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Matt;

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

		a	b	c	d	e	f
Sample #	**Water Name/Location/ Collection Date/ Collector**	**N**	**#Markers**	**Taxa ID**	**Power**	**%**	**# Fish**
4457	**Colonite Creek 47.57169 115.17845 9/26/2012 Mike Hensler**	**26**	**R19W20Y20**	**WCT X RBT**			
4458	**Deer Creek 48.20187 115.08952 9/25/2012 Mike Hensler**	**24**	**R19W20Y20**	**WCT X RBT**			
4459	**Richards Creek 48.28737 115.20374 9/24/2012 Mike Hensler**	**26**	**R19W20Y20**	**WCT X RBT**			
4460	**Owl Creek 47.43141-43058 113.59236-59151 11/10/2011 Leo Rosenthal**	**12 (22)**	**R19W20Y20**	**WCT**	**R97Y96**		

Sample #	Water Name/Location/ Collection Date/ Collector	a N	b #Markers	c Taxa ID	d Power	e %	f # Fish
4461	Upper Soup Creek T24N R17W S25 SW1/4 47.81412-81463 113.72426-72336 8/21/2012 Jim Bower	33	R19W20Y20	WCT X RBT		W97.7 X R2.3	
4462	Lower Youngs Creek Babcock to Hahn 47.3639971 113.267911 7/15/2012 Matt Boyer	15	R19W20Y20	WCT	R94Y95		
4463	Upper Youngs Creek Jenny to Big Slide 47.3426199-3040819 113.284739-291133 7/13-16/2012 Matt Boyer	29	R19W20Y20	WCT	R99Y99		
4464	Marshall Creek 47.319054 113.327343 7/14/2012 Matt Boyer	20 (46)	R19W20Y20	WCT	R99Y99		
4465	Gordon Creek- upstream of Doctor confluence 47.43006 113.46130 8/12/2012 Matt Boyer	29	R19W20Y20	WCT WCT X YCT	R99Y99		26 3
4466	Middle Fork Flathead River- from Granite to Dryad 48.08675 113.22583 8/1-2/2012 Matt Boyer	28 (173)	R19W20Y20	WCT	R99Y99		

Sample #	Water Name/Location/ Collection Date/ Collector	a N	b #Markers	c Taxa ID	d Power	e %	f # Fish
4467	Danaher Creek- 2013 Spawners 3/5/2013 Matt Boyer	331	R19W20Y20				
4468	Danaher Creek- 2012 Progeny 3/5/2013 Matt Boyer	74	R19W20Y20				
4470	Sanko Creek 48.41599 114.67724 6/28/2012 Matt Boyer	30	R19W20Y20	WCT WCT X RBT	R99Y99		29 1
4471	Good Creek Above Culvert 48.44658 114.86668 T31N R25W S13&14 7/12/2012 Matt Boyer	28	R19W20Y20	WCT	R99Y99		
4472	Robertson Creek 48.47439 114.83481 T31N R25W S6 7/8/2011 & 7/9/2012 Matt Boyer	26	R19W20Y20	WCT WCT X RBT			23 3
4473	Gregg Creek 48.42456-42581 114.75340-74952 T31N R25W S26 7/27/2011 & 6/27/2012 Matt Boyer	57	R19W20Y20	WCT X YCT			
4474	Martin Creek Above Falls 48.32997 114.43531 9/21/2011 Beth Gardner	29	R19W20Y20	WCT X YCT		W91.9 X Y8.1	
4475	Alder Creek 48.48822 114.79186 T32N R25W S4&33 7/10/2012 Matt Boyer	30	R19W20Y20	WCT?			

Sample #	Water Name/Location/ Collection Date/ Collector	a N	b #Markers	c Taxa ID	d Power	e %	f # Fish
4476	Sheppard Creek T30N R26W S13 Upstream of Forest Road 2474 7/23/2012 T30N R26W S23 100m below Forest Road 2885 7/30/2012 Matt Boyer	33	R19W20Y20	WCT	R99Y99		
4477	Griffin Creek 48.15868 114.45622 7/12/2010 Beth Gardner	17	R19W20Y20	WCT X RBT X YCT WCT X RBT X YCT		W53.4 X R6.8 X Y39.8	16 1

[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 16 individuals we have better than a 95 % chance to detect as little as a 0.5% rainbow (19 diagnostic loci) or Yellowstone cutthroat trout (20 diagnostic loci) genetic contribution to a hybrid swarm that once was a non-hybridized westslope cutthroat trout population. Likewise, with 16 individuals we have better than a 95% chance to detect as little as a 0.5% percent rainbow (20 diagnostic loci) or westslope cutthroat trout (20 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 in different years or different reaches of a stream, 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. In interpreting these results, it must be kept in mind that the power of this test to detect significant differences is generally weak. Samples could contain individuals from two or more populations, therefore, and appear not to especially when the amount of genetic divergence among the populations is relatively small.

Results and Discussion

Colonite Creek 4457

In the sample from Colonite Creek, alleles characteristic of rainbow trout were detected at all of the rainbow and westslope markers that were analyzed. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers. The allele frequencies were statistically heterogeneous (X^2_{38}=56.150; P<0.05) among the diagnostic loci and the rainbow trout alleles were not randomly distributed (X^2_{11}=459.380; P<0.001) among the fish in the sample. In contrast, the hybrid indices among the fish had a discontinuous range of zero to 39 (Figure 1) indicating a wide range in the amount of admixture among individuals. The fish with a hybrid index of 39 was heterozygous at all of the rainbow and westslope markers suggesting it was an F_1 hybrid. Its mtDNA was characteristic of westslope cutthroat trout suggesting it was produced from a cross between a female westslope cutthroat and male rainbow trout.

Although the hybrid indices indicate the sample very likely contained fish from two or more 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 63 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.

The above results are very different from those obtained from a previous allozyme analysis (#2833, col. 7/31/03, T25N R29W S2, N=25) of trout collected from Colonite Creek. This analysis detected no evidence of hybridization with either rainbow or Yellowstone cutthroat trout. This sample was basically collected from the same reach as the most recent sample. Thus, it appears that hybridization between westslope cutthroat and rainbow trout has only recently occurred in this reach of the creek and has not yet resulted in the formation of a hybrid swarm. Essentially all of the fish in the recent sample, however, were definitely of hybrid origin indicating the stream should simply be considered to contain hybrids.

Deer Creek 4458

Alleles characteristic of rainbow trout were detected at all of the rainbow and westslope markers analyzed in the sample from Deer Creek. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed. Although the allele frequencies were statistically homogeneous (X^2_{38}=40.303; P>0.10) among the rainbow and westslope markers, the rainbow trout alleles did not appear to be randomly distributed (X^2_{12}=518.596; P<0.001) among the fish in the sample. Rather, there was a broad but, discontinuous range of hybrid indices among the fish (Figure 2).

There is some indication that the observed genotypic distributions in the sample may not conform to expected random mating proportions. At the 68 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, five of the observed genotypic distributions significantly deviated from expected Hardy–Weinberg proportions. These differences remained significant at the modified level with one involving a deficit of observed heterozygotes and four an excess. Furthermore, considering all the loci 51 had more heterozygotes than expected by chance (X^2_1=17.000; P<0.001) Thus, there appeared to be a strong tendency for there to be an excess of heterozygotes compared to random mating expectations among the fish in the sample.

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 276 possible pair wise comparisons, 135 (49%) appeared to contain individuals with a relatively high degree of relationship. Of these pairs, 35 had a degree of relationship comparable to that of half-siblings, 81 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 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.

The results of a previous allozyme analysis (#831, col. 8/30/93, T28N R27W S19, N=13) of trout collected from lower Deer Creek were very different from those obtained from the recent sample. The previous sample appeared to contain mainly non-hybridized rainbow trout (N=11), a non-hybridized westslope cutthroat trout, and an F_1 hybrid between westslope cutthroat and rainbow trout. The recent sample was collected from about the same reach as the previous one. Thus, the genetic characteristics of the fish in this section of the stream appear to have dramatically changed since 1993 with most trout now being hybrids with a substantial to intermediate westslope cutthroat trout genetic contribution instead of rainbow trout.

Richards Creek 4459

In the sample from Richards Creek, alleles characteristic of rainbow trout were detected at all of the rainbow and westslope markers that were analyzed. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers. Although the allele frequencies were statistically homogeneous (X^2_{38}=38.209; P>0.10) among the diagnostic loci, the rainbow trout alleles were not randomly distributed (X^2_{13}=2765.254; P<0.001) among the fish in the sample. In contrast, the hybrid indices among the fish had a very broad but discontinuous range of zero to 75 (Figure 3) indicating a wide range in the amount of admixture among individuals. The majority of the fish in the sample had a major westslope cutthroat trout genetic contribution but, a few had a substantial amount of admixture or a predominant rainbow trout genetic contribution.

There is a good indication that this sample contained fish from two or more genetically divergent populations. First, there was the extremely broad range of hybrid indices. Next, out of 68 comparisons of

observed to expected random mating genotypic distributions 40 were statistically significant. These differences remained significant at the modified level and 39 of them involved a deficit of heterozygotes.

