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Mike Hensler
Montana Department of Fish Wildlife and Parks
475 Fish Hatchery Road
Libby, Montana 59923

Mike;

We have completed the protein electrophoretic analysis of the following samples collected from populations in the Yaak River drainage, Montana presumed to be Columbia River redband trout (redband), *Oncorhynchus mykiss gairdneri*:

Sample	T	R	S	Sample size	Col. Date	Status
Blacktail Creek (#2941)	37N	30W	18	40	8/25/04	R,RXW
Caribou Creek (#2942)	37N	30W	22	21	7/26/01	R
Caribou Creek (#2943)	37N	30W	22	40	8/25/04	R
East Fork Yaak River (#2944)	37N	30W	28	40	8/25/04	R,RXW
Solo Joe Creek (#2945)	37N	30W	32	40	8/25/04	R
West Fork Yaak River (#2946)	37N	31W	32	30	8/25/04	W,R, WXR

(R=redband, C=coastal rainbow, W=Westslope, Y=Yellowstone)

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 redband and coastal rainbow trout (collectively termed rainbow trout), *O. m. irideus*, 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 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.

Populations of redband and coastal rainbow trout are usually genetically distinguishable from each other. Redband populations usually possess *LDH-B2*76* at a frequency greater than 0.25 and *sSOD-1*152* at a frequency less than 0.10 (Knudsen et al. 2002).

In contrast, coastal rainbow trout populations usually possess *LDH-B2*76* at a frequency less than 0.10 and *sSOD-1*152* at a frequency greater than 0.15 (Knudsen et al. 2002). The frequency of these two alleles in a sample, therefore, can be used to help determine whether it came from a redband, coastal rainbow trout, or a population in which hybridization between these fishes has or is occurring.

Results and Discussion

Genetic variation at *sIDHP-1**

Previous analysis of samples from non-hybridized redband and westslope cutthroat trout populations in the Kootenai River, including the Yaak River, drainage indicates that many of them possess *sIDHP-1*71* at appreciable frequency (e.g. Sage et al. 1992). Because populations of both these taxa commonly share this allele, *sIDHP-1** was not used as a diagnostic locus in our analysis of hybridization in this study.

Hybridization between redband and westslope cutthroat trout

Alleles characteristic of only rainbow trout were detected in the two Caribou Creek samples (Table 3) and the Solo Joe Creek sample (Table 4). The allele frequencies at *LDH-B2** and *sSOD-1** were highly characteristic of redband trout in all of these samples (Tables 3 and 4). Thus, these three samples appear to have contained only non-hybridized redband trout.

Although only redband trout were detected in the Caribou and Solo Joe Creek samples, an earlier sample from each stream contained a mixture of redband trout and what appear to be early generation hybrids, probably backcrosses between first generation redband-westslope cutthroat trout hybrids and redband trout (redband backcrosses), because most of these fish possessed alleles characteristic of westslope cutthroat trout at more than one diagnostic locus and also possessed *LDH-B2*76* (Table 9). The most likely source of *LDH-B2*76* in the hybrid fish is redband trout since this allele is not common in coastal rainbow trout and we have not detected it in any westslope cutthroat trout population analyzed from the Kootenai River drainage (e.g. Sage et al. 1992). Contingency table chi-square analysis for heterogeneity of the proportion of hybrids among the samples from the same stream indicates they are statistically homogeneous ($P>0.05$) among the Caribou Creek samples but, are heterogeneous ($P<0.05$) among the Solo Joe Creek samples. Thus, the proportion of early generation hybrids between redband and westslope cutthroat trout appears to have been temporally stable in the Caribou Creek population but, has significantly decreased in the Solo Joe Creek population.

Like in two previous samples, the Blacktail Creek sample contained a mixture of redband trout (30) and redband backcrosses (10; Tables 5 and 9). The proportion of hybrids among the three Blacktail Creek samples is statistically homogeneous ($P>0.05$) suggesting the extent of hybridization in this population has been temporally stable.

Very similar results were obtained from the East Fork Yaak River sample. It contained a mixture of redband trout (38) and redband backcrosses (2; Tables 6 and 9). The proportion of hybrids among the three East Fork Yaak River samples also appears to have been temporally stable (Table 9).

