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

Sample	Collection Date	Location	N
Lake Creek	10/06/94	T31N R33W S30AD	25
Lower Keeler Creek	09/21/94	T30N R33W S17C	13
Upper Keeler Creek	09/22/94	T30N R34W S30BC	29
Ross Creek above falls	07/27/94	T28N R33W S7B	28
Dry Creek	07/27/94	T29N R33W S9B	1

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 samples collected from Ross Creek above the falls and Dry Creek, only alleles characteristic of westslope cutthroat trout were detected (Table 3). With a sample size of 28 fish from Ross Creek, there is better than a 95% chance of detecting as little as one percent rainbow trout genes and better than a 99% chance of detecting as little as one percent Yellowstone cutthroat trout genes in the sample. Thus, this sample was almost undoubtedly collected from a genetically pure westslope cutthroat trout population. With a sample size of only one fish from Dry Creek, however, we can only be 95% certain that no more than 22% rainbow trout genes and no more than 11.5% percent Yellowstone cutthroat trout genes are present within the population. Thus, to be able to more conclusively state whether this population is a pure westslope cutthroat trout population or a population that is hybridized with rainbow trout or Yellowstone cutthroat trout it will be necessary to analyze more fish from the population. Until additional fish are analyzed, however, this population should be treated as a pure westslope cutthroat trout population.

In the sample collected from Lake Creek, only alleles characteristic of rainbow trout were detected. 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. The Lake Creek sample, therefore, was almost undoubtedly collected from a genetically pure rainbow trout population.

Rainbow trout can further be divided into two genetically distinct groups based upon allele frequencies at the <u>LDH-B2*</u> and <u>sSOD-1*</u> loci. Coastal rainbow trout, or those native to waters west of the Cascade Mountain Range crest, usually possess <u>LDH-B2*100</u> at frequencies greater than 0.90 and <u>sSOD-1*152</u> 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 <u>LDH-B2*100</u> at frequencies less than 0.80. They also usually lack <u>sSOD-1*152</u> or possess it at a very low frequency. Thus, using this criteria, it appears that the Lake Creek sample was collected from a coastal rainbow trout population (Table 4).

In the remaining two samples taken from upper and lower Keeler Creek, alleles characteristic of both coastal rainbow trout and westslope cutthroat trout were detected (Table 5). The upper Keeler Creek population appears to be a westslope cutthroat trout by coastal rainbow trout population, while the lower Keeler Creek population appears to be a coastal rainbow trout by westslope cutthroat trout population.

Sincerely,

George K. Sage

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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 71,114,20	100,71	100,71 40,114	100,71 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 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 two putative populations of westslope cutthroat. All other loci analyzed but not listed here were genetically invariant for the allele characteristic of westslope cutthroat trout.

		Sample and allele frequencies	
Locus	Alleles	Ross Creek	Dry Creek
sAAT-1*	200	0.982	1.000
	250	0.018	-
CK-C1*	100	0.893	1.000
	38	0.107	-
GPI-A*	92	0.964	1.000
	100	0.036	-
IDDH*	40	0.964	1.000
	100	0.036	-
sIDHP-1*	86	0.857	1.000
	71	0.143	-
sIDHP-2*	100	0.982	1.000
	40	0.018	-
PGM-2*	100	0.500	1.000
	85	0.500	-

Table 4

Allele frequencies at the polymorphic loci in the putative coastal rainbow trout population from Lake Creek.

	Sample and allele frequencies	
Locus	Alleles	Lake Creek
CK-C1*	100	0.960
	120	0.040
mIDHP-2*	100	0.760
	140	0.240
sIDHP-1,2*	100	0.760
	71	0.130
	40	0.080
	114	0.030
LDH-B2*	100	0.960
	76	0.040
sMDH-B1,2*	100	0.930
	125	0.020
	83	0.040
	74	0.010
PGM-2*	100	0.880
	90	0.120
sSOD-1*	100	0.840
	152	0.160

Table 5

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.

		Sample and allele frequencies	
Locus	Alleles	Lower Keeler Creek	Upper Keeler Creek
sAAT-1*	100	0.577	-
	200	0.423	0.741
	250	-	0.259
CK-A2*	100	0.615	0.017
	84	0.385	0.983
GPI-A*	100	0.654	0.017
	92	0.346	0.983
IDDH*	100	0.654	0.017
	40	0.346	0.983
sIDHP-1,2*	100	0.731	0.526
	71	0.019	0.017
	40	0.077	-
	86	0.173	0.457
mMEP-1*	null	0.654	-
	88	0.346	1.000
LDH-B2*	100	1.000	1.000
	76	-	-
sSOD-1*	152	0.077	-
	100	0.923	1.000
Average coastal rainbow		0.635	0.020
Average westslope		0.365	0.980