GENETIC DIVERGENCE AMONG YELLOWSTONE CUTTHROAT TROUT POPULATIONS IN THE YELLOWSTONE RIVER DRAINAGE, MONTANA: UPDATE

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Population Genetics Laboratory Report 89/2
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ABSTRACT

We used enzyme variation at 46 gene loci to determine the genetic status of 34 trout populations in the Yellowstone River drainage, Montana. The results indicate that 22 (64.7%) of the populations appear to be genetically pure native Yellowstone cutthroat trout. The remaining populations are hybridized with introduced rainbow trout. Thus, about 30 to 40% of the Yellowstone cutthroat trout populations in the area appear to have been lost because of hybridization.

Among the Yellowstone cutthroat trout populations sampled, we detected evidence of intraspecific genetic variation at eleven loci. Only the pair of isoloci AAT-3,4, however, were highly variable in most populations. Variation at the other polymorphic loci was due to low frequency alleles that occurred in few populations. Thus, most of the total genetic variation detected was contained within local populations (96.1%), and very little was due to allele frequency differences among populations (3.9%).

INTRODUCTION

There are three fishes of the genus Oncorhynchus native to the waters of Montana. The westslope cutthroat trout, O. clarki lewisi, has a broad natural distribution. It is native to all major drainages west of the Continental Divide and the upper Missouri and South Saskatchewan drainages east of the Divide. (Trotter 1987). The Yellowstone cutthroat, O. c. bouvieri, and rainbow trout, O. mykiss, have narrow endemic distributions. The former being native only to the Yellowstone River drainage of south central Montana (Trotter 1987) and the latter to the Kootenai River drainage of northwestern Montana (Allendorf et al. 1980).

These trouts are all considered fishes of special concern by the Montana Department of Fish, Wildlife and Parks. Human exploitation of the environment has now made many waters unsuitable for the existence of trout. The major factor responsible for the loss of native trout populations, however, has been the introduction of trout into waters outside their natural range. These introductions have often resulted in interbreeding between the native and introduced fishes and the destruction of the genetic integrity of the native populations (Allendorf et al. 1980; Leary et al. 1984; Marnell et al. 1987; Allendorf and Leary 1988).

Preservation of remaining native trout populations is now the goal of state, federal, and Native American management agencies. Identification of native populations is the initial step in a preservation program. Historically, morphological comparisons were used to distinguish 'genetically pure' populations from those in which hybridization has or is occurring. These comparisons assume that hybridized populations or hybrid swarms will be morphologically intermediate to the parental taxa and have increased

morphological variance. A number of recent studies, however, have shown that these assumptions are not always valid for trout hybrid swarms (Busack and Gall 1981; Leary et al. 1984, 1985; Marnell et al. 1987). Thus, morphological comparisons can potentially provide misleading information about the genetic status of trout populations. That is, whether they represent a genetically pure population of a taxon or a hybrid swarm.

Electrophoretic analysis of proteins provides an extremely powerful means of determining the genetic status of a population when complete, or nearly complete, allele (form of a gene) frequency differences exist between taxa at several loci (genes). Because of this attribute, loci at which such differences exist are commonly termed diagnostic loci (Ayala and Powell 1972). Individuals in samples from a genetically pure population will possess alleles at all diagnostic loci characteristic of only that taxon. In contrast, first generation hybrids will be heterozygous for alleles characteristic of both parental taxa at all diagnostic loci between them (e.g. Leary et al. 1983). Hybrid swarms are created when first generation hybrids mate among themselves and with the parental taxa. Individuals from such populations will have highly variable genotypes at diagnostic loci. They will be homozygous at some diagnostic loci and heterozygous at others. Furthermore, the particular loci that are homozygous and heterozygous will differ widely among individuals when the genes from the parental taxa are randomly distributed throughout the population. In such situations, no individual is likely to be a genetically pure representative of either parental taxa.

Because electrophoretic analysis is now required to reliably identify remaining native trout populations, the genetic status of many populations is unknown. This situation hinders the efficacy of conservation programs and the

use of waters and adjacent lands for other uses such as grazing or logging. For example, with adequate information it could be possible to place emphasis on conservation and other compatible uses on drainages predominantly inhabited by native trout. In drainages where hybrid swarms predominate, other uses could be given priority.

Management agencies now recognize the value of having a knowledge of the genetic status of trout populations under their jurisdiction. Consequently, many populations are being sampled for electrophoretic analysis. This report presents the results of our electrophoretic analysis of trout samples collected from the upper Yellowstone River drainage, Montana. The data are used to address two issues: the proportion of native Yellowstone cutthroat trout populations in the drainage and the relative amount of genetic divergence among them. The latter issue has important implications for the conservation of the genetic diversity of a taxon.

