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University of Montana Conservation Genetics Laboratory
Division of Biological Sciences, University of Montana, Missoula, Montana 59812
Phone (406) 243-6749 or 6725; Fax (406) 243-4184

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Dave Moser
Montana Fish, Wildlife & Parks
P. O. Box 869
Great Falls, Montana 59403

Dave;

We have analyzed the DNA extracted from fin clips from trout collected from the following locations:

Sample #	Water Name/Location/ Collection Date/ Collector	a N	b #Markers	c Taxa ID	d Power	e %	f # Fish
4435	Elkhorn Creek x=438062 y=5198445 9/20/2012 Dave Moser	25	R19W20Y20	WCT X RBT WCT X RBT		W98.5 X R1.5	21 4
4436	Lightning Creek x=469726 y=4986015 9/10/2012 Dave Moser	27	R19W20Y20	WCT X YCT		W91.8XY8.2	
4437	Collar Gulch x=635900 y=5228819 7/12/2012 Dave Moser	50	R17W18Y20	WCT	R99Y99		
4438	North Fork Greenhorn Creek 45.11258-14169 112.04720-01029 9/19/2012 Matt Jaeger	23	R18W20Y20	WCT X RBT		W99.5 X R0.5	

Sample #	Water Name/Location/ Collection Date/ Collector	a N	b #Markers	c Taxa ID	d Power	e %	f # Fish
4439	South Fork Greenhorn Creek 45.10801-12297 112.03269-98857 9/20/2012 Matt Jaerger	10	R18W20Y20	WCT?			
4440	Meadow Fork Greenhorn Creek 45.16098-15800 111.97051-96037 8/7/2012 Matt Jaerger	25 (70)	R19W20Y20	WCT	R99Y99		
4441	Dark Hollow Creek (upper) 45.16201-15777 112.01172-01844 9/10/2012 Matt Jaerger	25	R19W20Y20	WCT	R99Y99		
4443	Dark Hollow Creek (lower) 45.12735-12901 112.03451-03582 8/8/2012 Matt Jaerger	25	R19W20Y20	WCT	R99Y99		
4442	Peet Creek (above pond) 44.57249-57213 112.07026-07077 7/17/2012 Matt Jaerger	25	R19W20Y20	WCT X YCT		W98.8Y1.2	
4444	Middle Fork Little Sheep Creek 44.50267-50234 112.62696-62648 8/2/2012 Matt Jaerger	25	R19W20Y19	WCT X YCT WCT X YCT		W96.3Y3.7	22 3

Sample #	Water Name/Location/ Collection Date/ Collector	a N	b #Markers	c Taxa ID	d Power	e %	f # Fish
4445	West Fork East Fork Sweetwater Creek 45.10459-10555 112.42162-42323 7/9/2012 Matt Jaerger	25	R17W19Y20	WCT X RBT		W99.8R0.2	
4446	Peterson Creek 45.22213-22285 112.18887-19230 7/10/2012 Matt Jaeger	25	R19W20Y20	WCT X YCT X RBT			
4447	Middle Fork Odell Creek 44.52157-52124 111.81712-81810 8/14/2012 Matt Jaeger	25	R19W20Y20	WCT X YCT X RBT			
4448	East Fork Odell Creek 44.54770-54975 111.78091-78426 8/15/2012 Matt Jaeger	25	R19W20Y20	WCT X YCT WCT X YCT		W99.5Y0.5	21 4
4449	East Fork East Fork Clover Creek 44.73960-74006 112.20971-20835 7/12/2012 Matt Jaeger	10	R18W19Y19	WCT?	R83Y85		
4450	Deadman Creek Mile 1.5 11/1/2012 Pat Clancey	8	R19W20Y20	WCT X YCT		W98.4Y1.6	
4451	Pine Butte Creek Mile 2.0-2.5 11/1/2012 Pat Clancey	22	R19W18Y19	WCT X YCT		W97.8Y2.2	

[a]Number of fish successfully analyzed. If combined with a previous sample, the number in parentheses indicates the combined sample size.

[b]Number of diagnostic loci analyzed for the taxon (R=rainbow trout *Oncorhynchus mykiss,* W=westslope cutthroat trout *O. clarkii lewisi,* Y=Yellowstone cutthroat trout *O. c. bouvieri*).
[c]Taxa: WCT = westslope cutthroat trout; RBT = rainbow trout; YCT = Yellowstone cutthroat trout . Only one taxon code is listed when the entire sample possessed alleles from that taxon only. It must be noted, however, that we cannot definitely 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 *d*). Taxa separated by "x" indicate hybridization between them was detected.
[d]Power: the number corresponds to the percent chance we have to detect 0.5% introgression in a hybrid swarm (a random mating population in which taxa markers are randomly distributed among individuals such that essentially all of them in the population are of hybrid origin) given the number of individuals successfully analyzed and the number of diagnostic markers used. For example, with 12 individuals we have better than a 99 % chance to detect as little as a 0.5% rainbow (39 diagnostic loci) or Yellowstone cutthroat trout (40 diagnostic loci) genetic contribution to a hybrid swarm that once was a non-hybridized westslope cutthroat trout population. Likewise, with 12 individuals we have better than a 99% chance to detect as little as a 0.5% percent rainbow (39 diagnostic loci) or westslope cutthroat trout (40 diagnostic loci) genetic contribution to a hybrid swarm that once was a non-hybridized Yellowstone cutthroat trout population. Not reported when hybridization is detected. Taxa as in b.
[e]Indicates the genetic contribution of the hybridizing taxa denoted as in b. This number is usually reported only if the sample appears to have come from a hybrid swarm.
[f]Indicates the number of individuals with genetic characteristics corresponding to the taxa ID code column when the sample contains individuals from two or more genetically distinct groups.

Methods and Data Analysis

We developed a 'chip' specifically for analysis of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) populations. This chip allows us to simultaneously genotype up to 95 single nucleotide polymorphic loci (SNPs) in 91 trout using a Fluidigm EP1 Genotyping System. Each SNP locus has only two states (alleles). Thus, considering hybridization among rainbow (*Oncorhynchus mykiss*), westslope cutthroat, and Yellowstone cutthroat trout (*O. c. bouvieri*) a single locus can only distinguish one of the taxa from the other two. In order to address hybridization issues among these fishes, therefore, each chip contained 19 loci that differentiate rainbow from westslope cutthroat and Yellowstone cutthroat trout (rainbow markers), 20 loci that distinguish westslope cutthroat from rainbow and Yellowstone cutthroat trout (westslope markers), and 20 loci that distinguish Yellowstone cutthroat from westslope cutthroat and rainbow trout (Yellowstone markers, Table 1). We verified the diagnostic property of each marker by analyzing them in reference samples that had previously been determined to be non-hybridized westslope cutthroat, Yellowstone cutthroat, or rainbow trout by analysis of allozymes, paired interspersed nuclear elements (PINEs), a combination of insertion/deletion (indel loci) events and microsatellite loci, or two or all of these techniques (Table 2).

If a sample possessed alleles characteristic of only westslope cutthroat trout at all westslope markers and had no alleles characteristic of rainbow trout at the rainbow markers or Yellowstone cutthroat trout at the Yellowstone markers, then it was considered to have come from a non-hybridized westslope cutthroat trout population. Evidence for potential hybridization between rainbow and westslope cutthroat trout was generally considered to be present when three criteria were met. First, the sample had to contain alleles characteristic of rainbow trout at, at least, some of the rainbow markers. Next, at least some of the westslope markers also had to be genetically variable (polymorphic). Finally, no Yellowstone cutthroat trout alleles were detected at the Yellowstone markers. In this situation, the alleles at the rainbow markers shared between westslope cutthroat and Yellowstone cutthroat trout can confidently be assigned to having originated from westslope cutthroat trout and the alleles shared between rainbow and Yellowstone cutthroat trout at the westslope markers can confidently be assigned to having originated from rainbow trout. Thus, in terms of hybridization between westslope cutthroat and rainbow trout the data set contains information from 39 diagnostic loci. Likewise, when evidence of hybridization was detected only between westslope and Yellowstone cutthroat trout (no rainbow alleles at rainbow markers, at least some westslope markers polymorphic, and Yellowstone cutthroat trout alleles present at, at least, some Yellowstone markers) the data set contains information from 40 diagnostic loci. When all three sets of markers were polymorphic, this generally indicates hybridization among all three taxa. In this situation, the rainbow markers (19) provide

information about rainbow trout hybridization and the Yellowstone markers (20) provide information about Yellowstone cutthroat trout hybridization.

An important aspect of SNPs is that they demonstrate a codominant mode of inheritance. That is, all genotypes are readily distinguishable from each other. Thus, at marker loci the genotype of individuals in a sample can directly be determined. From these data, the proportion of alleles from different taxa in the population sampled can be directly estimated at each marker locus analyzed. These values averaged over all marker loci yields an estimate of the proportion of alleles in the population that can be attributed to one or more taxa (proportion of admixture). In samples showing evidence of hybridization among all three taxa, we estimated the amount of rainbow trout admixture using only the 19 rainbow markers and the amount of Yellowstone cutthroat trout admixture using only the 20 Yellowstone markers. The amount of westslope cutthroat trout admixture was then estimated by subtracting the sum of the former two values from one. We used this procedure so the estimates would sum to one. Because of sampling error, it is unlikely that all three estimates from the marker loci would sum to one.

When evidence of hybridization is detected, the next issue to address is whether or not the sample appears to have come from a hybrid swarm. That is, a random mating population in which the alleles of the hybridizing taxa are randomly distributed among individuals such that essentially all of them are of hybrid origin.

A common, but not absolute, attribute of hybrid swarms is that allele frequencies at marker loci are similar among them because their presence can all be traced to a common origin or origins. Thus, one criterion we used for the assessment of whether or not a sample appeared to have come from a hybrid swarm was whether or not the allele frequencies among diagnostic loci reasonably conformed to homogeneity using contingency table chi-square analysis.

In order to determine whether or not alleles at the marker loci were randomly distributed among the fish in a sample showing evidence of hybridization, we calculated a hybrid index for each fish in the sample. The hybrid index for an individual was calculated as follows. At each marker locus, an allele characteristic of the native taxon was given a value of zero and an allele characteristic of the non-native taxon a value of one. Thus, at a single diagnostic locus the hybrid index for an individual could have a value of zero (only native alleles present, homozygous), one (both native and non-native alleles present, heterozygous), or two (only non-native alleles present, homozygous). These values summed over all diagnostic loci analyzed yields an individual's hybrid index. Considering westslope cutthroat and rainbow trout, therefore, non-hybridized westslope cutthroat trout would have a hybrid index of zero, non-hybridized rainbow trout a hybrid index of 78, F_1 (first generation) hybrids a hybrid index of 39, and post F_1 hybrids could have values ranging from zero to 78. The distribution of hybrid indices among the fish in a sample was statistically compared to the expected random binomial distribution based on the proportion of admixture estimated from the allele frequencies at the diagnostic loci. If the allele frequencies appeared to be statistically homogeneous among the marker loci and the observed distribution of hybrid indices reasonably conformed to the expected random distribution, then the sample was considered to have come from a hybrid swarm.

