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Joe:

The electrophoretic analysis of the trout samples collected from the Cabinet Wilderness lakes, Smith Lake, and the Kootenai River have been completed:

Sample	Date	Location	N
Baree Lake	07/23/95	T26N R31W S36	21
Lower Bear Lake	07/27/95	T26N R30W S30	23
Bramlet Lake	08/15/95	T26N R31W S11	25
Lower Cedar Lake	08/19/95	T30N R32W S07	14
Upper Cedar Lake	08/19/95	T30N R32&33W S7&12	25
Lower Geiger Lake	08/03/95	T26N R31W S13	20
Upper Geiger Lake	08/03/95	T26N R31W S14	17
Granite Lake	08/23/95	T29N R32W S27&28	25
Lower Hanging Valley Lake	09/08/94	T30N R32W S27&34	25
Upper Hanging Valley Lake	08/25/95	T30N R32W S33&34	25
Lower Sky Lake	09/04/95	T30N R32W S32	25
Snowshoe Lake	09/05/95	T29N R32W S32	25
Smith Lake	08/30/95	T59N R03E S32	25
Kootenai River	06/22/95	T30N R31W S03	26

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 westslope cutthroat trout, *Oncorhynchus clarki lewisi*, rarely share alleles (form of a gene) in common with Yellowstone cutthroat trout, *O. c. bouvieri*, or rainbow trout, *O. mykiss* (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 lower Bear Lake sample, only alleles characteristic of westslope cutthroat trout were detected (Table 3). With a sample size of 23 fish, we have a 95% chance of detecting as little as 1.1% rainbow trout genes and better than a 99% chance of detecting as little as 1.0% Yellowstone cutthroat trout genes in the sample. Thus, the lower Bear Lake population is almost undoubtedly a genetically pure westslope cutthroat trout population.

In the samples collected from upper and lower Cedar, upper and lower Geiger, and upper and lower Hanging Valley Lakes, only alleles characteristic of rainbow trout were detected. As you are aware, rainbow trout can be divided into two genetically distinct groups based upon the allele frequencies observed at the LDH-B2* and sSOD-1* loci. Coastal rainbow trout, or those native to waters west of the Cascade Mountain Range crest, usually possess LDH-B2*100 at frequencies greater than 0.90 and sSOD-1*152 at frequencies greater than 0.15. Most hatchery populations of rainbow trout were established from coastal populations. Interior rainbow trout, or those native to waters east of the Cascade Crest, usually possess LDH-B2*100 at frequencies less than 0.80. They also usually lack sSOD-1*152 or possess it at a very low frequency. Utilizing this criteria, it appears that the upper and lower Cedar Lake samples were collected from coastal rainbow trout populations (Table 4), while the upper and lower Geiger, and upper and lower Hanging Valley Lake samples were collected from hybridized coastal rainbow trout by interior rainbow trout populations (Table 5).

In the sample collected from Bramlet Lake, alleles characteristic of both interior rainbow trout and Yellowstone cutthroat trout were detected at all the loci that can be used to distinguish between these species (Table 6). In addition, the alleles characteristic of these fishes appear to be randomly distributed in the sample and suggests that this sample was collected from a hybrid swarm population. Thus, it is unlikely that any pure fish of either species still exist in Bramlet Lake.

In the Kootenai River sample, alleles characteristic of coastal rainbow trout, interior rainbow trout, and westslope cutthroat trout were detected. Unfortunately, it is not possible to determine the individual percentage of coastal and interior

rainbow trout genes in the population because we lack the pre-hybridization allele frequencies at the LDH-B2* and sSOD-1* loci. Rainbow trout, however, contributed a much larger proportion of the genes present in the population sample (Table 7). Of the 26 fish analyzed, only six had alleles characteristic of westslope cutthroat trout and of these, two appeared to be first generation hybrids. This population, therefore, is not a randomly mating population and pure fish from these taxa undoubtedly still exist in the Kootenai River.

In the remaining five samples, westslope cutthroat trout contributed a much larger proportion of the genes observed in each population. In the Granite and Smith Lake samples, alleles characteristic of both westslope cutthroat trout and rainbow trout were detected (Table 8). Because of the small genetic contribution of rainbow trout to these populations, however, it's not possible to determine whether coastal rainbow, interior rainbow, or both rainbow trout types contributed genes to the populations. Although the taxonomic origin of the rainbow trout genes observed in these populations can not be determined with certainty, their distribution among the fish sampled from each population appears to be random and suggests that these samples were also collected from hybrid swarm populations. Thus, as in Bramlet Lake, it is unlikely that any pure fish still exist in these lakes.

