Final report on genetics of catostomid suckers in the Muddy Creek drainage, WY

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Introduction:

Threatened fish in the western United States include several native species in the family Catostomidae. The purpose of this study was to assess the genetic structure of potential hybridization between two native species – the bluehead sucker *Catostomus* (*Pantosteus*) *discobolus*, and the flannelmouth sucker *Catostomus latipinnis* – and an introduced species, the white sucker *C. commersoni*. Here I report the results of using Amplified Fragment Polymorphism (AFLP) genetic analyses to reveal the patterns of introgression between the three species.

Muddy Creek, south of Rawlins, is a tributary of the Colorado River (Figure 1). The two native species have often been considered to be in different genera (*Pantosteus* vs. *Catostomus*), and natural hybrids between the two appear to have been rare or non-existent prior to the accidental introduction of the white sucker (*Catostomus commersoni*), which is native to drainages east of the continental divide (such as the Laramie River). The introduction of white sucker to the Muddy Creek drainage was likely an inadvertent by-product of bait-bucket transfer by anglers in the Saratoga Valley region. Similar inadvertent introductions appear to have occurred in Colorado. Since their introduction, white suckers are known to have hybridized readily with the native flannelmouth sucker, and it now appears that it may be difficult to find pure populations of flannelmouth sucker anywhere in their range in the western United States. Prior to this research, no genetic studies had been conducted to assess the genetic status of these three species where they co-occur.

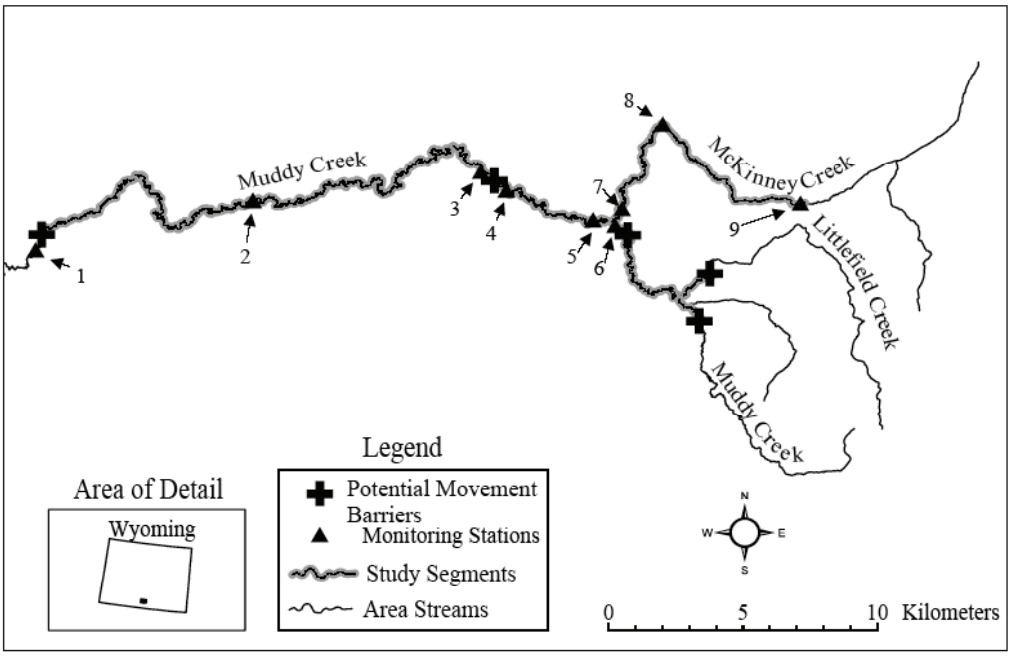

Fig. 1. Muddy Creek drainage in southern Wyoming, flowing eventually into the Colorado River. Above McKinney Creek is Bitter Creek, where a pure population of flannelmouth may be protected by natural barriers from the genetic introgression of white suckers.