In old or numerically small hybrid swarms, allele frequencies at marker loci can randomly diverge from homogeneity over time because of genetic drift. In this case, however, the observed distribution of hybrid indices is still expected to reasonably conform to the expected random distribution. Thus, if the allele frequencies were statistically heterogeneous among the marker loci in a sample but, the observed distribution of hybrid indices reasonably conformed to the expected random distribution the sample was also considered to have come from a hybrid swarm.

The strongest evidence that a sample showing evidence of hybridization did not come from a hybrid swarm is failure of the observed distribution of hybrid indices to reasonably conform to the expected random distribution. The most likely reasons for this are that the population has only recently become hybridized or

the sample contains individuals from two or more populations with different amounts of admixture. At times, the distribution of genotypes at marker loci and the observed distribution of hybrid indices can provide insight into which of these two factors appears mainly responsible for the nonrandom distribution of the alleles from the hybridizing taxa among individuals in the sample. At other times, the distribution of genotypes at marker loci and the observed distribution of hybrid indices may provide little or no insight into the cause of the nonrandom distribution of alleles among individuals. The latter situation is expected to be fairly common as the two factors usually responsible for the nonrandom distribution of alleles are not necessarily mutually exclusive. Regardless of the cause, when alleles at the marker loci do not appear to be randomly distributed among individuals in a sample, estimating the amount of admixture has little if any biological meaning and, therefore, is generally not reported.

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. When no evidence of hybridization was detected in a sample, we assessed the likelihood the population is non-hybridized by determining the chances of not detecting as little as a 0.5 percent genetic contribution of a non-native taxon to a hybrid swarm. This is simply 0.995^{2NX} where N is the number of fish in the sample and X is the number of marker loci analyzed.

The chip also contained 34 loci that are generally polymorphic within westslope cutthroat trout populations. Information from these loci can be used to address issues concerning the relative amount of genetic variation within and divergence among westslope cutthroat trout populations.

Finally, the chip contained two mitochondrial DNA (mtDNA) loci that differentiate cutthroat and rainbow trout. Data from these loci were used only if an individual appeared to be an F_1 hybrid. Because mtDNA is inherited only from females (maternal inheritance), in this situation we can determine the taxon of the female, and by default the taxon of the male, that produced the hybrid.

When two or more samples were collected from the same area we used the log likelihood G test of Goudet et al. (1996) in GENEPOP version 4.0 (Rousset 2008) to test for genetic differences among the samples. In instances where multiple loci were compared among samples and some demonstrated significant differences, significance was determined using Rice's (1989) method for correcting for multiple comparisons (modified level of significance). When no differences were detected at the modified level, any observed differences were considered to most likely represent chance departures from homogeneity and the samples were combined for further analysis. When evidence of genetic differences were detected between samples they were kept separate for analysis and the relative amount of divergence between them was estimated as F_{ST} using the method of Weir and Cockerham (1984) available in GENEPOP version 4.0.

It is possible that samples may have contained individuals from genetically divergent populations. If this is the case, there may be a significant deficit of heterozygotes compared to expected random mating (Hardy-Weinberg) proportions at some loci. In the samples, therefore, we tested for deviations from Hardy-Weinberg proportions at loci with more than one copy of a variant allele using the Markov chain method of Guo and Thompson (1992) in GENEPOP version 4.0. Again, when some loci indicated a significant deviation from Hardy-Weinberg proportions, significance was determined using the modified level.

Results and Discussion

Elkhorn Creek just Downstream of Confluence with North Fork 4435

In the sample from Elkhorn Creek collected just below the confluence with the North Fork, alleles characteristic of rainbow trout were detected at 13 of the rainbow markers and at ten of the westslope markers. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers.

The allele frequencies were statistically heterogeneous (X^2_{38}=120.658; P<0.001) among the diagnostic loci and the rainbow trout alleles were not randomly distributed (X^2_6=46.414; P<0.001) among the fish in the sample. In contrast, four fish had a hybrid index higher than expected (Figure1). When these four fish were eliminated from the data, the rainbow trout alleles appeared to be randomly distributed (X^2_3=4.938; P>0.10) among the remaining individuals. Thus, this sample appeared to contain a mixture of fish from a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (0.985) westslope cutthroat trout genetic component and a few hybrids with a higher amount of admixture.

Although the hybrid indices indicate the sample very likely contained fish from two genetically divergent groups in terms of the amount of admixture, this does not appear to have resulted in observed genotypic distributions significantly deviating from expected random mating proportions. At the 42 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, only three of the observed genotypic distributions significantly deviated from expected Hardy–Weinberg proportions. These differences, however, were not significant at the modified level suggesting that they very likely represented chance departures from homogeneity rather than actual deviations from expected random mating proportions.

When this reach of Elkhorn Creek was first sampled, allozyme analysis (#2718, col. 8/18/96, N=25) suggested it contained non-hybridized westslope cutthroat trout. A subsequent PINE analysis (#2342, col. 9/26/02, T14N R2W S26, N=25), however, indicated the fish were a mixture of non-hybridized westslope cutthroat trout and hybrids with rainbow trout. The most recent sample suggests that the genetic characteristics of the fish in this reach have continued to change. The fish now appear to be a mixture of hybrids with a relatively small rainbow trout genetic component and others with a higher amount of rainbow trout introgression. We suspect the latter fish are probably originating downstream of this reach as a microsatellite/indel analysis of fish collected downstream (#3743, col. 10/7/08, 46.9360 111.8321, N=50) indicated they were a hybrid swam between westslope cutthroat and rainbow trout with about a 12 percent rainbow trout contribution. In contrast, fish collected upstream of the reach based on microsatellite/indel analyses (#3948, col. 7/29/09, x=439974 y=5197373, N=26; #3949, col. 7/29/09, x=441646 y=5196335, N=49; #3951, col. 7/29/09, x=440232 y=5200548, N=50) possessed only a relatively small amount of admixture with rainbow trout.

Lightning Creek 4436

Alleles characteristic of Yellowstone cutthroat trout were detected at 17 of the Yellowstone markers and 15 of the westslope markers analyzed in the sample from Lightning Creek. No alleles characteristic of rainbow trout were detected at the rainbow markers. Although the allele frequencies were statistically heterogeneous (X^2_{39}=152.348; P<0.001) among the diagnostic loci, the Yellowstone cutthroat trout alleles appeared to be randomly distributed (X^2_{13}=13.008; P>0.10) among the fish in the sample. This sample, therefore, appears to have come from a hybrid swarm between westslope and Yellowstone cutthroat trout with a predominant (0.918) westslope cutthroat trout genetic contribution.

There is some indication that the observed genotypic distributions in the sample may not conform to expected random mating proportions. At the 51 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, three of the observed genotypic distributions significantly deviated from expected Hardy–Weinberg proportions. These differences remained significant at the modified level with two involving a deficit of observed heterozygotes and one an excess. Since there was no apparent tendency for there to be an excess or deficit of heterozygotes at loci showing significant deviations from expected random mating proportions, it is unclear biologically what these departures represent.

Collar Gulch 4437

In the sample from Collar Gulch, no alleles usually characteristic of rainbow trout were detected at the rainbow markers except *OmyRD_RAD_29252_Hoh* and *OmyRD_RAD_55820_Hoh*. These loci possessed the allele usually characteristic of rainbow trout at an unusually high frequency compared to the other markers. This could indicate hybridization with rainbow trout or it could simply represent westslope cutthroat trout genetic variation. In this case, we tend to favor the latter interpretation because the variation detected at *OmyRD_RAD_29252_Hoh* has been detected in other samples that otherwise appear to be non-hybridized westslope cutthroat trout (Table 3). Furthermore, all the westslope markers except *OmyWD_RAD_55391_Hoh* and *OclWD111312_Garza* possessed alleles characteristic of only westslope cutthroat trout. The allele usually characteristic of rainbow or Yellowstone cutthroat trout was detected at an unusually high frequency compared to the other markers at the latter two loci. Again we feel this variation more likely represents westslope cutthroat trout polymorphisms than evidence of hybridization especially since the variation detected at *OclWD111312_Garza* has also been detected in other samples that otherwise appear to be non-hybridized westslope cutthroat trout (Table 4). No alleles usually characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers. Thus, we consider the sample from Collar Gulch as having come from a non-hybridized westslope cutthroat trout population with very unusual genetic characteristics.

There is some indication that the observed genotypic distributions in the sample may not conform to expected random mating proportions. At the 12 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected Hardy-Weinberg genotypic proportions, three of the comparisons significantly deviated from the expected random mating proportions. These differences remained significant at the modified level with two involving a deficit of observed heterozygotes and one an excess. Thus, again it is unclear biologically what these departures from expected random mating proportions represent.

Greenhorn Creek Drainage

Samples were collected from the North Fork, South Fork, and Meadow Fork in the Greenhorn Creek drainage. The allele frequencies significantly differed at three of the 15 polymorphic loci detected between the South Fork and Meadow Fork samples. These differences remained significant at the modified level. Likewise, the allele frequencies significantly differed between the North Fork and Meadow Fork samples at 12 of the 27 polymorphic loci detected between them. These differences also remained significant at the modified level. Finally, the allele frequencies significantly differed at nine of the 27 polymorphic loci detected between the North Fork and South Fork samples and these differences were significant at the modified level. The amount of divergence between the samples was surprisingly high. F_{ST} between the South Fork and Meadow Fork samples was 0.181, between the North Fork and Meadow Fork samples 0.144, and between the North Fork and South Fork samples 0.188. Since there was good evidence of substantial genetic differences between all the samples, they were kept separate for further analysis.

North Fork Greenhorn Creek 4438

Data were unattainable from the rainbow marker *OmyRD_URO_302_May* in the sample from North Fork Greenhorn Creek. Alleles characteristic of rainbow trout were detected at only two of the remaining rainbow markers and one of the westslope markers in the sample. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed in the sample. Normally we would be uncertain whether the variation detected at the rainbow and westslope markers represented evidence of hybridization or westslope cutthroat trout genetic variation. In this situation, however, we strongly favor the former interpretation as previous allozyme (#1097, col. 8/30/95, T8S R4W S24 SW1/4, N=15), PINE (#3059, col. 7/26/04, T8S R4W S24, N=11), and microsatellite/indel (#3444, col. 10/5/06, x=418762 y=4997405, T8S

R4W S24, N=50) analyses of fish sampled from North Fork Greenhorn Creek indicated a slight amount of hybridization with rainbow trout.