Owl Creek 4460

No alleles characteristic of rainbow trout were detected at the rainbow markers, no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers, and only alleles characteristic of westslope cutthroat trout were detected at the westslope markers analyzed in the sample from Owl Creek. Thus, there was no evidence of hybridization in the sample. These results are concordant from those obtained from a previous microsatellite/indel analysis (#3396, col. 6/1/05, T19N R15W S12, N=10) of trout from Owl Creek which also detected no evidence of hybridization. With the combined sample size of 22, and 716 rainbow trout diagnostic alleles analyzed and 640 Yellowstone cutthroat trout diagnostic alleles analyzed we had a 97 percent chance of detecting as little as a 0.5 percent rainbow trout and a 96 percent chance of detecting as little as a 0.5 percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was non-hybridized westslope cutthroat trout. Owl Creek, therefore, almost certainly contains non-hybridized westslope cutthroat trout.

There is some indication that the observed genotypic distributions may not have conformed to expected Hardy-Weinberg proportions in the recent Owl Creek sample. Out of 21 comparisons, two were statistically significant. These differences remained significant at the modified level but, one involved a deficit and the other an excess of heterozygotes. Furthermore, considering all the loci 14 had an excess of heterozygotes which is not significantly more than expected by chance (X^2_1=2.333; P>0.10). Since there was no apparent tendency for there to be either an excess or deficit of heterozygotes, it is unclear biologically what the significant departures from expected random mating genotypic proportions in the sample indicate.

Upper Soup Creek 4461

Alleles characteristic of rainbow trout were detected at eight of the rainbow markers and seven of the westslope markers in the sample from upper Soup Creek. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed in the sample. Although the allele frequencies were statistically heterogeneous (X^2_{38}=204.947; P<0.001) among the rainbow and westslope markers, the rainbow trout alleles appeared to be randomly distributed (X^2_5=10.434; 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 (0.977) 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 43 polymorphic loci in the sample providing meaningful tests for conformity of observed to expected random mating genotypic proportions, four 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.

The above hybridization results differ from those obtained from a previous allozyme analysis (#59, col. 6/6/83, T24N R17W S27 SE1/4, N=25) of trout collected from lower Soup Creek. These results suggested the fish were a hybrid swarm between westslope and Yellowstone cutthroat trout with a predominant (0.977) westslope cutthroat trout genetic contribution. A subsequent microsatellite/indel analysis (#3607, col. 7/10/07, 47.814 113.731, N=30) collected upstream of the recent sample, however, indicated the trout were hybrids between westslope cutthroat and rainbow trout with a predominant westslope cutthroat trout genetic component. Thus, there are either spatial genetic differences among the trout in Soup Creek or there have been temporal genetic changes with the fish now being slightly hybridized with rainbow trout.

Youngs Creek

Samples were collected from lower (Babcock to Hahn Creek, N=15), middle (Marshall Creek to Big Slide, N=10) and upper (at Jenny Creek, N=20) Youngs Creek**.** Between the lower and middle samples, 30 loci were polymorphic. The allele frequencies significantly differed between the samples at 11 of these loci and the differences remained significant at the modified level. Between the lower and upper samples, 28 loci were polymorphic. At 13 of these loci, the allele frequencies significantly differed between the samples. These differences remained significant at the modified level. Finally, 30 loci were polymorphic between the middle and upper samples. Only three loci had significant allele frequency differences between the middle and upper samples but, these differences were not significant at the modified level suggesting they most likely represented chance departures from homogeneity. Overall, therefore, there was good evidence that genetic differences existed between the lower and both the middle and upper samples. There was no compelling evidence of genetic differences between the middle and upper samples. The lower sample, therefore, was treated separately and the middle and upper samples combined into a single upper sample for subsequent analysis.

Lower Youngs Creek 4462

There was no evidence of hybridization with either rainbow or Yellowstone cutthroat trout in the sample from lower Youngs Creek. No alleles characteristic of rainbow trout were detected at the rainbow markers analyzed in the sample. 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. This reach of Youngs Creek very likely contains non-hybridized westslope cutthroat trout. With the sample size of 15, we had about a 94 percent chance to detect as little as a 0.5 percent rainbow and about a 95 percent chance to detect as little as a 0.5 percent Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was non-hybridized westslope cutthroat trout.

There was also no evidence that the observed genotypic distributions in the sample significantly differed from expected random mating proportions. At the 26 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.

Upper Youngs Creek 4463

No alleles characteristic of rainbow trout were detected at the rainbow markers, no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers, and only alleles characteristic of westslope cutthroat trout were detected at the westslope markers analyzed in the sample from upper Youngs Creek. Thus, there was no evidence of hybridization in the sample. This reach of Youngs Creek very likely contains non-hybridized westslope cutthroat trout. With the sample size of 29, we had better than a 99 percent chance to detect as little as a 0.5 percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was non-hybridized westslope cutthroat trout.

There was also no evidence that the observed genotypic distributions in the sample significantly differed from expected random mating proportions. At the 23 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.

Youngs Creek drainage

A previous PINE analysis (#4120, col. 2001, N=30) of trout collected from Youngs Creek also detected no evidence of hybridization with either rainbow or Yellowstone cutthroat trout. A subsequent microsatellite/indel analysis (#3345, col. 7/11/05, 47.389 113.236, N=50) of a sample collected further downstream than the recent samples, however, indicated it was a mixture of non-hybridized westslope cutthroat trout and a few individuals with a small amount of hybridization with rainbow trout. The recent samples suggest that this hybridization has not now at least moved as far upstream as Hahn Creek. The presence of hybrids in the drainage, however, certainly compromises it attractiveness as a potential source of fish for conservation purposes.

Marshall Creek 4464

There was no evidence of hybridization with either rainbow or Yellowstone cutthroat trout in the sample from lower Marshall Creek. No alleles characteristic of rainbow trout were detected at the rainbow markers analyzed in the sample. 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. A previous allozyme analysis (#224, col. 8/26/87, T18N R14W S13, N=26) of fish collected from Marshall Creek also detected no evidence of hybridization with either rainbow or Yellowstone cutthroat trout. With the combined sample size of 46 and 1072 diagnostic rainbow trout and 1320 Yellowstone cutthroat trout diagnostic alleles analyzed, we had better than a 99 percent chance to detect as little as a 0.5 percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was non-hybridized westslope cutthroat trout. Marshall Creek, therefore, almost certainly contains non-hybridized westslope cutthroat trout.

There was some indication that the observed genotype distributions in the recent Marshall Creek sample may not strictly conform to expected random mating proportions. At the 31 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 was significant at the modified level and involved a deficit of heterozygotes. Considering all the polymorphic loci, however, there was no tendency (X^2_1=0.290, P>0.50) for there to be a deficit of heterozygotes among them. Since there was no apparent tendency for there to be either an excess or deficit of heterozygotes, it is unclear biologically what the single significant departure from expected random mating genotypic proportions in the sample indicates.

Gordon Creek Upstream of Doctor Creek Confluence 4465

Alleles characteristic of Yellowstone cutthroat trout were detected at 12 of the Yellowstone markers and 14 of the westslope markers in the sample from Gordon Creek collected upstream of the confluence with Doctor Creek. No alleles characteristic of rainbow trout were detected at the rainbow markers analyzed in the sample. Although the allele frequencies were statistically homogeneous (X^2_{39}=30.938; P>0.50) among the Yellowstone and westslope markers, the Yellowstone cutthroat trout alleles did not appear to be randomly distributed (X^2_4=91.475; P<0.001) among the fish in the sample. In contrast, all of the Yellowstone cutthroat trout alleles were detected in only three individuals with an appreciable amount of admixture (Figure 4). The remaining fish in the sample appeared to be non-hybridized westslope cutthroat trout.

Considering just the non-hybridized westslope cutthroat trout, there was no evidence this sample contained fish from two or more populations. Out of 29 meaningful comparisons of observed genotypic to expected Hardy-Weinberg proportions, none were significantly different.

When Gordon Creek was initially sampled further downstream than the most recent sample in the vicinity of the confluence with Gabe Creek, allozyme (#308, col. 8/2/89, T19N R13W S5, N=26) and PINE (#2126, col. 8/5/2000, T19N R13W S5, N=25; #2127, col. 8/5/2000, T19N R13W S7, N=25) analyses detected no evidence of hybridization with either rainbow or Yellowstone cutthroat trout. A subsequent PINE analysis (#2319, col. 8/27/02, T19N R14W S10, N=41) of fish collected further upstream than the previous samples but downstream of the recent sample provided ambiguous results. There was a suggestion that the fish may be slightly hybridized with both rainbow and Yellowstone cutthroat trout but, the evidence was far from conclusive. Microsatellite/indel analysis (#3344, col. 9/27/05, 47.419 113.393, N=61) of another sample from this reach provided more compelling evidence that the fish were very slightly (0.001) hybridized with rainbow trout. This level of hybridization could very easily not have been detected in the most recent (probability =0.372) and initial three (allozyme probability=0.732, PINE probability P=0.741) samples due to sampling error. Thus, the conclusion the fish with a hybrid index of zero in the recent sample and the initial three samples were truly non-hybridized westslope cutthroat trout is tentative. The big difference between the most recent and previous samples is the presence of hybrid individuals between westslope and Yellowstone cutthroat trout with an appreciable amount of admixture. Although these individuals are readily detectable and could be eliminated from spawning operations, we suggest Gordon Creek not be used as a source of fish for restoration and conservation efforts due to the uncertainty about whether or not the fish are slightly hybridized with rainbow trout.