METHODS

Samples for electrophoretic analysis were obtained from trout populations in the upper Yellowstone River drainage by electrofishing during the summer and autumn of 1986 through 1988 (Table 1). The populations were mainly sampled without regard to prior suspicions of their genetic status. Thus, they should represent a reliable estimate of the proportion of genetically pure populations in the region.

Horizontal starch gel electrophoresis was used to determine each fishes genotype at 46 loci coding for proteins present in muscle, liver, or eye tissue. Electrophoresis followed the procedures outlined by Allendorf and Utter (1979). The buffers used to make the gels and recipes for stains used

to reveal the position of particular proteins in the gels after electrophoresis are provided by Allendorf et al. (1977). Nomenclature of loci and alleles follows the terminology of Leary et al. (1987) with modifications to conform with the standard nomenclature for fish recently proposed by an American Fisheries Society panel. The following enzymes with the loci that encode them in parentheses were analyzed: adenylate kinase (AK-1,2), alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT-1,2,3,4), creatine kinase (CK-A1,2, CK-B, CK-C1,2), dipeptidase (PEPA-1,2), glucose-6-phosphate isomerase (GPI-A, GPI-B1,2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH-3,4), glycerol-3-phosphate dehydrogenase (G3PDH-1,2), iditol dehydrogenase (IDDH-1,2), isocitrate dehydrogenase (IDH-1,2, mIDH-1,2), lactate dehydrogenase (LDH-A1,2, LDH-B1,2, LDH-C), malate dehydrogenase (MDH-A1,2, MDH-B1,2, MDHp-1,2, mMDHp-1,2), phosphoglucomutase (PGM-1,2), 6-phosphogluconate dehydrogenase (PGDH), tripeptide aminopeptidase (PEPB), superoxide dismutase (SOD-1), and xanthine dehydrogenase (XDH).

A number of diagnostic loci are known to exist between the Yellowstone cutthroat trout and either the rainbow or westslope cutthroat trout (Leary et al. 1987, Table 2). We used the alleles detected and the genotypes of individuals at these loci to determine the genetic status of the populations from which the samples were obtained. We concentrated only on these fish because the rainbow and westslope cutthroat trout are the only fishes capable of interbreeding with Yellowstone cutthroat trout that are likely to have been introduced into the drainage.

Information from all the loci was used to estimate the amount of genetic divergence among the populations considered to be genetically pure Yellowstone cutthroat trout relative to the amount of genetic variation within the

populations. In Yellowstone cutthroat trout, some pairs of loci produce a protein with identical function and electrophoretic mobility. For example, AAT-3 and AAT-4 both produce an aspartate aminotransferase present in muscle and the proteins produced from the common allele 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 detected only when one or both loci are polymorphic (genetically variable). In these situations, however, it is not possible to determine at which particular locus of the pair a variant allele exists. In order to estimate allele frequencies at the isoloci in Yellowstone cutthroat trout (AAT-3,4, IDDH-1,2, MDH-A1,2, and MDH-B1,2), therefore, we treated each pair as a single gene with four instead of two copies per individual.

We estimated the proportional genetic contribution of Yellowstone cutthroat and introduced trout to hybrid swarms by averaging the alleles characteristic of each species over all diagnostic loci. In rainbow trout, IDH-1,2 constitute a pair of isoloci. Thus, we treated them as such in Yellowstone cutthroat-rainbow trout hybrid swarms. With this terminology, the frequency of IDH-1,2*71 in pure Yellowstone cutthroat trout populations is usually 0.500. Thus, the proportion of Yellowstone cutthroat trout genes at these loci in hybrid swarms with rainbow trout is estimated to be twice the frequency of IDH-1,2*71.

RESULTS AND DISCUSSION

Hybrid swarms

Of the 34 samples analyzed, 12 came from populations that had hybridized with rainbow trout. In these populations, we detected alleles characteristic of both the Yellowstone cutthroat and rainbow trout at all or practically all

the diagnostic loci between these species (Tables 1 and 3). In all of these samples except that from Coke Creek, at least one individual had a multiple locus genotype at the diagnostic loci indicative of matings between hybrids or hybrids and parental taxa. With the exception of Coke Creek, therefore, these populations are undoubtedly Yellowstone cutthroat-rainbow trout hybrid swarms.