Considering the recent sample, the allele frequencies significantly differed (X^2_{37}=104.165; P<0.001) among the rainbow and westslope markers, but the rainbow trout alleles appeared to be randomly distributed (X^2_2=0.994; P>0.50) 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 (0.995) westslope cutthroat trout genetic component.

There is little evidence that the observed genotypic distributions in the sample significantly differed from expected random mating proportions. At the 24 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, only two significantly deviated from expected Hardy–Weinberg proportions. These differences, however, were not significant at the modified level suggesting that they very likely represented chance departures from homogeneity rather than actual deviations from expected random mating proportions.

South Fork Greenhorn Creek 4439

In the sample from South Fork Greenhorn Creek, data were unattainable from the rainbow marker *OmyRD_URO_302_May*. All of the other rainbow markers except one lacked alleles characteristic of rainbow trout. The exception was *OmyRD_RAD_30423_Hoh* at which one copy of the allele usually characteristic of rainbow trout was detected. Alleles characteristic of only westslope cutthroat trout were detected at all the westslope markers and no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed in the sample. We are uncertain whether the variation detected at *OmyRD_RAD_30423_Hoh* represents hybridization with rainbow trout or westslope cutthroat trout genetic variation. The presence of hybridization with rainbow trout in other samples collected from the Greenhorn Creek drainage (e.g. #3407, col. 7/14/06, x=415674-418538 y=4994549-4997404, N=33 and #4438) lends some support to the hybridization interpretation but, this conclusion would be tentative. At this time, we conservatively consider the trout in South Fork Greenhorn Creek to be non-hybridized westslope cutthroat trout but, because of the uncertainty would suggest that they not be used as a source for broodstock or transfer purposes.

There is no evidence that the observed genotypic distributions in the sample significantly differed from expected random mating proportions. At the eight polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, none of the comparisons significantly deviated from expected Hardy–Weinberg proportions.

Meadow Fork Greenhorn Creek 4440

No alleles characteristic of rainbow trout were detected at the rainbow markers analyzed in the sample from Meadow Fork Greenhorn Creek. Furthermore, only alleles characteristic of westslope cutthroat trout were detected at the westslope markers and no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed in the sample. Previous PINE (#3010, col. 8/4/04, x=422289 y=5000155, T8S R3W S8, N=14) and microsatellite/indel (#3409, col. 9/25/06, 45.152 111.982, N=31) analyses also detected no evidence of hybridization in trout sampled from Meadow Fork Greenhorn Creek. With the combined sample size of 70 and a total of 1924 diagnostic rainbow trout alleles and 1608 diagnostic Yellowstone cutthroat trout alleles analyzed we have a 98 percent chance of detecting as little as a 0.2 percent rainbow trout and a 96 percent chance of detecting as little as a 0.2 percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was non-hybridized westslope cutthroat trout. Meadow Fork Greenhorn Creek, therefore, very likely contains non-hybridized westslope cutthroat trout.

There is good evidence that the observed genotypic distributions in the sample from Meadow Fork Greenhorn Creek did not conform to expected random mating proportions. At three of the 11 loci at which meaningful comparisons were possible, there was a significant excess of heterozygotes compared to expected Hardy-Weinberg proportions. These differences remained significant at the modified level. Furthermore, considering all the comparisons ten possessed an excess of heterozygotes (X^2_1=7.364, P<0.01). An excess of heterozygotes can arise in a sample if the individuals in it were produced from a relatively small number of parents (Balloux 2004; Pudovkin et al. 2010). Thus, we investigated this possibility by estimating the degree of relatedness between all possible pairs of individuals using the program ML-RELATE (Kalinowski et al. 2006). Out of 300 possible pair wise comparisons, 123 (41%) appeared to contain individuals with a relatively high coefficient of relationship. Of these pairs, 13 had a degree of relationship comparable to that of half-siblings, 88 a degree of relationship comparable to that of full-siblings, and 22 a degree of relationship comparable to that of a parent and offspring. The excess of heterozygotes in this sample, therefore, probably mainly results from the relatively high degree of relationship among the fish suggesting the population is being maintained by a relatively small number of parents. Because of the apparent relatively high degree of relationship among the fish in the population we would not recommend that it be used as a sole source for broodstock or transfer purposes unless the reason for the transfer is for "replication" in a secure stream.

Dark Hollow Creek

Samples were collected from two reaches of Dark Hollow Creek. Between the samples, evidence of genetic variation was detected at 12 loci. The allele frequencies significantly differed between the upper and lower samples at four of these loci. These differences remained significant at the modified level indicating that genetic differences existed between the samples. These differences were far from trivial as F_{ST} between the samples was 0.115. Thus, they were treated separately for subsequent analysis.

Upper Dark Hollow Creek 4441

In the sample from upper Dark Hollow Creek, no alleles characteristic of rainbow trout were detected at the rainbow markers. Furthermore, only alleles characteristic of westslope cutthroat trout were detected at the westslope markers and no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed in the sample. Thus, there was no evidence of hybridization with either rainbow or Yellowstone cutthroat trout in the sample.

There is no evidence that the observed genotypic distributions in the sample significantly differed from expected random mating proportions. At the five polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, none of the comparisons significantly deviated from expected Hardy–Weinberg proportions.

Lower Dark Hollow Creek 4443

No alleles characteristic of rainbow trout were detected at the rainbow markers in the sample from lower Dark Hollow Creek. Furthermore, only alleles characteristic of westslope cutthroat trout were detected at the westslope markers and no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed in the sample. Like the sample from upper Dark Hollow Creek, therefore, there was no evidence of hybridization with either rainbow or Yellowstone cutthroat trout in the lower sample.

There is little evidence that the observed genotypic distributions in the sample significantly differed from expected random mating proportions. At the eight polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, only one significantly deviated from expected Hardy–Weinberg proportions. This difference, however, was not significant at the

modified level suggesting that it most likely represented a chance departure from homogeneity rather than an actual deviation from expected random mating proportions.

Dark Hollow Creek Summary

No evidence of hybridization was detected in the two samples from Dark Hollow Creek. These results are very similar to those obtained from a previous PINE analysis (#3011, col. 8/4/04, T8S R4W S13, x=418350 y=4999314, N=15) of trout sampled from the creek. This analysis also detected no evidence of hybridization with either rainbow or Yellowstone cutthroat trout. Thus, Dark Hollow Creek very likely contains non-hybridized westslope cutthroat trout. The trout in the stream, therefore, would be a suitable source for broodstock or transfer purposes. If used for such purposes, however, some consideration should be given to the presence of apparently at least two genetically divergent groups of fish in the stream. If the purpose of the broodstock or transfer action is to capture the genetic diversity of the fish in the creek, then fish or gametes will have to be collected from at least the lower and upper reaches.

Peet Creek Above Pond 4442

Allele characteristic of Yellowstone cutthroat trout were detected at five of the Yellowstone markers and three of the westslope markers analyzed in the sample from Peet Creek collected from above the pond. No alleles characteristic of rainbow trout were detected at the rainbow markers. Although the allele frequencies were statistically heterogeneous (X^2_{39}=172.664; P<0.001) among the diagnostic loci, the Yellowstone cutthroat trout alleles appeared to be randomly distributed (X^2_3=2.183; P>0.50) among the fish in the sample. This sample, therefore, appears to have come from a hybrid swarm between westslope and Yellowstone cutthroat trout with a predominant (0.988) westslope cutthroat trout genetic contribution.

There is good evidence that the observed genotypic distributions in the sample from Peet Creek did not conform to expected random mating proportions. At three of the 20 loci at which meaningful comparisons were possible, there was a significant excess of heterozygotes compared to expected Hardy-Weinberg proportions. These differences remained significant at the modified level. Furthermore, considering all the comparisons 16 possessed an excess of heterozygotes (X^2_1=7.200, P<0.01). We investigated the possibility that this apparent excess of heterozygotes may be indicative of a relatively high amount of relatedness among the fish in the sample. Out of 300 possible pair wise comparisons, 71 (23.7%) appeared to contain individuals with a relatively high degree of relationship. Of these pairs, 27 had a degree of relationship comparable to that of half-siblings, 25 a degree of relationship comparable to that of full-siblings, and 19 a degree of relationship comparable to that of a parent and offspring. The excess of heterozygotes in this sample, therefore, probably reflects the relatively high degree of relationship among the fish suggesting the population is being maintained by a relatively small number of parents.

The hybridization results obtained from the recent Peet Creek sample are different from those obtained from a previous allozyme analysis (#694, col. 8/27/92, T14S R4W S34 NE1/4, N=10) of trout collected from Peet Creek. The allozyme analysis indicated the sample, also collected above the pond (Matt Jaeger, Montana Fish, Wildlife & Parks, personal communication), came from a hybrid swarm between westslope and Yellowstone cutthroat trout with a substantially higher (0.121) Yellowstone cutthroat trout genetic contribution than observed in the recent sample. This difference could represent a temporal change in the genetic characteristics of the fish in the stream because of its relatively small effective population size. This explanation is purely speculative but, regardless the genetic characteristics of the fish in the stream appear not to have been temporally stable.

Middle Fork Little Sheep Creek 4444

In the sample from Middle Fork Little Sheep Creek, data were unattainable from the Yellowstone marker *OclYGD117286_Garza*. Alleles characteristic of Yellowstone cutthroat trout were detected in the sample at 13 of the remaining Yellowstone markers and 15 of the westslope markers that were analyzed. No alleles characteristic of rainbow trout were detected at the rainbow markers that were analyzed. The allele frequencies were statistically heterogeneous (X^2_{38}=78.241; P<0.001) among the Yellowstone and westslope markers and the Yellowstone cutthroat trout alleles were not randomly distributed (X^2_8=28.424; P<0.001) among the fish in the sample. The nonrandom distribution of the Yellowstone cutthroat trout alleles appeared to mainly be due to the presence of three individuals in the sample with a hybrid index of nine or ten (Figure 2). When these fish were eliminated from the data, the Yellowstone cutthroat trout alleles appeared to be randomly distributed (X^2_6=6.659; P>0.10) among the remaining fish. This sample, therefore, appeared to contain a mixture of fish from a hybrid swarm between westslope and Yellowstone cutthroat trout with a predominant (0.963) westslope cutthroat trout genetic component and a few hybrids with a higher amount of admixture.

