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We have completed the microsatellite analyses of the fin clips taken from presumed walleye, *Sander vitreus*, collected from the following locations:

Sample Number	Location	Collected	N	Species	N
4432	Fort Peck Reservoir Nelson Creek	3/28 & 29/12	43	Walleye	43
4433	Yellowstone-Tongue River	3/22-24/20/12	38	Walleye Back Cross to Walleye	37 1
4434	Lake Sakakawea Parshall Bay	4/25/2012	50	Walleye	50

The Fort Peck Reservoir and Lake Sakakawea samples were taken during the 2012 spawning operations and the Yellowstone-Tongue River sample during the spawning season. All of the fish in the Yellowstone-Tongue River sample were tagged for future identification.

Methods

Each fish's genotype was determined at eleven microsatellite loci that distinguish walleye, sauger, *S. canadensis*, and their hybrids (Bingham et al. 2012). We first used the data from these loci to determine whether each individual in the sample appeared to be a walleye, sauger, or hybrid using the reference samples of Bingham et al. (2012) and the program STRUCTURE (Pritchard et al. 2000, 2007) with the number of groups (K) set to two. We then examined the likelihood each sample may have contained individuals from two or more populations by comparing observed genotypic proportions to expected random mating proportions (Hardy-Weinberg proportions) using the Markov chain method of Guo and Thompson (1992) available in GENEPOP version 4.0 (Rousett 2008). Samples containing individuals from two or more fairly divergent populations are expected to contain fewer observed heterozygotes than expected based on Hardy-Weinberg proportions. Since multiple comparisons were performed between samples, in order to account for chance departures from homogeneity due to sampling error we compared the probability values at loci showing significant deviations from Hardy-Weinberg proportions to the modified level of significance proposed by Rice (1989). Next we used the log likelihood G test of Goudet et al. (1996) also available in

GENEPOP version 4.0 to test for genetic differences between pairs of samples. Since multiple comparisons were performed between samples, probability values at loci showing significant allele frequency differences were compared to the modified level of significance. We then used the method of Weir and Cockerham (1984) also available in GENEPOP version 4.0 to partition the total amount of genetic variation between samples showing significant allele frequency differences into genetic variation within the samples and genetic differences between them (F_{ST}). Finally, we used the procedures of Rannala and Mountain (1997) available in GENECLASS2 (Piry et al. 2004) to examine how well individuals based on their multiple locus genotypes could be assigned to their sample of origin and to examine the possibility that the samples may have contained first generation immigrants.

Results and Discussion

Hybridization

STRUCTURE identified all individuals in the Fork Peck Reservoir and Lake Sakakawea samples as being walleye (Figure 1). In contrast, one individual (tag number 7498) collected from the Tongue River was identified as being about 75% walleye and 25% sauger (Figure 1). This fish, therefore, appears to have been a first generation back cross to a walleye. The remaining fish in the sample all appeared to be walleye. The hybrid individual was removed from the data in all subsequent analyses.

Our hybridization results are very similar to those obtained from a range wide population genetic structure analysis of sauger in the upper Missouri River drainage. Using the same eleven microsatellite loci, Bingham et al. (2012) analyzed 954 presumed sauger from 21 different locations. With the same reference samples and K set to two, STRUCTURE identified 875 (91.7%) of the fish to be sauger, 61 (6.4%) to be walleye, and only 18 (1.9%) to be of hybrid origin. The majority (86.9%) of the walleye were collected from Yellowtail Reservoir on the Big Horn River and probably represented stocked fish. Hybrids had a broad distribution but, most (83.3%) were collected from the Tongue River and downstream in the Yellowstone River.

Hardy-Weinberg Proportions

In the samples from Fort Peck Reservoir and Lake Sakakawea, observed genotypic proportions statistically conformed to expected random mating distributions at all the loci analyzed. In the Yellowstone-Tongue River sample, one locus had a significant (P=0.047) deficit of heterozygotes. This difference, however, was not significant at the modified level (0.005). Thus, it most likely represented a chance departure from homogeneity rather than an actual deviation from expected random mating proportions. There was no compelling evidence, therefore, that observed genotypic distributions deviated from Hardy-Weinberg expectations in any of the samples.

Genetic Differences Between Samples

There were significant allele frequency differences between the Fort Peck Reservoir and Lake Sakakawea samples at four loci and between the Yellowstone-Tongue River and Lake Sakakawea samples at one locus. These differences remained significant at the modified level indicating that genetic differences existed between the Lake Sakakawea sample and the others. There was a significant allele frequency difference between the Fort Peck Reservoir and Yellowstone-Tongue River samples at one locus. At the modified level, however, this difference was not significant suggesting it most likely represented a chance departure from homogeneity. Thus, there was no conclusive evidence of genetic differences between the Fort Peck Reservoir and Yellowstone-Tongue River samples.

The lack of detectable genetic divergence between the Fort Peck Reservoir and Yellowstone-Tongue River samples probably reflects that the latter was mainly established from the former. Walleye produced from Fort Peck Reservoir fish have been extensively stocked into the Yellowstone River drainage (Bingham et al.

2012). Furthermore, the Miles City Fish Hatchery has commonly raised walleye of Fort Peck Reservoir origin and certainly could periodically serve as a source of inadvertent stocking of the Yellowstone River.

