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January 26, 2013

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Jim;

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

		а	b	С	d	e	f
Sample #	Water Name/Location/ Collection Date/ Collector	N	#Markers	Taxa ID	Power	%	# Fish
4392	Lower Dunn 48.38303-38297 115.32122-31950 7/6/2011 Jim Dunnigan	17	R19W18Y20	IRT X CRT X WCT	Y97		
4393	Upper Dunn 48.38342-38268 115.31603-31652 7/6/2011 Jim Dunnigan	15	R19W18Y20	IRT X CRT X WCT	Y96		
4394	Fisher-Wolf 48.27415-31419 115.30469-30502 7/7 & 11/2011 Jim Dunnigan	28	R18W18Y20	IRT X CRT X WCT	Y99		
4395	Wolf 48.24611-23227 115.22243-27130 7/7&8/2011 Jim Dunnigan	31	R18W18Y20	IRT X CRT X WCT	Y99		

		а	b	С	d	е	f
Sample #	Water Name/Location/ Collection Date/ Collector	Ν	#Markers	Taxa ID	Power	%	# Fish
4396	Fisher-Silver Butte 48.02857-07372 115.35633-37695 7/7/2011 Jim Dunnigan	30	R17W18Y20	IRT X CRT X WCT	Y99		
4397	Libby Below Big Cherry 48.36412-37875 115.52733-53240 7/15/2011 Jim Dunnigan	30	R19W18Y20	IRT X CRT X WCT	Y99		
4398	Big Cherry 48.32720-35267 115.52835-52615 7/20/2011 Jim Dunnigan	29	R19W18Y20	IRT X CRT X WCT	Y99		
4399	Libby Near Highway 2 48.22450-22482 115.48008-47783 8/19/2011 Jim Dunnigan	30	R19W18Y20	IRT X CRT X WCT	Y99		
4400	Lower Flower 48.39215-39748 115.55980-56042 7/13/2011 Jim Dunnigan	14	R19W18Y20	IRT X CRT X WCT	Y93		
4401	Middle Flower 48.38585-38697 115.56425-56335 7/12/2011 Jim Dunnigan	15	R19W18Y20	IRT X CRT X WCT	Y96		
4402	Upper Flower 48.35660-35283 115.56723-57065 7/12/2011 Jim Dunnigan	11	R19W18Y20	IRT X CRT X WCT WCT	Y89		10 1

		а	b	С	d	е	f
Sample #	Water Name/Location/ Collection Date/ Collector	N	#Markers	Taxa ID	Power	%	# Fish
4403	Lower Parmenter 48.39723-39877 115.57548-57277 7/13/2011 Jim Dunnigan	9	R19W18Y20	IRT X CRT X WCT	Y84		
4404	Upper Parmenter 48.39188-39265 115.57998-57748 7/13/2011 Jim Dunnigan	19	R19W18Y20	IRT X CRT X WCT	Y98		
4405	Pipe 48.42838-42743 115.59490-59595 7/20/2011 Jim Dunnigan	28	R19W18Y20	IRT X CRT X WCT	Y99		
4406	Bobtail 48.46155-42757 115.59253-60370 7/13/2011 Jim Dunnigan	29	R18W17Y20	IRT X CRT X WCT	Y99		
4407	Cedar 48.42393-43045 115.63588-62900 7/13/2011 Jim Dunnigan	30	R18W17Y20	IRT X CRT X WCT	Y99		
4408	Lower Quartz 48.445657-44307 115.63055-63163 7/28/2011 Jim Dunnigan	14	R19W17Y20	IRT X CRT X WCT	Y93		
4409	Upper Quartz 48.44850-445657 115.63150-63055 8/12/2011 Jim Dunnigan	15	R19W17Y20	IRT X CRT X WCT	Y96		

