The University of
**Montana**

Division of Biological Sciences
Missoula, Montana 59812-1002
(406) 243-5122
FAX (406) 243-4184

November 13, 1995

Doug Perkinson
Fisheries Division
Kootenai National Forest
506 Highway 2 West
Libby, Montana 59923

Doug:

The electrophoretic analysis of the trout samples collected from the Big Cherry Creek drainage in the Kootenai National Forest have been completed:

| Sample | Collection Date | Location | N |
|---|---|---|---|
| Lower Big Cherry Creek | 10/05/94 | T29N R31W S11A | 25 |
| Middle Big Cherry Creek | 09/30/94 | T29N R31W S27C | 25 |
| Deep Creek | 09/29/94 | T29N R31W S21B | 25 |
| Granite Creek | 09/29/94 | T29N R31W S3C | 25 |
| Leigh Creek | 09/30/94 | T28N R31W S4A | 25 |

Horizontal starch gel electrophoresis was used to determine the genetic characteristics of each fish at 45 loci (genes) coding for proteins present in eye, liver, or muscle tissue (Table 1). At some of these loci the rainbow trout, *Oncorhynchus mykiss*, rarely share alleles (form of a gene) in common with westslope cutthroat trout, *O. clarki lewisi*, or Yellowstone cutthroat trout, *O. c. bouvieri*, (Table 2). These loci are generally termed diagnostic loci, as the alleles detected at them can be used to determine the genetic status of a population. That is, whether a sample came from a genetically pure population of one of these fishes, or one where hybridization has or is occurring.

In the middle Big Cherry Creek sample, only alleles characteristic of rainbow trout were detected (Table 3). With a sample size of 25 fish, we have a 95% chance of detecting as little as one percent westslope cutthroat trout genes and a 99% chance of detecting as little as one percent Yellowstone cutthroat trout genes in the sample. Thus, this population is almost undoubtedly a genetically pure rainbow trout population.

Rainbow trout can further be divided into two genetically distinct groups based upon the allele frequencies observed at the LDH-B2* and sSOD-1* loci. Coastal rainbow trout, or those native to waters west of the Cascade Mountain Range crest, usually possess LDH-B2*100 at frequencies greater than 0.90 and sSOD-1*152 at frequencies greater than 0.15. Most hatchery populations of rainbow trout were established from coastal populations. Interior rainbow trout, or those native to waters east of the Cascade Crest, usually possess LDH-B2*100 at frequencies less than 0.80. They also usually lack sSOD-1*152 or possess it at a very low frequency. Thus, the middle Big Cherry Creek sample appears to have been collected from an interior rainbow trout population.

In the samples collected from lower Big Cherry, Deep, Granite, and Leigh Creeks, alleles characteristic of both rainbow trout and westslope cutthroat trout were detected at some or all of the loci that can be used to distinguish between these fishes (Table 4). In the lower Big Cherry and Granite Creek samples, rainbow trout contributed a much larger proportion of the genes present, while in the Deep and Leigh Creek samples westslope cutthroat trout contributed a much larger proportion of the genes present in the populations. The rainbow and westslope cutthroat trout alleles also appear to be randomly distributed in the samples collected from lower Big Cherry, Deep, and Granite Creeks. These three populations, therefore, appear to be hybrid swarms and it is unlikely that any pure fish still exist within them. In contrast, the rainbow and westslope cutthroat trout alleles do not appear to be randomly distributed among the fish sampled from Leigh Creek. It is possible, therefore, that some pure fish still exist within this creek. Unless steps are taken to prevent further hybridization, however, this population will almost undoubtedly become a hybrid swarm in the future.