In the Coke Creek sample, 22 fish contained alleles at all diagnostic loci characteristic of only Yellowstone cutthroat trout. The other three fish were heterozygous at all diagnostic loci for alleles characteristic of both Yellowstone cutthroat and rainbow trout. Thus, in July 1986 this population appeared to largely contain pure Yellowstone cutthroat trout and a few first generation Yellowstone cutthroat-rainbow trout hybrids.

The average frequency of rainbow and Yellowstone cutthroat genes in hybrid swarms can be used to obtain an insight into whether the genes from these species are randomly or not randomly distributed among individuals in the populations. In the Coke Creek population, the genes are not randomly distributed among individuals since all rainbow trout genes are apparently confined to only first generation hybrids. The genes from the two taxa also do not appear to be randomly distributed among individuals in the East and North Fork Bear Creek, Mol Heron Creek, Big Creek above Cliff, and Big Creek below Cliff populations. In all of these samples except the last one, there is an excess of individuals with alleles at all diagnostic loci characteristic of only Yellowstone cutthroat trout. Although these populations are hybrid swarms, they apparently still contain some genetically pure Yellowstone cutthroat trout.

The Big Creek samples represent an interesting situation. The sample from below Cliff Creek contained an excess of individuals that appear to be

genetically pure rainbow trout. The sample from above Cliff Creek contained an excess of individuals that appear to be genetically pure Yellowstone cutthroat trout. Thus, Big Creek is inhabited by at least two genetically distinct hybrid swarms; one with a predominant rainbow trout genetic contribution and one above it with a predominant Yellowstone cutthroat trout genetic contribution.

In contrast to the above populations, the genes from the rainbow and Yellowstone cutthroat trout appear to be randomly distributed among individuals in the hybrid swarms in Area, Cinnabar, Little Trail, Mission, and West Pine Creek. These populations, therefore, probably do not contain any genetically pure individuals of the parental taxa.

We can only speculate why in some hybrid swarms the Yellowstone cutthroat and rainbow trout genes are randomly distributed among individuals and in others they are not. When first generation hybrids are produced in only a single spawning season, it takes about five generations of random mating before the genes of the parental taxa are randomly distributed among individuals in a hybrid swarm. Thus, one explanation for the nonrandom distribution of genes in some hybrid swarms is that they have existed only for a few generations. This certainly pertains to the Coke Creek population which in 1986 apparently contained only Yellowstone cutthroat trout and first generation hybrids.

The attainment of a random distribution of genes can be delayed with nonrandom mating or the migration of individuals from genetically divergent populations into hybrid swarms. Thus, it is also possible that the hybrid swarms with a nonrandom distribution of Yellowstone cutthroat and rainbow trout genes have existed for an appreciable amount of time. Either nonrandom

mating or migration could be of sufficient magnitude to prevent attaining a random distribution of genes. We suspect the migration factor to be at least a partial explanation for the Big Creek hybrid swarms. The samples were obtained only a short distance apart and there is no barrier to migration in this region. Fish, therefore, could frequently migrate from one population to the other. It is not possible to determine at this time whether the nonrandom distribution of genes in the East and North Fork Bear Creek and Mol Heron Creek, is mainly due to recent hybridization, migration, or nonrandom mating.

Genetically pure Yellowstone cutthroat trout populations

In 22 of the samples, we detected alleles characteristic of only Yellowstone cutthroat trout at all or practically all of the diagnostic loci (Tables 4 and 5). In a few of these samples, we detected an allele characteristic of either the westslope cutthroat or rainbow trout at one or two diagnostic loci (Table 5). There are two possible explanations for these situations. The samples may have come from hybrid swarms with a small percentage of westslope cutthroat or rainbow trout genes. Conversely, these alleles may represent intraspecific Yellowstone cutthroat genetic variation that is identical to the common allele at these loci characteristic of the other taxa. If we assume the frequency of these alleles represents the proportional genetic contribution of westslope cutthroat or rainbow trout to a hybrid swarm, then the probability we would detect no other alleles characteristic of these fishes at all the other diagnostic loci is less than 0.0002 in all cases. Thus, we conclude these situations most likely represent instances of Yellowstone cutthroat trout genetic variation that is identical to the common allele at these loci in westslope cutthroat or rainbow trout.

With a sample size of 15 or more individuals, we have better than a 95% chance of detecting as little as one percent westslope cutthroat or rainbow trout genes in a hybrid swarm with Yellowstone cutthroat. This assumes the genes of the parental taxa are randomly distributed among individuals. All of the sample sizes from the populations in Table 4 are equal to or greater than 15 except those from East Fork Smith Creek (N=9), Lodgepole Creek (N=4), and Turkey Creek (N=13). Thus, with the exception of these populations we can be reasonably certain that all populations in Table 4 represent genetically pure Yellowstone cutthroat trout.

