



University of Montana

Biological Sciences, Department of Zoology • Missoula, Montana 59812-1201 • (406) 243-5122

December 3, 1987

Joe Huston
Montana Department of Fish,
Wildlife and Parks
490 North Meridian
Kalispell, MT 59901

Joe:

We have completed the electrophoretic analysis of the samples of Salmo collected during September 1987 from the following waters in the Kootenai River drainage: Arbo Creek (N=30), East Fork Yaak River (22), Pete Creek (6), West Fork Yaak River (3), and lower (26) and upper (19) 17 Mile Creek. Each fishes genotype was determined at 45 loci coding for enzymes present in muscle, liver, or eye tissue (Table 1). We detected alleles characteristic of only westslope cutthroat trout, S. clarki lewisi, at those loci that differentiate this fish and the rainbow, S. gairdneri, and Yellowstone cutthroat trout, S. c. bouvieri, in the samples from Pete Creek, West Fork Yaak River, and upper 17 Mile Creek (Tables 2 and 3). Because of the small sample sizes from the former two waters, no reasonable conclusion can be made about the genetic status of the populations from which the samples were collected. Similarly, since we can detect as little as one percent rainbow trout genes in a population with a probability of only 0.890 in a sample of 19 fish, the possibility that the upper 17 Mile Creek population contains a small amount of rainbow trout genes cannot reasonably be excluded. It is unlikely, however, that this population contains Yellowstone cutthroat trout genes as we have a 98 percent chance of detecting a one percent genetic contribution of this fish to a population in a sample of 19 fish.

We detected genes characteristic of both the westslope cutthroat and rainbow trout at those loci that differentiate these fishes in samples from Arbo Creek, East Fork Yaak River, and lower 17 Mile Creek. In the East Fork Yaak River sample, 19 fish had genotypes at all the loci in Table 4 characteristic of rainbow trout and the other fish had genotypes unquestionably of hybrid origin. Considering the proportion of rainbow trout genes in the sample, the number of individuals in these categories conforms to that expected if the genes from the parental taxa are randomly distributed among the fish (chi-square, $P > 0.50$). This sample, therefore, most likely came from a rainbow-westslope cutthroat trout introgressed population that contains no individuals that are genetically pure representatives of the parental taxa. The small percentage of westslope cutthroat trout genes in the population, however, severely weakens the power of a statistical test to demonstrate a departure from a random distribution of these genes among individuals so the above conclusion must be viewed as tentative.

The genes from the rainbow and westslope cutthroat trout do not appear to be randomly distributed among the fish in the Arbo and lower 17 Mile Creek

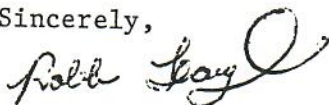
Joe Huston
December 3, 1987
Page 2

populations. There is an excess ($P < 0.001$) of individuals (4) with genotypes at all the loci characteristic of westslope cutthroat trout in the Arbo Creek population. This suggests that although most of the fish in this population are of hybrid origin it may contain a low proportion of genetically pure westslope cutthroat trout. In the lower 17 Mile Creek sample, only two fish had genotypes conclusively indicative of hybrid origin ($P < 0.05$). The remaining fish all had genotypes characteristic of rainbow trout at all the loci. Although this is certainly an introgressed population, it appears that it may contain an appreciable frequency of genetically pure rainbow trout.

Your next question, of course, is what type of rainbow trout (i.e., coastal or interior) have contributed genes to the three introgressed populations. We must examine the allele frequencies at the Ldh4 and Sod1 loci in order to address this issue. Populations of interior rainbow trout generally have a high frequency (> 0.20) of the Ldh4(76) allele and a low frequency (< 0.10) of the Sod1(152) allele. The reverse condition usually pertains to populations of coastal rainbow trout. We must correct the frequencies of these alleles in the samples for the presence of westslope cutthroat trout genes in the population to obtain estimates of their frequencies in the rainbow trout contributing to the populations. Fortunately, this presents no problem because we have never seen the Ldh4(76) allele in populations of westslope cutthroat trout and we rarely observe the Sod1(152) allele. Thus, the corrected allele frequency is simply that observed in the sample divided by the average percentage of rainbow trout genes in the sample.

In the Arbo Creek sample, the Ldh4(76) allele frequency is characteristic of interior rainbow trout and the Sod1(152) allele frequency is similar to that usually observed in coastal rainbow trout (Table 5). Thus, it appears that both 'races' of rainbow trout have contributed genes to this population. In contrast, the allele frequencies at both loci in the East Fork Yaak River and lower 17 Mile Creek samples are characteristic of interior rainbow trout (Table 5). Coastal rainbow trout, therefore, apparently have contributed no or a very low proportion of genes to these two populations.