The final question to address, is whether the rainbow trout alleles observed in the hybridized populations originated from interior or coastal rainbow trout. Using the criteria listed above, it appears that the rainbow trout genes observed in the Granite Creek sample were derived solely from interior rainbow trout. In the lower Big Cherry Creek sample, it also appears that the majority of rainbow trout alleles observed were derived from interior rainbow trout. However, the presence of the sSOD-1*152 allele suggests that a small percentage of coastal rainbow trout genes may also occur in this population. In the Deep and Leigh Creek samples it is not possible to determine whether coastal or interior rainbow trout contributed the rainbow alleles observed in the samples because only a small percentage of the genes present in those samples were derived from rainbow trout. Given the large proportion of interior rainbow trout genes observed throughout the drainage, however, it seems likely that the rainbow genes observed in the Deep and Leigh Creek samples were also derived from interior rainbow trout.

Sincerely,

George K. Sage

George K. Sage

Table 1

Enzymes and loci examined. Tissues: E = eye, L = liver, M = muscle.

| Enzyme | Loci | Tissue |
|---|---|---|
| Adenylate kinase | AK-1*, AK-2* | M |
| Alcohol dehydrogenase | ADH* | L |
| Aspartate aminotransferase | sAAT-1*, sAAT-2* | L |
| | sAAT-3,4* | M |
| Creatine Kinase | CK-A1*, CK-A2* | M |
| | CK-B*, CK-C1*, CK-C2* | E |
| Dipeptidase | PEPA-1*, PEPA-2* | E |
| Glucose-6-phosphate isomerase | GPI-A*, GPI-B1*, GPI-B2* | M |
| Glyceraldehyde-3-phosphate dehydrogenase | GAPDH-3*, GAPDH-4* | E |
| Isocitrate dehydrogenase | mIDHP-1*, mIDHP-2* | M |
| | sIDHP-1*, sIDHP-2* | E |
| L-Iditol dehydrogenase | IDDH* | L |
| L-Lactate dehydrogenase | LDH-A1*, LDH-A2* | M |
| | LDH-B1*, LDH-B2*, LDH-C* | E |
| Malate dehydrogenase | sMDH-A1,2* | L |
| | sMDH-B1,2* | M |
| Malic enzyme | mMEP-1*, mMEP-2*, sMEP-1* | M |
| | sMEP-2* | L |
| Phosphoglucomutase | PGM-1*, PGM-2* | M |
| Phosphogluconate dehydrogenase | PGDH* | M |
| Superoxide dismutase | sSOD-1* | L |
| Tripeptide aminopeptidase | PEPB* | E |
| Xanthine dehydrogenase-like | XDH1 | L |

Note: In westslope cutthroat trout some pairs of loci produce a protein with identical function and electrophoretic mobility. For example, sAAT-3* and sAAT-4* both produce an aspartate aminotransferase in muscle tissue. The proteins produced from the common alleles at these loci occupy the same position in the gels after electrophoresis. Such pairs of loci are commonly termed isoloci and their existence can be confirmed only when one or both loci are genetically variable. In such situations, however, it is not possible to determine at which locus of the pair a variant allele exists. In order to estimate allele frequencies at the isoloci in westslope cutthroat trout populations (sAAT-3,4*, sMDH-A1,2*, sMDH-B1,2*), therefore, each pair was considered to be a single gene with four instead of two copies per individual.

Table 2

Diagnostic loci and characteristic alleles between westslope cutthroat trout, Yellowstone cutthroat trout, coastal rainbow trout, and interior rainbow trout. When more than one allele exists at a locus within a taxon the most common allele is listed first.

| Locus | Westslope | Yellowstone | Coastal Rainbow | Interior Rainbow |
|---|---|---|---|---|
| sAAT-1* | 200,250 | 165 | 100 | 100 |
| CK-A2* | 84,100 | 84 | 100,76 | 100,76 |
| CK-C1* | 100,38 | 38 | 100,38 | 100 |
| GPI-A* | 92,100 | 100 | 100 | 100 |
| IDDH* | 40,100 | 100,-63 | 100,200,40 | 100,200,40 |
| mIDHP-1* | 100 | -75 | 100 | 100 |
| sIDHP-1,2* | 86,100,40<br>71,114,20 | 100,71 | 100,71<br>40,114 | 100,71<br>40,114 |
| LDH-B2* | 100,112,76,24 | 100 | 100,76 | 76,100 |
| mMEP-1* | 88 | null | null | null |
| sMEP-1* | 100 | 90 | 100,75 | 100,75 |
| sMEP-2* | 100 | 110 | 100 | 100 |
| PEPA-1* | 100 | 101 | 100,115,90 | 100,115<br>90,101 |
| PEPB* | 100 | 135,100 | 100,135 | 100,135 |
| PGM-1* | 100,null | null | 100,null | 100,null |
| sSOD-1* | 100 | 100 | 100,152 | 100,152 |