Although the hybrid indices indicate the sample very likely contained fish from two genetically divergent groups in terms of the amount of admixture, this does not appear to have resulted in observed genotypic distributions significantly deviating from expected random mating proportions. At the 45 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, only three comparisons significantly deviated from expected Hardy–Weinberg proportions. These differences, however, were not significant at the modified level suggesting that they most likely represented chance departures from homogeneity rather than actual deviations from expected random mating proportions.

The hybridization results from the recent sample are fairly similar to those obtained from previous allozyme analyses (#582, col. 10/3/91, T15S R9W S16, N=6; #674, col. 8/12/92, T15S R9W S23, N=11) of trout collected from Middle Fork Little Sheep Creek. Both analyses indicated the samples came from a hybrid swarm between westslope and Yellowstone cutthroat trout with about a five percent Yellowstone cutthroat trout genetic component. Thus, the main difference between the recent and former samples was the presence of a few hybrids with a relatively higher amount of introgression with Yellowstone cutthroat trout in the recent sample. The potential origin of these fish cannot be determined from the available data.

West Fork East Fork Sweetwater Creek 4445

Compared to the other rainbow markers, *OmyRD_RAD_29252_Hoh* and *OmyRD_RAD_77157_Hoh* possessed alleles usually characteristic of rainbow trout at frequencies substantially higher than observed at the other rainbow markers. This situation also pertained to the westslope marker *OclWD_ Tnsf_387Kal*. We believe these anomalies most likely represent westslope cutthroat trout genetic variation rather than evidence of hybridization. This interpretation is supported the existence of genetic variation at *OmyRD_RAD_29252_Hoh* in samples that otherwise appeared to have come from non-hybridized westslope cutthroat trout (Table 3). These loci, therefore, were not considered to be diagnostic in the analysis of hybridization in the sample.

At all of the remaining rainbow markers except one, no alleles characteristic of rainbow trout were detected in the sample. The exception involved *OmyRD_RAD_48301_Hoh* where a single copy of the allele usually characteristic of rainbow trout was detected. Likewise, with two exceptions, only alleles characteristic of westslope cutthroat trout were detected at the westslope markers analyzed in the sample. The exceptions involved *OmyWD_RAD_52968_Hoh* and *OclWD_129170L_Garza* where one and two copies, respectively, of the allele characteristic of rainbow or Yellowstone cutthroat trout was observed. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed in the sample.

Normally we would be somewhat uncertain whether the above situation indicated the presence of hybridization between westslope cutthroat and rainbow trout or the existence of westslope cutthroat trout genetic variation. In this situation, however, we tend to favor the former interpretation as previous allozyme analyses of samples collected from North Fork Sweetwater Creek (#1016, col. 9/8/94, T8S R6W S15C, N=10; #1098, col. 8/17/95, T8S R6W S15 SW1/4, N=15) and West Fork Sweetwater Creek (#4452, col. 9/14/94, T8S R7W S19D, N=10) either suggested or conclusively indicated the presence of hybridization with rainbow trout in the Sweetwater Creek drainage. Thus, we conclude that the sample from West Fork East Fork Sweetwater Creek most likely came from a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (0.998) westslope cutthroat trout genetic component.

There is little evidence that the observed genotypic distributions in the sample significantly differed from expected random mating proportions. At the ten polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, only one significantly deviated from expected Hardy–Weinberg proportions. This difference, however, was not significant at the modified level suggesting that it most likely represented a chance departure from homogeneity rather than an actual deviation from expected random mating proportions.

Peterson Creek 4446

In the sample from Peterson Creek, alleles characteristic of rainbow trout were detected at 11 of the rainbow markers analyzed. Alleles characteristic of Yellowstone cutthroat trout were detected at five of the Yellowstone markers analyzed in the sample. Finally, 12 of the westslope markers analyzed in the sample were polymorphic. This sample, therefore, definitely contained evidence of hybridization among westslope cutthroat, Yellowstone cutthroat, and rainbow trout.

Considering the Yellowstone markers, although the allele frequencies were statistically homogeneous (X^2_{19}=23.614; P>0.10) among them the Yellowstone cutthroat trout alleles did not appear to be randomly distributed (X^2_2=10.996; P<0.01) among the fish in the sample. The nonrandom distribution, however, appeared to mainly be due to the presence of one fish (#22) with a hybrid index of four (Figure 3). When this fish was removed from the data, the Yellowstone cutthroat trout alleles appeared to be randomly distributed (X^2_1=2.478; P>0.10) among the remaining fish.

Similar results were obtained from the rainbow markers. The allele frequencies were statistically homogeneous (X^2_{18}=18.432; P>0.10) among them but, the rainbow trout alleles did not appear to be randomly distributed (X^2_3=21.742; P<0.001) among the fish in the sample. The nonrandom distribution, however, appeared to mainly be due to the presence of one fish (#1) with a hybrid index of six (Figure 4). When this fish was removed from the data, the rainbow trout alleles appeared to be randomly distributed (X^2_3=7.233; P>0.05) among the remaining fish. Considering all the data, therefore, this sample appears to have consisted of mainly hybrids among westslope cutthroat, Yellowstone cutthroat, and rainbow trout with a predominant (>0.950) westslope cutthroat trout genetic contribution and a few fish with a higher amount of admixture with either Yellowstone cutthroat or rainbow trout.

There is some indication that the observed genotypic distributions in the sample may not conform to expected random mating proportions. At the 35 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected Hardy-Weinberg genotypic proportions, three of the comparisons significantly deviated from the expected random mating proportions. These differences remained significant at the modified level with two involving an excess of observed heterozygotes and one a deficit. Thus, it is unclear biologically what these departures from expected random mating proportions indicate.

Odell Creek Forks

Samples were collected from East Fork and West Fork Odell Creek. Between the samples evidence of genetic variation was detected at 65 loci. The allele frequencies significantly differed between the samples at 15 of these loci and the differences remained significant at the modified level. The amount of divergence between the samples was far from trivial (F_{ST}=0.090) so they were treated separately for subsequent analysis.

Middle Fork Odell Creek 4447

Alleles characteristic of rainbow trout were detected at seven of the rainbow markers analyzed in the sample from Middle Fork Odell Creek. Alleles characteristic of Yellowstone cutthroat trout were detected at 19 of the Yellowstone markers analyzed in the sample. Finally, 15 of the westslope markers analyzed in the sample were polymorphic. This sample, therefore, definitely contained evidence of hybridization among westslope cutthroat, Yellowstone cutthroat, and rainbow trout.

The allele frequencies were statistically heterogeneous (X^2_{19}=34.436; P<0.01) among the Yellowstone markers and the Yellowstone cutthroat trout alleles were not randomly distributed (X^2_5=63.626; P<0.001) among the individuals in the sample. The nonrandom distribution was mainly due to the presence of six fish (#10, 11, 13, 15, 18, 19) in the sample with a hybrid index of five or more (Figure 5). When these fish were eliminated from the data, the Yellowstone cutthroat trout alleles appeared to be randomly distributed (X^2_3=2.345; P>0.50) among the remaining individuals.

Among the rainbow markers analyzed, the allele frequencies were statistically homogeneous (X^2_{18}=15.924; P>0.50) but, the rainbow trout alleles were not randomly distributed (X^2_1=3.945; P<0.05) among the fish in the sample. The nonrandom distribution appeared to mainly be due to the presence of one fish (#18) with a hybrid index of four (Figure 6). When this fish was eliminated from the data, the rainbow trout alleles appeared to be randomly distributed (X^2_1=2.735; P>0.05) among the remaining individuals. Considering all the data, therefore, this sample appears to have consisted of mainly hybrids among westslope cutthroat, Yellowstone cutthroat, and rainbow trout with a predominant (>0.950) westslope cutthroat trout genetic contribution and a few fish with a higher amount of admixture with Yellowstone cutthroat trout and one with a higher amount of admixture with rainbow trout.

There is some indication that the observed genotypic distributions in the sample may not conform to expected random mating proportions. At the 55 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected Hardy-Weinberg genotypic proportions, five of the comparisons significantly deviated from the expected random mating proportions. These differences remained significant at the modified level with three involving an excess of observed heterozygotes and two a deficit. Thus, it is unclear biologically what these departures from expected random mating proportions indicate.

East Fork Odell Creek 4448

In the sample from East Fork Odell Creek, alleles characteristic of Yellowstone cutthroat trout were detected at seven of the Yellowstone markers and two of the westslope markers. No alleles characteristic of rainbow trout were detected at the rainbow markers. Among the westslope and Yellowstone markers, the allele frequencies were statistically heterogeneous (X^2_{39}=117.062; P<0.001) and the Yellowstone cutthroat trout alleles did not appear to be randomly distributed (X^2_3=9.927; P<0.05) among the fish in the sample. The nonrandom distribution, however, appeared to mainly be due to the inclusion of four fish in the sample with a hybrid index of three or more (Figure 7). When these four fish were eliminated from the data, the Yellowstone cutthroat trout alleles appeared to be randomly distributed (X^2_2=0.576; P>0.50) among the remaining individuals. Thus, this sample appeared to contain a mixture of fish from a hybrid swarm between

westslope and Yellowstone cutthroat trout with a predominant (0.995) westslope cutthroat trout genetic component and a few hybrids with a higher amount of admixture.

There is good evidence that the observed genotypic distributions in the sample from East Fork Odell Creek did not conform to expected random mating proportions. At two of the 29 loci at which meaningful comparisons were possible, there was a significant excess of heterozygotes compared to expected Hardy-Weinberg proportions. These differences remained significant at the modified level. Furthermore, considering all the comparisons 25 possessed an excess of heterozygotes (X^2_1=15.207, P<0.001). We investigated the possibility that this apparent excess of heterozygotes may be indicative of a relatively high amount of relatedness among the fish in the sample. Out of 300 possible pair wise comparisons, 99 (33%) appeared to contain individuals with a relatively high degree of relationship. Of these pairs, 34 had a degree of relationship comparable to that of half-siblings, 35 a degree of relationship comparable to that of full-siblings, and 30 a degree of relationship comparable to that of a parent and offspring. The excess of heterozygotes in this sample, therefore, probably reflects the relatively high degree of relationship among the fish suggesting the population is being maintained by a relatively small number of parents.

