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Joe:

The electrophoretic analysis of the following trout samples have been completed:

Sample	Date	Location	N
Alexander Creek	08/02/94	T30N R29W S5A	9
Pinkham Creek	07/12/94	T35N R27W S5C	26
Sutton Creek	07/12/94	T35N R28W S33B	26
Ten Mile Creek	07/12/94	T33N R23W S27A	12

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 Pinkham and Sutton creeks, only alleles characteristic of rainbow trout were detected (Table 3). With sample sizes of 26 fish, we have a 96% chance of detecting as little as one percent westslope cutthroat trout genes and better than a 99% chance of detecting as little as one percent Yellowstone cutthroat trout genes in the samples. Thus, these populations are almost undoubtedly genetically pure rainbow trout populations.

Graduate Degree Programs

Biochemistry  
 Biological Sciences  
 (Teaching)  
 Botany

Microbiology  
 Wildlife Biology  
 Zoology



Rainbow trout can further be divided into two genetically distinct groups based upon allele frequencies 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. Using this criteria, it appears that both the Pinkham and Sutton creek samples were collected from pure coastal rainbow trout populations.

In the samples collected from Alexander and Ten Mile creeks alleles characteristic of both westslope cutthroat trout and rainbow trout were detected at some or all of the loci that can be used to distinguish between these fishes (Table 4). Rainbow trout, however, contributed a much larger proportion of genes to these populations. In addition, it appears that the rainbow trout genes present were derived solely from coastal rainbow trout stocks with no apparent genetic contribution from interior rainbow trout.

Sincerely,



George K. Sage

Table 1

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

Enzyme	Loci	Tissue
Adenylate kinase	<u>AK-1*</u> , <u>AK-2*</u>	M
Alcohol dehydrogenase	<u>ADH*</u>	L
Aspartate aminotransferase	<u>sAAT-1*</u> , <u>sAAT-2*</u> <u>sAAT-3, 4*</u>	L M
Creatine Kinase	<u>CK-A1*</u> , <u>CK-A2*</u> <u>CK-B*</u> , <u>CK-C1*</u> , <u>CK-C2*</u>	M E
Dipeptidase	<u>PEPA-1*</u> , <u>PEPA-2*</u>	E
Glucose-6-phosphate isomerase	<u>GPI-A*</u> , <u>GPI-B1*</u> , <u>GPI-B2*</u>	M
Glyceraldehyde-3-phosphate dehydrogenase	<u>GAPDH-3*</u> , <u>GAPDH-4*</u>	E
Isocitrate dehydrogenase	<u>mIDHP-1*</u> , <u>mIDHP-2*</u> <u>sIDHP-1*</u> , <u>sIDHP-2*</u>	M E
L-Iditol dehydrogenase	<u>IDDH*</u>	L
L-Lactate dehydrogenase	<u>LDH-A1*</u> , <u>LDH-A2*</u> <u>LDH-B1*</u> , <u>LDH-B2*</u> , <u>LDH-C*</u>	M E
Malate dehydrogenase	<u>sMDH-A1, 2*</u> <u>sMDH-B1, 2*</u>	L M
Malic enzyme	<u>mMEP-1*</u> , <u>mMEP-2*</u> , <u>sMEP-1*</u> <u>sMEP-2*</u>	M L
Phosphoglucomutase	<u>PGM-1*</u> , <u>PGM-2*</u>	M
Phosphogluconate dehydrogenase	<u>PGDH*</u>	M
Superoxide dismutase	<u>sSOD-1*</u>	L
Tripeptide aminopeptidase	<u>PEPB*</u>	E
Xanthine dehydrogenase-like	<u>XDH1</u>	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
<u>sAAT-1*</u>	<u>200, 250</u>	<u>165</u>	<u>100</u>	<u>100</u>
<u>CK-A2*</u>	<u>84, 100</u>	<u>84</u>	<u>100, 76</u>	<u>100, 76</u>
<u>CK-C1*</u>	<u>100, 38</u>	<u>38</u>	<u>100, 38</u>	<u>100</u>
<u>GPI-A*</u>	<u>92, 100</u>	<u>100</u>	<u>100</u>	<u>100</u>
<u>IDDH*</u>	<u>40, 100</u>	<u>100, -63</u>	<u>100, 200, 40</u>	<u>100, 200, 40</u>
<u>mIDHP-1*</u>	<u>100</u>	<u>-75</u>	<u>100</u>	<u>100</u>
<u>sIDHP-1, 2*</u>	<u>86, 100, 40</u> <u>71, 114, 20</u>	<u>100, 71</u>	<u>100, 71</u> <u>40, 114</u>	<u>100, 71</u> <u>40, 114</u>
<u>LDH-B2*</u>	<u>100, 112, 76, 24</u>	<u>100</u>	<u>100, 76</u>	<u>76, 100</u>
<u>mMEP-1*</u>	<u>88</u>	<u>null</u>	<u>null</u>	<u>null</u>
<u>sMEP-1*</u>	<u>100</u>	<u>90</u>	<u>100, 75</u>	<u>100, 75</u>
<u>sMEP-2*</u>	<u>100</u>	<u>110</u>	<u>100</u>	<u>100</u>
<u>PEPA-1*</u>	<u>100</u>	<u>101</u>	<u>100, 115, 90</u>	<u>100, 115</u> <u>90, 101</u>
<u>PEPB*</u>	<u>100</u>	<u>135, 100</u>	<u>100, 135</u>	<u>100, 135</u>
<u>PGM-1*</u>	<u>100, null</u>	<u>null</u>	<u>100, null</u>	<u>100, null</u>
<u>sSOD-1*</u>	<u>100</u>	<u>100</u>	<u>100, 152</u>	<u>100, 152</u>