The West Fork Yaak River sample contained what appear to be nine non-hybridized redband trout (Table 7), 20 non-hybridized westslope cutthroat trout (Table 8), and one individual that appears to be a backcross produced from a mating between a first generation redband-westslope cutthroat trout hybrid and a westslope cutthroat trout (westslope backcross; Table9). This is the only sample we have analyzed from this portion of the West Fork Yaak River but, analysis of previous samples collected further upstream and an upstream tributary Garver Creek indicated they appeared to contain only non-hybridized westslope cutthroat trout (Sage et al. 1992). The previous samples were collected during August and September. The available data, therefore, indicate at least during this time of the year the upper portion of the West Fork Yaak River drainage is occupied mainly, if not solely, by westslope cutthroat trout and the lower portion contains a mixture of westslope cutthroat trout, redband trout, and a small proportion of early generation hybrids between them.

Considering all the data, we feel the following conclusions can be made. Redband and westslope cutthroat trout hybridize in waters in the upper Yaak River drainage, with the exception of Blacktail Creek the proportion of hybrids in the samples is generally low (<5%) and with the exception of Solo Joe Creek statistically temporally stable, and hybridization does not appear to proceed beyond the first generation backcross. Because hybridization is confined only to the very early generations, it is not resulting in the formation of hybrid swarms; that is, populations in which essentially all individuals are of hybrid origin. Rather despite occasional hybridization events redband and westslope cutthroat trout continue to represent genetically very divergent gene pools in the drainage and should be considered distinct groups of fish.

Any explanation for why fish of hybrid origin appear to be more prevalent in Blacktail Creek than the other regions sampled presently is strictly speculative. Possibilities include habitat differences such that spawning habitat is more limited or there is a longer overlap in spawning time between redband and westslope cutthroat trout than in other streams. Another possibility is that population densities between redband and westslope cutthroat trout on the spawning grounds are highly skewed, and as Hubbs (1955) noted long ago this can promote hybridization events. Regardless of the explanation, the data clearly indicate hybrids between redband and westslope cutthroat trout are consistently about five times more frequent in Blacktail Creek than the other stream reaches sampled containing hybrid individuals.

An enigma of the data is that although fish of hybrid origin have been detected in the East Fork Yaak River drainage as far back as 1986 none of the stream samples collected from the drainage have contained what appear to be non-hybridized westslope cutthroat trout (Sage et al. 1992). The continued production of only early generation hybrids over a span of about 20 years, however, strongly indicates non-hybridized westslope cutthroat trout must be present in the drainage during the spawning season. At this point in time, the origin of these fish is unknown. The West Fork Yaak River drainage data suggests that maybe during the summer non-hybridized westslope cutthroat trout only occupy very headwater stream reaches in the East Fork and thus, have not been collected. This possibility of course is purely speculative and may not be valid as Sage et al. (1992) analyzed samples collected from further upstream in the East Fork Yaak River drainage than the samples addressed here and found them to contain only redband trout.

Why hybridization between redband and westslope cutthroat trout apparently does not proceed beyond the first generation backcross in the upper Yaak River drainage is unknown. It is possible there is some genetic incompatibility between these fishes but, we find this hard to believe. Redband and coastal rainbow trout are genetically much more similar to each other than either is to westslope cutthroat trout and hybridization between coastal rainbow and westslope cutthroat trout often results in the formation of hybrid swarms (e.g. Leary et al. 1984; Allendorf and Leary 1988). Basically, on this issue we haven't a clue.

Temporal genetic characteristics of redband trout populations in the East Fork Yaak River drainage

Contingency table chi-square analysis for heterogeneity of allele frequencies indicates they are statistically homogeneous at the genetically variable loci among the three samples of redband trout collected from Blacktail Creek (Table 5), Caribou Creek (Table 3), and the East fork Yaak River (Table 6). Since the genetic characteristics of these populations appear to have been temporally stable, the three samples from each area were combined into a single sample for analysis of genetic divergence among the East Fork Yaak River drainage populations.

In contrast to the above results, the allele frequencies at *sIDHP-1,2** are statistically heterogeneous among the three Solo Joe Creek samples (table 4). This could indicate that genetic differences exist among the samples or this could simply be a chance departure from homogeneity due to the number of comparisons performed. In order to distinguish between these possibilities, we compared the chi-square statistic at *sIDHP-1,2** to that associated with the modified level of significance proposed by Rice (1989). At the modified level, the statistic remains significant indicating genetic differences exist among the samples. Since the genetic characteristics of the Solo Joe Creek population do not appear to have been temporally stable, it is not appropriate to combine the samples into one for analysis of genetic divergence among populations. In this analysis, therefore, we used only the 2004 Solo Joe Creek sample.