Methods:

Field sampling: The analyses are based on 122 fish collected by BLM biologist Mike Bower along the course of Muddy Creek, plus a sample of 10 apparently pure bluehead suckers from Ringdahl Reservoir in western Wyoming, where white suckers do not occur. At the time of capture, these individuals were classified by phenotype (appearance) as one of six forms: 1) "pure" flannelmouth (color-coded red in all figures), "pure" bluehead (color-coded blue in all figures), "pure" white (color-coded yellow in all figures) or any of the three crosses (bluehead/white,

flannelmouth/white, bluehead/white; color-coded with the appropriate combinations of red, yellow and blue in all figures). All analyses are based on comparing the phenotype to the genotype.

Laboratory genetic approach: All analyses were by Amplified Fragment Polymorphism (AFLP). AFLP uses short DNA sequences to amplify regions of the nuclear DNA with the polymerase chain reaction (PCR). The amplified fragments can then be scored with an automated DNA sequencer/genotyper. The resulting DNA fragment profiles provide a large array of independent, dominant markers from which to assess the genetic variability within and among the species. All samples were genotyped in the Nucleic Acid Exploration Facility at the University of Wyoming, using an *ABI 310*™ capillary sequencer. Tom Parchman of the Dept. of Zoology & Physiology conducted all the laboratory analyses.

Tree-building analyses: We created a pairwise matrix of relatedness, *r*, among individuals using the method of Lynch and Milligan (1994) with the program *AFLP-Survey* (Vekemans et al., 2002). The measure 1-*r* served as the basis for a distance matrix. We also used the 0/1 matrix as a binary gene frequency measure for individuals in order to create a matrix of Cavalli-Sforza chord distances (Cavalli-Sforza and Edwards, 1967) among individuals. From each of these distance matrices we created an unrooted neighbor-joining tree with individuals as the operational taxonomic units (OTU), using the *Neighbor* routine of *PHYLIP* (Felsenstein 1995). We also used the gene frequencies for each of the six forms to create 1,000 bootstrapped replicates of Nei's standard distance (Nei, 1972) from which we calculated neighbor-joining trees in *Neighbor*. We then used PHYLIP's *Consense* routine to calculate the unrooted majority rule consensus tree and the bootstrap support for the nodes.

Assignment tests: The Bayesian approach of Pritchard et al. (2000), implemented in the program *Structure* (Pritchard, 2005), assesses whether the sampled genotypes are substructured into multiple ($K > 1$) clusters or constitute a single Hardy-Weinberg population ($K = 1$). Log-likelihood ratios from Monte Carlo Markov chain sampling provide the basis for deciding what number of clusters best fits the data. We used *AFLP-Survey* to create the input file for *Structure*. Using the gene frequencies for each of the three sets of phenotypically pure types (white, flannelmouth, bluehead) we calculated genotypic probability scores as the natural logarithm of the product of the 190 gene frequencies (Paetkau et al. 1995). As a correction for zero-frequency alleles, we assumed a frequency of 0.005 (Paetkau et al. 1995). Each individual thus had a likelihood score for each of the three parent forms. The resulting three-dimensional plot, whose points occurred in an oblique plane, was then reduced to two dimensions by principal components analysis using *Mathematica*™ software.

Results:

We generated AFLP genotypes for 132 individuals at 189 polymorphic loci using 2 selective primer combinations. Allelic frequency differentials averaged 0.27 for flannelmouth versus white and greater than 0.45 for bluehead versus either of the other species. For the flannelmouth white comparison, no loci showed fixed differences (present in all of one species while absent in the other), with a maximum differential of 0.86. In contrast, 15 loci were fixed between bluehead and flannelmouth, and 21 between bluehead and white. Using distances calculated as 1-*r* from a matrix of individual relatedness (Lynch and Milligan, 1994) we constructed a neighbor-joining dendrogram for the 132 individuals (Fig. 2). With the caveat that individual relatedness should often be expected to be more reticulate than should higher-level relationships, we note that the bluehead suckers fall out as a monophyletic unit, whereas the flannelmouth suckers are interleaved with flannelmouth/white hybrids. An unrooted neighbor-joining tree for the six forms (three parentals and the crosses among them) places the introduced white sucker between the two native species, and makes the white sucker the nearest neighbor of the bluehead/flannelmouth crosses (Fig. 3).

