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Lee:

The paired interspersed nuclear DNA elements (PINE) technique has been used to analyze DNA from the following trout samples:

Summary of results.

		a	b	c	d	e	f
Sample #	**Water Name/Location/Collection Date/ Collector**	**N**	**# markers**	**Species ID**	**Power (%)**	**% WCT**	**Individuals**
3237	**Simpson Creek** 9/22/2005 Lee Nelson	50(67)	R6Y4	WCT	R99Y99	100	xx
3238	**North Fork Everson Creek** 9/28/2005 Lee Nelson	50(60)	R6Y4	WCT	R99Y99	100	xx
3239	**Middle Fork Cabin Creek** 10/11/2005 Pat Clancey	8	R6Y4	WCT X RBT			xx
3240	**Middle Fork Cabin Creek** 10/11/2005 Pat Clancey	17	R6Y4	WCT X RBT			xx
3241	**Cabin Creek** 10/17/2005 Pat Clancey	15	R6Y4	WCT X RBT		97W3R	xx
3242	**WF Dyce Creek** 6S12W14NW1/4 2/25/2005 Paul Hutchinson	25	R6Y4	WCT X RBT			xx
3243	**Arasta Creek** 7/14/2005 Chris Riley	25	R6Y4	WCT X YCT X RBT		87W8R5Y	xx
3244	**Rose Creek** 7/14/2005 Bruce Roberts	12	R6Y4	WCT X RBT		97W3R	xx
3246	**Rape Creek** 10S13W21 8/12/2005 Paul Hutchinson	25	R6Y4	WCT	R95Y87	100	xx

Sample #	Water Name/Location/Collection Date/ Collector	a N	b # markers	c Species ID	d Power (%)	e % WCT	f Individuals
3248	**East Fork Cabin Gulch** 10/13/2005 Lee Nelson	25	R6Y4	WCT X YCT		82W18Y	xx
3249	**Twelvemile Creek** 8/19/2005 Dan Downing	17	R6Y4	WCT	R87Y75	100	xx
3250	**Soap Creek** 6/8/2005 Chris Riley	10	R6Y4	WCT X RBT		94W6R	xx
3251	**Moose Creek** 7/16/2002 Bruce Roberts	7	R6Y4	WCT X RBT		94W6R	xx
3252	**Upper Whitetail Deer Creek** 6/29/2005 Chris Riley	25(70)	R6Y4	WCT	R99Y99	100	xx
3253	**Little Wapiti Creek** 7/26/2005 Bruce Roberts	23	R6Y4	WCT X RBT		81W19R	xx
3254	**Lump Gulch** 9/1/2004 Lee Nelson	10	R6Y4	YCT	R70W33	100	xx
3255	**Unnamed trib2 South Fork Sixteenmile Creek** 9/8/2003 Brad Shepard	20	R6Y4	WCT X YCT X RBT		82W9Y9R	xx
3256	**LF Deadhorse Creek** 9S3E03SE1/4NW1/4 10/7/2003 Scott Barndt	10	R6Y4	WCT X RBT		86W14R	xx
3257	**Unnamed trib South Fork Sixteenmile Creek** 6/24/2005 Bruce Roberts	9	R6Y4	WCT X RBT		97W3R	xx
3258	**Cottonwood Creek** 10S07W27 6/1/2004 Pat Clancey	33	R6Y4	WCT	R98Y93	100	xx
3259	**Cottonwood Creek** 10S07W27 6/1/2005 Pat Clancey	19	R6Y4	WCT X RBT			xx
3260	**Upper Jack Creek** 07N06W13NW1/4NW1/4 9/19/2003 Tim LaMarr	11	R6Y4	WCT	R98Y99	100	xx
3261	**Upper German Gulch** 03N10W33SE1/4SE1/4 7/17/2003 Tim LaMarr	30	R6Y4	WCT X RBT			xx
3262	**Beefstraight Creek** 03N11W24SW1/4SE1/4 8/20/2003 Tim LaMarr	25	R6Y4	WCT X RBT		98W2R	xx

Sample #	Water Name/Location/Collection Date/ Collector	N [a]	# markers [b]	Species ID [c]	Power (%) [d]	% WCT [e]	Individuals [f]
3263	**Minnesota Gulch** 03N10W31NW1/4SW1/4 6/26/2003 Tim LaMarr	29(70)	R6Y4	WCT	R97Y90	100	xx
3264	**Whitetail Deer Creek** 6/21/2005 Chris Riley	20	R6Y4	WCT	R99Y99	100	xx
3265	**Wild Horse Creek** 04S06E11SW1/4 6/15/2005 Bruce Roberts	7(30)	R6Y4	WCT	R97Y92	100	xx
3266	**Seymour Creek** 8/18/2005 Dan Downing	6	R6Y4	WCT X YCT?			xx
3267	**Little Elk River** 7/19/2005 Chris Riley	10	R6Y4	YCT	R70W33	100	xx
3268	**Hellroaring Creek** 7/26/2005 Chris Riley	10	R6Y4	WCT X YCT X RBT		27W17Y56R	xx
3269	**Magpie Creek** 7/1/2004 Lee Nelson	20	R5Y4	WCT	R87Y80	100	xx
3270	**Cooney Gulch** 7/1/2004 Lee Nelson	20	R6Y4	WCT X RBT		95W5R	xx