Middle Fork Flathead River from Granite Creek to Dryad Creek 4466

In the sample from the Middle Fork Flathead River collected between Granite Creek and Dryad Creek, no alleles characteristic of rainbow trout were detected at the rainbow markers, no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers, and only alleles characteristic of westslope cutthroat trout were detected at the westslope markers analyzed. Thus, there was no evidence of hybridization in the sample. These results a very similar to those obtained from previous samples of trout analyzed from the Middle Fork Flathead River (Table 3).

We do not feel the above results should be interpreted as indicating that the Middle Fork Flathead River does not contain, at least periodically, hybrids between westslope cutthroat and rainbow trout. For example, Hitt et al. (2003) presented data indicating that hybridization with rainbow trout was spreading into tributaries to the river that previously contained non-hybridized westslope cutthroat trout. Thus, hybrids or rainbow trout must at least occasionally occur in the river but, they appear relatively sparse.

Despite the sample being collected from a mainstem river with tributaries containing fish expressing a migratory life history, there was no indication that observed genotypic proportions in the sample significantly deviated from random mating expectations. None of the 31 meaningful comparisons were statistically significant. We do not feel this result should be interpreted to indicate that the westslope cutthroat trout in the Middle Fork Flathead River originated from only one population. Rather, we suspect the failure to detect significant departures from Hardy-Weinberg proportions is due to the very weak power of this test unless sample sizes are large or the populations are markedly divergent from each other.

Danaher Creek 2013 Spawners 4467

These fish were captured from Danaher Creek in 2011 and subsequently raised at Sekokoni Springs. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed in the sample. At the rainbow marker *OmyRD_22111_Hoh*, individuals 2, 302, and 326 were heterozygous for alleles characteristic of rainbow trout. No alleles characteristic of rainbow trout were detected at the other rainbow markers analyzed. At the westslope marker *OclWD_109651_Garza*, individuals 105 and 224 were heterozygous for alleles characteristic of rainbow trout. Only alleles characteristic of westslope cutthroat trout were detected at the other westslope markers analyzed. We are uncertain whether the variation detected

at *OmyRD_22111_Hoh* and *OclWD_109651_Garza* represents a very small (0.001) amount of hybridization with rainbow trout or westslope cutthroat trout genetic variation. We have comparable data from 76 individuals collected from Danaher Creek in 2009 that were spawned in 2011 and 250 fish collected from Danaher Creek in 2010 that were spawned in 2012. Given the frequency of the "rainbow alleles" at *OmyRD_22111_Hoh* and *OclWD_109651_Garza* in the 2011 fish the chances we would not have detected them in the previous two samples is 0.005. Thus, these data support the hybridization interpretation. In contrast, if the alleles detected at *OmyRD_22111_Hoh* and *OclWD_109651_Garza* represent hybridization given the low level it is highly unlikely (Poisson distribution, X^2_1=106.036; P<0.001) that the "rainbow alleles" would have been detected at only two loci. Thus, this analysis lends some support to the westslope cutthroat trout genetic variation interpretation.

The question now is with this uncertainty should these fish be spawned and the progeny used to stock lakes in the South Fork Flathead River drainage. An argument for stocking is that when we first attempted to convert some of the lakes to westslope cutthroat trout by stocking the criterion for success was considered to be 0.990 or more westslope cutthroat trout. Even if the 2011 Danaher Creek fish are hybridized they easily meet this criterion. An argument against would be we really do not purposely want to stock hybrids and some individuals may consider this to be what was done and use it to argue for stocking hybrids in other situations. This probably needs some discussion with others. Our suggestion at this time would be to spawn the fish except numbers 2, 91 (no data were available from this fish), 105, 224, 302, and 326 and subsequently decide their fate.

Danaher Creek 2012 Progeny 4468

In 2012, fish collected from Danaher Creek in 2010 were spawned at Sekokoni Springs seven times. For rearing, the progeny from the early and late spawning (B6 and B4, respectively) dates were pooled but, the middle spawning date (B3) fish were kept separate. Between the B4-B6 (N=49) and B3 (N=25) samples, 29 loci were polymorphic. The allele frequencies were statistically homogeneous between the samples at all of these loci. Since there was no evidence of genetic differences between the samples, they were combined for further analysis.

We have comparable data from the parents that produced these fish (#4376). Between the parents and offspring, evidence of genetic variation was detected at 32 loci. In the parents, a low frequency variant at *OclVar113772_Garza* (0.006), *OclVar_Carpa1_45NC* (0.006), and *OclVar_impa1_189_NC* (0.020) was not detected in the progeny. The difference between the parents and progeny at these loci, however, was not statistically significant suggesting that the apparent absence of these alleles in the progeny may simply represent sampling error. At two other loci, there was a statistically significant allele frequency difference between the parents and offspring. These differences remained significant at the modified level indicating that genetic differences existed between parents and offspring. These differences are small (F_{ST}=0.002) and probably mainly reflect a combination of variation in reproductive success among individuals and random mortality among families. Furthermore, it appears the progeny were produced from a reasonably large number of parents as the effective number of breeders estimated using the procedure of Waples and Do (2008) was 180 (95% confidence interval 81-9141). Thus, there appears to be no reason from a genetics perspective not to use the progeny to stock Lick Lake and Necklace Chain of Lakes as planned.

Sanko Creek 4470

Alleles characteristic of rainbow trout were detected at two of the rainbow and four of the westslope markers analyzed in the sample from Sanko Creek. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers that were analyzed. Although the allele frequencies were statistically homogeneous (X^2_{38}=31.665; P>0.50) among the rainbow and westslope markers, the rainbow trout alleles did not appear to be randomly distributed (X^2_4=91.475; P<0.001) among the fish in the sample. In contrast, all of

the rainbow trout alleles were detected in one fish (Figure 5). The other fish in the sample appeared to be non-hybridized westslope cutthroat trout.

Considering just the non-hybridized westslope cutthroat trout, there is good evidence that the observed genotypic distributions in the sample do not conform to expected random mating proportions. Of the 28 meaningful comparisons, eight were statistically significant. These differences remained significant at the modified level and six involved an excess and two a deficit of heterozygotes suggesting the fish tended to possess more heterozygotes than expected by chance. This is supported by the occurrence of an excess of heterozygotes at 20 of the 28 loci (X^2_1=5.143; P<0.05). Thus, we examined the degree of relationship among the fish in the sample. Out of 378 possible pair wise comparisons, 118 (31%) appeared to contain individuals with a relatively high degree of relationship. Of these pairs, 38 had a degree of relationship comparable to that of half-siblings, 70 a degree of relationship comparable to that of full-siblings, and 10 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.

Sanko Creek was previously sampled three times and the fish were analyzed using microsatellite and insertion/deletion loci. The fish in these samples mainly came from further downstream than those in the recent sample. The sample collected furthest downstream (#3609, col. 6/15/07, 48.414 114.653, N=28) contained what appeared to be one non-hybridized westslope cutthroat trout and the other fish in the sample appeared to be hybrids between westslope cutthroat and rainbow trout with a predominant rainbow trout genetic contribution. The other two samples came from the same reach between the first and most recent samples. Of these, the first (#3389, col. 8/2/06, T31N R24W S28 SW1/4 SW1/4, N=25) appeared to contain 23 non-hybridized westslope cutthroat trout and two hybrids between westslope cutthroat and rainbow trout. The second sample (#3446, col. 6/13/07, 48.416 114.667, N=30) contained only westslope cutthroat trout but, we could not exclude the possibility the reach may have contained a small proportion of hybrids because of sampling error. Thus, it appears there may be spatial differences in terms of the genetic characteristics among the trout in Sanko Creek. Fish in the lower reach appear to mainly be hybrids between westslope cutthroat and rainbow trout with a predominant rainbow trout genetic contribution. Those further upstream appear to be mainly westslope cutthroat trout with a few hybrids with rainbow trout with a predominant westslope cutthroat trout genetic component.

The source of the hybrids in the samples upstream of #3609 is not clear. We initially thought it may be immigrants from this reach but, if this was the case given the high proportion of rainbow trout genetic material in the downstream fish the hybrids in the upstream samples would have to be about three generations old. If this was the case, then we would expect the fish in these reaches to more approximate a hybrid swarm than they do. We previously did not consider this when we suggested the downstream fish were likely the source of the hybrids. Taking this into consideration, therefore, leaves open the possibility that the source of the apparent hybrid immigrants into the upper reaches of Sanko Creek may be the small lake in the drainage headwaters.