Because the samples were largely collected without prior suspicion of their genetic status, they should provide a reliable estimate of the percentage of Yellowstone cutthroat trout populations remaining in the sampled area. At best, only 22 of 34 populations sampled have not become hybridized with rainbow trout. Excluding the three populations whose genetic status is uncertain because of small sample sizes reduces the percentage of genetically pure Yellowstone cutthroat trout populations remaining only from 64.7% (binomial 95% confidence interval 48.3 to 81.1%) to 61.3% (46.0 to 76.6%). Thus, approximately 40 to 30% of the populations of Yellowstone cutthroat trout in the drainage appear to have been lost because of hybridization with introduced rainbow trout.

Genetic divergence among Yellowstone cutthroat trout populations

The total amount of genetic diversity within a taxon usually has a hierarchical geographic structure commonly referred to as its population genetic structure. For example, a certain proportion of the total variation may be due to genetic differences among populations inhabiting particular regions, among populations within the regions, and finally among individuals

within a population. An understanding of the population genetic structure of a taxon is crucial to formulating a genetically sound conservation program.

We used the procedure of Nei (1972) to estimate the population genetic structure of Yellowstone cutthroat trout in the area sampled. In the analysis, we excluded the Lodgepole Creek population because the extremely small sample size precludes obtaining accurate allele frequency estimates. The total amount of genetic variation among the 21 remaining populations (H=0.0129) was divided into that due to allele frequency differences among them (H=0.0005, 3.9%) and genetic variation within them (H=0.0124, 96.1%). Thus, the vast majority of the genetic variation is contained within local populations of Yellowstone cutthroat trout.

The population genetic structure of Yellowstone cutthroat trout arises from two distinct factors. We detected genetic variation at eleven loci (Tables 4 and 5). The only highly variable locus, however, is the pair of isoloci AAT-3,4. This variation is present in all populations resulting in relatively little between population variation but substantial within population variation. In contrast, variation at the other loci is due to alleles that usually occur at low frequency (less than 0.05) and in only a few populations. These alleles result in little divergence among and little variation within populations. Thus, the population genetic structure of Yellowstone cutthroat trout largely reflects the widespread distribution of three alleles at AAT-3,4 that occur at appreciable frequency (greater than 0.100) in practically all populations sampled and a number of sporadically distributed low frequency alleles. Conservation of the genetic diversity of Yellowstone cutthroat trout, therefore, may effectively be accomplished by

ensuring the continued existence of only a few populations. From other perspectives, however, this may not constitute a wise management policy.

Data are available on the population genetic structure of seven other salmonid taxa (Table 6). With the exception of Yellowstone cutthroat trout, those taxa inhabiting interior drainages show substantial genetic divergence among populations. In contrast, the population genetic structure of Yellowstone cutthroat trout generally resembles that of anadromous species. This suggests that the migratory nature of Yellowstone cutthroat trout in the Yellowstone River drainage results in substantially more gene flow among populations than is usual for interior salmonid fishes. This gene flow and the presence of hybrid swarms threatens the genetic integrity of the remaining Yellowstone cutthroat trout populations in the drainage. Conservation programs, therefore, should place emphasis on practically all populations known to be genetically pure Yellowstone cutthroat trout that are isolated from migration due to barriers to upstream dispersal.

ACKNOWLEDGEMENTS

Samples were collected by Chris Clancy of the Montana Department of Fish, Wildlife, and Parks. Financial support was provided by the United States Forest Service.

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TABLE 1

Trout populations sampled in the upper Yellowstone River drainage and their genetic status. Y = pure Yellowstone cutthroat trout, YxR = hybird swarm of Yellowstone cutthroat and rainbow trout.