Odell Creek Drainage Summary

The results obtained from the East Fork and Middle Fork Odell Creek samples are fairly similar to those obtained from samples collected from Odell Creek. A previous allozyme analysis (#1000, col. 8/11/94, T14S R1W S31, N=10) of trout from Odell Creek suggested they constituted a hybrid swarm between westslope and Yellowstone cutthroat trout with a predominant (0.950) westslope cutthroat trout genetic contribution. A subsequent PINE analysis (#3016, col. 7/22/02, T14S R1W S31, N=10) provided no evidence of hybridization with either Yellowstone cutthroat or rainbow trout. From these data, however, we cannot exclude the possibility that the fish may have been slightly hybridized with Yellowstone cutthroat trout but, this was not detected because of sampling error. In this sample, there was only a 95 percent chance of detecting as little as a three percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm. This amount of introgression is well within the 95 percent confidence interval (0.081-0.019) obtained from the allozyme analysis. Thus, it appears that hybridization between westslope and Yellowstone cutthroat trout exists throughout the drainage. Based on the available data, hybridization with rainbow trout, however, has only been detected in Middle Fork Odell Creek.

East Fork East Fork Clover Creek 4449

Data were unattainable from the rainbow marker *OmyRD_URO_302May* in the East Fork East Fork Clover Creek sample. At the remaining rainbow markers, no alleles characteristic of rainbow trout were detected in the sample. With one exception, alleles characteristic of only westslope cutthroat trout were detected at all the westslope markers analyzed in the sample. The exception involved *OclWD111312_Garza* where the allele usually characteristic of Yellowstone cutthroat or rainbow trout was detected at high frequency (Table 4). Given this allele has been detected in other populations that otherwise appear to be non-hybridized westslope cutthroat trout we interpret this polymorphism to more likely indicate westslope cutthroat trout genetic variation rather than evidence of hybridization. A similar situation to the westslope markers existed among the Yellowstone markers. All of them except one lacked alleles characteristic of Yellowstone cutthroat trout. At *OclYSD129870_Garza*, the allele usually characteristic of Yellowstone cutthroat trout was detected at appreciable frequency (Table 5). Again, given this allele has been detected in other populations that otherwise appear to be non-hybridized westslope cutthroat trout we interpret this polymorphism to more likely indicate westslope cutthroat trout genetic variation rather than evidence of hybridization. Thus, we conclude that it appears East Fork East Fork Clover Creek contains non-hybridized westslope cutthroat trout with fairly unusual genetic characteristics.

There is little evidence that the observed genotypic distributions in the sample significantly differed from expected random mating proportions. At the 12 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, only two significantly deviated from expected Hardy–Weinberg proportions. These differences, however, were not significant at the modified level suggesting that they most likely represented chance departures from homogeneity rather than actual deviations from expected random mating proportions.

The results obtained from East Fork East Fork Clover Creek are similar to those obtained from East Fork Clover Creek above the cascades but, different from those obtained from fish collected below the cascades. SNP analysis (#4364, col. 9/27/11, 44.73956 112.21339, N=15) of samples from above the cascades indicated the fish were non-hybridized westslope cutthroat trout. In contrast, PINE (#3174, col. 8/7/02, T13S R5W S4, N=15) and SNP (#4363, col. 9/26/11, 44.73463 112.22555, N=20) analyses indicated the trout below the cascades were hybrids between westslope and Yellowstone cutthroat trout with about a 95 percent westslope cutthroat trout genetic contribution.

Deadman Creek 4450

In the sample from Deadman Creek, alleles characteristic of Yellowstone cutthroat trout were detected at two of the Yellowstone markers analyzed. Likewise, two of the westslope markers were polymorphic. Finally, no alleles characteristic of rainbow trout were detected at the rainbow markers that were analyzed. Although the allele frequencies were statistically heterogeneous (X^2_{39}=111.908; P<0.001) among the westslope and Yellowstone markers, the Yellowstone cutthroat trout alleles appeared to be randomly distributed (X^2_3=2.899; P>0.10) among the individuals in the sample. This sample, therefore, appears to have come from a hybrid swarm between westslope and Yellowstone cutthroat trout with a predominant (0.984) westslope cutthroat trout genetic contribution.

There is no evidence that the observed genotypic distributions in the sample significantly differed from expected random mating proportions. At the eight polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, none significantly deviated from expected Hardy–Weinberg proportions.

Pine Butte Creek 4451

Compared to the other Yellowstone markers, *OclYGD110571_Garza* possessed the allele usually characteristic of Yellowstone cutthroat trout at an unusually high frequency (0.455) in the Pine Butte Creek sample. This situation also pertained to the westslope markers *OmyWD_RAD_76689_Hoh* (0.386) and *OclWD_105075L_Garza* (0.273) where the allele usually characteristic of rainbow or Yellowstone cutthroat trout was detected at a frequency much higher than that observed at the other westslope markers. We suspect these anomalies most likely represent westslope cutthroat trout genetic variation rather than evidence of hybridization and these loci were not considered to be markers in the analysis of potential hybridization.

At the remaining 19 Yellowstone markers analyzed, alleles characteristic of Yellowstone cutthroat were detected at seven. Likewise, alleles characteristic of Yellowstone cutthroat trout were detected in the sample at six of the remaining 18 westslope markers analyzed. No alleles characteristic of rainbow trout were detected at the rainbow markers analyzed in the sample. Although the allele frequencies were statistically heterogeneous (X^2_{36}=85.361; P<0.001) among the westslope and Yellowstone markers, the Yellowstone cutthroat trout alleles appeared to be randomly distributed (X^2_4=1.619; P>0.50) among the individuals in the sample. This sample, therefore, appears to have come from a hybrid swarm between westslope and Yellowstone cutthroat trout with a predominant (0.978) westslope cutthroat trout genetic contribution.

There is little evidence that the observed genotypic distributions in the sample significantly differed from expected random mating proportions. At the 25 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, only one significantly deviated from expected Hardy–Weinberg proportions. This difference, however, was not significant at the modified level suggesting that it most likely represented a chance departure from homogeneity rather than an actual deviation from expected random mating proportions.

Robb Leary

Sally Painter

Angela Lodmell

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Table 1

SNP loci that differentiate rainbow from westslope and Yellowstone cutthroat trout (rainbow markers), westslope cutthroat from rainbow and Yellowstone cutthroat trout (westslope markers), and Yellowstone cutthroat from westslope cutthroat and rainbow trout (Yellowstone markers).

Rainbow Markers			Reference
	Taxa and characteristic alleles		
Locus	Rainbow	Westslope/Yellowstone	
OmyRD_RAD_29252_Hoh	11	22	Amish et al. 2012
OmyRD_RAD_77157_Hoh	11	22	Amish et al. 2012
OmyRD_RAD_30378_Hoh	11	22	Amish et al. 2012
OclRD_P53T7R1_Har	11	22	Harwood and Phillips 2011
OmyRD_RAD_30423_Hoh	11	22	Amish et al. 2012
OmyRD_RAD_59515_Hoh	11	22	Amish et al. 2012
OclRD_Thymo_320Kal	11	22	Kalinowski et al. 2011
OmyRD_RAD_48301_Hoh	11	22	Amish et al. 2012
OmyRD_RAD_49759_Hoh	11	22	Amish et al. 2012
OclRD_P53T7R2_Har	11	22	Harwood and Phillips 2011
OmyRD_URO_302May	11	22	Finger et al. 2009
OmyRD_RAD_20663_Hoh	22	11	Amish et al. 2012
OmyRD_RAD_51740_Hoh	11	22	Amish et al. 2012
OmyRD_RAD_22111_Hoh	22	11	Amish et al. 2012
OmyRD_RAD_55820_Hoh	22	11	Amish et al. 2012
OmyRD_RAD_5666_Hoh	11	22	Amish et al. 2012
OmyRD_F5_136May	22	11	Finger et al. 2009
OmyRD_RAD_42014_Hoh	22	11	Amish et al. 2012
OmyRD_RAD_54584_Hoh	22	11	Amish et al. 2012
Westslope Markers			
	Taxa and characteristic alleles		
Locus	Westslope	Rainbow/Yellowstone	
OclRD_CLK3W5_Har	22	11	Harwood and Phillips 2011
OclWD_CLK3W1_Har	22	11	Harwood and Phillips 2011
OclWD101119_Garza	22	11	Campbell et al. 2012
OmyWD_RAD_76689_Hoh	22	11	Amish et al. 2012
OclWD_114315L _Garza	22	11	Campbell et al. 2012
OclWD_Tnsf_387Kal	22	11	Kalinowski et al. 2011
OmyWD_RAD_55391_Hoh	22	11	Amish et al. 2012
OclWD_P53_307Kal	22	11	Kalinowski et al. 2011
OclWD111312_Garza	22	11	Campbell et al. 2012
OclWD_107031L _Garza	22	11	Campbell et al. 2012
OclWD_PrLcW1_Har	22	11	Harwood and Phillips 2011
OmyWD_RAD_54516_Hoh	22	11	Amish et al. 2012
OclWD_105075L_Garza	22	11	Campbell et al. 2012
OmyWD_RAD_52968_Hoh	22	11	Amish et al. 2012
OclWD114336_Garza	11	22	Campbell et al. 2012
OclWD103713_Garza	22	11	Campbell et al. 2012
OclWD107074_Garza	22	11	Campbell et al. 2012
OclWD109651_Garza	22	11	Campbell et al. 2012
OclWD_129170L _Garza	11	22	Campbell et al. 2012
OclWD_ppie_32NC	11	22	Campbell et al. 2012

Table 1-continued

Locus	Yellowstone Markers Taxa and characteristic alleles		Reference
	Yellowstone	Westslope/Rainbow	
OclYD_CLK3Y1_Har	22	11	Harwood and Phillips 2011
OclYGD100974_Garza	22	11	Campbell et al. 2012
OclYGD110571_Garza	22	11	Campbell et al. 2012
OclYSD117432_Garza	22	11	Campbell et al. 2012
OclYGD1127236_Garza	22	11	Campbell et al. 2012
OclYGD112820_Garza	22	11	Campbell et al. 2012
OclYGD104216_Garza	22	11	Campbell et al. 2012
OclYGD113600_Garza	22	11	Campbell et al. 2012
OclYSD129870_Garza	22	11	Campbell et al. 2012
OclYGD104569_Garza	22	11	Campbell et al. 2012
OclYGD117286_Garza	22	11	Campbell et al. 2012
OclYGD117370_Garza	22	11	Campbell et al. 2012
OclYSD107607_Garza	22	11	Campbell et al. 2012
OclYGD106457_Garza	22	11	Campbell et al. 2012
OclYSD106367_Garza	11	22	Campbell et al. 2012
OclYGD107031_Garza	11	22	Campbell et al. 2012
OclYGD106419_Garza	11	22	Campbell et al. 2012
OclYSD123205_Garza	11	22	Campbell et al. 2012
OclYGD109525_Garza	11	22	Campbell et al. 2012
OclYSD113109_Garza	11	22	Campbell et al. 2012

Table 2

Reference samples used for the identification of marker SNPs among westslope cutthroat, rainbow, and Yellowstone cutthroat trout. Taxa: WCT=westslope cutthroat trout, YCT=Yellowstone cutthroat trout, IRT=redband trout, CRT=coastal rainbow trout. N=sample size.