Good Creek Above Culvert 4471

There was no evidence of hybridization with either rainbow or Yellowstone cutthroat trout in the sample from Good Creek collected above a culvert modified in 2011 to prevent upstream dispersal of brook trout, *Salvelinus fontinalis*. No alleles characteristic of rainbow trout were detected at the rainbow markers, 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. With the sample size of 28 and 1064 diagnostic rainbow and 1120 Yellowstone cutthroat trout diagnostic alleles analyzed, we had better than a 99 percent chance to detect as little as a 0.5 percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was non-hybridized

westslope cutthroat trout. This reach of Good Creek, therefore, almost certainly contains non-hybridized westslope cutthroat trout.

There is little evidence that the observed genotypic distributions significantly differed from expected Hardy-Weinberg proportions in the sample. Out of 24 meaningful comparisons, two were statistically significant. These apparent deviations, however, were not significant at the modified level. Thus, we cannot reasonably exclude the possibility that they simply represent chance departures from homogeneity.

Good Creek below the culvert was previously sampled two times. Allozyme analysis of the first sample (#243, col. 7/26/88, T31N R26W S13, N=25) also detected no evidence of hybridization with either rainbow or Yellowstone cutthroat trout. A subsequent PINE analysis (#2146, col. 8/1/01, 48.4516 114.84, N=26), however, indicated the trout were a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (0.960) westslope cutthroat trout genetic contribution. It appears, therefore, that hybridization in this reach of Good Creek first occurred sometime after 1988 but, did not progress above the culvert.

Robertson Creek 4472

Robertson Creek was sampled in 2011 and 2012. Between the samples, 35 loci were polymorphic. The allele frequencies significantly differed between the samples at only one of these loci. This difference, however, was not significant at the modified level suggesting it might simply represent a chance departure from homogeneity. Since there was no conclusive evidence of genetic differences between the samples, they were combined for further analysis.

Robertson Creek was historically fishless. The trout population in it was established in 2000 by the transfer of 73 fish collected from below the culvert in Good Creek. Not surprisingly, there was evidence of hybridization between westslope cutthroat and rainbow trout in the Robertson Creek sample. Alleles characteristic of rainbow trout were detected at three of the rainbow markers and two of the westslope markers analyzed in the sample. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed. The allele frequencies were statistically heterogeneous (X^2_{38}=59.092; P<0.05) among the rainbow and westslope markers and the rainbow trout alleles did not appear to be randomly distributed (X^2_1=10.619; P<0.01) among the fish in the sample. In contrast, they were detected in only three fish definitely of hybrid origin (Figure 6). The remaining fish in the sample all appeared to be non-hybridized westslope cutthroat trout.

Considering just the apparent non-hybridized westslope cutthroat trout, there is no compelling evidence that the observed genotypic distributions deviated from expected random mating proportions in the sample. There were 25 meaningful comparisons of observed to expected Hardy-Weinberg proportions. Of these, five were statistically significant but, these differences were not significant at the modified level suggesting they might simply be chance departures from homogeneity.

Our hybridization results are slightly different than those obtained from trout sampled in Good Greek below the culvert in 2001 (#2146). PINE analysis of this sample indicated the trout were a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (0.960) westslope cutthroat trout genetic contribution. Considering our results, the Good Greek sample may have only appeared to have come from a hybrid swarm because of the reduced power of PINEs compared to SNPs to separate non-hybridized from hybridized individuals. This is especially the case with low levels of admixture as only six diagnostic PINE loci were analyzed compared to the 39 diagnostic SNP loci.

Gregg Creek 4473

Samples were collected from upper Gregg Creek in 2011 and lower Gregg Creek in 2012. Between the samples, 39 loci were polymorphic. The allele frequencies significantly differed between the samples at two of these loci. These differences, however, were not significant at the modified level suggesting they might simply represent chance departures from homogeneity. Since there was no conclusive evidence of genetic differences between the samples, they were combined for further analysis.

This was a very odd sample. No alleles characteristic of rainbow trout were detected at the rainbow markers and only alleles characteristic of westslope cutthroat trout were detected at the westslope markers analyzed in it. In contrast, alleles characteristic of Yellowstone cutthroat trout were detected at nine of the Yellowstone markers analyzed. Based on the westslope markers, the fish appear to be non-hybridized westslope cutthroat trout but, the Yellowstone markers suggests hybridization with Yellowstone cutthroat trout is present. Because numerous Yellowstone markers were polymorphic, we conservatively have considered this sample to contain evidence of hybridization with Yellowstone cutthroat trout.

The sample does not appear to have come from a hybrid swarm between westslope and Yellowstone cutthroat trout. The allele frequencies were statistically heterogeneous (X^2_{39}=60.473; P<0.05) among the Yellowstone and westslope markers and the Yellowstone cutthroat trout alleles did not appear to be randomly distributed (X^2_2=42.257; P<0.01) among the fish in the sample. Rather, they were detected in only six fish (Figure 7). The sample, therefore, may have contained some non-hybridized westslope cutthroat trout but, with the available data such fish cannot reliably be identified on an individual basis. Thus, Gregg Creek should simply be considered as containing hybrids between westslope and Yellowstone cutthroat trout with a predominant (>0.990) westslope cutthroat trout genetic component.

Besides the sample not appearing to have come from a hybrid swarm, there is other evidence suggesting it may have contained fish from more than one population. In the sample, there were 32 meaningful comparisons of observed genotypic distributions to expected Hardy-Weinberg proportions. Of these comparisons, four were statistically significant. These differences remained significant at the modified level and all involved a deficit of heterozygotes.

When Gregg Creek was first sampled, PINE analysis (#1920, col. 7/13/98, N=25) indicated the trout to be non-hybridized westslope cutthroat trout. This sample, however, was collected about 5km downstream of the recent sample. Thus, we cannot determine from the available data whether the difference, in terms of hybridization, between the samples represents a spatial or temporal genetic change or both.

Martin Creek Above Falls 4474

Alleles characteristic of Yellowstone cutthroat trout were detected at 16 of the Yellowstone markers and 15 of the westslope markers in the sample from Martin Creek. 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_{39}=172.224; P<0.001) among the Yellowstone and westslope markers, the Yellowstone cutthroat trout alleles appeared to be randomly distributed (X^2_{11}=14.127; P>0.10) among the fish in the sample. Thus, this sample appears to have come from a hybrid swarm between westslope and Yellowstone cutthroat trout with a predominant (0.919) westslope cutthroat trout genetic component.

There is no evidence that the observed genotypic distributions deviated from expected random mating proportions in the sample. There were 58 meaningful comparisons of observed to expected Hardy-Weinberg proportions. Of these, two were statistically significant but, these differences were not significant at the modified level suggesting they might simply represent chance departures from homogeneity.

The above hybridization results are somewhat similar to those obtained from a sample from Martin Creek collected below the falls (#2145, col. 5/1/01, T32N R24W S5, N=25). PINE analysis of these fish suggested they were a hybrid swarm among westslope cutthroat (0.690), rainbow (0.020), and Yellowstone cutthroat trout (0.290).

Alder Creek 4475

No alleles characteristic of rainbow trout were detected at the rainbow markers and no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed in the sample from Alder Creek. At the westslope marker *OclWD_105075L_Garza*, one individual was heterozygous for the allele characteristic of rainbow and Yellowstone cutthroat trout. All the other westslope markers analyzed in the sample possessed only alleles characteristic of westslope cutthroat trout. The variation at *OclWD_105075L_Garza* could indicate a small amount of hybridization with either rainbow or Yellowstone cutthroat trout or it could be westslope cutthroat trout genetic variation. In this case, we tend to favor the latter interpretation as a previous PINE analysis (#1988, col. 10/1/99, N=25) of trout from Alder Creek detected no evidence of hybridization. Thus, conservatively we consider the trout in Alder Creek to be non-hybridized westslope cutthroat trout but, recommend that they not be used for conservation and restoration purposes until their status is known with better certainty.

There is good evidence that the observed genotypic distributions may not have conformed to expected Hardy-Weinberg proportions in the recent Alder Creek sample. Out of 25 comparisons, seven were statistically significant. These differences remained significant at the modified level and six involved an excess of heterozygotes. Furthermore, considering all the loci 19 had an excess of heterozygotes which is significantly more than expected by chance (X^2_1=6.760; P<0.01). Since there was a strong tendency for there to be an excess of heterozygotes in the sample, we estimated the degree of relationship between pairs of individuals. Out of 378 possible pair wise comparisons, 127 (34%) appeared to contain individuals with a relatively high degree of relationship. Of these pairs, 46 had a degree of relationship comparable to that of half-siblings, 67 a degree of relationship comparable to that of full-siblings, and 14 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.

Sheppard Creek Upstream of FR 2474 and 100m Below FR 2885 4476

Samples were collected from two reaches of Sheppard Creek . Between the samples, 26 loci were polymorphic. The allele frequencies were statistically homogeneous between the samples at all of these loci. Since there was no evidence of genetic differences between the samples, they were combined for further analysis.

