Genetic analysis of ancestry in Minnesota's stocked muskellunge populations

A Preliminary Report by

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Abstract

The Minnesota Department of Natural Resources (MNDNR) has stocked several strains of muskellunge into native or introduced waters, but the contributions of the various strains to current populations was unknown. The strains derived from two Minnesota Lakes, Shoepack Lake and Leech Lake, and Wisconsin and Iowa hatcheries. The Shoepack strain was of particular interest because MNDNR discontinued stocking it when it displayed poor growth in stocked waters. We used 13 microsatellite DNA markers to determine the ancestry of muskellunge in 12 stocked populations and assessed the effect of Shoepack strain ancestry on fish size. Shoepack strain ancestry was not detected in almost half of the lakes despite years of stocking up to the early 1980's. When present, Shoepack strain ancestry was relatively low (0.03-0.18 of overall ancestry), but appeared to affect fish size. For example, in three lakes, fish with Shoepack strain ancestry had a 7-50% lower percentage of legal-sized individuals (> 40" or 1016 mm total length) than fish without Shoepack strain ancestry. Wisconsin strain ancestry was relatively high, given the few years the strain was stocked, but was not detected in one lake in which it was stocked. Iowa strain ancestry, deriving from a single year of stocking, was present in Lake Mille Lacs. In addition to assessment of stocking impacts, our study revealed considerable genetic diversity among the native muskellunge populations in Minnesota.

Background

Minnesota has one of the nation's premier muskellunge fisheries, due to prudent use of management tools—restrictive seasonal and size regulations, voluntary catch and release, and the creation of new muskellunge waters by stocking [see Wingate and Younk (2007) for a review of Minnesota's muskie program]. But for muskies, like no other Minnesota fish species, size matters. The history of muskie stocking in Minnesota includes a period when Shoepack Lake broodstock was the primary source of stocked fish. The MNDNR developed the Shoepack broodstock in the 1950s and stocked their descendents for over 30 years in many Minnesota lakes (Younk and Strand 1992). Shoepack Lake was chosen as a source because of its abundance of muskies that enabled capture of adequate numbers of spawners to establish a robust broodstock. The spawners collected in Shoepack Lake were typically small, but it was originally assumed that this was due to stunting in the dense population (Wingate and Younk 2007). By the 1970s, indirect evidence suggested that Shoepack Lake fish may have smaller size potential than other native and stocked muskie populations. Lakes with introduced Shoepack populations produced few fish over 40 inches, and there were perceived declines in numbers of large fish in lakes that had native populations (Minnesota Department of Natural Resources (MNDNR) unpublished data; Wingate and Younk 2007). In response to the observed poor size structure in lakes stocked with Shoepack muskies, the MNDNR conducted a growth experiment by stocking Shoepack, Leech Lake and Wisconsin strain muskies simultaneously into two lakes. The experiments supported a genetic basis for slower growth and smaller predicted maximum size by Shoepack muskies (Younk and Strand 1992). Shoepack muskies are no longer stocked but the genetic legacy of their introductions may be a reduction in size potential of their descendents, if they exist, in current muskie populations.

Shoepack Lake has not been the only source of muskies stocked in Minnesota. When the lower growth potential of Shoepack fish was recognized, the MNDNR transitioned to a new source population. For a few years, generally in the mid-1980s, MNDNR stocked fish derived from Wisconsin hatchery sources. Wisconsin strain stocking occurred as MNDNR developed its current muskie broodstock derived from Leech Lake spawners. Leech Lake muskies were first stocked in the 1980s and they have been the only source stocked since 1990. Muskie populations in Minnesota may now descend from various combinations of native, Shoepack, Leech and Wisconsin ancestors.

Recently developed molecular genetic tools (Sloss et al. 2008) allowed us to determine the ancestry of muskies in populations stocked with fish from one or more source populations. With these tools we could estimate both the ancestry from multiple sources in the overall population and the ancestry of each individual fish, so that size and ancestry could be compared. This report describes preliminary results based on 21 samples from 14 Minnesota muskellunge populations with past or ongoing stocking, plus three source populations. We included lakes with various histories; those stocked with one, two, or all three source populations (Shoepack, Leech, and Wisconsin). Our study had two main objectives:

Objective 1: to determine the ancestry of muskellunge in stocked populations
throughout Minnesota
Objective 2: to assess the effect of Shoepack strain ancestry on fish size

This report is intended to document the significant progress we have made in examining muskellunge ancestry in Minnesota and provide an initial, tentative assessment of our findings. We are still adding a few populations and additional samples for some populations. All results and interpretations should be considered preliminary. The power of these techniques is that they use information from all samples to best infer the genetic differences among populations and the ancestry of individuals. A new evaluation when all data are collected will be required to make a final evaluation of ancestry. That said, our experience has been that many aspects of our results change little as new data are included. This has held true especially for the estimation of Shoepack ancestry.