Genetic divergence among redband trout populations in the lower East Fork Yaak River drainage

At the genetically variable loci, the allele frequencies at *bGLUA**, *sIDHP-1,2**, and *LDH-B2** are statistically heterogeneous among the Blacktail Creek, Caribou Creek, East Fork Yaak River, and Solo Joe Creek samples (Table 10). These differences are significant at the modified level indicating genetic differences exist among the populations. The amount of genetic divergence among the populations, however, is relatively small. Using the procedure of Chakraborty (1980), the differences account for only about two percent of the total genetic variation detected. This relatively small amount of divergence suggests that historically these populations have been exchanging an appreciable amount of migrants. From a genetics perspective, therefore, it would not be inappropriate to consider these four redband trout populations as a single management unit.

Sincerely,

Robb Leary

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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
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, 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*
	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 loci showing evidence of genetic variation in samples of Columbia River redband trout collected from Caribou Creek in 1986 (N=23), 2001 (21), and 2004 (40). X^2= contingency table chi-square statistic for heterogeneity of allele frequencies among the samples. D.f.= degrees of freedom. NS= locus not scoreable in the sample.

		Sample and Allele Frequencies				
Locus	Alleles	1986	2001	2004	X^2	D.f.
*sIDHP-1,2**	*100*	0.500	NS	0.581	3.488	2
	71	0.500		0.406		
	40	--		0.013		
*LDH-B2**	*100*	0.022	0.071	0.125	4.172	2
	76	0.978	0.929	0.875		

Table 4

Allele frequencies at the loci showing evidence of genetic variation in samples of Columbia River redband trout collected from Solo Joe Creek in 1990 (24), 2000 (25), and 2004 (40). X^2 and D.f. as in Table 3. **= P<0.01. To avoid inflating the chi-square statistic, the *sIDHP-1,2*114* and *40* alleles were combined into a single category.

		Sample and Allele Frequencies				
Locus	Alleles	1990	2000	2004	X^2	D.f.
*sIDHP-1,2**	*100*	0.542	0.817	0.605	15.322**	4
	114	--	--	0.007		
	71	0.406	0.183	0.316		
	40	0.054	--	0.072		
*LDH-B2**	*100*	0.146	0.220	0.200	0.949	2
	76	0.854	0.780	0.800		

Table 5

Allele frequencies at the loci showing evidence of genetic variation in samples of Columbia River redband trout collected from Blacktail Creek in 1991 (20), 2000 (22), and 2004 (30). X^2, D.f., and NS as in Table 3.

		Sample and allele frequencies				
Locus	Alleles	1991	2000	2004	X^2	D.f.
*bGLUA**	*100*	NS	0.955	1.000	3.057	1
	80		0.045	--		
*sIDHP-1,2**	*100*	0.558	NS	0.525	4.600	2
	71	0.442		0.438		
	40	--		0.038		
*LDH-B2**	*100*	0.075	0.045	0.100	1.008	2
	76	0.925	0.955	0.900		
*sMDH-B1,2**	*100*	0.988	1.000	1.000	2.341	2
	125	0.012	--	--		
*PGM-2**	*100*	1.000	0.977	1.000	2.349	2
	90	--	0.023	--		

Table 6

Allele frequencies at the loci showing evidence of genetic variation in samples of Columbia River redband trout collected from the East Fork Yaak River in 1987 (21), 2000 (29), and 2004 (38). X^2, D. f., and NS as in Table 3. In order to avoid inflating the chi-square statistic, the *sIDHP-1,2*71* and *40* alleles were combined into a single category.

		Sample and Allele Frequencies				
Locus	Alleles	1987	2000	2004	X^2	D.f.
*sIDHP-1,2**	*100*	0.512	NS	0.539	0.165	2
	71	0.488		0.441		
	40	--		0.020		
*LDH-B2**	*100*	0.048	0.086	0.092	0.782	2
	76	0.952	0.914	0.908		
*sMDH-B1,2**	*100*	1.000	1.000	0.987	2.667	2
	125	--	--	0.013		

Table 7

Allele frequencies at the loci showing evidence of genetic variation in a sample of Columbia River redband trout collected from West Fork Yaak River.