In the Baree Lake sample, alleles characteristic of westslope cutthroat, Yellowstone cutthroat, and rainbow trout were observed at all the diagnostic loci that can be used to distinguish between these fishes (Table 9). However, as in the Granite and Smith Lakes samples it is not possible to determine which rainbow trout type contributed genes to the population. In addition, the alleles at the diagnostic loci do not appear to be randomly distributed among the fish sampled. Thus, it is possible that some pure westslope cutthroat trout still exist in Baree Lake.

Lastly, in the samples collected from lower Sky Lake and Snowshoe Lake, alleles characteristic of westslope cutthroat and Yellowstone cutthroat trout were detected at some or all the diagnostic loci that can be used to distinguish between these fishes (Table 10). The westslope and Yellowstone cutthroat trout alleles also appear to be randomly distributed among the fish in each sample, and this suggests that these samples were also collected from hybrid swarm populations. Thus, it is unlikely that any pure fish of either species exist in these lakes as well.

Sincerely,

George K. Sage

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

Locus	Westslope	Yellowstone	Coastal Rainbow	Interior Rainbow
sAAT-1*	200,250	165	100	100
CK-A2*	84,100	84	100,76	100,76
CK-C1*	100,38	38	100,38	100
GPI-A*	92,100	100	100	100
IDDH*	40,100	100,-63	100,200,40	100,200,40
mIDHP-1*	100	-75	100	100
sIDHP-1,2*	86,100,40 71,114,20	100,71	100,71 40,114	100,71 40,114
LDH-B2*	100,112,76,24	100	100,76	76,100
mMEP-1*	88	null	null	null
sMEP-1*	100	90	100,75	100,75
sMEP-2*	100	110	100	100
PEPA-1*	100	101	100,115,90	100,115 90,101
PEPB*	100	135,100	100,135	100,135
PGM-1*	100,null	null	100,null	100,null
sSOD-1*	100	100	100,152	100,152

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 polymorphic loci in the sample of westslope cutthroat collected from lower Bear Lake. All other loci analyzed but not listed here were genetically invariant for the alleles characteristic of westslope cutthroat trout.

		Sample and allele frequencies
Locus	Alleles	Lower Bear Lake
AAT-1*	100	0.935
	250	0.065
CK-C1*	100	0.875
	38	0.125
GAPDH-4*	100	0.978
	null	0.022
sIDHP-2*	100	0.565
	40	0.435
PGM-2*	100	0.978
	85	0.022

Table 4

Allele frequencies at the polymorphic loci in two putative coastal rainbow trout populations. All other loci analyzed but not listed here were genetically invariant for the allele characteristic of coastal rainbow trout.

		Sample and allele frequencies	
Locus	Alleles	Upper Cedar Lake	Lower Cedar Lake
AAT-3,4*	100	0.990	0.982
	83	0.010	0.018
CK-C1*	100	0.920	1.000
	38	0.080	-
mIDHP-2*	100	0.800	0.929
	140	0.200	0.071
sIDHP-1,2*	100	0.760	0.678
	114	0.090	0.054
	71	0.130	0.161
	40	0.020	0.107
LDH-B2*	100	0.900	0.929
	76	0.100	0.071
LDH-C*	100	0.920	0.893
	95	0.080	0.107
sMDH-A1,2*	100	0.930	0.875
	40	0.070	0.125
sMDH-B1,2*	100	0.780	0.750
	125	0.050	-
	83	0.040	0.143
	74	0.130	0.107
sMEP-1*	100	0.820	0.714
	90	0.180	0.286
PGM-2*	100	0.940	0.929
	90	0.060	0.071
PEP-A1*	100	0.980	1.000
	115	0.020	-
sSOD-1*	100	0.400	0.536
	152	0.600	0.464

Table 5

Allele frequencies at the polymorphic loci in four putative coastal rainbow x interior rainbow trout populations. All other loci analyzed but not listed here were genetically invariant for the allele characteristic of rainbow trout.