No alleles characteristic of rainbow trout were detected at the rainbow markers, no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers, and only alleles characteristic of westslope cutthroat trout were detected at the westslope markers analyzed in the sample from Sheppard Creek. Thus, there was no evidence of hybridization in the sample. With the sample size of 33, and 1254 rainbow trout diagnostic alleles analyzed and 1320 Yellowstone cutthroat trout diagnostic alleles analyzed we had better than a 99 percent chance of detecting as little as a 0.5 percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was non-hybridized westslope cutthroat trout. Sheppard Creek, therefore, almost certainly contains non-hybridized westslope cutthroat trout.

There is good evidence that the observed genotypic distributions may not have conformed to expected Hardy-Weinberg proportions in the Sheppard Creek sample. Out of 19 comparisons, three were statistically significant. These differences remained significant at the modified level and all involved an excess of

heterozygotes. Furthermore, considering all the loci 14 had an excess of heterozygotes which is significantly more than expected by chance (X^2_1=4.263; P<0.05). Since there was a strong tendency for there to be an excess of heterozygotes in the sample, we estimated the degree of relationship between pairs of individuals. Out of 528 possible pair wise comparisons, 164 (31%) appeared to contain individuals with a relatively high degree of relationship. Of these pairs, 36 had a degree of relationship comparable to that of half-siblings, 91 a degree of relationship comparable to that of full-siblings, and 37 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.

Griffin Creek 4477

In the sample from Griffin Creek, alleles characteristic of rainbow trout were detected at 13 of the rainbow markers and alleles characteristic of Yellowstone cutthroat trout were detected at all of the Yellowstone markers. All of the westslope markers were polymorphic. Thus, this sample contained evidence of hybridization among westslope cutthroat, rainbow, and Yellowstone cutthroat trout.

This sample appears to have come from a hybrid swarm between westslope cutthroat and rainbow trout with a small (0.068) rainbow trout genetic contribution. The allele frequencies were not homogeneous (X^2_{18}=47.248; P<0.001) among the rainbow and westslope marker loci but, the rainbow trout alleles appeared to be randomly dispersed (X^2_6=11.699; P>0.05) among the fish in the sample.

Compared to the above results, the allele frequencies were also not homogeneous (X^2_{19}=37.314; P<0.001) among the westslope and Yellowstone markers but, the Yellowstone cutthroat trout alleles were not randomly distributed (X^2_{18}=127.969; P<0.001) among the fish in the sample. The nonrandom distribution, however, was mainly due to one fish with a hybrid index of four (Figure 8). When this fish is removed from the data, the Yellowstone cutthroat trout alleles appear to be randomly (X^2_{15}=20.219; P>0.10) distributed among the remaining fish. With the exception of one fish with an unusually small Yellowstone cutthroat trout contribution, this sample appears to have come from a hybrid swarm among westslope cutthroat (0.534), Yellowstone cutthroat (0.398), and rainbow trout (0.068).

Comparison of observed to expected random mating genotypic proportions also suggests the Griffin Creek sample mainly contained individuals from a hybrid swarm. When the individual with an unusually low Yellowstone cutthroat trout genetic contribution was eliminated from the data, there were 76 meaningful comparisons to expected Hardy-Weinberg proportions. Of these, seven were statistically significant. These differences, however, were not significant at the modified level suggesting they most likely indicate chance departures from homogeneity.

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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

Location of various samples of westslope cutthroat trout collected from the Middle Fork Flathead River. N=sample size. Date=collection date

Sample Number	Analysis	Location	Date	N
358	Allozyme	T27N R13W S23 SE1/4	9/27/1989	18
871	Allozyme	T27N R13W S23 SW1/4	10/1/1993	8
978	Allozyme	T30N R16W S6-8	8/2/1994	26
1282	PINE	T28N R15W S29 at Spruce Park	8/11/1998	25
4030	Microsatellite/Indel	48.15914-16990 113.51077-5400 at Spruce Park	7/28/2009	39
4294	SNPs	Headwaters Junction to Cox Creek	8/21/2011	29
4466	SNPs	48.08675 113.22583 From Granite Creek to Dryad Creek	8/1&2/2012	28

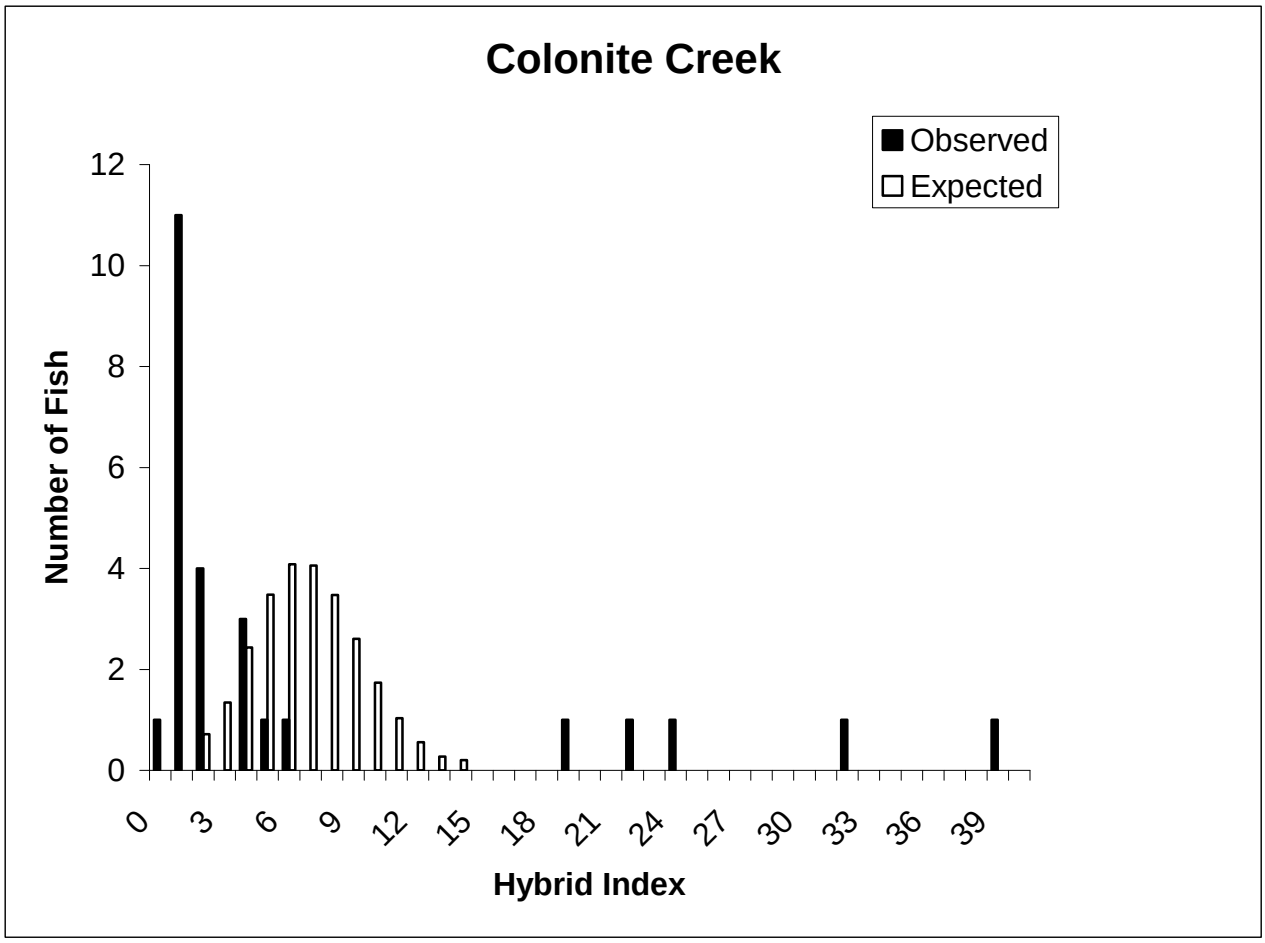

Figure 1. Observed and expected random distribution of hybrid indices in a sample showing evidence of hybridization between westslope cutthroat and rainbow trout in a sample from Colonite Creek. Note the observed distribution significantly differs ($P<0.001$) from the expected indicating the sample did not come from a hybrid swarm.

