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Travis;

We have completed the protein electrophoretic analysis of the suspected rainbow trout, *Oncorhynchus mykiss*, collected from the following tributaries to Hebgen Lake:

		a	b	c	d	e	
Sample #	Water Name/Location/ Collection Date/ Collector	N	#Markers	Taxa ID	Power	%	Individual
3792	Madison River above Hebgen Lake 07/01/09 Travis Lohrenz	19	W6Y10	RBT			
3793	Duck Creek 7/15/2008 Travis Lohrenz	41	W6Y10	RBT			
3794	Grayling Creek 7/1/2009 Travis Lohrenz	26	W6Y10	RBT			

[a]Number of fish successfully analyzed. If combined with a previous sample, the number in parentheses indicates the combined sample size.
[b]Number of diagnostic loci analyzed for the non-native taxa (R=rainbow trout *Oncorhynchus mykiss,* W=westslope cutthroat trout *O. clarkii lewisi,* Y=Yellowstone cutthroat trout *O. c. bouvieri*).
[c]Codes: WCT = westslope cutthroat trout; RBT = rainbow trout; YCT = Yellowstone cutthroat trout . Only one taxon code is listed when the entire sample possessed alleles from that taxon only. It must be noted, however, that we cannot definitely 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 %). Taxa codes separated by "x" indicate hybridization between those taxa.
[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, with 25 individuals we have better than a 99 % chance to detect as little as 1% hybridization with rainbow trout or a 98% chance to detect as little as 1% hybridization with Yellowstone cutthroat trout in a hybrid swarm (a random mating population in which taxa markers are randomly distributed among individuals such that essentially all of them in the population are of hybrid origin) that once was a westslope cutthroat trout population. Likewise, with 25

individuals we have better than a 99% chance to detect as little as a 1% rainbow trout genetic contribution in a hybrid swarm that once was a Yellowstone cutthroat trout population. Not reported when hybridization is detected. Taxa as in b.
[e]Indicates the genetic contribution of the hybridizing taxa denoted as in b. This number is usually reported only if the sample appears to have come from a hybrid swarm.
[f]Indicates number of individuals with genetic characteristics corresponding to the taxa ID code column when the sample can be analyzed at the individual level. This occurs when marker alleles are not randomly distributed among individuals and hybrids and non-hybrids can be reliably distinguished.

Horizontal starch gel electrophoresis was used to determine each fish's genotype (genetic characteristics) at 47 loci (genes) coding for proteins present in muscle, liver, or eye tissue (Table 1). At some of these loci, rainbow and westslope cutthroat trout, *O. clarkii lewisi*, rarely share alleles (form of a gene) in common (Table 2). This situation also pertains to a comparison of rainbow and Yellowstone cutthroat trout, *O. c. bouvieri* (Table 2). Loci at which such "fixed genetic differences" exist between taxa are commonly called 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 one in which hybridization between two or all of them has or is occurring.

Among the samples, evidence of genetic variation was detected at 14 loci (Table 3). Contingency table chi-square analysis indicated that the allele frequencies were statistically heterogeneous among the samples only at *bGLUA** and *sIDHP-1,2** (Table 3). This could indicate that genetic differences exist among the samples or these apparent differences could simply be chance departures from homogeneity due to the number of comparisons performed. In order to distinguish between these possibilities, we compared the chi-square statistics at *bGLUA** and *sIDHP-1,2** to that associated with the modified level of significance proposed by Rice (1989). The differences are not significant at the modified level indicating they most likely represent chance departures from homogeneity. Thus, there was no compelling evidence of allele frequency differences among the samples so they were combined for further analysis.

With the exception of *GPI-A**, alleles characteristic of only rainbow trout were detected at all the loci analyzed in the combined sample. The single copy of *GPI-A*92* in the sample is rarely detected in rainbow trout but, it is highly characteristic of westslope cutthroat trout (Tables 2 and 3). Its presence, therefore, could indicate the fish are very slightly hybridized with westslope cutthroat trout or it could simply be unusual rainbow trout genetic variation. With the available data, we cannot distinguish between these possibilities. Thus, it is uncertain whether these fish are non-hybridized rainbow trout or slightly hybridized with westslope cutthroat trout. Despite this uncertainty, since the potential amount of hybridization with westslope cutthroat trout is so slight from a management perspective these fish should simply be considered to be rainbow trout.