Sample	Taxa	N	Location
Washoe Park State Trout Hatchery	WCT	12	Anaconda, Montana
Big Foot Creek	WCT	2	Upper Kootenai River, Montana
Runt Creek	WCT	3	Yaak River, Montana
Hawk Creek	WCT	2	North Fork Flathead River, Montana
Werner Creek	WCT	3	North Fork Flathead River, Montana
Morrison Creek	WCT	3	Middle Fork Flathead River, Montana
Sixmile Creek	WCT	3	Swan River, Montana
South Fork Jocko River	WCT	3	Lower Flathead River, Montana
Cottonwood Creek	WCT	3	Upper Clark Fork River, Montana
Copper Creek	WCT	2	Flint-Rock Creek, Montana
Gillispie Creek	WCT	3	Flint-Rock Creek, Montana
Davis Creek	WCT	4	Bitterroot River, Montana
Humbug Creek	WCT	2	Blackfoot River, Montana
Ringeye Creek	WCT	2	Blackfoot River, Montana
Flat Creek	WCT	3	Middle Clark Fork River, Montana
McGinnis Creek	WCT	3	Lower Clark Fork River, Montana
Bear Creek	WCT	1	Red Rock River, Montana
McVey Creek	WCT	1	Big Hole River, Montana
McClellan Creek	WCT	1	Upper Missouri River, Montana
Yellowstone River State Trout Hatchery-Goose Lake	YCT	6	Big Timber, Montana
Slough Creek	YCT	4	Yellowstone River, Montana
Lake Koocanusa	IRT	4	Upper Kootenai River, Montana
North Fork Yahk River	IRT	5	Yahk River, British Columbia
Jocko River State Trout Hatchery Arlee Rainbow	CRT	7	Arlee, Montana

Table 3

Frequency of the allele usually characteristic of rainbow trout at the rainbow marker *OmyRD_RAD_29252_Hoh* in samples from what otherwise appear to be non-hybridized westslope cutthroat trout. Number in parentheses represents sample number.

Sample	Allele Frequency
Spruce (#4337)	0.383
S Child (#4285)	0.036
Collar Gulch (#4437)	0.190

Table 4

Frequency of the allele usually characteristic of rainbow or Yellowstone cutthroat trout at the westslope marker *OclWD111312_Garza* in samples that otherwise appear to be non-hybridized westslope cutthroat trout. Number in parentheses represents sample number.

Sample	Allele Frequency
EF Clover (#4364)	0.333
Sidney (#4384)	0.010
NF Highwood Trib. (#4374)	0.052
Collar Gulch (#4437)	0.090
EF EF Clover (#4449)	0.450

Table 5

Frequency of the allele usually characteristic of Yellowstone cutthroat trout at the Yellowstone marker *OclYSD129870_Garza* in samples that otherwise appear to be non-hybridized westslope cutthroat trout. Number in parentheses represents sample number.

Sample	Allele Frequencies
EF Clover (#4364)	0.200
Spruce (#4337)	0.100
Yodkin (#4299)	0.022
EF EF Clover (#4449)	0.300

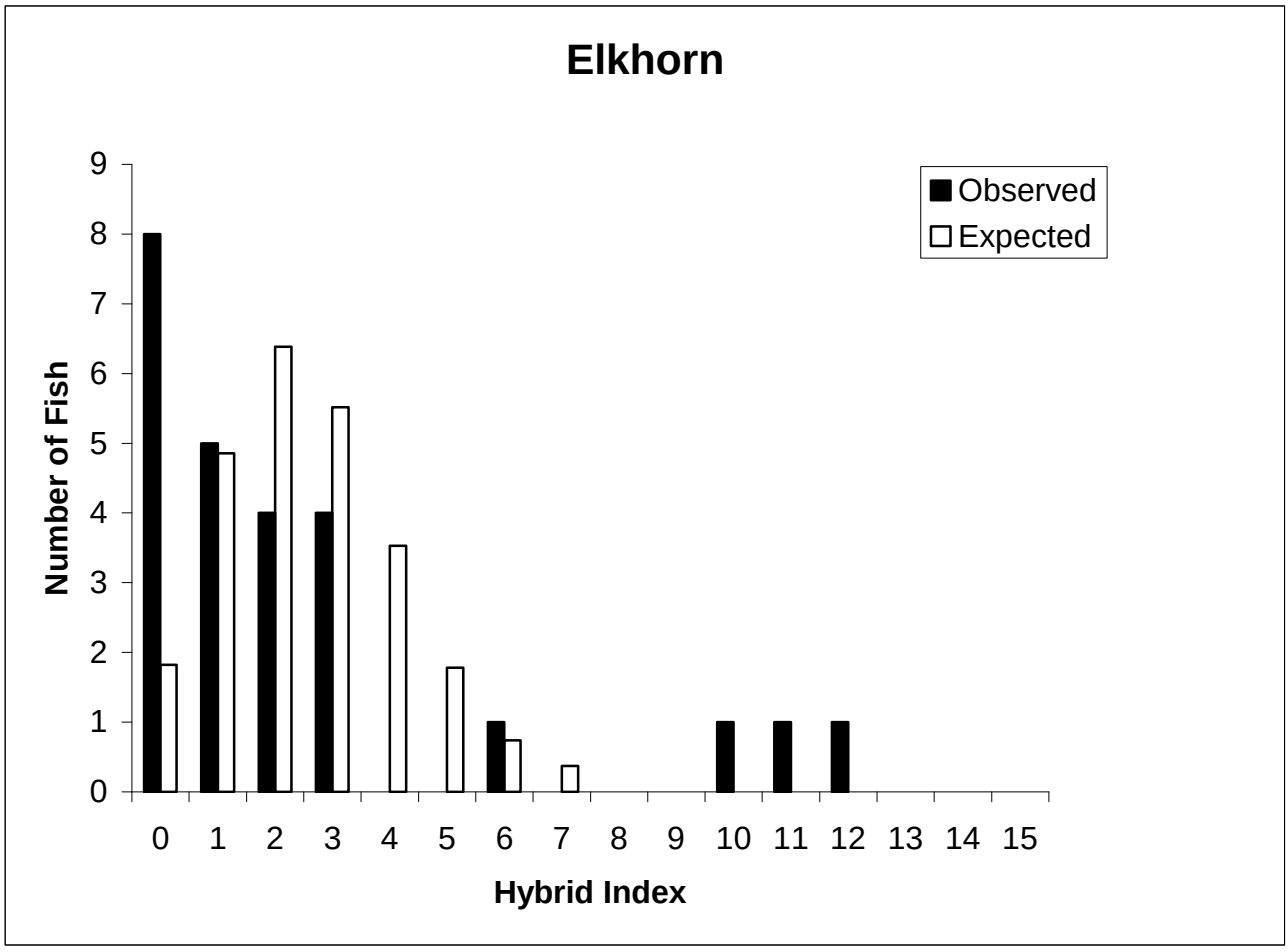

Figure 1. Observed and expected random distribution of hybrid indices in a sample from Elkhorn Creek showing evidence of hybridization between westslope cutthroat and rainbow trout. Note the observed distribution significantly differs ($P<0.001$) from the expected random distribution.

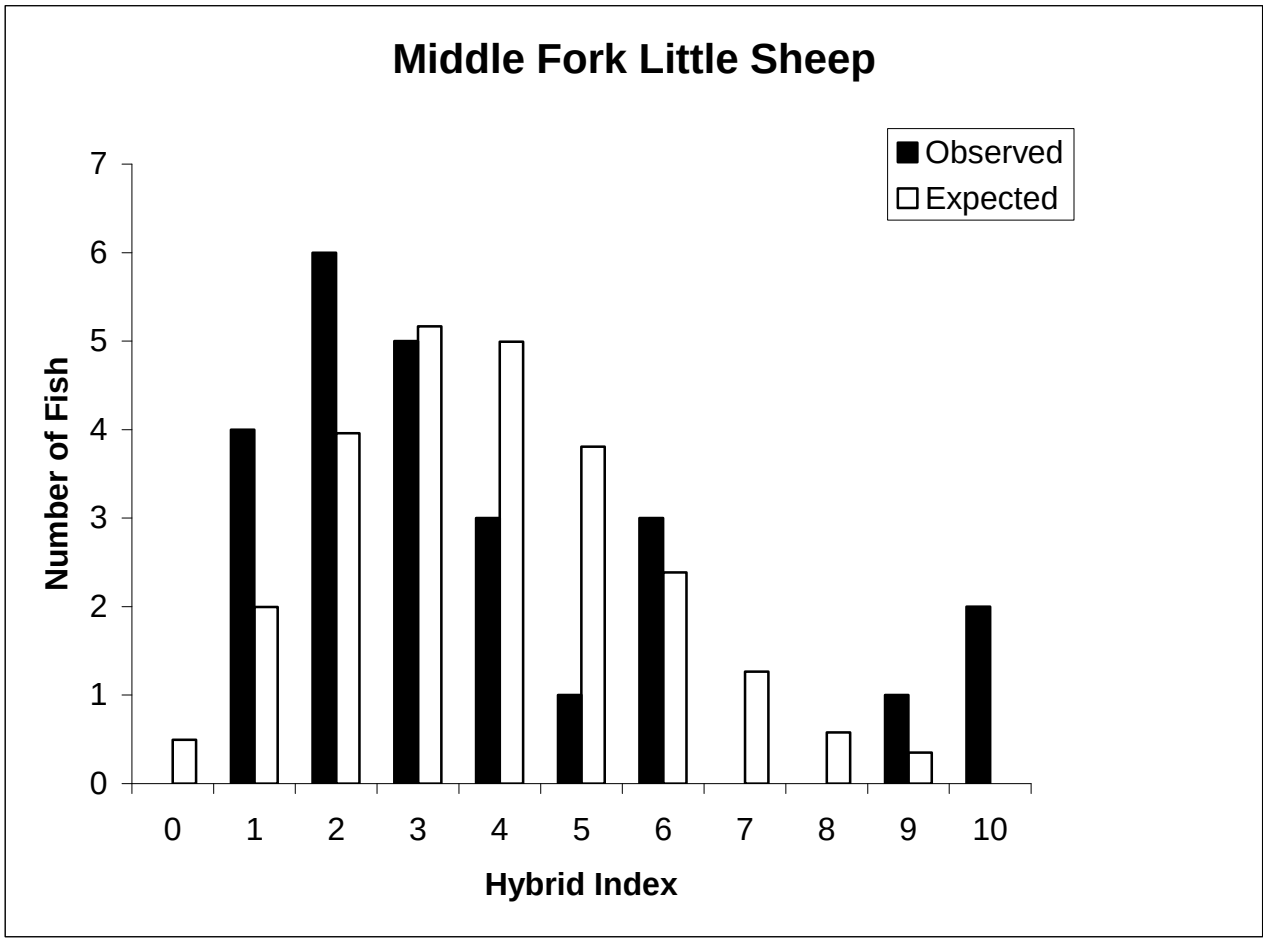

Figure 2. Observed and expected random distribution of hybrid indices in a sample from Middle Fork Little Sheep Creek showing evidence of hybridization between westslope and Yellowstone cutthroat trout. Note the observed distribution significantly differs ($P<0.001$) from the expected random distribution.