Note: In rainbow trout, sIDHP-1,2* constitute a pair of isoloci. For comparative purposes to rainbow trout, therefore, these loci are also considered isoloci in westslope cutthroat trout. The 86 allele at these loci usually exists at a frequency of 0.500 in westslope populations but is absent from rainbow trout populations. The proportional genetic contribution of westslope to a hybridized population of these fishes at these loci, therefore, is the observed 86 allele frequency divided by 0.500.

Table 3

Allele frequencies at the polymorphic loci in the putative interior rainbow trout population collected from middle Big Cherry Creek. All other loci analyzed but not listed here were genetically invariant for the allele characteristic of rainbow trout.

| | Sample and Allele frequencies | |
|---|---|---|
| Locus | Alleles | Middle Big Cherry Creek |
| AAT-1* | 100 | 0.960 |
| | 200 | 0.040 |
| CK-A2* | 100 | 0.960 |
| | 200 | 0.040 |
| IDDH* | 100 | 0.960 |
| | 200 | 0.040 |
| sIDHP-1,2* | 100 | 0.380 |
| | 71 | 0.450 |
| | 40 | 0.170 |
| LDH-B2* | 100 | 0.400 |
| | 76 | 0.600 |
| PGM-1* | 100 | 0.717 |
| | null | 0.283 |
| PGM-2* | 100 | 0.980 |
| | 90 | 0.020 |

Table 4

Allele frequencies at the six diagnostic loci between rainbow and westslope cutthroat trout, and at the two loci that differentiate coastal and interior rainbow trout. At the diagnostic loci, the allele characteristic of rainbow trout is listed first. At the last two loci the allele characteristic of coastal rainbow trout is listed first.

| Locus | Alleles | Lower Big Cherry Creek | Deep Creek | Granite Creek | Leigh Creek |
|---|---|---|---|---|---|
| sAAT-1* | 100 | 0.980 | 0.080 | 0.980 | 0.060 |
| | 200 | 0.020 | 0.920 | 0.020 | 0.940 |
| CK-A2* | 100 | 0.940 | 0.040 | 0.940 | 0.080 |
| | 84 | 0.060 | 0.960 | 0.060 | 0.920 |
| GPI-A* | 100 | 0.940 | 0.080 | 0.980 | 0.040 |
| | 92 | 0.060 | 0.920 | 0.020 | 0.960 |
| IDDH* | 100 | 0.960 | 0.100 | 0.900 | 0.120 |
| | 40 | 0.040 | 0.900 | 0.100 | 0.880 |
| sIDHP-1,2* | 100 | 0.410 | 0.500 | 0.470 | 0.520 |
| | 71 | 0.400 | 0.040 | 0.350 | 0.020 |
| | 40 | 0.190 | 0.040 | 0.180 | - |
| | 86 | - | 0.420 | - | 0.460 |
| mMEP-1* | null | 0.960 | 0.020 | 0.980 | 0.080 |
| | 88 | 0.040 | 0.980 | 0.020 | 0.920 |
| LDH-B2* | 100 | 0.280 | 0.960 | 0.560 | 0.980 |
| | 76 | 0.720 | 0.040 | 0.440 | 0.020 |
| sSOD-1* | 152 | 0.040 | - | - | - |
| | 100 | 0.960 | 1.000 | 1.000 | 1.000 |
| Average rainbow | | 0.973 | 0.080 | 0.980 | 0.077 |
| Average westslope | | 0.027 | 0.920 | 0.020 | 0.923 |