

Montana Conservation Genetics Laboratory

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Dave;

We have completed the protein electrophoretic analysis of the presumed westslope cutthroat trout, *Oncorhynchus clarki lewisi*, collected from Lost Creek.

Sample #	Water Name/Location/Collection Date/ Collector	a N	# marker	s Species l	c ID	Power	1 (%)
3150	Lost Creek	17(22)	R6Y1	0 WCT		R99.9Y100	100
	2005 Dave Moser						

a) Number of fish in the sample. When more than one sample was collected from a water body, the number in parentheses represents the combined sample size.

b) Number of diagnostic loci. W=westslope cutthroat trout, Y=Yellowstone cutthroat trout, R=rainbow trout

c) CBRT=Columbia Basin redband trout, RBT=coastal rainbow trout, WCT= westslope cutthroat trout, YCT= Yellowstone cutthroat trout. Taxa separated by X indicate hybridization between them was detected.

d) Probability of detecting one percent hybridization with the indicated taxa. Taxa indicated as in b.

Horizontal starch gel electrophoresis was used to determine each fishes genetic characteristics (genotype) at 46 loci (genes) coding for proteins present in muscle, liver, or eye tissue (Table 1). At some of these loci, westslope cutthroat and rainbow trout, *O. mykiss*, rarely share alleles (form of a gene) in common (Table 2). This situation also pertains to a comparison of westslope and Yellowstone cutthroat trout, *O. c. bouvieri* (Table 2). Loci at which such fixed genetic differences exist between taxa are commonly termed diagnostic loci because the alleles detected at them can be used to help determine whether a sample came from a non-hybridized population of one of these fishes or a population in which hybridization between two or all three of them has or is occurring.

Lost Creek 3150

Alleles characteristic of only westslope cutthroat trout were detected in the sample (Table 3). These results are highly concordant with those obtained from a previous allozyme analysis of trout collected from Lost Creek (sample # 1886, N=5, collected 7/9/96). In contrast to these results, PINE analysis of a previous sample (#2299, N=49, 7/18/02) detected DNA fragments usually characteristic of Yellowstone cutthroat trout at two of the four diagnostic loci analyzed that usually distinguish Yellowstone from westslope cutthroat trout.

As discussed previously, there are two possible interpretations for the apparent discrepancy between the allozyme and PINE results (Leary 2005). Since the allele frequencies are statistically highly heterogeneous (contingency table chi-square, P<0.001) among the diagnostic PINE loci, one possibility is the Lost Creek population is an old westslope-Yellowstone cutthroat trout hybrid swarm in which the allele frequencies at diagnostic loci have diverged from homogeneity over time due to genetic drift. Another possibility is that the variation detected at the two PINE loci represents westslope cutthroat trout genetic variation that is electrophoretically indistinguishable from that usually characteristic of Yellowstone cutthroat trout.

In this situation, we strongly favor the latter interpretation for two reasons. First, the allozyme data suggest when the population was sampled in 1996 that it was not hybridized with Yellowstone cutthroat and, therefore, these data are not compatible with the population being an old hybrid swarm. Second, if the population contained a 5.6% Yellowstone cutthroat trout genetic contribution as suggested by the PINE data, then it is unlikely we would not have detected this in the first allozyme sample (P=0.003) and highly unlikely we would not have detected it in the combined allozyme samples (P=9.7X10⁻¹²). Thus, we conclude the Lost Creek population is not hybridized with Yellowstone cutthroat trout. Furthermore, with the combined allozyme and PINE samples, it is highly unlikely (P= $2.0X10^{-4}$) we would not detect as little as a one percent rainbow trout genetic contribution to a hybrid swarm. The lost Creek population, therefore, is almost certainly non-hybridized westslope cutthroat trout with unusual PINE genetic variation.

Robb Leary

Literature Cited

Leary, R. 2005. Letter to Anne Tews, Montana Department of Fish, Wildlife, and Parks, June 28, 2005.

Table 1

Enzyme	Loci	Tissue
Adenylate Kinase	AK-1*, AK-2*	М
Alcohol Dehydrogenase	ADH*	L
Aspartate Aminotransferase	sAAT-1*, sAAT-2* sAAT-3,4*	L M
Creatine Kinase	CK-A1*, CK-A2* CK-B*, CK-C1*, CK-C2*	M E
Dipeptidase	PEPA-1*, PEPA-2*	Е
N-acetyl-beta-Glucosaminidase	bGLUA*	L
Glucose-6-phosphate Isomerase	GPI-A* GPI-B1*, GPI-B2*	E M
Glyceraldehyde-3-phosphate Dehydroge	nase GAPDH-3*, GAPDH-4*	Е
Glycerol-3-phosphate Dehydrogenase	G3PDH-1*, G3PDH-2*	L
Iditol Dehydrogenase	IDDH*	L
Isocitrate Dehydrogenase	mIDHP-1*, mIDHP-2* sIDHP-1*, sIDHP-2*	M L
Lactate Dehydrogenase	LDH-A1*, LDH-A2* LDH-B1*, LDH-B2*, LDH-C*	M E
Malate Dehydrogenase	sMDH-A1,2* sMDH-B1,2*	L M
Malic Enzyme	mMEP-1*, mMEP-2*, sMEP-1* sMEP-2*	M L
Phosphoglucomutase	PGM-1*, PGM-2*	М

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

Table	1-continued
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Enzyme	Loci	Tissue	
Phosphogluconate Dehydrogenase	PGDH*	М	
Superoxide Dismutase	sSOD-1*	L	
Tripeptide Aminopeptidase	PEPB*	E	
Xanthine Dehydrogenase-like	XDHl*	L	

Table 2

Alleles at the diagnostic loci that differentiate westslope cutthroat trout and rainbow trout, westslope and Yellowstone cutthroat trout, and rainbow and Yellowstone cutthroat trout. When more than one allele exists at a locus within a taxon, the most common allele is listed first.

Locus	Taxa and char	acteristic alleles
	Westslope	Rainbow
sAAT-1*	200,250	100
CK-A2*	84	100
GPI-A*	92,100	100
IDDH*	40,100	100,200,40
sIDHP-1*	86,71	100,114,71,40
mMEP-1*	100	null
	Westslope	Yellowstone
sAAT-1*	200,250	165
CK-C1*	100,38	38
GPI-A*	92,100	100
IDDH*	40,100	100
mIDHP-1*	100	-75
sIDHP-1*	86,71	71
mMEP-1*	100	null
sMEP-1*	100	90
sMEP-2*	100	110
PEPB*	100	135
	Rainbow	Yellowstone
sAAT-1*	100	165
CK-A2*	100	84
CK-C1*	100,38,150	38
mIDHP-1*	100	-75
sIDHP-1*	100,114,71,40	71
sMEP-1*	100	90
sMEP-2*	100,75	110
PEPA-1*	100,115	101
PEPB*	100,120	135
PGM-1*	100,null	null

Table 3

Allele frequencies at the allozyme loci showing evidence of genetic variation in the combined 1666 and 2005 samples of westslope cutthroat trout collected from Lost Creek.

Loci	Alleles	Allele frequencies		
PGM-1*	100	0.787		
	null	0.213		