Methods

Lake selection and sample collection

We chose muskie populations to study based on the availability of scales samples collected during MNDNR spring assessment netting (Table 1). We included lakes with the following stocking histories: those with a single source (Shoepack) introduced or stocked over native populations; those with two sources stocked, some combination of Shoepack, Leech and Wisconsin; and those with all three sources stocked. Some lakes had multiples samples, beginning as early as 1981, to assess changes in ancestry over time. All lakes had at least one sample collected from 1995-2008. Jerry Younk, Steve Mero, and Rick Brusewitz provided scales from archived MNDNR collections.

Genetic data collection and analysis

We genotyped individual fish at 13 microsatellite DNA markers developed specifically for muskellunge (Sloss et al. 2008). We used single fish scales as our source of DNA. The 13 microsatellite markers were screened using the DNA amplifying technique polymerase chain reaction (PCR). The DNA fragments produced by PCR were submitted to a core facility on the UMN campus for electrophoresis. We processed the resulting data files to score fragment sizes, and thus determine genotypes, for each individual.

We used the genetics software Structure (Pritchard et al. 2000) to determine the ancestry of individual fish based on their genotypes at the 13 microsatellite markers. The genotypes we resolve correspond to variation in DNA sequences on the fish's chromosome pairs. Each variant sequence is called an allele. Fish from the same population tend to share the same alleles, while different populations often have different alleles or different frequencies of the same alleles. When distinct populations are mixed, for example through stocking, it can be possible to determine the ancestry of individuals by tracing their alleles back to their ancestral source populations. Structure uses mathematical rules to estimate the number of populations contributing to a sample of individuals and the ancestry of each individual in the sample.

For each group of lake with a common stocking history, we included genotype data for samples from the lakes themselves and from known source populations stocked into the lakes, when available. We collected genotype data for Shoepack Lake (N = 40) and Leech Lake (N = 29), two of the major known muskie stocking sources in Minnesota lakes. We also obtained data from Tomahawk Lake, Wisconsin, which is thought to be in the area from which Wisconsin strain fish originated. Genotypes from Leech Lake and Tomahawk Lake were provided by B. Sloss (USGS Fisheries Cooperative Research Unit – Steven's Point, WI). Genotype data from known populations "help" the program estimate the true genetic profile of these populations and increases the accuracy of ancestry estimates where they are stocked.

Length comparisons by ancestry

We evaluated the relationship between individual ancestry and fish size, with an emphasis on the effect of Shoepack strain ancestry. We did not directly compare growth rates (i.e., length at the same age), because older muskies are very difficult to age using scales. Instead, we compared length distributions of fish with different ancestries, grouping those with and without Shoepack strain ancestry. For lakes with sufficient numbers of Shoepack descendents, we compared median lengths and the percentage of fish with and without Shoepack ancestry among those >1015 mm (40 inches), a common size limit at one time in Minnesota.

Results

We have genotypes of 1257 muskellunge from 24 samples of 17 populations, including three stocking source populations. To determine ancestry, we ran STRUCTURE on combined sets of samples with common stocking histories, and included just the corresponding source populations known to be stocked in the lakes. We present a series of figures that depict the estimated ancestry of individuals in our samples. These are the color images produced by STRUCTURE to visualize results, where each color corresponds to genetically-distinct ancestry. Each vertical bar represents an individual fish, and the different colors represent distinct genetic ancestries. Bars all of one color represent "pure" individuals, which could be stocked fish or pure offspring produced in the lake. Bars with multiple colors represent mixed ancestry, i.e., naturally-produced descendents from multiple sources intermating in the stocked lakes. Colors can not be chosen within the program, so note the correspondence of colors and ancestries given below each figure. The figures capture the big picture of overall genetic differences in Minnesota muskie populations and the effects of stocking various source populations. More detailed analyses of ancestry will be provided in the final project report. We also provide estimates of overall Shoepack strain ancestry in these populations and evaluate the relationship between Shoepack ancestry and fish size for select lakes.

Ancestry by lake

Lakes stocked with Shoepack or Shoepack and Wisconsin

Four lakes were stocked only with Shoepack strain muskies or Shoepack and Wisconsin strain and the time our samples were taken, including Big Mantrap in 1984, prior to subsequent stocking of the Leech strain (Figure 1). Spider Lake has been stocked only with Shoepack strain, and shows a perfect correspondence with the Shoepack sample (they are the same color, red), indicating that our analysis can distinguish populations and ancestry. Big Mantrap had a native population, but was stocked numerous times with Shoepack fish to create a broodstock lake. It too shows a strong correspondence with Shoepack. Only Shoepack strain had been stocked at the time of our 1981 Moose Lake and Deer Lake samples, and both Shoepack and native fish (green) were present. The Wisconsin strain (blue) had been stocked by the time of our later samples, and all three strains survived and reproduced in both lakes, as indicated by the mixed individuals between all strains.

Lakes stocked with Shoepack or Shoepack and Leech

The lakes stocked with Shoepack and Leech include four not discussed previously (Figure 2). The repeated Big Mantrap, Spider and the early Moose Lake and Deer Lake samples all show the same population correspondence as in Figure 1. Of the new samples, Little Boy Lake and Lake Wabedo form a genetically distinct group (yellow) with some Leech strain influence and a small amount of Shoepack strain ancestry in Lake Wabedo. Cass Lake shows no Shoepack influence and also forms a genetically distinguishable group (blue). Big Lake is essentially indistinguishable from Leech Lake, indicating either that it is entirely descendents of Leech strain stocking, or that its native population was similar to that of Leech Lake. The latter seems less likely because most other lakes have distinguishable populations, except those in very close proximity (e.g., Little Boy and Wabedo). Baby Lake was removed from analysis because of its small sample size. This population appeared to have some, but not entire, Shoepack ancestry.