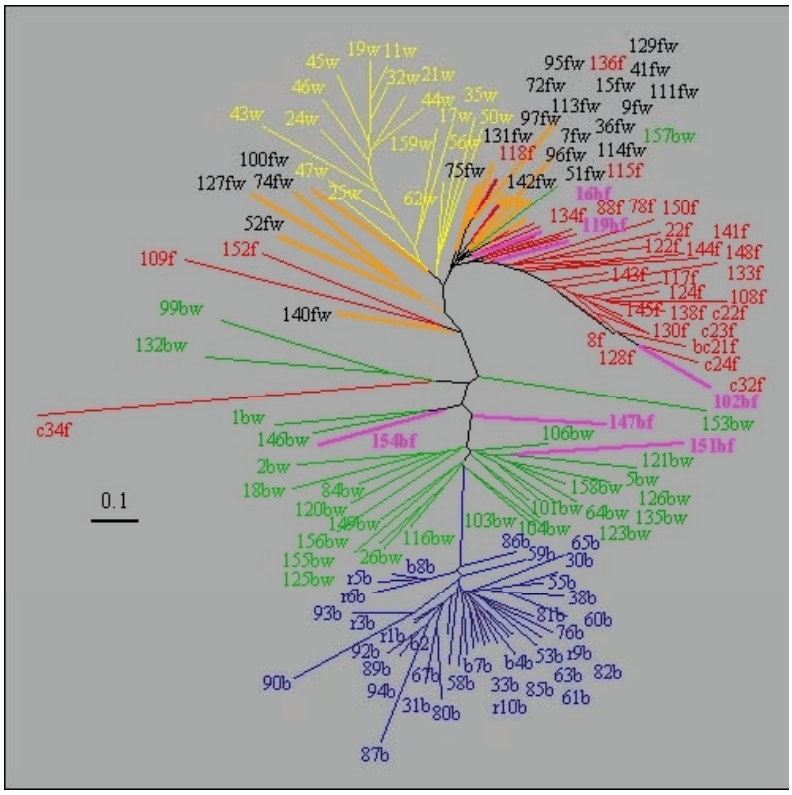

Fig. 2. Dendrogram of genetic relationships among phenotypically pure native bluehead and flannelmouth suckers and introduced white suckers, as well as hybrids among those forms, from the Muddy Creek drainage of the Colorado in south-central Wyoming. Assessment of pure versus hybrid phenotype occurred in the field, largely on the basis of mouthpart morphology. Note that bluehead phenotypes (blue) form a cohesive monophyletic unit (all individuals descend from a single common branch), that bluehead/white crosses (green) also occur as a fairly cohesive unit, but that flannelmouth/white crosses (orange) and especially flannelmouth/bluehead crosses (purple) are more scattered in their genetic relationships. The unrooted neighbor-joining tree was constructed from a matrix of pairwise relatedness (1-*r*) calculated according to Lynch and Milligan (1994) using the software *AFLP Survey*. The genotypes were for 189 AFLP loci arising from two primer pair combinations.