Sincerely,



Robb Leary

TABLE I

Loci and enzymes examined (E=eye, L=liver, M=muscle)

Enzyme	Loci	Tissue
Adenylate kinase (AK)	Ak1,2	M
Alcohol dehydrogenase (ADH)	Adh	L
Aspartate aminotransferase (AAT)	Aat1,2 Aat(3,4)	L M
Creatine kinase (CK)	Ck1,2 Ck3,CkCl,2	M E
Glucose phosphate isomerase (GPI)	Gpi1,2,3	M
Glyceraldehyde-3-phosphate dehydrogenase (GAP)	Gap3,4	E
Glycerol-3-phosphate dehydrogenase (G3P)	G3p1,2	L
Glycyl-leucine Peptidase (GL)	G11,2	E
Isocitrate dehydrogenase (LDH)	Idh1,2 Idh3,4	M L
Lactate dehydrogenase (LDH)	Ldh1,2 Ldh3,4,5	M E
Leucyl-glycyl-glycine peptidase (LGG)	Lgg	E
Malate dehydrogenase (MDH)	Mdh(1,2) Mdh(3,4)	L M
Malic enzyme (ME)	Me1,2,3 Me4	M L
Phosphoglucomutase (PGM)	Pgml,2	M
6-Phosphogluconate dehydrogenase (6PG)	6Pg	M
Sorbitol dehydrogenase (SDH)	Sdh	L
Superoxide dismutase (SOD)	Sod	L
Xanthine dehydrogenase (XDH)	Xdh	L

Note: The protein products of the pairs of loci in () are electrophoretically indistinguishable. Thus, they are considered to be single tetrasomic loci in all analyses.

TABLE 2

Loci that can be used to differentiate rainbow, westslope cutthroat, and Yellowstone cutthroat trout. Alleles are designated as the proportional migration distance in the gel relative to the distance traveled by the common allele in rainbow trout which is given a mobility of 100.

Loci	Alleles		
	Rainbow	Westslope	Yellowstone
Aat1	100	200,250	165
Ck2	100	84	84
CkC1	100,38	100,38	38
G11	100,115,90	100	101
Gpi3	100	92	100
Idh1	100	100	-75
Idh3,4	100,114,71,40	100,86,71,40,Null	100,71
Lgg	100,135	100	135
Me1	100,55	88	100
Me3	100,75	100,75	90
Me4	100	100	110
Pgml	100,Null	100,Null	Null
Sdh	100,200,40	40,100	100

TABLE 3

Allele frequencies at the loci that showed evidence of being genetically variable in three potential populations of westslope cutthroat trout in the Kootenai River drainage.

Locus	Alleles	Sample and allele frequencies		
		Pete	West Yaak	upper 17 Mile
Aat1	200	0.833	1.000	1.000
	250	0.167	-	-
Gp11	100	1.000	1.000	0.974
	null	-	-	0.026
Idh3	86	1.000	0.667	0.974
	71	-	0.333	0.026
Mdh3,4	100	1.000	1.000	0.974
	125	-	-	0.026
Proportion polymorphic loci		0.024	0.024	0.071
Average heterozygosity		0.008	0.016	0.005

TABLE 4

Allele frequencies at the loci that differentiate westslope cutthroat and rainbow trout in three introgressed populations of these fishes in the Kootenai River drainage. The allele characteristic of the westslope cutthroat trout at each locus is listed first.

Locus	Alleles	Sample and allele frequencies		
		Arbo	West Yaak	lower 17 Mile
Aat1	200	0.533	-	0.038
	100	0.467	1.000	0.962
Ck2	84	0.600	0.023	0.019
	100	0.400	0.977	0.981
Gpi3	92	0.500	0.023	0.019
	100	0.500	0.977	0.981
Idh3,4	86	0.183	-	0.010
	114	0.017	-	-
	100	0.467	0.511	0.577
	71	0.125	0.489	0.404
	40	0.208	-	0.010
Mel	88	0.633	0.045	0.038
	100	0.367	0.955	0.962
Sdh	40	0.550	0.023	0.019
	100	0.450	0.977	0.981
Average westslope		0.530	0.019	0.026
Average rainbow		0.470	0.981	0.974

Note: The frequency of the Idh3,4(86) allele is usually 0.500 in populations of westslope cutthroat trout. The proportion of westslope genes at these loci, therefore, is estimated to be twice the frequency of the Idh3,4(86) allele.

TABLE 5

Allele frequencies at the Ldh4 and Sod1 loci in the rainbow trout contributing genes to three rainbow-westslope cutthroat trout introgressed populations in the Kootenai River drainage.

Population	Ldh4(100)	allele frequencies		
		Ldh4(76)	Sod1(100)	Sod1(152)
Arbo Creek	0.681	0.319	0.717	0.283
East Fork Yaak River	0.050	0.950	1.000	-
17 Mile Creek (lower)	0.270	0.730	0.941	0.059