[a]Number of fish successfully analyzed. If combined with a previous sample, the number in parentheses indicates the combined sample size
[b]Number of markers analyzed that are diagnostic for the non-native species (R=rainbow trout, W=westslope cutthroat trout, Y=Yellowstone cutthroat trout).
[c]Codes: WCT = westslope cutthroat trout (*Oncorhynchus clarki lewisi*); RBT = rainbow trout (*O. mykiss*); YCT = Yellowstone cutthroat trout (*O. clarki bouvieri*). Only one species code is listed when the entire sample possessed alleles from that species only. However, it must be noted that we cannot definitively 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 %). Species codes separated by "x" indicate hybridization between those species.
[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, 25 individuals are required to yield a 95% chance to detect as little as 1% hybridization with rainbow or an 87% chance to detect as little as 1% hybridization with Yellowstone cutthroat trout into what once was a westslope cutthroat trout population. Not reported when hybridization is detected.
[e]Indicates the genetic contribution of the hybridizing taxa in the order listed under c to the sample assuming Hardy-Weinburg proportions. This number is reported if the sample appears to have come from a hybrid swarm. That is, a random mating population in which species markers are randomly distributed among individuals.
[f]Indicates number of individuals with genetic characteristics corresponding to the species code column when the sample can be analyzed on the individual level. This occurs when marker alleles are not randomly distributed among individuals and hybridization appears to be recent and/or if the sample appears to consist of a mixture of populations and hybrids and non-hybrids can be reliably distinguished.

Methods and Data Analysis

The PINE technique uses short synthetically made segments of DNA called primers, in pairs, to search for relatively small segments of organismal DNA flanked by particular, often viral, DNA inserts. During the polymerase chain reaction (PCR), the primers bind to the ends of the inserts and many copies of the organismal DNA between the primers are made. While the DNA from some organisms may have two appropriately spaced inserts to which the primers can attach, the DNA from other organisms may have only one or none of the

appropriately spaced inserts in particular regions. During PCR we will fail to copy DNA in the latter two cases. Thus, the PINE technique coupled with PCR is used to search for evidence of genetic variation based on the presence or absence of particular DNA fragments. The fragments are labeled by the primers used to produce them and their length in terms of the number of nucleotides in the fragment.

The fragments are made using dye labeled nucleotides and after PCR are separated from each other via electrophoresis in polyacrylamide gels. Smaller fragments move through the gels at a faster rate than larger fragments. The use of dye labeled nucleotides allows one to visualize the position of the fragments in the gels after electrophoresis using a spectrophotometer and the size of the fragments is determined by comparison to the position of synthetic fragments of known size that were also migrated into the gel.

When DNA from westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and rainbow trout, *O. mykiss*, is compared with PINE analysis and three different pairs of primers seven fragments are usually characteristic of westslope cutthroat trout and six fragments are usually characteristic of rainbow trout (Table 1). Likewise, when DNA from westslope and Yellowstone cutthroat trout, *O. c. bouvieri*, is compared using the same procedure two fragments are usually characteristic of westslope cutthroat trout and four fragments are usually characteristic of Yellowstone cutthroat trout (Table 1).

Fragments produced from the DNA of one taxon and not another are commonly termed diagnostic or marker loci because they can be used to help determine whether a sample came from a non-hybridized population of one of the taxa or a population in which hybridization between them has or is occurring. Individuals from a non-hybridized population will possess fragments characteristic of only that taxon. In contrast, since half the DNA of first generation hybrids comes from each of the parental taxa the DNA from such individuals will yield all the fragments characteristic of the two parental taxa. In later generation hybrids, the amount and particular regions of DNA acquired from the parental taxa will vary among individuals. Thus, DNA from later generation hybrid individuals will yield only a subset of the parental fragments and the particular subset will vary among individuals.

In a sample from a random mating hybrid swarm, that is a population in which the genetic material (i.e. fragments) of the parental taxa is randomly distributed among individuals such that essentially all of them are of hybrid origin, the frequency of the fragment producing allele from the non-native taxon is expected to be nearly equal among the diagnostic loci since their presence can all be traced to a common origin or origins. Thus, if a sample contains substantial variation at only a single marker locus where the presence of the fragment is usually characteristic of a non-native taxon and lacks such fragments at all other markers this is probably not indicative of hybridization. Rather, it much more likely represents the existence of genetic variation for the presence or absence of the fragment within this particular population of the native taxon.

An important aspect of PINE marker loci is that individuals homozygous for the presence allele (*pp*) or heterozygous (*pa*) will both yield the fragment. That is, *p* is dominant to *a*. Thus, in order to estimate the genetic contribution of the native taxon to a hybrid swarm we concentrate on the marker loci at which the *p* allele is characteristic of the non-native taxon. Furthermore, we must assume that genotypic distributions in the population reasonably conform to expected random mating proportions. Under this assumption the frequency of the native *a* allele is approximately the square root of the frequency of individuals in the population lacking the fragment (*aa).* The frequency of the non-native allele then is one minus this value. We focus on the *p* alleles characteristic of the non-native taxon because with low levels of hybridization it is the presence of these alleles that are likely to provide evidence of hybridization. With low levels of hybridization, it is likely all individuals in the sample will genotypically be *pp* or *pa* where the *p* allele is characteristic of the native taxon. Thus, like in non-hybridized populations all individuals in the sample will yield the fragment providing no evidence of hybridization.