Creek	Sample Location	Collection Date	Sample Size	Genetic Status
Anderson	T6S R10E S34	1986	25	Y
Area	T2S R8E S11	1986	25	YxR
Big (above Cliff)	T6S R6E S23	July 2, 1987	14	YxR
Big (below Cliff)	T6S R6E S24	July 2, 1987	11	YxR
Billman	T2S R8E S13	1986	19	Y
Brackett	T1N R7E S5	June 19, 1987	20	Y
Cinnabar (headwaters)	T8S R7E S32	1986	15	YxR
Coke	T2S R8E S26	July 31, 1986	25	YxR
Eagle	T9S R8E S13	1986	25	YxR
East Fork Bear	T9S R9E S4	1986	10	YxR
East Fork Mill	T6S R10E S18	July 22, 1987	20	Y
East Fork Smith	T6N R10E S6	October 20, 1988	9	Y
Little Mission	T3S R11E S14	June 12, 1987	21	Y
Little Trail	T9S R8E S9	October 1, 1986	4	YxR
Lodgepole	T5N R11E S16	1986	4	Y
Middle Fork Brackett	T1N R7E S7	July 23, 1987	21	Y
Mill	T6S R10E S32+34	July 8, 1986(N=8) Sept. 29, 1988(N=15)	23	Y
Mill Fork Mission	T3S R11E S4	1986	21	Y
Miner	T2S R8E S27	1986	28	Y
Mission	T2S R11E S33	Sept. 29, 1988	12	YxR
Mol Heron	T8S R7E S25	October 3, 1986	29	YxR
North Fork Bear	T9S R9E S4	October 1, 1986	10	YxR
North Fork Brackett	T1N R7E S5	July 23, 1987	21	Y
Passage	T7S R10E S8	Sept. 29, 1988	23	Y
Rock (1)	T7S R7E S19	Sept. 30, 1986	25	Y
Rock (2)	T2N R11E S8+9	October 6, 1988	19	Y
Six Mile	T7S R8E S9	1986	25	Y
Shields	T5N R11E S18	October 20, 1988	22	Y
Smith	T6N R10E S6	October 20, 1988	23	Y
Suce	T3S R10E S16	June 12, 1987	16	Y
Tom Miner	T8S R6E S9	Sept. 30, 1986	25	Y
Turkey	T5N R11E S21	1986	13	Y
West Fork Mill	T6S R9E S35	July 22, 1987	20	Y
West Pine	T4S R8E S5	Sept. 29, 1988	21	YxR

TABLE 2

Alleles at the diagnostic loci between Yellowstone cutthroat and rainbow trout and Yellowstone and westslope cutthroat trout. When more than one allele exists at a locus within a taxon, the most common allele is listed first.

	Characteristic alleles			Characteristic alleles	
Locus	Yellowstone	Rainbow	Locus	Yellowstone	Westslope
AAT-1	165	100	AAT-1	165	200,250
CK-A2	84	100	CK-C1	38	100,38
CK-C1	38	100,38	GPI-A	100	92,100
IDH-1,2	71,100	100,114	IDDH-1,2	100,200	40,100, 71,40
mIDH-1	-75	100	IDH-1	71	86,71
MDHp-1	90	100,75	mIDH-1	-75	100
MDHp-2	110	100	MDHp-1	90	100
PEPA-1	101	100,115,90	MDHp-2	110	100
PEPB	135,100	100,135	mMDHp-1	null	88
PGM-1	null	100,null	PEPA-1	101	100
			PEPB	135,100	100
			PGM-1	null	100,110, null

TABLE 3

Allele frequencies at the diagnostic loci between Yellowstone cutthroat and rainbow trout in hybridized populations of these fishes in the Yellowstone River drainage. At each locus, the allele characteristic of Yellowstone cutthroat trout is listed first.

		Sample and allele frequencies					
Locus	Alleles	Area	Big (above Cliff)	Big (below Cliff)	Cinnibar	Coke	Eagle
AAT-1	165	1.000	0.536	0.227	1.000	0.940	0.900
	100	-	0.464	0.773	-	0.060	0.100
CK-A2	84	0.980	0.571	0.182	1.000	0.940	0.980
	100	0.020	0.429	0.818	-	0.060	0.020
CK-C1	38	1.000	0.500	0.050	1.000	0.940	0.980
	100	-	0.500	0.950	-	0.060	0.020
IDH-1,2	71	0.500	0.339	0.091	0.484	0.470	0.430
	100	0.500	0.589	0.727	0.516	0.530	0.440
	114	-	0.018	-	-	-	-
	40	-	0.054	0.182	-	-	0.130
mIDH-1	-75	1.000	0.571	0.045	1.000	0.940	0.960
	100	-	0.429	0.955	-	0.060	0.040
MDHp-1	90	1.000	0.607	0.091	0.967	0.940	0.840
	100	-	0.393	0.909	0.033	0.060	0.160
MDHp-2	110	1.000	0.571	0.045	1.000	0.940	1.000
	100	-	0.429	0.955	-	0.060	-
PEPA-1	101	1.000	0.464	0.091	1.000	0.940	0.959
	100	-	0.536	0.909	-	0.060	0.041
PEPB	135	1.000	0.536	-	0.933	0.940	0.900
	100	-	0.464	1.000	0.067	0.060	0.100
PGM-1	null	0.980	0.679	0.136	1.000	0.940	0.894
	100	0.020	0.321	0.864	-	0.060	0.106
Average Yellowstone		0.996	0.571	0.105	0.987	0.940	0.929
Average Rainbow		0.004	0.429	0.895	0.013	0.060	0.071

