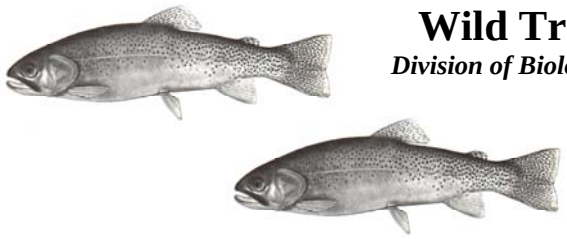

Wild Trout and Salmon Genetics Laboratory

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Jim:

We have completed the protein electrophoretic analysis of the presumed California golden trout, *Oncorhynchus mykiss aguabonita*, collected from Sylvan Lake in the East Rosebud River drainage.

Summary of results.

		a	b	c	d	e	f
Sample #	**Water Name/Location/Collection Date/ Collector**	**N**	**# markers**	**Species ID**	**Power (%)**	**%**	**Individuals**
3065	**Sylvan Lake**	32	W6Y10	CGT	W98Y99	100	xx
	9/1/2004 Jim Olsen						

[a]Number of fish successfully analyzed. If combined with a previous sample (Indicated in "Location" column), the number indicates the combined sample size. If present, the number in () is the average number of individuals successfully analyzed per locus if not all individuals were scoreable at all loci.
[b]Number of markers analyzed that are diagnostic for the non-native species (R=rainbow trout, W=westslope cutthroat trout, Y=Yellowstone cutthroat trout).
[c]Codes: WCT = westslope cutthroat trout (*Oncorhynchus clarki lewisi*); RBT = rainbow trout (*O. mykiss*); YCT = Yellowstone cutthroat trout (*O. clarki bouvieri*); CGT=California golden trout *(O. m. aguabonita).* Only one species code is listed when the entire sample possessed alleles from that species only. However, it must be noted that we cannot definitively rule out the possibility that some or all of the individuals are hybrids. We may not have detected any non-native alleles at the loci examined because of sampling error (see Power %). Species codes separated by "x" indicate hybridization between those species.
[d]Number corresponds to the percent chance we have to detect 1% hybridization given the number of individuals successfully analyzed and the number of diagnostic markers used. For example, 25 individuals are required to yield a 95% chance to detect 1% hybridization with westslope or an 99% chance to detect 1% hybridization with Yellowstone cutthroat trout into what once was a rainbow or California golden trout population. Not reported when hybridization is detected.
[e]Indicates the genetic contribution of the hybridizing taxa in the order listed under c to the sample assuming Hardy-Weinburg proportions. This number is reported if the sample appears to have come from a hybrid swarm. That is, a random mating population in which species markers are randomly distributed among individuals.

[f]Indicates number of individuals with genetic characteristics corresponding to the species code column when the sample can be analyzed on the individual level. This occurs when marker alleles are not randomly distributed among individuals and hybridization appears to be recent and/or if the sample appears to consist of a mixture of populations.

Methods and Data Analysis

Horizontal starch gel electrophoresis was used to determine each fishes genetic characteristics (genotype) at 45 loci (genes) coding for proteins present in muscle, liver, or eye tissue (Table 1). At some of these loci rainbow trout, *Oncorhynchus mykiss*, and California golden trout rarely share alleles (form of a gene) in common with westslope cutthroat trout, *O. clarki lewisi* (Table 2). This situation also pertains to a comparison of rainbow and California golden trout 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.

Phelps (1982) obtained protein electrophoretic data from 38 loci, all of which were analyzed by us, from samples from six golden trout populations in Montana. The data indicated that among these populations the only locus that was genetically variable was *sSOD-1**. In contrast, rainbow trout populations usually possess genetic variation at eight or more of the loci analyzed by Phelps and us (Leary et al. 1983). Thus, if the Sylvan Lake population represents non-hybridized California golden trout we would expect to detect evidence of genetic variation at only *sSOD-1** or at no loci. If it is hybridized with rainbow trout, or is simply rainbow trout, then we would expect to find evidence of genetic variation at loci other than *sSOD-1**.

Results and Discussion

Sylvan Lake 3065

Alleles characteristic of only rainbow and California golden trout were detected in the sample. Furthermore, the only genetically variable locus was *sSOD-1** (Table 3). The Sylvan Lake population, therefore, is almost undoubtedly non-hybridized California golden trout.

Sincerely,

Robb Leary

Literature Cited

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1983. Genetic variation in eight strains of rainbow trout maintained by the USFWS in Montana. University of Montana Population Genetics Laboratory Report 83/5.

Phelps, S. R. 1982. Genetic analysis of Montana golden trout. University of Montana Population genetics Laboratory Report 82/2.

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
N-acetyl-beta-Glucosaminidase	*bGLUA**	L
Glucose-6-phosphate Isomerase	*GPI-A**	E
	GPI-B1, GPI-B2**	M
Glyceraldehyde-3-phosphate Dehydrogenase	*GAPDH-3*, GAPDH-4**	E
Glycerol-3-phosphate Dehydrogenase	*G3PDH-1*, G3PDH-2**	L
Iditol Dehydrogenase	*IDDH**	L
Isocitrate Dehydrogenase	*mIDHP-1*, mIDHP-2**	M
	*sIDHP-1,2**	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	*sMEP-1**	M
	*sMEP-2**	L
Phosphoglucomutase	*PGM-1*, PGM-2**	M
	*PGM-1r**	L

Table 1-continued

Enzyme	Loci	Tissue
Phosphogluconate Dehydrogenase	*PGDH**	M
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 or California golden trout, westslope and Yellowstone cutthroat trout, and rainbow trout or California golden trout 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 characteristic 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*
	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*
*sMEP-1**	*100*	*90*
*sMEP-2**	*100*	*110*
*PEPA-1**	*100*	*101*
*PEPB**	*100*	*135*
*PGM-1**	*100,null*	*null*
	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 only locus showing evidence of genetic variation in a sample from a California golden trout population in Sylvan Lake, Montana.

Locus	Alleles	Allele frequencies
*sSOD-1**	*100*	0.891
	152	0.109