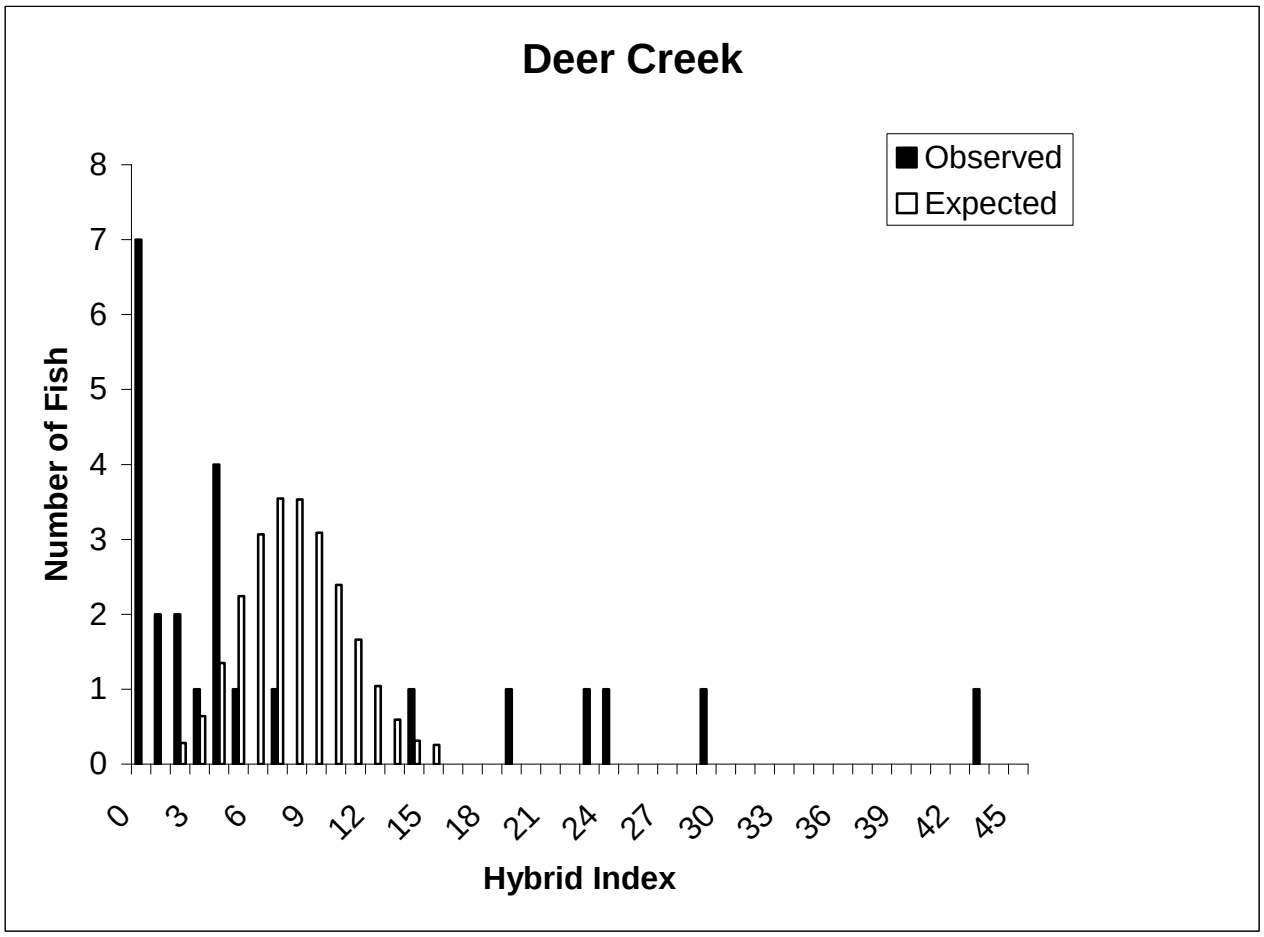

Figure 2. Observed and expected random distribution of hybrid indices in a sample showing evidence of hybridization between westslope cutthroat and rainbow trout in a sample from Deer Creek. Note the observed distribution significantly differs ($P<0.001$) from the expected indicating the sample did not come from a hybrid swarm.

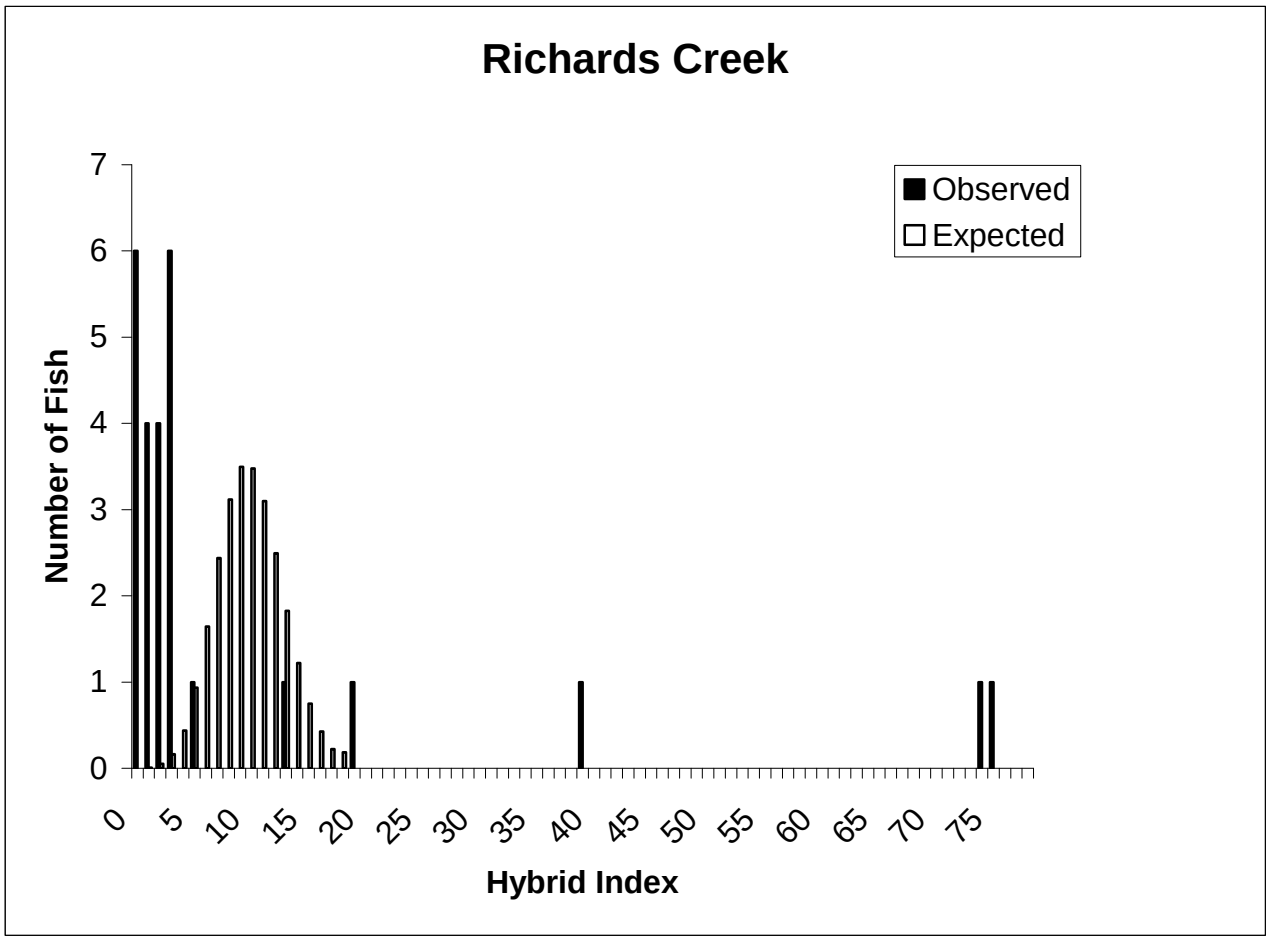

Figure 3. Observed and expected random distribution of hybrid indices in a sample showing evidence of hybridization between westslope cutthroat and rainbow trout in a sample from Richards Creek. Note the observed distribution significantly differs ($P<0.001$) from the expected indicating the sample did not come from a hybrid swarm.