Over the past years Hebgen Lake has mainly been stocked with Eagle Lake rainbow trout. Among the numerous rainbow trout populations from which we have protein electrophoretic data, Eagle Lake is unusual by having a relatively high frequency of *G3PDH-1*140*, *sMEP-2*75*, and *PEPA-1*115*. Most other populations actually lack these alleles. The presence of these alleles in the combined sample, therefore, strongly suggests these fish have an appreciable Eagle Lake genetic component. We have never detected *LDH-B2*76* and *sMDH-B1,2*125* in Eagle Lake rainbow trout and these alleles are usually at very low frequency in other coastal rainbow trout, *O. m. irideus*, populations. They are common, however, in interior rainbow trout, *O. m. gairdneri*, populations such as Kamloops. Hebgen Lake has been stocked with Kamloops rainbow trout in the past and the presence of these alleles in the sample suggests the sampled fish also have a significant Kamloops genetic contribution. Finally, we have never detected *ADH*0*, *CK-C1*150*, *CK-C1*38*, and *PGM-2*90* in Eagle Lake or Kamloops rainbow trout. The presence of these alleles in the sample, therefore, indicates these fish have a genetic component from at least one other source of rainbow trout than Eagle Lake or Kamloops.

Robb Leary

P. S. The sample from Grayling Creek contained one brown trout so the sample size reported is one less than what you sent in.

Literature Cited

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

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, sIDHP-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	*mMEP-1*, mMEP-2*, sMEP-1**	M
	*sMEP-2**	L
Phosphoglucomutase	*PGM-1*, PGM-2**	M
	*PGM-r**	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, 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 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*
*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*
*PEPA-1**	*100*	*101*
*PEPB**	*100*	*135*
*PGM-1**	*100,null*	*null*

Table 2- continued

Locus	Taxa and characteristic alleles	
	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 loci showing evidence of genetic variation in samples from what appear to be rainbow trout collected from the Madison River above Hebgen Lake, Grayling Creek, Duck Creek, and the combined samples. X^2=contingency table chi-square statistic for heterogeneity of allele frequencies among the Madison River, Grayling Creek, and Duck Creek samples. D.f.=degrees of freedom. *=P<0.05. **=P<0.01.

		Sample and allele frequencies					
Locus	Alleles	Duck	Grayling	Madison	Combined	X^2	D.f.
*ADH**	*100*	0.988	1.000	1.000	0.994	0.283	1
	0	0.012			0.006		
*CK-C1**	*100*	0.963	0.942	1.000	0.965	1.753	2
	150	0.024	0.038		0.023		
	38	0.012	0.019		0.012		
*G3PDH-1**	*100*	0.939	0.904	1.000	0.942	3.012	1
	140	0.061	0.096		0.058		
*bGLUA**	*100*	0.817	0.846	0.974	0.860	5.203*	1
	80	0.183	0.154	0.026	0.140		
*GPI-A**	*100*	1.000	0.981	1.000	0.994	0.283	1
	92		0.019		0.006		
*mIDHP-2**	*100*	0.645	0.646	0.529	0.620	1.569	1
	140	0.355	0.354	0.471	0.380		
*sIDHP-1,2**	*100*	0.738	0.712	0.737	0.730	14.110**	3
	114	0.006	0.019	0.026	0.015		
	71	0.128	0.163	0.013	0.113		
	40	0.128	0.106	0.224	0.142		
*LDH-B2**	*100*	0.866	0.981	0.816	0.890	2.492	1
	76	0.134	0.019	0.184	0.110		
*sMDH-B1,2**	*100*	0.909	0.933	0.921	0.919	0.283	1
	125	0.006			0.003		
	83	0.085	0.067	0.079	0.078		
*sMEP-2**	*100*	0.866	1.000	1.000	0.936	3.332	1
	75	0.134			0.064		
*PEPA-1**	*100*	0.976	0.942	1.000	0.971	1.469	1
	115	0.024	0.058		0.029		

Table 3-continued

Locus	Alleles	Sample and allele frequencies				X^2	D.f.
		Duck	Grayling	Madison	Combined		
*PGM-1**	*100*	1.000	0.723	1.000	0.848	0.577	1
	null		0.277		0.152		
*PGM-2**	*100*	0.963	0.942	0.974	0.959	0.261	1
	90	0.037	0.058	0.026	0.041		
*sSOD-1**	*100*	0.695	0.769	0.684	0.715	0.226	1
	152	0.305	0.231	0.316	0.285		