TABLE 3 - CONTINUED

Locus	Alleles	East Fork Bear	Little Trail	Mission	Mol Heron	North Fork Bear	West Pine
		Sample and allele frequencies					
AAT-1	165	0.850	1.000	1.000	0.638	0.500	0.976
	100	0.150	-	-	0.362	0.500	0.024
CK-A2	84	0.900	1.000	1.000	0.638	0.700	0.976
	100	0.100	-	-	0.362	0.300	0.024
CK-C1	38	0.800	1.000	1.000	0.619	0.600	0.810
	100	0.200	-	-	0.381	0.400	0.190
IDH-1,2	71	0.400	0.375	0.479	0.405	0.325	0.500
	100	0.600	0.438	0.521	0.569	0.625	0.476
	114	-	0.188	-	-	-	0.024
	40	-	-	-	0.026	0.050	-
mIDH-1	-75	0.750	0.625	0.958	0.707	0.550	0.952
	100	0.250	0.375	0.042	0.293	0.450	0.048
MDHp-1	90	0.750	0.625	1.000	0.638	0.800	0.952
	100	0.250	0.375	-	0.362	0.200	0.048
MDHp-2	110	0.850	1.000	0.958	0.672	0.450	1.000
	100	0.150	-	0.042	0.328	0.550	-
PEPA-1	101	0.900	1.000	1.000	0.743	0.700	1.000
	100	0.100	-	-	0.257	0.300	-
PEPB	135	0.900	1.000	0.958	0.672	0.600	0.952
	100	0.100	-	0.042	0.328	0.400	0.048
PGM-1	null	0.850	1.000	1.000	0.766	0.707	0.976
	100	0.150	-	-	0.234	0.293	0.024
Average Yellowstone		0.835	0.900	0.983	0.690	0.626	0.959
Average Rainbow		0.165	0.100	0.017	0.310	0.374	0.041

TABLE 4

Allele frequencies at the highly variable pair of isoloci AAT-3,4 in populations of Yellowstone cutthroat trout in the Yellowstone River drainage, Montana.

	Alleles and their frequencies			
Sample	100	110	90	H_e
Anderson	0.590	0.260	0.150	0.0137
Billman	0.697	0.105	0.197	0.0113
Brackett	0.788	0.125	0.088	0.0099
East Fork Mill	0.413	0.350	0.238	0.0182
East Fork Smith	0.917	0.028	0.056	0.0037
Little Mission	0.655	0.310	0.036	0.0116
Lodgepole	0.750	-	0.250	0.0089
Middle Fork Brackett	0.786	0.119	0.095	0.0110
Mill	0.684	0.152	0.163	0.0115
Mill Fork Mission	0.488	0.179	0.333	0.0173
Miner	0.634	0.250	0.116	0.0153
North Fork Brackett	0.810	0.131	0.060	0.0090
Passage	0.848	0.065	0.087	0.0064
Rock (1)	0.530	0.280	0.190	0.0147
Rock (2)	0.700	0.225	0.075	0.0120
Six Mile	0.850	0.090	0.060	0.0065
Shields	0.614	0.205	0.182	0.0151
Smith	0.652	0.141	0.207	0.0179
Suce	0.594	0.281	0.125	0.0135
Tom Miner	0.580	0.250	0.170	0.0140
Turkey	0.712	0.173	0.115	0.0110
West Fork Mill	0.513	0.400	0.088	0.0162

Notes: H_e = average expected heterozygosity based on all loci analyzed. The two Mill Creek samples are combined into one because the allele frequencies are statistically homogenous between them (chi-square $P > 0.05$).

TABLE 5

Allele frequencies at rarely polymorphic loci in populations of Yellowstone cutthroat trout from the upper Yellowstone River drainage, Montana. Populations in Table 4 not listed here were monomorphic for the common allele at all these loci.