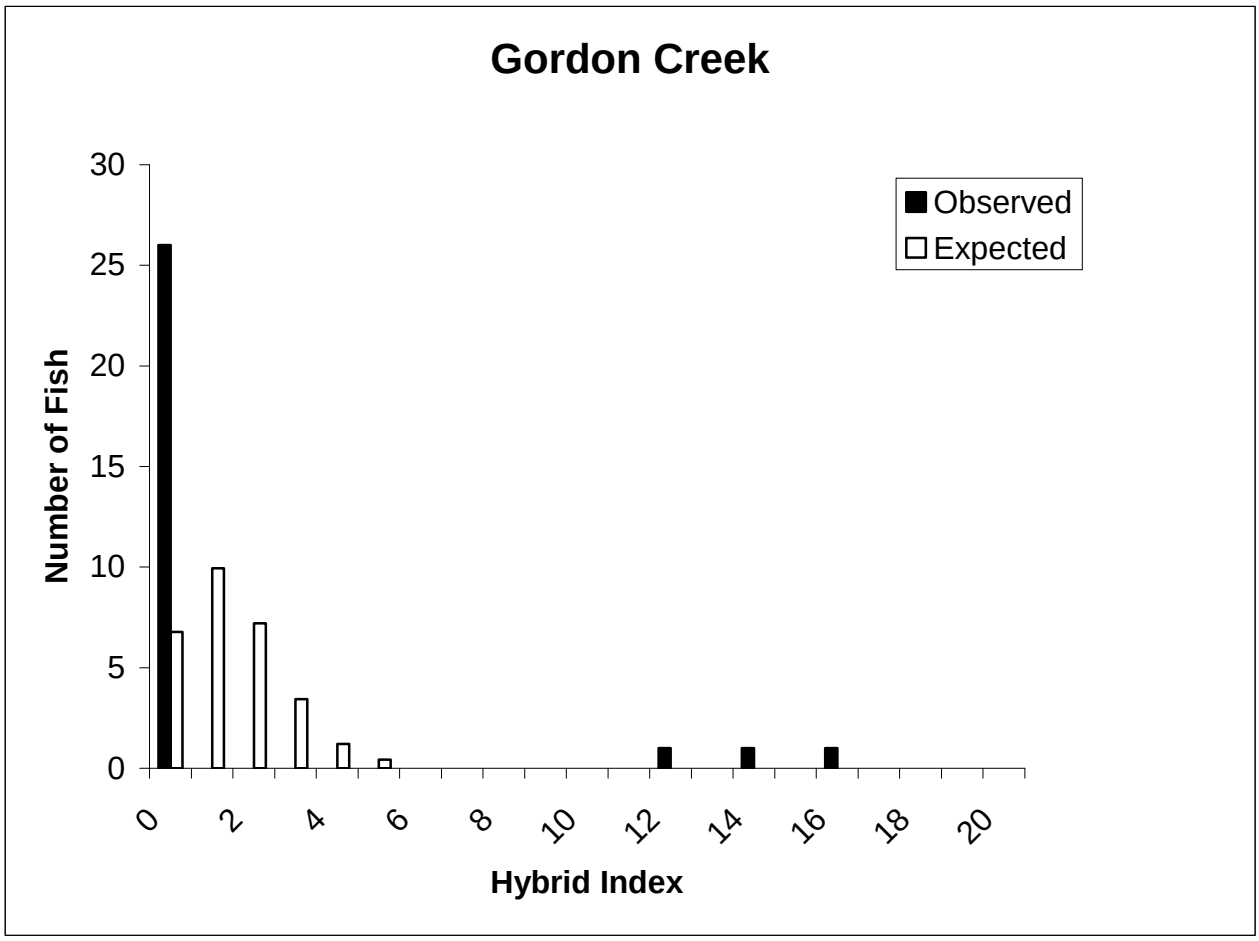

Figure 4. Observed and expected random distribution of hybrid indices in a sample showing evidence of hybridization between westslope and Yellowstone cutthroat trout in a sample from Gordon Creek. Note the observed distribution significantly differs ($P<0.001$) from the expected indicating the sample did not come from a hybrid swarm.

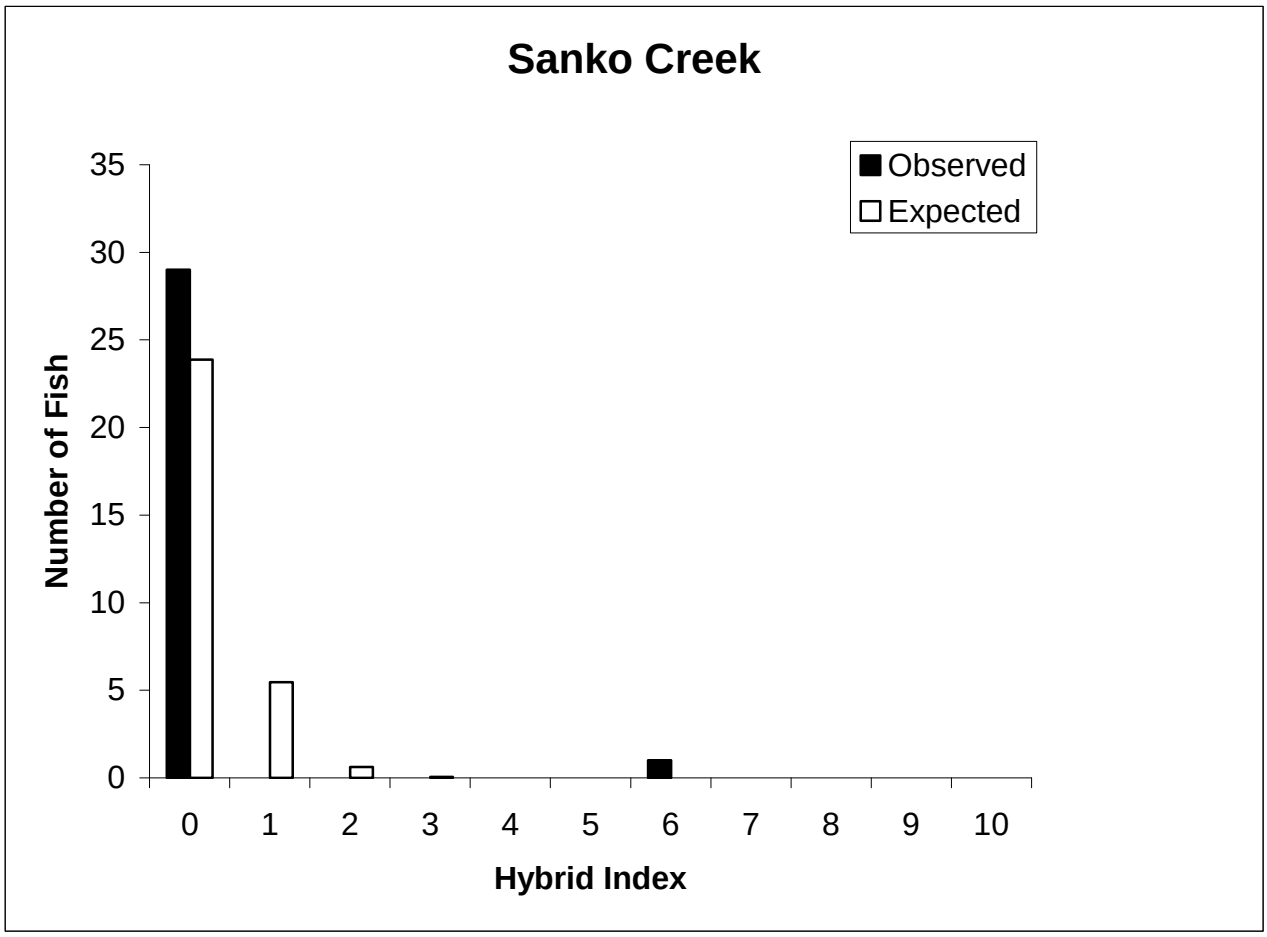

Figure 5. Observed and expected random distribution of hybrid indices in a sample showing evidence of hybridization between westslope cutthroat and rainbow trout in a sample from Sanko Creek. Note the observed distribution significantly differs ($P<0.001$) from the expected indicating the sample did not come from a hybrid swarm.