Locus	Alleles	Allele frequencies
*sIDHP-1,2**	*100*	0.500
	71	0.469
	40	0.031
*LDH-B2**	*100*	0.056
	76	0.944
*sMDH-B1,2**	*100*	0.972
	83	0.028

Table 8

Allele frequencies at the loci showing evidence of genetic variation in a sample of westslope cutthroat trout from the West Fork Yaak River.

Locus	Alleles	Allele frequencies
*sAAT-1**	*200*	0.025
	250	0.975
*bGLUA**	*100*	0.875
	90	0.125
*sIDHP-1,2**	*86*	0.361
	71	0.639
*PGM-2**	*100*	0.975
	85	0.025

Table 9

Genotypes at the diagnostic loci between Columbia River redband (redband) and westslope cutthroat trout and at *LDH-B2** analyzed in fish of hybrid origin between these fishes collected from Blacktail Creek, Caribou Creek, East Fork Yaak River, Solo Joe Creek, and the West Fork Yaak River. At the diagnostic loci, R= homozygous for redband trout alleles, R/W= heterozygous for redband and westslope cutthroat trout alleles, and W= homozygous for westslope cutthroat trout alleles. At *LDH-B2**, 100= homozygous for the *100* allele, 100/76= heterozygous for the *100* and *76* alleles, and 76= homozygous for the *76* allele. The *76* allele at this locus is highly characteristic of redband trout.

	Locus and Genotype				
Sample	*sAAT-1**	*CK-A2**	*GPI-A**	*IDDH**	*LDH-B2**
Blacktail 1991	W/R	W/R	W/R	W/R	100/76
	W/R	R	W/R	W/R	76
	R	W/R	R	W/R	76
	R	R	R	W/R	76
	R	R	R	W/R	76
Blacktail 2000	W/R	R	R	W/R	76
	W/R	R	W/R	W/R	100/76
	W/R	W/R	W/R	W/R	100/76
Blacktail 2004	R	R	W/R	R	100/76
	W/R	R	R	W/R	76
	W/R	W/R	R	R	76
	W/R	R	W/R	R	76
	R	R	W/R	W/R	100/76
	W/R	W/R	R	R	100/76
	W/R	R	R	W/R	100/76
	R	R	R	W/R	100/76
	W/R	W/R	R	R	76
	W/R	R	R	W/R	76
Caribou 1986	W/R	R	W/R	W/R	100/76
East Fork Yaak 1987	R	W/R	W/R	W/R	100/76
East Fork Yaak 2000	W/R	R	W/R	R	76
East Fork Yaak 2004	W/R	R	W/R	R	76
	W/R	R	R	W/R	76

Table-9 continued

Sample	*sAAT-1**	*CK-A2**	*GPI-A**	*IDDH**	*LDH-B2**
	Locus and Genotype				
Solo Joe 1990	R	R	R	W/R	100/76
	W/R	R	W/R	W/R	76
	W/R	W/R	R	W/R	76
West Fork Yaak 2004	W	W/R	W/R	W/R	100/76

Table 10

Allele frequencies at the loci showing evidence of genetic variation in the Blacktail Creek combined, Caribou Creek combined, East Fork Yaak River combined, and 2004 Solo Joe Creek Columbia River redband trout samples. X^2 and D.f. as in Table 3. *= P<0.05, **= P<0.01, ***= P<0.001.

		Sample and Allele Frequencies					
Locus	Alleles	Blacktail	Caribou	E. F. Yaak	Solo Joe	X^2	D.f.
*bGLUA**	*100*	0.978	1.000	1.000	1.000	8.288*	3
	80	0.022	--	--	--		
*sIDHP-1,2**	*100*	0.542	0.541	0.526	0.605	32.284***	9
	114	--	--	--	0.007		
	71	0.440	0.453	0.465	0.316		
	40	0.019	0.007	0.010	0.072		
*LDH-B2**	*100*	0.073	0.073	0.075	0.200	11.362**	3
	76	0.927	0.927	0.925	0.800		
*sMDH-B1,2**	*100*	0.996	1.000	0.996	1.000	2.624	3
	125	0.004	--	0.004	--		
*PGM-2**	*100*	0.999	1.000	1.000	1.000	3.006	3
	90	0.001	--	--	--		