Failure to detect evidence of hybridization in a sample does not necessarily mean the population is non-hybridized because there is always the possibility that we would not detect evidence of hybridization because of sampling error. In order to assess the likelihood the population is non-hybridized, we determine the chances of not detecting as little as a one percent genetic contribution of a non-native taxon to a hybrid swarm. This is simply 0.99^{2NX} where N is the number of fish in the sample and X is the number of marker loci where the *p* allele is characteristic of the non-native taxon.

In samples showing evidence of hybridization, that is; fragments characteristic of a non-native taxon were detected at two or more marker loci, we used two approaches to determine if the population appeared to be a hybrid swarm. First, contingency table chi-square analysis was used to test for heterogeneity of allele frequencies among the marker loci. Next, we computed a hybrid index for each individual in the sample. Each diagnostic locus at which an individual possessed a PINE fragment characteristic of the non-native taxon was given a value of one. Each diagnostic locus at which an individual did not possess a PINE fragment characteristic of the non-native taxon was given a value of zero. These values summed over all diagnostic loci represent an individual's hybrid index. The observed distribution of hybrid index scores was then statistically compared to the expected random binomial distribution based on the estimated native and non-native genetic contributions to the sample. If the allele frequencies were statistically homogeneous among the diagnostic loci and the observed distribution of hybrid indices statistically conformed to the expected random binomial distribution, then the sample was considered to have come from a hybrid swarm.

Heterogeneity of allele frequencies among marker loci can arise in very old hybrid swarms as the frequencies over time diverge from each other due to genetic drift. In this case, however, the non-native fragments will still be randomly distributed among individuals. Thus, samples with these characteristics were also considered to have come from hybrid swarms.

There are two likely reasons why a non-random distribution of non-native fragments may be observed among individuals in a sample. It may contain individuals from genetically divergent populations with different amounts of hybridization or hybridization may have only recently occurred in the population. Based on PINE data alone, these two situations will generally be difficult to distinguish from each other. Regardless of the explanation, when the non-native fragments are not randomly distributed among individuals in a sample estimating a mean level of hybridization has little, if any, biological meaning and, therefore, is often not estimated.

Results and Discussion

Simpson Creek 3237

PINE fragments characteristic of only westslope cutthroat trout were detected in the sample. Previous allozyme (#685, N=10) and PINE analyses (#3020, N=7) of fish and fin clips, respectively, collected from Simpson Creek also detected alleles characteristic of only westslope cutthroat trout With the combined sample size of 67, we have better than a 99% chance of detecting as little as a one percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The Simpson Creek population, therefore, is almost undoubtedly non-hybridized westslope cutthroat trout.

North Fork Everson Creek 3238

PINE fragments characteristic of only westslope cutthroat trout were detected in the sample. A previous allozyme analysis (#679, N=10) of fish collected from North Fork Everson Creek also detected alleles characteristic of only westslope cutthroat trout. With the combined sample size of 60, we have better than a 99% chance of detecting as

little as a one percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The North Fork Everson Creek population, therefore, is almost undoubtedly non-hybridized westslope cutthroat trout.

Middle Fork Cabin Creek 3239

PINE fragments usually characteristic of rainbow trout were detected at four of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The fragments characteristic of rainbow trout, however, were not randomly distributed (Poisson distribution, $P<0.001$) among the fish in the sample. In contrast, they were detected in only one of the eight fish. Thus, like in previous samples collected from Middle Fork Cabin Creek (e. g. #2744) this sample appears to have been a mixture of non-hybridized westslope cutthroat trout and hybrids between westslope cutthroat and rainbow trout. Because many previously detected hybrids possessed PINE fragments characteristic of rainbow trout at only one or two diagnostic loci in the Middle Fork Cabin Creek we can not reliably separate on an individual basis hybrids from non-hybridized fish. From a management perspective, therefore, Middle Fork Cabin Creek should simply be considered to contain a hybridized population between westslope cutthroat and rainbow trout.

Middle Fork Cabin Creek 3240

PINE fragments usually characteristic of rainbow trout were detected at one of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout fragments were detected in three of the 17 fish in the sample. In many situations like this, we would generally be uncertain as to whether the observed PINE variation represented evidence of hybridization or it was simply westslope cutthroat trout genetic variation that was electrophoretically indistinguishable from that usually characteristic of rainbow trout. In this case, however, we strongly favor the former interpretation because fish definitely of hybrid origin between westslope cutthroat and rainbow trout have been detected in other samples collected from Middle Fork Cabin Creek (e. g. #2744 and #3239). Furthermore, the hybrids have been collected throughout the drainage and as discussed previously (#3239) we can not reliably separate on an individual basis hybrids from non-hybridized fish. Thus, from a management perspective Middle Fork Cabin Creek should simply be considered to contain a hybridized population between westslope cutthroat and rainbow trout.