Locus	Alleles	Brackett	East Fork Mill	Middle Fork Brackett	Mill Fork	Miner
ADH	-100	1.000	1.000	1.000	0.952	1.000
	0	-	-	-	0.048	-
CK-C1	38	1.000	1.000	1.000	1.000	1.000
	100	-	-	-	-	-
IDDH-1,2	100	1.000	1.000	1.000	1.000	0.982
	200	-	-	-	-	0.018
IDH-2	100	1.000	1.000	1.000	1.000	1.000
	71	-	-	-	-	-
mIDH-1	-75	0.975	1.000	1.000	1.000	1.000
	100	0.025	-	-	-	-
LDH-B1	100	1.000	1.000	1.000	1.000	1.000
	null	-	-	-	-	-
LDH-B2	100	1.000	0.975	1.000	1.000	1.000
	112	-	0.025	-	-	-
PEPA-1	101	1.000	1.000	1.000	1.000	0.982
	88	-	-	-	-	0.018
PEPB	135	1.000	0.975	1.000	1.000	0.982
	100	-	0.025	-	-	0.018
PGM-2	100	1.000	1.000	0.952	1.000	1.000
	90	-	-	0.048	-	-

TABLE 5 - CONTINUED

Locus	Alleles	Samples and allele frequencies: North Fork Brackett	Rock (2)	Shields	Smith	West Fork Mill
ADH	-100	1.000	1.000	1.000	1.000	1.000
	0	-	-	-	-	-
CK-C1	38	1.000	1.000	1.000	0.977	1.000
	100	-	-	-	0.023	-
IDDH-1,2	100	1.000	1.000	1.000	1.000	1.000
	200	-	-	-	-	-
IDH-2	100	1.000	1.000	0.955	1.000	1.000
	71	-	-	0.045	-	-
mIDH-1	-75	1.000	0.975	1.000	1.000	1.000
	100	-	0.025	-	-	-
LDH-B1	100	0.976	1.000	1.000	1.000	1.000
	null	0.023	-	-	-	-
LDH-B2	100	1.000	1.000	1.000	1.000	1.000
	112	-	-	-	-	-
PEPA-1	101	1.000	1.000	1.000	1.000	1.000
	88	-	-	-	-	-
PEPB	135	1.000	1.000	1.000	0.891	0.950
	100	-	-	-	0.109	0.050
PGM-2	100	1.000	1.000	1.000	1.000	1.000
	90	-	-	-	-	-

TABLE 6

Population genetic structure of eight salmonid taxa H_T = total amount of genetic variation, H_S = average variation within populations.

Taxa	H_T	H_S	Percent total variation: Between drainages	Between pop. within drainage	Within population
Coastal cutthroat*	0.101	0.095	2.2	3.6	94.2*
Lahontan cutthroat	0.065	0.036	-	44.5	55.5
Westslope cutthroat	0.029	0.019	16.7	15.7	67.6
Yellowstone cutthroat	0.013	0.012	-	3.9	96.1
Atlantic salmon*	0.040	0.023	37.4	3.6	59.0*
Brown trout	0.040	0.025	7.5	29.2	63.3
Rainbow trout*	0.069	0.058	7.3	7.7	85.0*
Sockeye salmon*	0.046	0.044	2.5	3.1	94.4*

Notes: * = anadromous taxa. Data are from the following sources: Atlantic salmon (*Salmo salar*), rainbow trout, and sockeye salmon (*O. nerka*) Ryman (1983); Lahontan cutthroat (*O. c. henshawi*) Loudenslager and Gall (1980); coastal cutthroat (*O. c. clarki*) Campton and Utter (1987); westslope cutthroat Allendorf and Leary (1988); Yellowstone cutthroat this report.

University of Montana
Missoula, Montana 59812

February 28, 1989

Wayne Hadley
Montana Department of Fish
Wildlife, and Parks
P.O. Box 835
Deer Lodge, MT 59722

Dear Wayne:

During a recent freezer inventory/cleanup we discovered a sample of trout (N=23) collected from Wyman Creek (T8N, R13W, S22) June 30, 1986. After all these years, we have finally completed the electrophoretic analysis of these fish. Each fishes genotype was determined at 45 loci (genes) coding for proteins present in muscle, liver, or eye tissue (Table 1). At some of these loci, the westslope, Oncorhynchus clarki lewisi, and Yellowstone cutthroat trout, O. c. bouvieri, rarely possess alleles (form of a gene) in common (Table 2). At other loci, this same situation exists between the westslope cutthroat and rainbow trout, O. mykiss. Loci at which such fixed allele frequency differences exist between taxa are commonly termed diagnostic loci because the alleles detected at them can be used to determine the genetic status of a population. That is, whether it is a genetically pure population of one of these taxa or a hybridized population of two or all of these fishes.