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 two putative populations of coastal rainbow trout. All other loci analyzed but not listed here were genetically invariant for the allele characteristic of coastal rainbow trout.

Locus	Alleles	Sample and allele frequencies	
		Pinkham Creek	Sutton Creek
<u>CK-A1*</u>	<u>100</u>	0.962	1.000
	<u>76</u>	0.038	-
<u>IDDH*</u>	<u>100</u>	0.981	1.000
	<u>200</u>	0.019	-
<u>sIDHP-1,2*</u>	<u>100</u>	0.827	0.510
	<u>114</u>	0.029	-
	<u>71</u>	0.019	0.490
	<u>40</u>	0.125	-
<u>sMDH-B1,2*</u>	<u>100</u>	0.913	0.904
	<u>83</u>	0.077	0.029
	<u>74</u>	0.010	0.067
<u>sMEP-1,2*</u>	<u>100</u>	0.990	1.000
	<u>75</u>	0.010	-
<u>PGM-1*</u>	<u>100</u>	0.561	1.000
	<u>null</u>	0.439	-
<u>PGM-1r*</u>	<u>100</u>	0.723	1.000
	<u>B</u>	0.177	-
<u>PGM-2*</u>	<u>100</u>	0.827	1.000
	<u>90</u>	0.173	-
<u>sSOD-1*</u>	<u>100</u>	0.865	1.000
	<u>152</u>	0.135	-

Table 4

Allele frequencies at the six diagnostic loci between rainbow trout 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	Alexander Creek	Ten Mile Creek
<u>sAAT-1*</u>	<u>100</u>	0.667	0.583
	<u>200</u>	0.333	0.417
<u>CK-A2*</u>	<u>100</u>	0.667	0.708
	<u>84</u>	0.333	0.292
<u>GPI-A*</u>	<u>100</u>	0.889	0.667
	<u>92</u>	0.111	0.333
<u>IDDH*</u>	<u>100</u>	0.611	0.583
	<u>40</u>	0.389	0.417
<u>sIDHP-1,2*</u>	<u>100</u>	0.444	0.458
	<u>71</u>	0.334	0.125
	<u>40</u>	0.222	0.313
	<u>86</u>	-	0.104
<u>mMEP-1*</u>	<u>null</u>	0.500	0.708
	<u>88</u>	0.500	0.292
<u>LDH-B2*</u>	<u>100</u>	1.000	0.916
	<u>76</u>	-	0.042
	<u>24</u>	-	0.042
<u>sSOD-1*</u>	<u>152</u>	0.111	0.083
	<u>100</u>	0.889	0.917
Percent coastal rainbow		0.667	0.673
Percent westslope cutthroat		0.333	0.327