Cabin Creek 3241

PINE fragments usually characteristic of rainbow trout were detected at three of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout fragments were randomly distributed (Poisson distribution, $P>0.05$) among the fish in the sample. Thus, this sample appears to have come from a hybrid swarm between westslope cutthroat and rainbow trout with a predominant westslope cutthroat trout genetic contribution (97%). These results are highly concordant with those obtained from previous PINE analyses of fish collected from Cabin Creek (#1333 and #1931).

West Fork Dyce Creek 3242

PINE fragments usually characteristic of rainbow trout were detected at two of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. At one of the variable diagnostic loci, 10 fish in the sample possessed a fragment usually characteristic of rainbow trout. At the other variable diagnostic locus, however, only one fish in the sample possessed the rainbow trout fragment. The highly heterogeneous (Contingency table chi-

square, $P<0.001$) allele frequencies among the diagnostic loci suggests that the variation detected at the highly variable diagnostic locus mainly represents westslope cutthroat trout genetic variation that is electrophoretically indistinguishable from that usually characteristic of rainbow trout rather than evidence of hybridization with rainbow trout. Taking this into consideration, one fish in the sample possessed rainbow trout fragments at two diagnostic loci definitely indicating it to be of hybrid origin. All the other fish in the sample possessed PINE fragments characteristic of only westslope cutthroat trout suggesting they were non-hybridized westslope cutthroat trout. Thus, like in a previous sample from West Fork Dyce Creek (#2947) this sample appears to have been a mixture of non-hybridized westslope cutthroat trout and hybrids between westslope cutthroat and rainbow trout. The hybrids appear to be post first generation which on an individual basis makes reliable identification of non-hybridized fish from hybridized ones problematic. Thus, although this population probably does contain non-hybridized westslope cutthroat trout from a management perspective it should simply be considered hybridized.

Arasta Creek 3243

PINE fragments usually characteristic of rainbow trout were detected at all six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. PINE fragments usually characteristic of Yellowstone cutthroat trout were also detected at all four of the diagnostic loci analyzed in the sample that usually distinguish Yellowstone from westslope cutthroat trout. The PINE fragments characteristic of rainbow and Yellowstone cutthroat trout appeared to be randomly distributed (Poisson distribution, $P>0.05$) among the fish in the sample. Thus, although previous analyses of fish from Arasta Creek produced ambiguous results because of small sample sizes (#1091, #1945, and #2851) the results from this sample clearly indicate the population to be a hybrid swarm among westslope cutthroat, rainbow, and Yellowstone cutthroat trout with a predominant (87%) westslope cutthroat trout genetic contribution.

Rose Creek 3244

PINE fragments usually characteristic of rainbow trout were detected at two of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout PINE fragments appeared to be randomly distributed (Poisson distribution, $P>0.05$) among the fish in the sample. This population, therefore, appears to be a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (97%) westslope cutthroat trout genetic contribution.

PINE fragments usually characteristic of Yellowstone cutthroat trout were detected at one of the four diagnostic loci analyzed in the sample that usually distinguish Yellowstone from westslope cutthroat trout. The Yellowstone cutthroat trout PINE fragments were detected in three fish. This variation, therefore, could indicate hybridization with Yellowstone cutthroat trout or it could simply be westslope cutthroat trout genetic variation that is electrophoretically indistinguishable from that usually characteristic of Yellowstone cutthroat trout. Regardless of what this variation represents the population is clearly hybridized with rainbow trout and should simply be considered to be hybridized.

Rape Creek 3246

PINE fragments characteristic of only westslope cutthroat trout were detected in this sample. With a sample size of 25, we have a 95% chance of detecting as little as a one percent rainbow trout and an 87% chance of detecting as little as a one percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The Rape Creek population, therefore, is most likely non-hybridized westslope cutthroat trout.

East Fork Cabin Gulch 3248

PINE fragments usually characteristic of Yellowstone cutthroat trout were detected at two of the four diagnostic loci that were analyzed in the sample that distinguish Yellowstone from westslope cutthroat trout. The Yellowstone cutthroat trout fragments appeared to be randomly distributed (Poisson distribution, P>0.05) among the fish in the sample. The East Fork Cabin Gulch population, therefore, clearly appears to be a hybrid swarm between westslope and Yellowstone cutthroat trout with a predominant (82%) westslope cutthroat trout genetic contribution.

PINE fragments usually characteristic of rainbow trout were detected at one of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout PINE fragments were detected in six fish. This variation, therefore, could indicate hybridization with rainbow trout or it could simply be westslope cutthroat trout genetic variation that is electrophoretically indistinguishable from that usually characteristic of rainbow trout. Regardless of what this variation represents the population is clearly hybridized with Yellowstone cutthroat trout and should simply be considered to be hybridized.

Twelvemile Creek 3249

PINE fragments characteristic of only westslope cutthroat trout were detected in this sample. With a sample size of 17, we have an 87% chance of detecting as little as a one percent rainbow trout and a 75% chance of detecting as little as a one percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The Twelvemile Creek population, therefore, is most likely non-hybridized westslope cutthroat trout.

Soap Creek 3250

PINE fragments usually characteristic of rainbow trout were detected at two of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout PINE fragments appeared to be randomly distributed (Poisson distribution, P>0.05) among the fish in the sample. This population, therefore, appears to be a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (94%) westslope cutthroat trout genetic contribution. These results are highly concordant with a previous PINE analysis (#2144) of fish collected from Soap Creek which indicated the population to be a hybrid swarm between rainbow and westslope cutthroat trout with about a 98% westslope cutthroat trout genetic contribution.