We detected alleles characteristic of the westslope cutthroat trout at all diagnostic loci except AAT-1 (Table 3). At AAT-1, one fish in the sample was heterozygous for the alleles characteristic of westslope and rainbow trout. The latter allele could indicate a small percentage of rainbow trout genes in the population. Conversely, it could be intraspecific westslope cutthroat trout genetic variation that is electrophoretically identical to the allele characteristic of rainbow trout. Assuming that the frequency of AAT-1*100 in the sample represents the proportion of rainbow trout genes in a hybridized population, then the probability we would not detect any rainbow trout alleles at other diagnostic loci is 0.006. Thus, we conclude that it is more likely AAT-1*100 is intraspecific westslope cutthroat trout genetic variation than evidence of hybridization with rainbow trout. Wyman Creek, therefore, apparently contains a genetically pure popultion of westslope cutthroat trout.

Sincerely,

Robb Leary

TABLE 1

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

Enzyme	Loci	Tissue
Adenylate kinase	AK-1,2	M
Alcohol dehydrogenase	ADH	L
Aspartate aminotransferase	AAT-1,2 AAT-(3,4)	L M
Creatine kinase	CK-A1,2 CK-B, CK-C1,2	M E
Dipeptidase	PEPA-1,2	E
Glucose-6-phosphate isomerase	GPI-A, GPI-B1,2	M
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH-3,4	E
Glycerol-3-phosphate dehydrogenase	G3PDH-1,2	L
Iditol dehydrogenase	IDDH	L
Isocitrate dehydrogenase	IDH-1,2 mIDH-1,2	L M
Lactate dehydrogenase	LDH-A1,2 LDH-B1,2, LDH-C	M E
Malate dehydrogenase	MDH-A(1,2) MDH-B(1,2) MDHp-1,2 mMDHp-1,2	L M L M
Phosphoglucomutase	PGM-1,2	M
6-Phosphogluconate dehydrogenase	PGDH	M
Tripeptide aminopeptidase	PEPB	E
Superoxide dismutase	SOD-1	L
Xanthine dehydrogenase	XDH	L

Notes: The common alleles at the pairs of loci in parentheses produce a protein with identical function and electrophoretic mobility. Such pairs of loci are usually termed isoloci and their existence can be detected only when one or both loci are genetically variable. In these 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, therefore, each pair is considered to be a single gene with four instead of two copies per individual.

TABLE 2

Diagnostic loci between westslope and Yellowstone cutthroat trout and between westslope cutthroat and rainbow trout. When more than one allele exists at a locus within a taxon the most common allele is listed first.

	Characteristic alleles			Characteristic alleles	
Locus	Westslope	Yellowstone	Locus	Westslope	Rainbow
AAT-1	200,250	165	AAT-1	200,250	100
CK-C1	100,38	38	CK-A2	84,100	100,75
GPI-A	92,100	100	GPI-A	92,100	100
IDDH	40,100	100,-63	IDDH	40,100	100,200,40
IDH-1	86,114,71	71	IDH-1,2	86,114,100, 71, 40	100,114, 71,40
mIDH-1	100	-75	mMDHp-1	88	null
MDHp-1	100	90			
MDHp-2	100	110			
mMDHp-1	88	null			
PEP-A1	100	101			
PEP-B	100	135			
PGM-1	100, null	null			

Notes: In rainbow trout, IDH-1,2 constitute a pair of isoloci. In hybridized populations between westslope cutthroat and rainbow trout, therefore, these loci are treated as isoloci. The 86 allele at these loci is characteristic of westslope cutthroat trout and usually exists in a genetically pure population at a frequency of 0.500. Thus, in hybridized populations the percentage of westslope cutthroat trout genes at these loci is estimated to be twice the frequency of the 86 allele.

Table 3

Allele frequencies at the genetically variable loci in westslope cutthroat trout from Wyman Creek. All other loci analyzed were genetically invariant for alleles characteristic of westslope cutthroat trout.

Locus	Alleles	Alleles frequencies
AAT-1	200	0.978
	100	0.022
Ck-C1	100	0.913
	38	0.087
GAPDH-4	100	0.957
	null	0.043
IDH-1	86	0.804
	71	0.196
IDH-2	100	0.348
	40	0.652
LDH-B2	100	0.957
	112	0.043
PGM-2	100	0.978
	62	0.022
Proportion polymorphic loci		0.167
Average expected heterozygosity		0.028