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Doug:

The electrophoretic analysis of the trout samples collected from upper O'Brien Creek (N=19; col. 09/94; T32N R33W S10AB), and lower O'Brien Creek (N=25; col. 09/94; T31N R33W S5A) in the Kootenai National Forest have been completed. 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 upper O'Brien Creek sample, alleles characteristic of both westslope cutthroat trout and Yellowstone cutthroat trout were detected at all diagnostic loci and in all nineteen fish analyzed (Table 3). This population, therefore, represents a hybrid swarm where no fish is likely to be genetically pure. In the lower O'Brien Creek sample, alleles characteristic of westslope cutthroat, Yellowstone cutthroat, and rainbow trout were detected at all the diagnostic loci that can be used to distinguish between these taxa and in 18 of the 25 fish analyzed (Table 4). Given the percentage of rainbow and Yellowstone cutthroat trout genes present in this sample (36.3%) it is highly unlikely that any fish would look like a pure westslope cutthroat if the genes from these three fishes were randomly distributed throughout the population. Thus, while it appears that some pure westslope cutthroat trout still exist in the lower section of this creek, it is unlikely that they will persist in the future given the current genetic status of the fish population.

It also appears that the rainbow trout genes observed in the sample were derived from coastal rainbow trout. The allele frequencies observed at the LDH-B2* and sSOD-1* loci are indicative of the allele frequencies expected if the rainbow trout genes present were contributed by coastal rainbow trout.

Sincerely,

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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 between westslope and Yellowstone cutthroat trout and between westslope cutthroat and rainbow trout. When more than one allele exists at a locus within a taxon the most common allele is listed first.

	Characteristic alleles			Characteristic alleles	
Locus	Westslope	Yellowstone	Locus	Westslope	Rainbow
sAAT-1*	200, 250	165	sAAT-1*	200, 250	100
CK-C1*	100, 38	38	CK-A2*	84	100
GPI-A*	92, 100	100	GPI-A*	92, 100	100
IDDH*	40, 100	100, -63	IDDH*	40, 100	100, 200 40
mIDHP-1*	100	-75	sIDHP-1,2*	86, 114 100, 71 40, 20	100, 114 71, 40
sIDHP-1*	86, 114 71	71	mMEP-1*	88	null
mMEP-1*	88	null			
sMEP-1*	100	90			
sMEP-2*	100	110			
PEPA-1*	100	101			
PEPB*	100	135, 100			
PGM-1*	100, 110 null	null			

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 diagnostic loci between westslope cutthroat and Yellowstone cutthroat trout in the hybridized population of these fishes from upper O'Brien Creek. The allele characteristic of westslope cutthroat is listed first at each locus.

	Sample and allele frequencies	
Locus	Alleles	Upper O'Brien Creek
sAAT-1*	200	0.842
	165	0.158
CK-C1*	100	0.789
	38	0.211
GPI-A*	92	0.921
	100	0.079
IDDH*	40	0.684
	100	0.316
mIDHP-1*	100	0.711
	-75	0.289
sIDHP-1*	86	0.368
	71	0.632
mMEP-1*	88	0.737
	null	0.263
sMEP-1*	100	0.711
	90	0.289
sMEP-2*	100	0.868
	110	0.132
PEPA-1*	100	1.000
	101	-
PEPB*	100	0.947
	135	0.053
PGM-1*	100	1.000
	null	-
Average westslope		0.798
Average Yellowstone		0.202

Table 4

Allele frequencies at the diagnostic loci between westslope cutthroat, Yellowstone cutthroat and coastal rainbow trout in the hybridized population of these fishes from lower O'Brien Creek. The allele characteristic of westslope cutthroat is listed first at each locus.

	Sample and allele frequencies	
Locus	Alleles	Lower O'Brien Creek
sAAT-1*	200	0.660
	165	0.040
	100	0.300
CK-A2*	84	0.660
	100	0.340
CK-C1*	100	0.920
	38	0.080
GPI-A*	92	0.600
	100	0.400
IDDH*	40	0.640
	100	0.360
mIDHP-1*	100	0.900
	-75	0.100
sIDHP-1,2*	86	0.240
	100	0.480
	114	0.040
	71	0.160
	40	0.080
mMEP-1*	88	0.700
	null	0.300
sMEP-1*	100	0.920
	90	0.080
sMEP-2*	100	1.000
	110	-
PEPA-1*	100	1.000
	101	-
PEPB*	100	0.980
	135	0.020
PGM-1*	100	1.000
	null	-
Average westslope		0.637
Average coastal rainbow		0.331
Average Yellowstone		0.032