Moose Creek 3251

PINE fragments usually characteristic of rainbow trout were detected at two of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout PINE fragments appeared to be randomly distributed (Poisson distribution, P>0.05) among the fish in the sample. This population, therefore, appears to be a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (94%) westslope cutthroat trout genetic contribution.

In this sample, we were only able to obtain information from two of the four diagnostic PINE loci that usually distinguish Yellowstone from westslope cutthroat trout. At one of these loci, fragments characteristic of only westslope cutthroat trout were detected. At the other locus, only fragments usually characteristic of Yellowstone cutthroat trout were detected. This situation is not at all what one normally expects to observe from hybridization. Thus, we conclude the latter variation very likely represents westslope cutthroat trout PINE genetic variation that is electrophoretically indistinguishable from that usually characteristic of Yellowstone cutthroat trout and there is no compelling evidence of hybridization with Yellowstone cutthroat trout in the Moose Creek population.

Whitetail Deer Creek (above reservoir) 3252

PINE fragments characteristic of only westslope cutthroat trout were detected in this sample. When this sample is combined with two other samples obtained from the Whitetail Deer Creek drainage (#3176 and #3264) we have a total sample size of 70 individuals. With this sample size, we have better than a 99% chance of detecting as little as a one percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The Whitetail Deer Creek population, therefore, is almost certainly non-hybridized westslope cutthroat trout.

Little Wapiti Creek 3253

PINE fragments usually characteristic of rainbow trout were detected at all six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout fragments were randomly distributed (Poisson distribution, $P>0.05$) among the fish in the sample. The Little Wapiti Creek population, therefore, is almost undoubtedly a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (81%) westslope cutthroat trout genetic contribution.

There also may be a slight amount of hybridization with Yellowstone cutthroat trout in the Little Wapiti Creek population. We were only able to obtain data from two diagnostic loci that usually distinguish Yellowstone from westslope cutthroat trout. At one of these loci, one fish in the sample possessed a PINE fragment usually characteristic of Yellowstone cutthroat trout. This could indicate hybridization or it could simply be westslope cutthroat trout PINE genetic variation that is electrophoretically indistinguishable from that usually characteristic of Yellowstone cutthroat trout. In this situation, which explanation is correct is largely irrelevant as the population is clearly hybridized with rainbow trout and should be treated as a hybrid swarm.

Lump Gulch 3254

PINE fragments characteristic of only Yellowstone cutthroat trout were detected in the sample. With the sample size of 10, however, we have only a 70% chance of detecting as little as a one percent rainbow trout and only a 33% chance of detecting as little as a one percent westslope cutthroat trout genetic contribution to a hybrid swarm. Thus, we can not reasonably exclude the possibility that the Lump Creek population may be slightly hybridized with rainbow trout, westslope cutthroat trout, or both.

Regardless of whether or not the present sample came from a non-hybridized Yellowstone cutthroat trout population, genetically it is very different from a previous sample (#370) collected from Lump Gulch. Allozyme analysis indicated the previous sample came from a hybrid swarm among westslope cutthroat, Yellowstone cutthroat, and rainbow trout with a predominant (65%) westslope cutthroat trout genetic contribution. The disparity between the samples suggests that the genetic characteristics of the Lump Gulch population either have not been temporally stable and the apparent absence of evidence of hybridization in the recent sample is due to sampling error or that Lump Gulch contains two genetically divergent populations one of which is hybridized and the other may be non-hybridized Yellowstone cutthroat trout.

Un-named Tributary 2 to South Fork Sixteenmile Creek 3255

We were able to obtain only low quality DNA from this sample. Thus, we were able to obtain reliable information from only four diagnostic loci that distinguish rainbow from westslope cutthroat trout and three diagnostic loci that

distinguish Yellowstone from westslope cutthroat trout. Despite this drawback interpretation of the results is straightforward.

PINE fragments usually characteristic of rainbow trout were detected at all four diagnostic loci analyzed that distinguish rainbow from westslope cutthroat trout. The rainbow trout fragments appeared to be randomly distributed (Poisson distribution, $P>0.05$) among the fish in the sample. This population, therefore, is almost undoubtedly a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (82%) westslope cutthroat trout genetic contribution.

PINE fragments usually characteristic of Yellowstone cutthroat trout were detected at two of the three diagnostic loci analyzed that distinguish Yellowstone from westslope cutthroat trout. In contrast to the rainbow trout PINE fragments, however, the Yellowstone cutthroat trout PINE fragments are not randomly distributed (Poisson distribution, $P<0.05$) among the fish in the sample. Rather, significantly more fish possessed Yellowstone cutthroat trout PINE fragments at two diagnostic loci (3 of 16) and significantly fewer possessed them at only one diagnostic locus (2 of 16) than expected by chance. Thus, although this population is clearly hybridized with Yellowstone cutthroat trout it does not appear to be a hybrid swarm between westslope and Yellowstone cutthroat trout when it was sampled. The simplest explanation for the difference between the results obtained from the rainbow trout and Yellowstone cutthroat trout diagnostic loci is that the population has been hybridized with rainbow trout longer than it has been hybridized with Yellowstone cutthroat trout.