		Sample and allele frequencies			
Locus	Alleles	Lower Geiger Lake	Upper Geiger Lake	Upper Hanging Valley Lake	Lower Hanging Valley Lake
CK-A2*	100	1.000	1.000	0.920	1.000
	75	-	-	0.080	-
CK-C1*	100	1.000	1.000	1.000	0.980
	120	-	-	-	0.020
GPI-A*	100	1.000	0.971	1.000	0.940
	92	-	0.029	-	0.060
mIDHP-2*	100	0.875	0.853	0.960	0.900
	140	0.125	0.147	0.040	0.100
sIDHP-1,2*	100	0.575	0.588	0.660	0.640
	114	-	0.015	0.010	0.030
	71	0.038	0.162	0.030	0.030
	40	0.387	0.235	0.300	0.300
LDH-A1*	100	0.675	0.882	0.820	1.000
	null	0.325	0.118	0.180	-
LDH-B2*	100	0.375	0.471	0.420	0.540
	76	0.625	0.529	0.580	0.460
sMDH-B1,2*	100	1.000	0.912	0.840	0.920
	83	-	0.059	0.150	0.070
	74	-	0.029	0.010	0.010
PGM-2*	100	0.950	0.912	1.000	0.940
	90	0.050	0.088	-	0.060
sSOD-1*	100	0.725	0.853	0.880	0.840
	152	0.275	0.147	0.120	0.160

Table 6

Allele frequencies at the diagnostic loci between interior rainbow trout and Yellowstone cutthroat trout in the sample collected from Bramlet Lake.

		Sample and allele frequencies
Locus	Alleles	Bramlet Lake
AAT-1*	100	0.860
	165	0.140
CK-A2*	100	0.780
	84	0.220
CK-C1*	100	0.980
	38	0.020
mIDHP-1*	100	0.740
	-75	0.260
sMEP-1*	100	0.800
	90	0.200
sMEP-2*	100	0.960
	110	0.040
PEP-A1*	100	0.900
	101	0.100
PEP-B*	100	0.880
	135	0.120
Average interior rainbow trout		0.863
Average Yellowstone cutthroat trout		0.137

Table 8

Allele frequencies at the diagnostic loci between westslope cutthroat and rainbow trout, and at the two loci that differentiate coastal and interior rainbow trout. At the diagnostic loci, the allele characteristic of westslope cutthroat trout is listed first. At the last two loci the allele characteristic of coastal rainbow trout is listed first.

		Sample and allele frequencies	
Locus	Alleles	Granite Lake	Smith Lake
sAAT-1*	200	1.000	1.000
	100	-	-
CK-A2*	84	1.000	0.940
	100	-	0.060
GPI-A*	92	1.000	0.960
	100	-	0.040
IDDH*	40	0.980	0.960
	100	0.020	0.040
mMEP-1*	88	0.980	0.960
	null	0.020	0.040
LDH-B2*	100	1.000	1.000
	76	-	-
sSOD-1*	152	-	0.040
	100	1.000	0.920
	55	-	0.040
Average westslope		0.992	0.964
Average rainbow		0.008	0.036

Table 10

Allele frequencies at the diagnostic loci between westslope cutthroat and Yellowstone cutthroat trout in the samples collected from Lower Sky Lake and Snowshoe Lake. The alleles characteristic of westslope cutthroat trout are listed first at each locus.

		Sample and Allele frequencies	
Locus	Alleles	Lower Sky Lake	Snowshoe Lake
AAT-1*	200	0.860	0.800
	250	0.140	0.100
	165	-	0.100
GPI-A*	92	0.980	0.760
	100	0.020	0.240
IDDH*	40	1.000	0.620
	100	-	0.380
mIDHP-1*	100	0.980	0.980
	-75	0.020	0.020
mMEP-1*	88	0.960	0.660
	null	0.040	0.340
sMEP-1*	100	0.980	0.840
	90	0.020	0.160
sMEP-2*	100	1.000	0.600
	110	-	0.400
PEP-A1*	100	0.940	0.780
	101	0.060	0.220
PEP-B*	100	1.000	0.820
	135	-	0.180
Average westslope		0.982	0.773
Average Yellowstone		0.018	0.227

Table 9

Allele frequencies at the diagnostic loci between westslope cutthroat, Yellowstone cutthroat, and rainbow trout in the sample collected from Baree Lake.

		Sample and allele frequencies
Locus	Alleles	Baree Lake
AAT-1*	200	0.929
	165	-
	100	0.071
CK-A2*	84	0.952
	100	0.048
GPI-A*	92	0.912
	100	0.088
IDDH*	40	0.786
	100	0.214
mIDHP-1*	100	0.929
	-75	0.071
sIDHP-1*	86	0.690
	71	0.143
	100	0.167
mMEP-1*	88	0.763
	null	0.237
sMEP-1*	100	0.905
	90	0.095
sMEP-2*	100	0.952
	110	0.048
PEP-A1*	100	1.000
	101	-
PEP-B*	100	0.952
	135	0.048
Average westslope cutthroat trout		0.881
Average rainbow trout		0.070
Average Yellowstone cutthroat trout		0.049