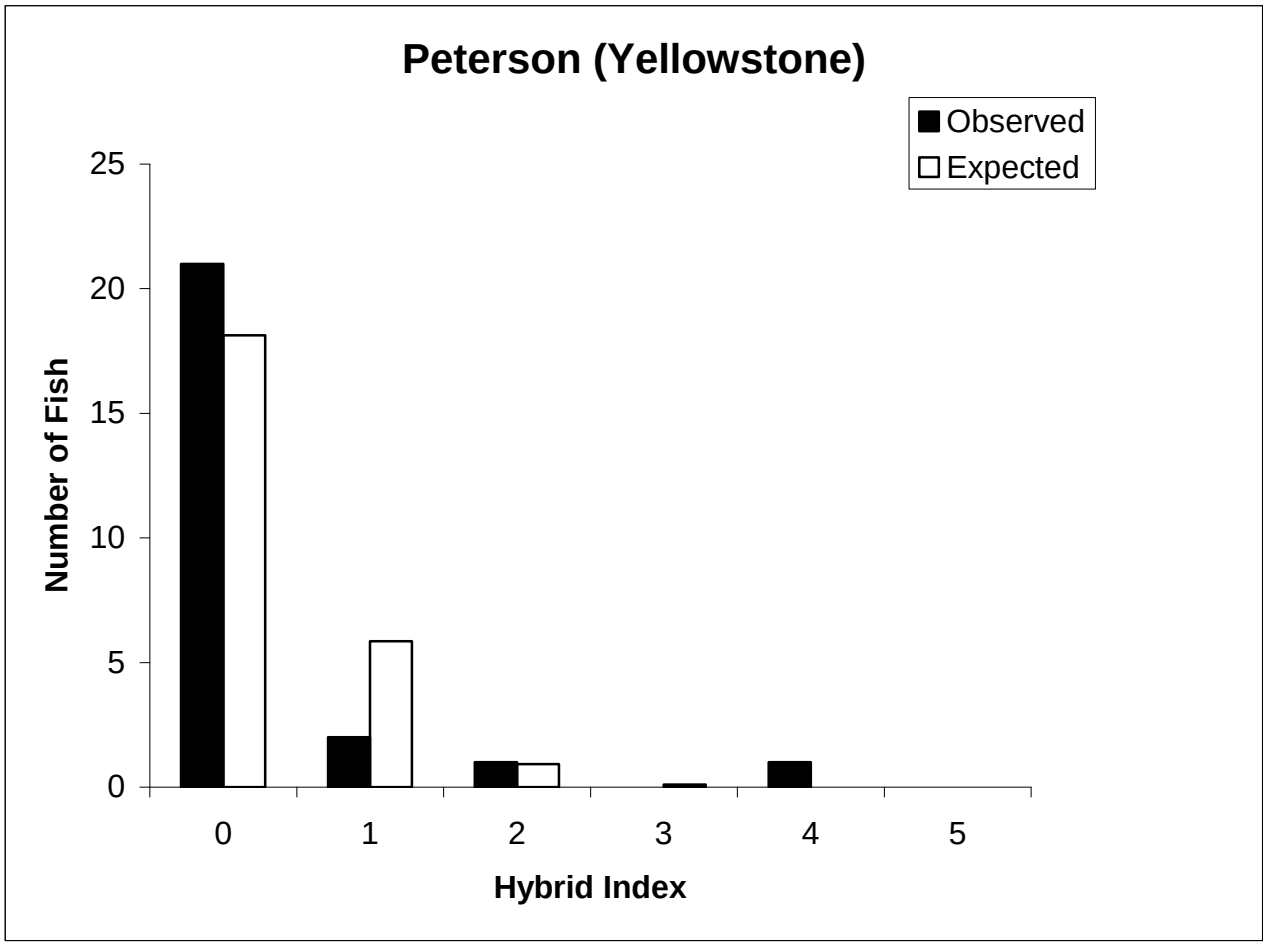

Figure 3. Observed and expected random distribution of hybrid indices in a sample from Peterson Creek showing evidence of hybridization between westslope and Yellowstone cutthroat trout. Note the observed distribution significantly differs ($P<0.01$) from the expected random distribution.

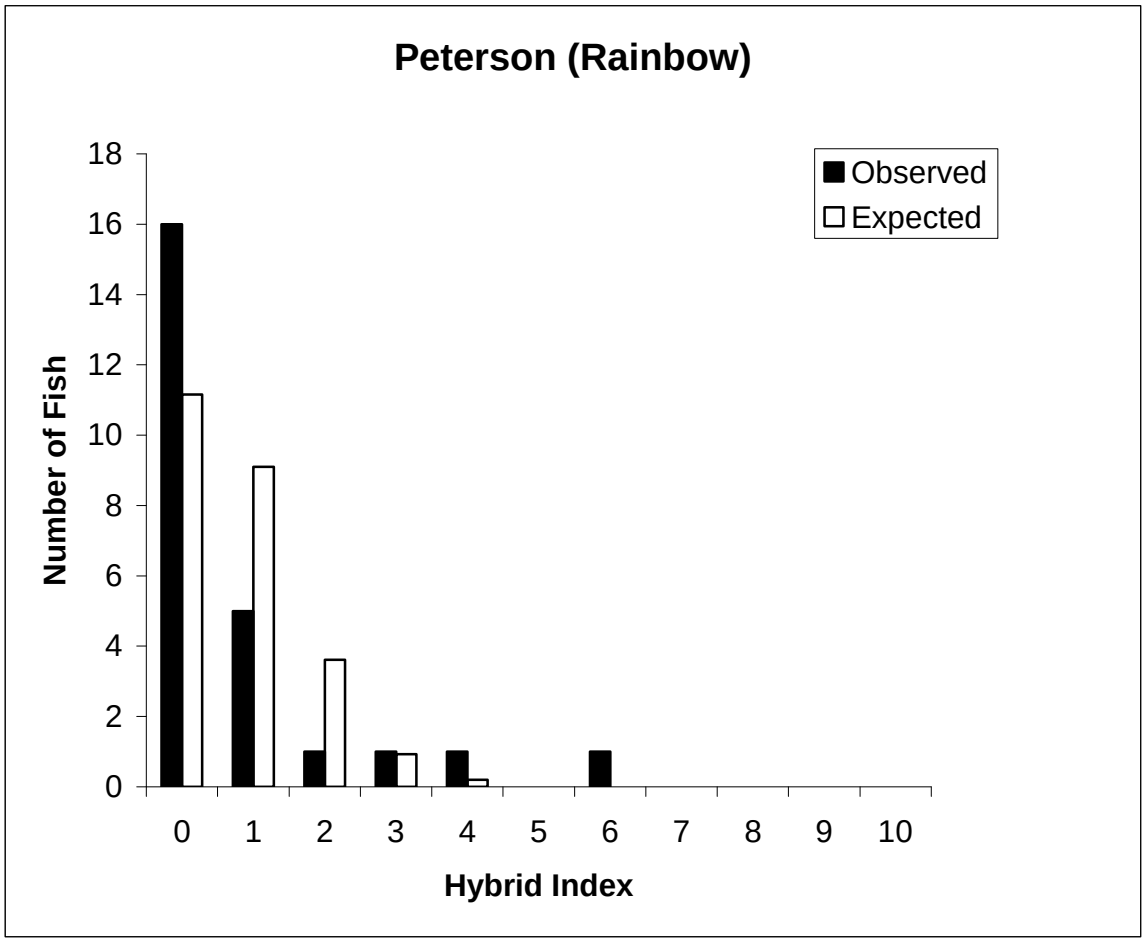

Figure 4. Observed and expected random distribution of hybrid indices in a sample from Peterson Creek showing evidence of hybridization between westslope cutthroat and rainbow trout. Note the observed distribution significantly differs ($P<0.001$) from the expected random distribution.