Left Fork Deadhorse Creek 3256

PINE fragments usually characteristic of rainbow trout were detected at five of the six diagnostic loci that were analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout PINE fragments were not randomly distributed (Poisson distribution, $P<0.001$) among the fish in the sample. This deviation from a random distribution of the rainbow trout PINE markers was do to the presence of one individual in the sample that possessed rainbow trout fragments at five loci (Figure 1). Among the remaining fish the rainbow trout fragments appeared to be randomly distributed among individuals (Figure 1). Thus, this population appeared to be a mixture of individuals from a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (86%) westslope cutthroat trout genetic contribution and hybrids with a much higher rainbow trout genetic contribution.

The results from this sample are fairly similar to those obtained from one collected in 1989 (#331). Allozyme analysis of this sample indicated it to be a hybrid swarm among westslope cutthroat (85%), Yellowstone cutthroat (5%), and rainbow trout (10%). The apparent absence of a Yellowstone cutthroat trout genetic contribution to the more recent sample may well be do to sampling error and not the actual absence of such a contribution. With a sample size of 10 fish, we have a 13% chance of not detecting a five percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm.

Un-named Tributary to South Fork Sixteenmile Creek 3257

PINE fragments usually characteristic of rainbow trout were detected at two of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout PINE fragments appeared to be randomly distributed (Poisson distribution, $P>0.05$) among the fish in the sample. This population, therefore, appears to be a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (97%) westslope cutthroat trout genetic contribution.

Cottonwood Creek 3258

PINE fragments characteristic of only westslope cutthroat trout were detected in this sample. With the sample size of 33, we have a 98% chance of detecting as little as a one percent rainbow trout and a 93% chance of detecting as little as a one percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm. When Cottonwood Creek was sampled in 2004 , the population was almost certainly non-hybridized westslope cutthroat trout.

Cottonwood Creek 3259

PINE fragments usually characteristic of rainbow trout were detected at three of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout PINE fragments, however, do not appear to be randomly distributed (Poisson distribution, $P<0.05$) among the fish in the sample. In contrast, one individual (#12) possessed rainbow trout PINE fragments at three diagnostic loci and the other 18 possessed PINE fragments characteristic of only westslope cutthroat trout. Thus, when Cottonwood Creek was sampled in 2005 it appears to have been a mixture of non-hybridized westslope cutthroat trout and fish definitely of hybrid origin between westslope cutthroat and rainbow trout. Because only one individual definitely of hybrid origin was collected we can not reasonably ascertain how well at an individual basis non-hybridized and hybrid individuals in the population can be distinguished. Because of this uncertainty, from a management perspective we suggest the conservative approach to take at this time would simply be to consider the Cottonwood Creek population to be hybridized with westslope cutthroat trout.

Upper Jack Creek 3260

PINE fragments characteristic of only westslope cutthroat trout were detected in the sample. When this sample is combined with two other samples obtained from Jack Creek (#952 and #1217) we have a total sample size of 31 individuals. With this sample size, we have a 98% chance of detecting as little as a one percent rainbow trout and better than a 99% chance of detecting as little as a one percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The Jack Creek population, therefore, is almost certainly non-hybridized westslope cutthroat trout.

Upper German Gulch 3261

PINE fragments usually characteristic of rainbow trout were detected at two of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. The rainbow trout PINE fragments, however, did not appear to be randomly distributed (Poisson distribution, $P<0.05$) among the fish in the sample. Rather, one fish (#8) possessed rainbow trout PINE fragments at two diagnostic loci and the remaining 29 fish possessed PINE fragments characteristic of only westslope cutthroat trout. Thus, when German Gulch was sampled in 2003 it appeared to be a mixture of non-hybridized westslope cutthroat trout and individuals of hybrid origin between westslope cutthroat and rainbow trout.

German Gulch was first sampled in 1984 (#75). Allozyme analysis of this sample indicated the population to be non-hybridized westslope cutthroat trout. When German Gulch was next sampled in 2002 (#2392), PINE analysis provided ambiguous results. PINE fragments characteristic of only westslope cutthroat trout were detected at all the loci analyzed except one. At one diagnostic locus that usually distinguishes rainbow from westslope cutthroat trout, one individual possessed a PINE fragment usually characteristic of rainbow trout. We were uncertain whether this

represented a small amount of hybridization with rainbow trout or if it was simply westslope cutthroat trout PINE genetic variation that was electrophoretically indistinguishable from that characteristic of rainbow trout. The present results strongly support the former interpretation especially considering the PINE fragment possessed by the fish in the 2002 sample was also possessed by the fish definitely of hybrid origin in the 2003 sample.

Considering all the samples, it appears German Gulch was a non-hybridized westslope cutthroat trout population in the mid 1980's and subsequently it has become hybridized with rainbow trout. Hybridization probably occurred fairly recently as the rainbow trout PINE fragments do not appear to be randomly distributed among the fish in the population and it, therefore, appears to be a mixture of non-hybridized westslope cutthroat trout and hybrids. Although the German Gulch population probably contains non-hybridized westslope cutthroat trout, it clearly contains hybrids well beyond the first generation which will make reliably distinguishing the non-hybridized fish from the hybrids on an individual basis problematic. From a management perspective, therefore, the German Gulch population should simply be considered to be hybridized.