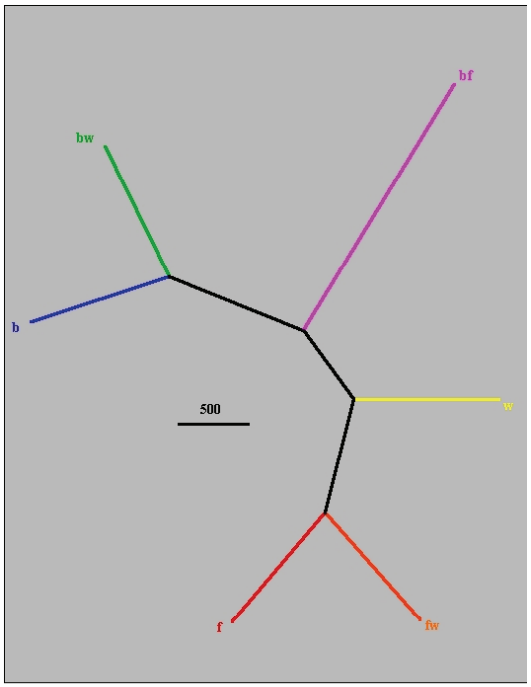

Fig. 3. Dendrogram of genetic relationships among six forms of suckers from the Muddy Creek drainage of south-central Wyoming. The neighbor-joining tree was the consensus majority rule tree from 1000 bootstraps of a Nei's (1972) distance matrix. Note that bluehead/flannelmouth crosses (purple) have white sucker (yellow) as the nearest neighbor, consistent with the individual genotypes and other results suggesting that bluehead/flannelmouth crosses arise from crosses between a pure form and an intermediate hybrid form (either bluehead/white or flannelmouth/white).

Using gene frequency likelihood method for three-way assignment to the three parent species, we assessed the likelihoods of the 132 genotypes, With principal components analysis, the first principal components explained 90.4% of the variance in the data, and the second explained an additional 8.7%, combining to explain 99.1% of the variance. This supports the observation that in the full three-dimensional probability plot, the points lie close to a plane, meaning that two-dimensional plotting causes minimal loss of information content (Fig. 4).

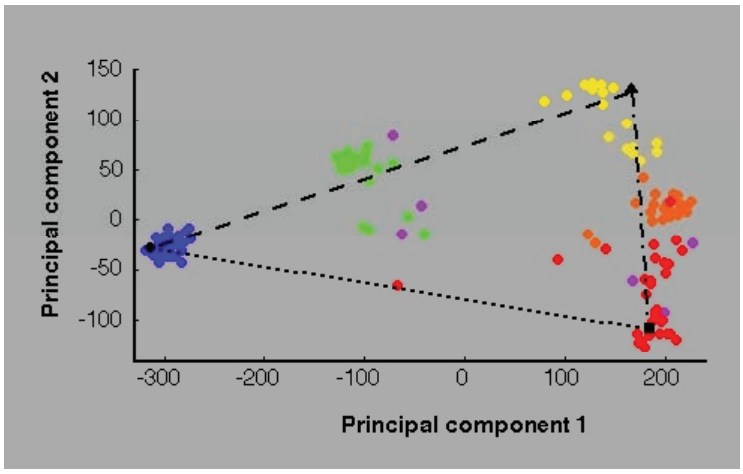

Fig. 4. Genetic variation, based on gene frequencies for AFLP genotypes, for bluehead (blue), flannelmouth (red), and white suckers (yellow) plus the three types of crosses among those species (green, purple and orange). The "pure" forms (bluehead, flannelmouth and white) should