The amount of genetic divergence between the Lake Sakakawea and the other samples was not large. F_{ST} was only 0.009 between the Fort Peck Reservoir and Lake Sakakawea samples and only 0.002 between the Lake Sakakawea and Yellowstone-Tongue River samples. These values are generally much smaller than that usually observed at microsatellite loci among native walleye populations (e.g. White et al. 2005; Strange and Stepien 2007; Stepien et al. 2008, 2009). This suggests that the Lake Sakakawea and Fort Peck Reservoir/Yellowstone-Tongue River walleye may share a very recent common ancestor or there is significant gene flow between the groups preventing substantial genetic divergence. These two possibilities are certainly not mutually exclusive.

There is some evidence supporting the existence of movement and possible gene flow between the Fort Peck Reservoir/Yellowstone-Tongue River and Lake Sakakawea populations. Of the fish in the Yellowstone-Tongue River sample that were tagged, eleven (tag numbers 7642, 7752, 7829, 7854, 7860, 7871, 7881, 6911, 6928, 6929, 6937) were subsequently recaptured in Lake Sakakawea (Caleb Bollman, Montana Fish, Wildlife & Parks, personal communication) conclusively indicating movement of fish from the Yellowstone River into Lake Sakakawea. Furthermore, three (4%) individuals from the Fort Peck Reservoir/Yellowstone-Tongue River sample were assigned to Lake Sakakawea and six (12%) individuals from the Lake Sakakawea sample were assigned to the Fort Peck Reservoir/Yellowstone-Tongue River sample. Finally, one individual (tag number 7604) in the Fort Peck Reservoir/Yellowstone-Tongue River sample was identified as being a possible first generation immigrant from Lake Sakakawea and two individuals in the Lake Sakakawea sample were identified as being possible first generation immigrants from the Fort Peck Reservoir/Yellowstone-Tongue River population.

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Literature Cited

Bingham, D. M., R. F. Leary, S. Painter, and F.W. Allendorf. 2012. Near absence of hybridization between sauger and introduced walleye despite massive releases. Conservation Genetics 13:509-523.

Goudet, J., M. Raymond, T. deMeeus, and F. Rousset. 1996. Testing differentiation in diploid populations. Genetics 144:1933-1940.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372.

Piry, S., A. Alapetite, J.-M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity 95: 536-539.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Pritchard, J. K., W. Wen, and D. Faulsh. 2007. Documentation for *structure* software: version 2.2. http://pritch.bsd.uchicago.edu/software.

Rannala, B., and J. L. Mountain. 1997. Detecting immigration using multilocus genotypes. Proceedings National Academy of Sciences 94:9197-9221.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103-106.

Stepien, C. A., D. J. Murphy, and R. N. Lohner. 2008. Status and delineation of walleye genetic stock structure across the Great Lakes. Great Lakes Fishery Commission Report 08-22-08.

Stepien, C. A., D. J. Murphy, R. N. Lohner, O. J. Sepulveda-Villet, and A. E. Haponski. 2009. Signatures of vicariance, postglacial dispersal and spawning philopatry: genetics of the walleye *Sander vitreus*. Molecular Ecology 18:3411-3428.

Strange, R. M., and C. A. Stepien. 2007. Genetic divergence and connectivity among river and reef spawning groups of walleye (*Sander vitreus vitreus*) in Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 64:437-448.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

White, M. W., T. W. Kassler, D. P. Philipp, and S. A. Schell. 2005. A genetic assessment of Ohio River walleyes. Transactions of the American Fisheries Society 134:661-675.

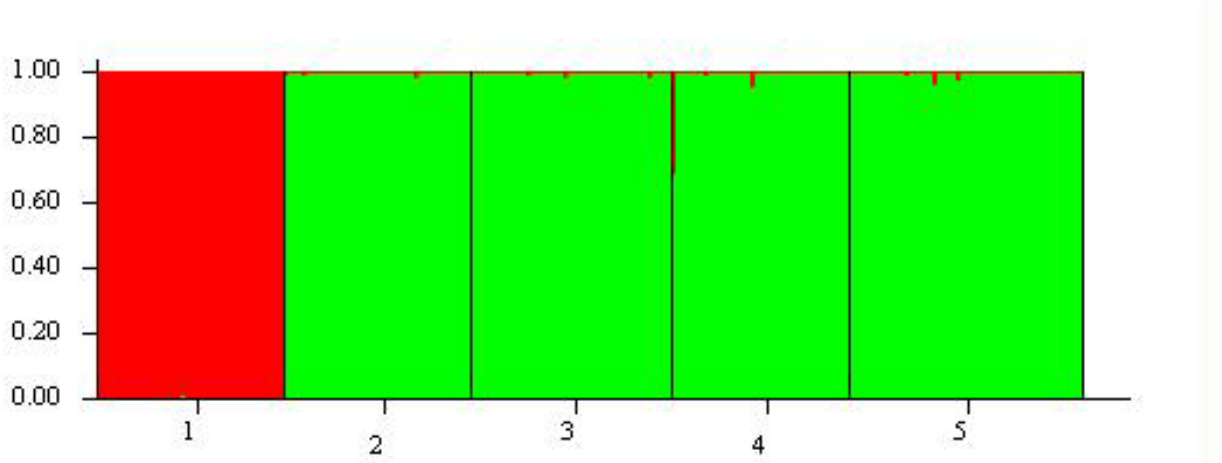

Figure 1. Results of STRUCTURE when the number of groups was set to two. 1=reference sauger sample. 2=reference walleye sample. 3=Fort Peck Reservoir sample. 4=Yellowstone-Tongue River sample. 5=Lake Sakakawea sample. Note all individuals in the latter three samples except one (#1) in the Yellowstone-Tongue River appear to be walleye. The exception appears to be a first generation back cross to walleye.