Beefstraight Creek 3262

When Beefstraight Creek was sampled in 2002 (#2390), it appeared to be a mixture of non-hybridized westslope cutthroat trout and hybrids between westslope cutthroat and rainbow trout. In the present sample, PINE fragments usually characteristic of rainbow trout were detected at three of the six diagnostic loci analyzed that distinguish rainbow from westslope cutthroat trout. The rainbow trout PINE fragments appeared to be randomly distributed (Poisson distribution, $P>0.05$) among the fish in the sample. The Beefstraight Creek population, therefore, appears to have become a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (98%) westslope cutthroat trout genetic contribution when it was sampled in 2003.

Minnesota Gulch 3263

PINE fragments characteristic of only westslope cutthroat trout were detected in the sample. With a sample size of 29, we have a 97% chance of detecting as little as a one percent rainbow trout and a 90% chance of detecting as little as a one percent Yellowstone cutthroat trout genetic contribution to the population. The Minnesota Gulch population, therefore, is most likely non-hybridized westslope cutthroat trout.

Whitetail Deer Creek (below reservoir) 3264

PINE fragments characteristic of only westslope cutthroat trout were detected in this sample. When this sample is combined with two other samples obtained from the Whitetail Deer Creek drainage (#3176 and #3252) we have a total sample size of 70 individuals. With this sample size, we have better than a 99% chance of detecting as little as a one percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The Whitetail Deer Creek population, therefore, is almost certainly non-hybridized westslope cutthroat trout.

Wild Horse Creek 3265

PINE fragments characteristic of only westslope cutthroat trout were detected in this sample. When this sample is combined with three other samples obtained from the Wild Horse Creek drainage (#1111, #1112, and #2940) we have a total sample size of 30 individuals. With this sample size, we have better than a 97% chance of detecting as little as a one percent rainbow trout and a 92% chance of detecting as little as a one percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The Wild Horse Creek population, therefore, is almost certainly non-hybridized westslope cutthroat trout.

Seymour Creek 3266

Seymour Creek was first sampled in 1989 (#354). Allozyme analysis indicated the population to be a hybrid swarm between westslope and Yellowstone cutthroat trout with a predominant (99%) westslope cutthroat trout genetic contribution. Thus, although PINE fragments characteristic of only westslope cutthroat trout were detected in the present sample we are hesitant to conclude the population is now non-hybridized westslope cutthroat trout. With the sample size of six, we have about a 62% chance of not detecting a one percent Yellowstone cutthroat trout genetic contribution to the population. It is very likely, therefore, that the population may be still slightly hybridized but, evidence of this was not detected because of sampling error. In this case of uncertainty, we suggest the conservative management approach to take is to consider the population to still be slightly hybridized with Yellowstone cutthroat trout unless future data conclusively indicate otherwise.

Little Elk River 3267

PINE fragments characteristic of only Yellowstone cutthroat trout were detected in the sample. With the sample size of 10, we have only about a 70% chance to detect as little as a one percent rainbow trout and about a 33% chance to detect as little as a one percent westslope cutthroat trout genetic contribution to a hybrid swarm. Although we can not reasonably exclude the possibility that the Little Elk River population may be slightly hybridized with rainbow trout, westslope cutthroat trout, or both this is clearly irrelevant as it is obvious that genetically the population is not even close to being native westslope cutthroat.

Hellroaring Creek 3268

PINE fragments usually characteristic of rainbow trout were detected at all six of the diagnostic loci analyzed in the sample that usually distinguish rainbow from westslope cutthroat trout. Furthermore, PINE fragments usually characteristic of Yellowstone cutthroat trout were detected at three of the four diagnostic loci analyzed in the sample that distinguish Yellowstone from westslope cutthroat trout. The rainbow and Yellowstone cutthroat trout PINE fragments were not randomly (Poisson distribution, $P<0.05$) distributed among the individuals in the sample. All of the fish in the sample, however, were definitely of hybrid origin. This population, therefore should simply be considered to be hybridized among rainbow, Yellowstone cutthroat, and westslope cutthroat trout with a predominant rainbow trout genetic contribution (56%) and a substantial genetic contribution from Yellowstone (17%) and westslope cutthroat trout (27%)

Magpie Creek 3269

PINE fragments usually characteristic of rainbow trout were detected at one of the six diagnostic loci analyzed in the sample that distinguish rainbow from westslope cutthroat trout. This fragment was possessed by seven individuals in

the sample and its frequency (0.194) was statistically different (Contingency table chi-square, $P<0.001$) from the allele frequencies observed at the other diagnostic loci. Thus, we conclude the presence of this fragment in the sample more likely indicates westslope cutthroat trout PINE genetic variation that is electrophoretically indistinguishable from that usually characteristic of rainbow trout than evidence of hybridization with rainbow trout. Conservatively, therefore, this population should be considered to be non-hybridized westslope cutthroat trout unless future data indicate otherwise.