lie in the corners of the triangle. Hybrids (green, purple and orange) would be expected to fall along the connectors (dashed or dotted lines) between the centroids (solid black symbols). While many of the bluehead/white hybrids (green) lie close to the white/bluehead connector (dashed line), others lie closer to the bluehead/flannelmouth connector (dotted line), suggesting that those individuals may have a complex pedigree involving repeated backcrossing. Likewise, some phenotypically pure flannelmouths (red points) lie far from either their centroid (black square) or the white/flannelmouth connector (dot-dash line). Especially notable is that no bluehead/flannelmouth hybrids (purple points) lie close to the bluehead/flannelmouth connector (dotted line), instead showing strong deviations toward the white sucker apex (yellow points and black triangle centroid). That suggests that flannelmouth and bluehead cannot hybridize directly, but require complex backcrossing involving hybrids with white sucker. Note also that all the bluehead points cluster close to their centroid (black circle) and are very distinct along the *X*-axis (*PC1*, which explains 90.4% of the variance). In contrast, the scattering for white suckers (yellow points) away from their centroid (black triangle) suggests that most white suckers may descend from lineages introgressed with the genomes of the two native species. Finally, note that the transition from white sucker (yellow) to hybrids (orange) to phenotypically pure flannelmouth (red) is a continuous spectrum paralleling the short *Y*-axis (*PC2*, which explains 8.7% of the variance) along the dot-dash line. Taken together, the evidence suggests that introduced white suckers have acted as a "bridge" that allows gene flow between two native species that previously had strong isolating barriers. The plot is based on genetic likelihood of assignment for each fish to one of the three "parental" species. Dimensionality of the three likelihoods was reduced by principal components analysis for plotting; the raw three-dimensional likelihoods lie very close to a triangular plane shaped like the plot above.

Discussion:

The continuous genetic variation between phenotypically pure flannelmouth and phenotypically pure white suckers suggests ongoing hybridization between the two species resulting in a hybrid swarm. Many of the "white" suckers from Muddy Creek appeared to be hybrids genetically, whereas all the phenotypically pure bluehead suckers appeared to be distinct from any of the crosses. Further, the blueheads from Muddy Creek (white sucker present) were genetically indistinguishable from the Ringdahl Reservoir blueheads (white sucker absent). Taken together, these data suggest that flannelmouth suckers may be in danger of extinction via hybridization (Rhymer and Simberloff, 1996).

Some individuals (those in the center of the triangle in Fig. 4) appear to have elements of the genomes of all three species. These multi-generation hybrids may represent a novel threat to the genetic integrity of the bluehead sucker. We suggest that these hybrids may represent a breakdown of the isolating mechanisms that previously separated the flannelmouth and white suckers. Thus, the introduced white sucker may eventually act as a bridge that permits a hybrid swarm involving all three species. Such a pattern has strong conservation implications. The continued presence of white suckers, and of hybrids between white and flannelmouth suckers may eventually result in a higher frequency of hybridization with bluehead suckers, thus presenting a threat to a hitherto distinct species sometimes placed in its own genus. Any such three-way hybridization would exacerbate the ongoing "homogenization" of western fishes (Rahel 2000).

Three major conclusions emerge from the genetic analyses

- First, phenotype is generally a fairly reliable guide to genotype. That is, individuals that looked like a bluehead/white cross, for example, were generally genetically intermediate between bluehead and white. Nevertheless, several interesting exceptions emerge, as discussed below and in the legend for Figure 4.

- Second, the ongoing hybridization between the white sucker and the flannelmouth sucker (both in the genus *Catostomus*) has led to a "hybrid swarm" in which a continuous spectrum exists from pure flannelmouth to pure white and everything in between (see right-hand edge of Fig. 4).
- Third, only six individuals were found that appeared to be bluehead/flannelmouth crosses. All such individuals were retained in the genetic sample, whereas the specimens collected for the other crosses and for the "pure" forms were only a subsample of the total set of captures. This suggests that the bluehead/flannelmouth cross occurs only under special circumstances. That conclusion is borne out by the genetic analyses. Contrary to expectation, and contrary to what we found for bluehead/white crosses, the bluehead/flannelmouth crosses were **not** genetically intermediate between bluehead and flannelmouth (see Fig. 4). Instead they show clear evidence of also containing genetic material from a white sucker ancestor. That suggests that these crosses arise from a complicated cross between one of the other crosses. For example, some might be crosses between a bluehead/white and a flannelmouth/white. The path may even be more complex, involving multiple generations before it is possible to get the mixing of genetic material from the flannelmouth and the blueheads. The genetic pathway to bluehead/flannelmouth crosses is at the core of one part of the ongoing genetic research.

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