Spl-60	0.0072
Total	0.075*

Table 3. Assignment testing results presented for two techniques, Model-based and likelihood. CI identity represents the initial identification of species. Number of genetic identifications that match the initial identification provided in number of individual fish, with percentage of agreement calculated for each species and each method.

Method	CI Identity	# Genetic / # CI	Agreement
Model-based	Pallid	47 / 53	89 %
	Shovelnose	64 / 69	97 %
Likelihood			
	Pallid	52 / 53	98 %
	Shovelnose	64 / 69	93 %