Cooney Gulch 3270

PINE fragments usually characteristic of rainbow trout were detected at two of the six diagnostic loci analyzed in the sample that usually distinguish rainbow from westslope cutthroat trout. The rainbow trout PINE fragments, however, were not randomly distributed (Poisson distribution, $P<0.001$) among the fish in the sample. In contrast, significantly more individuals (6) in the sample possessed rainbow trout PINE fragments at two diagnostic loci than expected by chance. Furthermore, more individuals (14) possessed PINE fragments characteristic of only westslope cutthroat trout at all the loci analyzed than expected by chance. The results, therefore, suggest that the sample contained a mixture of hybrids between rainbow and westslope cutthroat trout and non-hybridized westslope cutthroat trout. Since the hybrids are definitely later than first generation, reliably distinguishing hybrids from non-hybridized westslope cutthroat trout on an individual basis will be problematic. Thus, from a practical perspective the Cooney Gulch population should simply be considered to be hybridized between rainbow and westslope cutthroat trout with a predominant (95%) westslope cutthroat trout genetic contribution.

Robb Leary

Ben Wright

TABLE 1

Diagnostic PINE markers for westslope cutthroat, Yellowstone cutthroat, and rainbow trout. **X** indicates the fragment is present in the particular taxon.

Markers	Yellowstone	Westslope	Rainbow
Hpa1 5'/Hpa1 3'			
232	x		
153		x	
72	x	x	
70			x
69	x	x	
66			x
Fok1 5'/Tc1			
369			x
366	x	x	
230			x
159	x		
138	x		
110		x	
Hpa1 5'/33.6+2			
395			x
388	x	x	
266			x
248	x		
148	x	x	

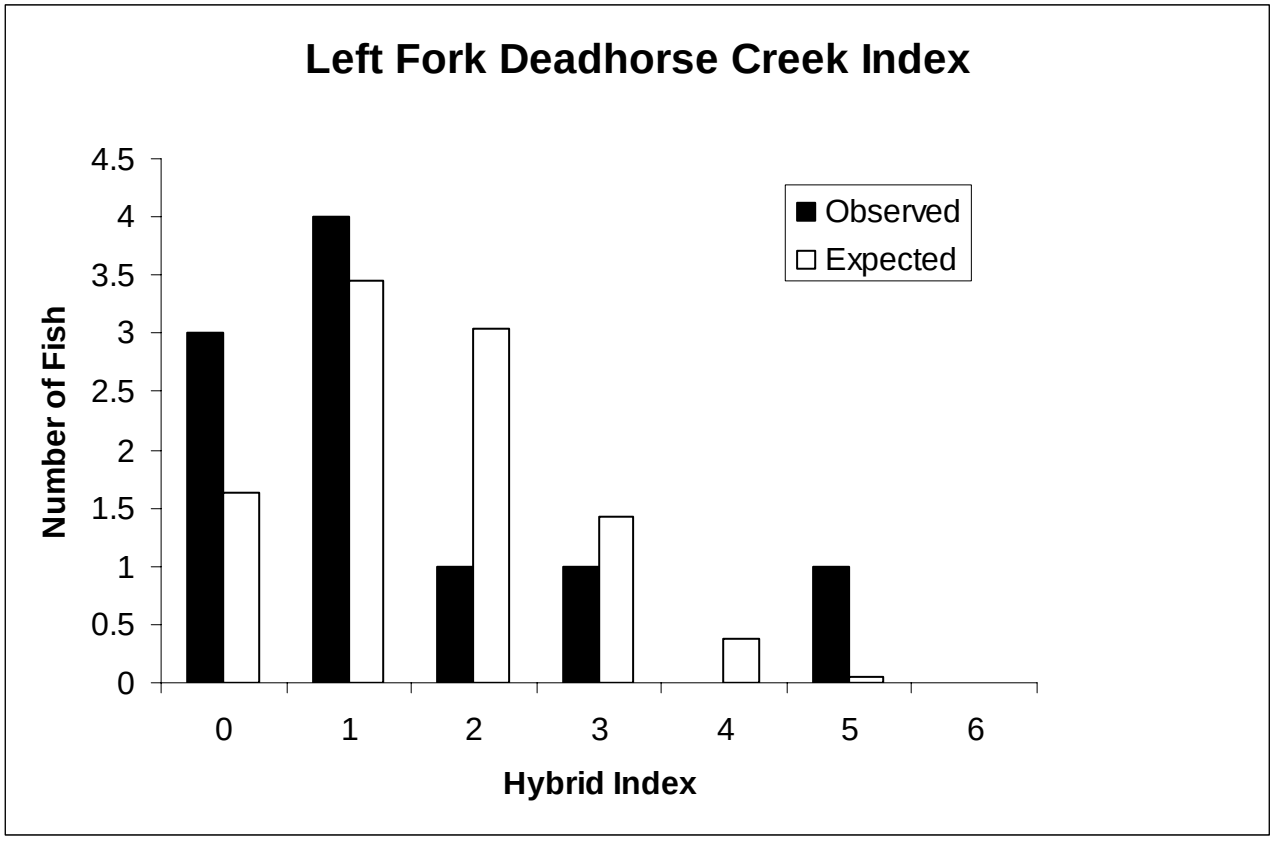

Figure 1. Observed and expected distribution of hybrid index scores in a sample collected from a hybridized population between rainbow and westslope cutthroat trout from Left Fork Deadhorse Creek