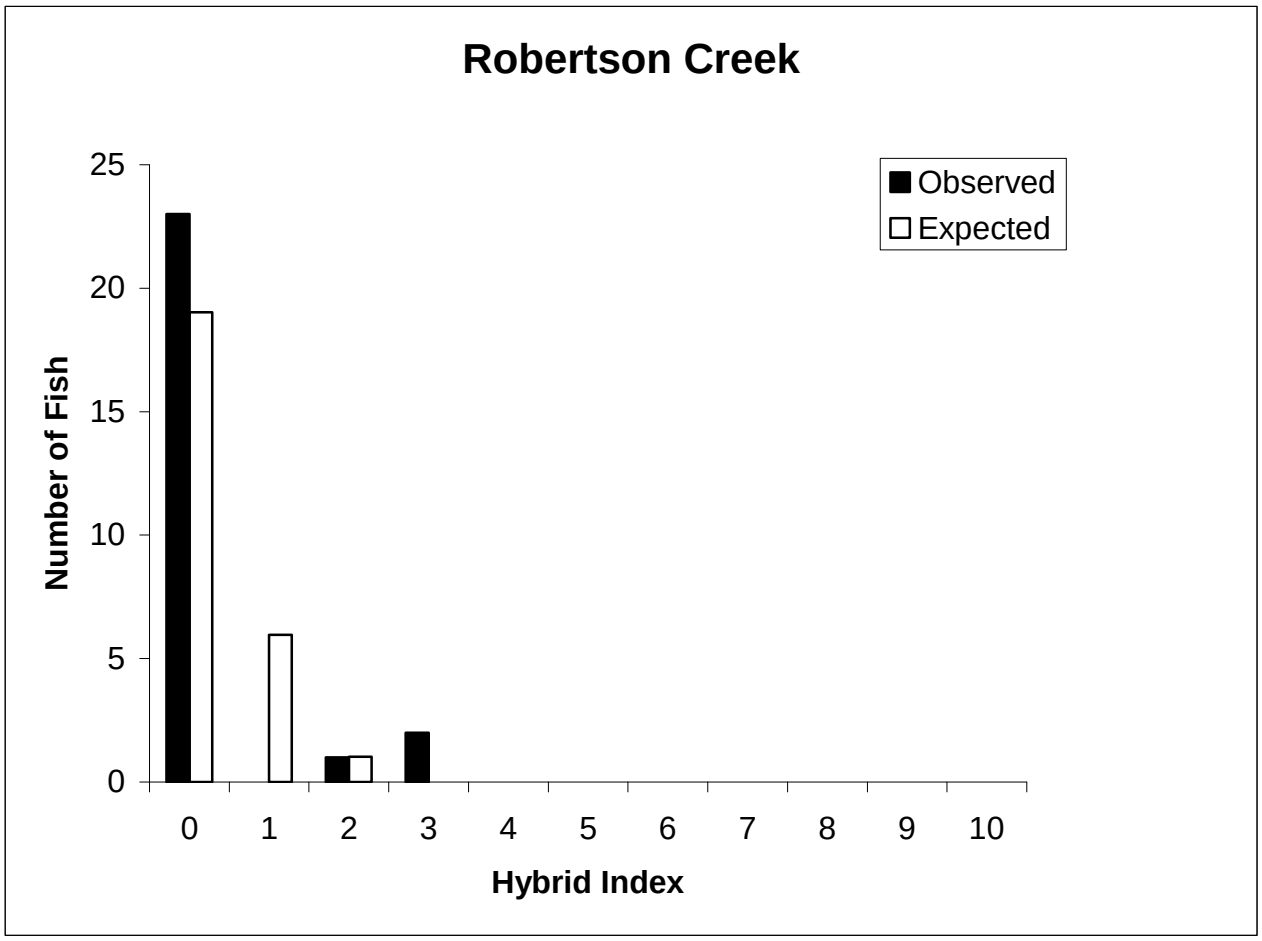

Figure 6. Observed and expected random distribution of hybrid indices in a sample showing evidence of hybridization between westslope cutthroat and rainbow trout in a sample from Robertson Creek. Note the observed distribution significantly differs ($P<0.01$) from the expected indicating the sample did not come from a hybrid swarm.

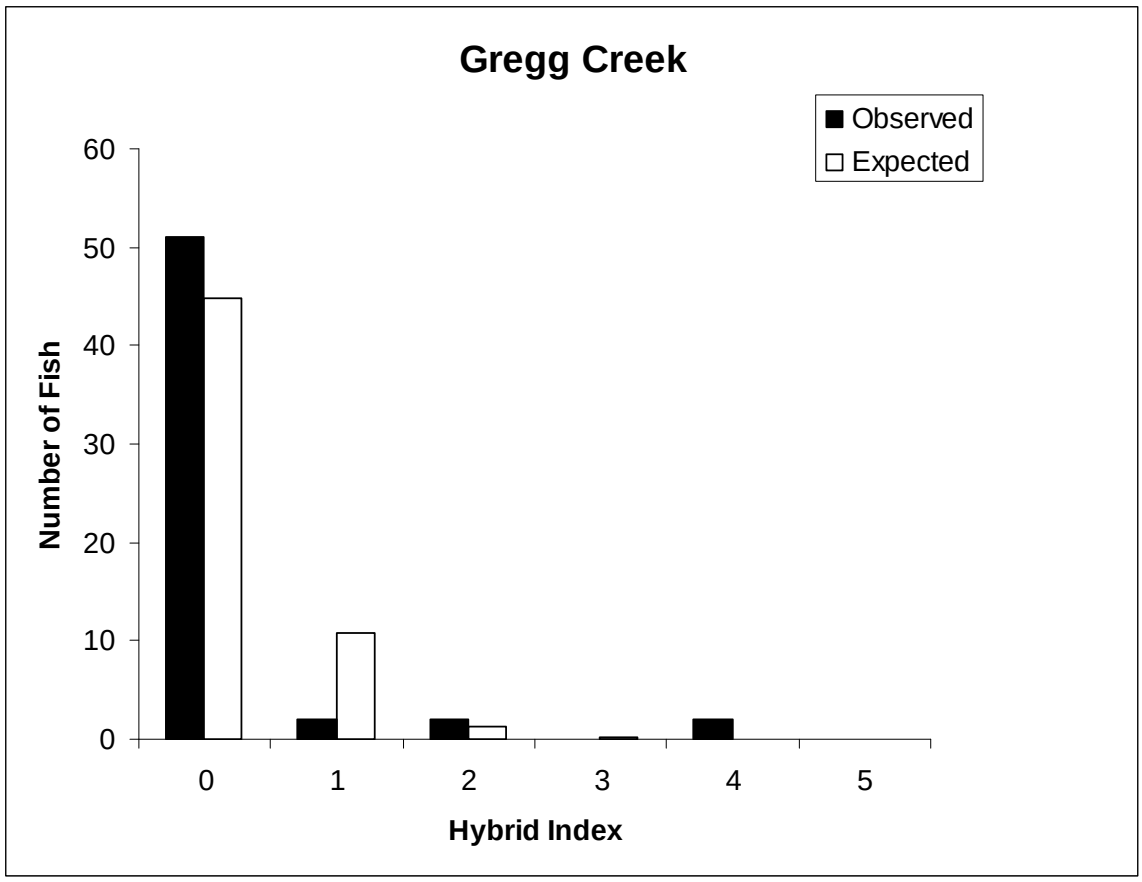

Figure 7. Observed and expected random distribution of hybrid indices in a sample showing evidence of hybridization between westslope and Yellowstone cutthroat trout in a sample from Gregg Creek. Note the observed distribution significantly differs ($P<0.01$) from the expected indicating the sample did not come from a hybrid swarm.

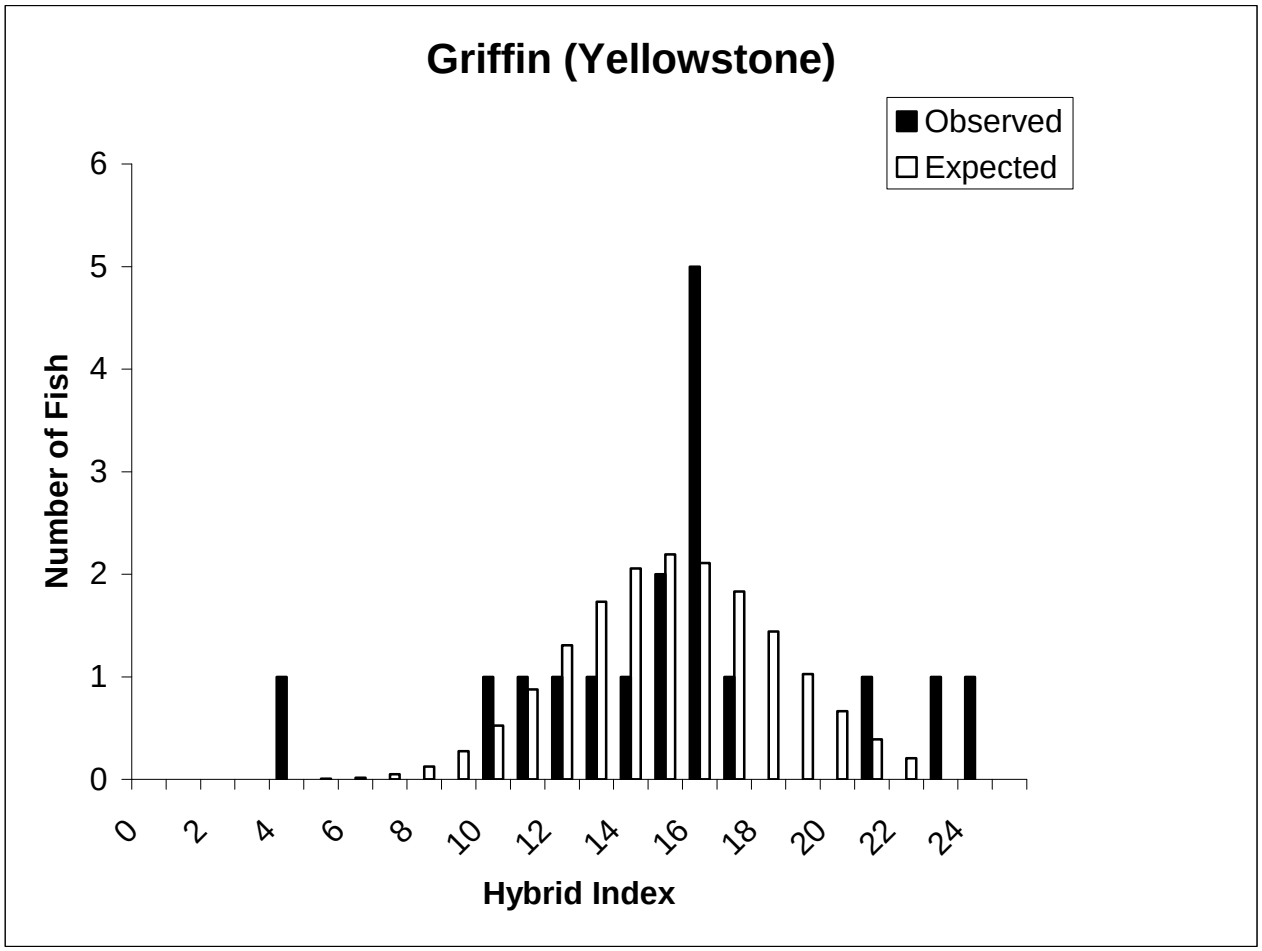

Figure 8. Observed and expected random distribution of hybrid indices in a sample showing evidence of hybridization between westslope and Yellowstone cutthroat trout in a sample from Griffin Creek. Note the observed distribution significantly differs ($P<0.001$) from the expected indicating the sample did not come from a hybrid swarm.