Lakes stocked with Shoepack, Leech and Wisconsin

Five new lakes are included in this analysis, along with a Big Mantrap Lake sample taken after the Wisconsin and Leech strains were stocked (Figure 3). The 2004 Big Mantrap Lake sample shows that both strains have survived and reproduced, largely swamping out the previously predominant Shoepack ancestry. Lake Bemidji appears to be entirely Leech strain ancestry, although like Big Lake it also could have a native population similar to Leech. A one-time Wisconsin strain stocking appeared to leave no descendents. Sugar Lake, Beers Lake and West Battle Lake all have introduced populations. Almost all individuals appear pure, including a single Shoepack fish in Beers Lake, indicating that these may likely be stocked fish. Only one fish in West Battle Lake appears to have mixed ancestry, which would indicate natural reproduction.

Mille Lacs had ancestry from additional origins besides the known Leech, Wisconsin and Shoepack strains. A fourth ancestry (pink) presumably corresponds to one year of stocking fish obtained from a Spirit Lake, Iowa hatchery, which may have derived from some unknown Wisconsin muskellunge population. The sample labeled 1991-94 is composed of fish assigned to year-classes by aging (it is not a random sample of the population). All fish assigned to the year-class of Iowa stocking had the pink color. A fifth unknown strain (yellow) occurs at a relatively low percentage.

Shoepack ancestry and fish size

All but one of our study lakes was stocked with Shoepack strain fish but the remaining Shoepack ancestry was generally low, excepting Spider Lake, which was introduced with Shoepack strain only (Table 2). We found no Shoepack ancestry in five lakes, and only one Shoepack fish in another lake. The five remaining lakes had 0.06-0.18 Shoepack ancestry. Note that the actual proportion of fish with at least partial Shoepack strain ancestry is higher in these populations because most Shoepack descendents had mixed ancestry (see Figures 1-3); and for example, two fish with 50% Shoepack ancestry would only have the ancestry contribution of one pure (100%) Shoepack fish.

Shoepack strain ancestry was generally associated with smaller size (total length) in three of the lakes with enough fish for comparisons (Figure 4). In the 2004 sample from Moose Lake, few fish >40” (1015 mm) had Shoepack strain ancestry, and it was mixed ancestry in all cases. Fish >40” comprised 30% of all fish without Shoepack strain ancestry but only 9% of fish with Shoepack ancestry. In the 2003 Deer Lake sample, the size differences were not as evident, but fish >40” comprised 31% and 24% of fish without and with Shoepack ancestry, respectively. The 2004 Big Mantrap Lake sample showed the greatest disparity, 73% versus 15%. Only eight fish exceeded 48” (1219 mm) in all three samples, and only one of these had any Shoepack strain ancestry, and Deer Lake individual with approximately 25% Shoepack and 75% Moose/Deer ancestry.

Discussion

Using microsatellite DNA genetic markers to determine ancestry, we have shown widely varying impacts of stocking muskellunge in Minnesota. Shoepack strain ancestry was not detected in almost half of the lakes despite years of stocking. When present, Shoepack strain ancestry was not prevalent (0.03-0.18 of overall ancestry). Shoepack strain ancestry does appear to limit the size potential of muskellunge in stocked waters, as Younk and Strand (1992) found in side-by-side stocking experiments, but this only appears to be an issue for introduced Shoepack populations, such as Spider Lake, and the few other lakes we identified with some remaining Shoepack ancestry.

Wisconsin strain ancestry was usually relatively high (see Figures 1 and 2), given the few years they were stocked, but was not detected in one lake (Lake Bemidji). Individuals with Wisconsin strain ancestry were common among the larger fish (data not shown). The Iowa strain, also thought to have originally derived from Wisconsin, was stocked just one year in Mille Lacs but was fairly common in the 2006 sample.

In addition to assessment of stocking impacts, our other key finding was the considerable genetic diversity among the native muskellunge populations in Minnesota. The genetic differences among populations are what made the identification of stocked ancestry possible because “non-native genes” could be traced in stocked populations. It is important to note, however, that these are differences in genetic markers, not necessarily genetic traits (for example: growth, survival, disease-resistance, etc.). The differences we see indicate that the populations form common gene pools, reproductively isolated from each other. When gene pools become isolated, the potential for genetic trait differences among populations becomes possible. This is apparently what happened with Shoepack Lake muskies and growth. Genetic trait differences among other Minnesota populations are possible, but have not been evaluated.

We are continuing to add a few samples and will then analyze the full data set to form final results and conclusions. Our final report will include more details on the lake-by-lake ancestry from all sources and fish size related to all ancestries. A manuscript on our early work with the temporal samples from Moose Lake is in revision for a fisheries journal (Miller et al. submitted).

References

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