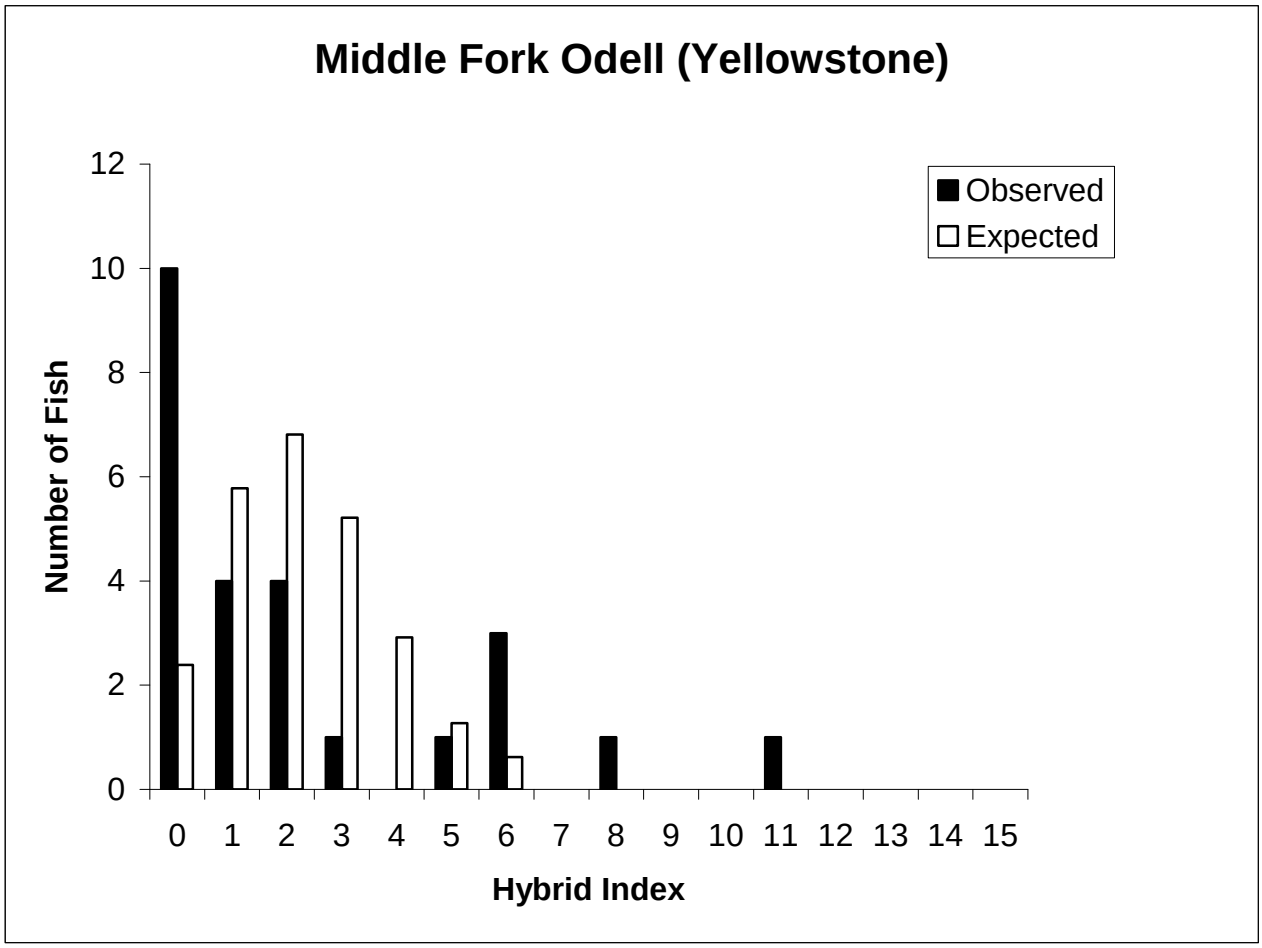

Figure 5. Observed and expected random distribution of hybrid indices in a sample from Middle Fork Odell Creek showing evidence of hybridization between westslope and Yellowstone cutthroat trout. Note the observed distribution significantly differs ($P<0.001$) from the expected random distribution.

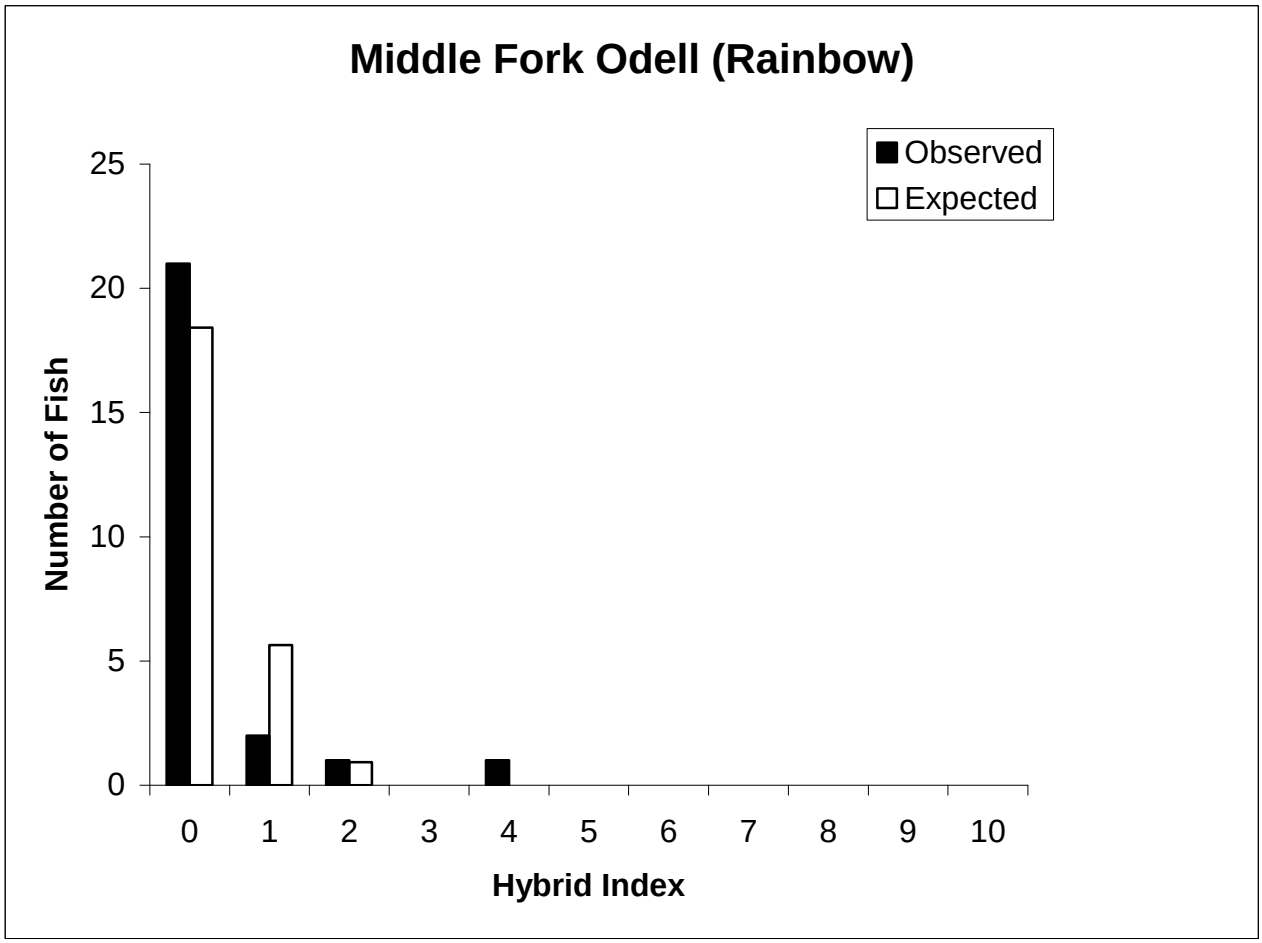

Figure 6. Observed and expected random distribution of hybrid indices in a sample from Middle Fork Odell Creek showing evidence of hybridization between westslope cutthroat and rainbow trout. Note the observed distribution significantly differs ($P<0.05$) from the expected random distribution.

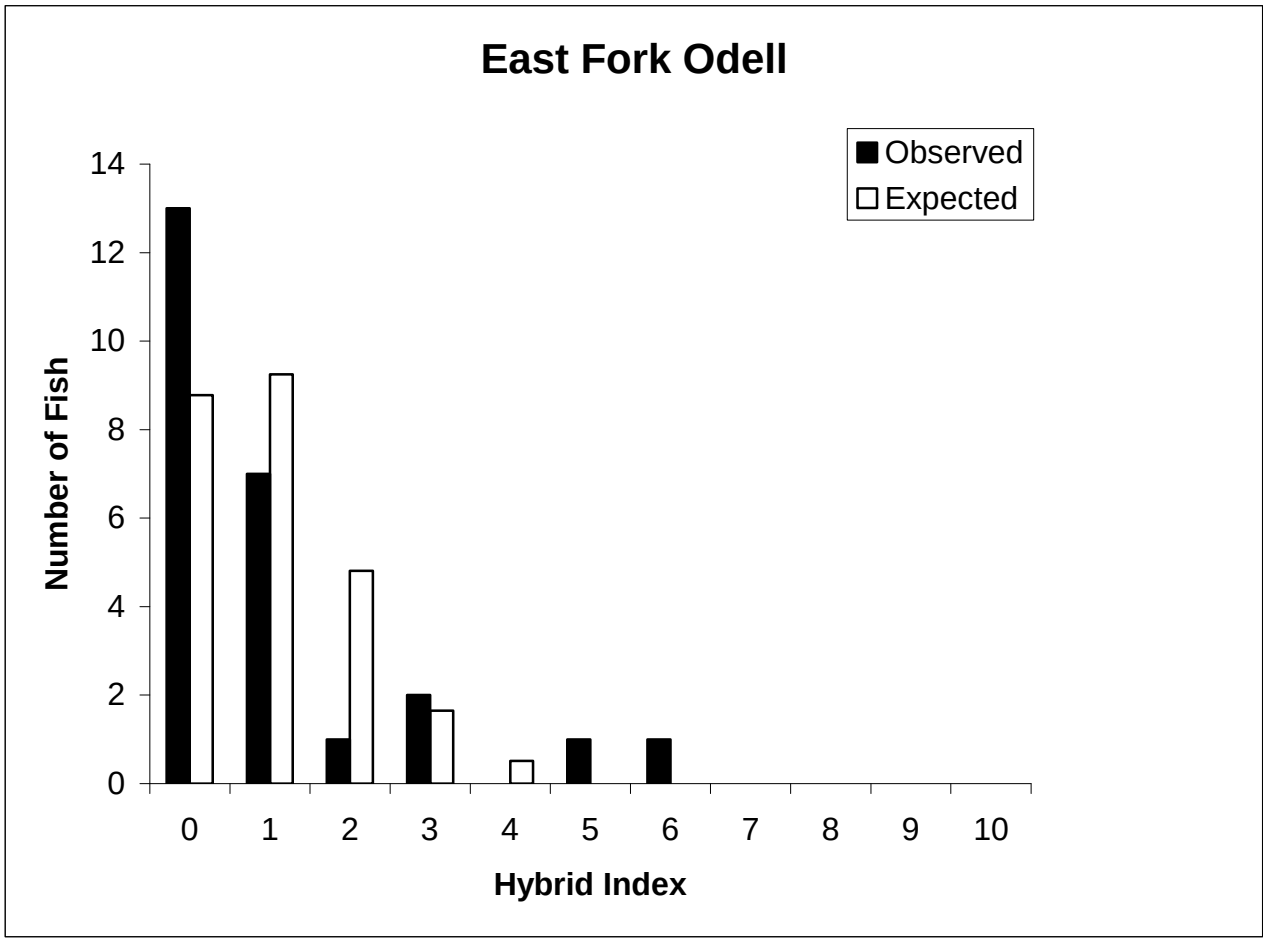

Figure 7. Observed and expected random distribution of hybrid indices in a sample from East Fork Odell Creek showing evidence of hybridization between westslope and Yellowstone cutthroat trout. Note the observed distribution significantly differs ($P<0.05$) from the expected random distribution.