		а	b	С	d	е	f
Sample #	Water Name/Location/ Collection Date/ Collector	Ν	#Markers	Taxa ID	Power	%	# Fish
4410	Lower Obrien 48.45945-45772 115.84952-85073 7/21/2011 Jim Dunnigan	15	R19W18Y20	IRT X CRT X WCT	Y96		
4411	Upper Obrien 48.48197-48113 115.84660-84682 7/21/2011 Jim Dunnigan	15	R19W18Y20	IRT X CRT X WCT	Y96		
4412	Lake Below Falls 48.44405-44997 115.87548-87927 7/22/2011 Jim Dunnigan	29	R18W17Y20	IRT X CRT X WCT WCT	Y99		28 1
4413	Lake Above Falls- Lower 48.39840 115.84603 8/30/2011 Jim Dunnigan	13	R19W18Y20	CRT X WCT CRT X WCT	Y93	C99.3 X W0.7	10 3
4414	Lake Above Falls- Upper 48.36805-37727 115.85652-86122 9/26/2011 Jim Dunnigan	5	R19W18Y20	IRT X CRT IRT X CRT X WCT WCT	Y63		1 2 2
4415	Callahan 48.45300-45565 115.89503-89292 8/19/2011 Jim Dunnigan	30	R17W18Y20	IRT X CRT	W99Y99		
4416	Star Below Falls 48.55848-55878 115.98412-98043 7/14/2011 Jim Dunnigan	11	R18W18Y20	IRT X CRT	W87Y99		

		а	b	С	d	е	f
Sample #	Water Name/Location/ Collection Date/ Collector	N	#Markers	Taxa ID	Power	%	# Fish
4417	Yaak Below Falls- Lower 48.56268-56188 115.96935-97123 7/14/2011 Jim Dunnigan	9	R17W18Y20	IRT X CRT	W96Y96		
4418	Yaak Below Falls- Upper 48.64418-64295 115.88570-88813 7/14/2011 Jim Dunnigan	19	R18W18Y20	IRT X CRT WCT	Y98	194.8 X C5.2	18 1
4419	Yaak Above Falls 48.81553-74190 115.86923-89033 7/18/2011 Jim Dunnigan	30	R19W18Y20	IRT X CRT X WCT	Y99		
4420	Kootenai-Libby Dam 48.40939-36608 115.31477-32416 9/6/2011 Jim Dunnigan	30	R18W18Y20	IRT X CRT X WCT	Y99		
4421	Kootenai-Flower to Pipe 48.40846-42161 115.57555-60605 9/7/2011 Jim Dunnigan	30	R19W18Y20	IRT X CRT X WCT	Y99		
4422	Kootenai-Troy 48.47009-49516 115.88801-91770 9/8/2011 Jim Dunnigan	30	R19W18Y20	IRT X CRT X WCT	Y99		

^aNumber of fish successfully analyzed. If combined with a previous sample, the number in parentheses indicates the combined sample size.

^bNumber of marker loci analyzed for rainbow *Oncorhynchus mykiss* (R), westslope cutthroat *O. clarkii lewisi* (W), and Yellowstone cutthroat trout *O. c. bouvieri* (Y).

^cTaxa: WCT = westslope cutthroat trout; IRT = redband rainbow trout *O. m. gairdneri*; CRT = coastal rainbow trout *O. m. irideus*; 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 evidence of hybridization because of sampling error (see *d*). Taxa separated by "x" indicate hybridization between them was detected.

^dPower: the number corresponds to the percent chance we have to detect 0.5% introgression in a hybrid swarm (a random mating population in which alleles at marker loci 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 marker loci used. For example, with 12 individuals we have better than a 99% chance to detect as little as a 0.5% westslope (38 marker loci) or Yellowstone cutthroat trout (39 marker loci) genetic contribution to a hybrid swarm that once was a non-hybridized redband rainbow trout population. Not reported when hybridization is detected. R = rainbow trout, W = westslope cutthroat trout, Y = Yellowstone cutthroat trout, I = redband rainbow trout, C = coastal rainbow trout.

^eIndicates the genetic contribution of the hybridizing taxa denoted as in d. This number is usually reported only if the sample appears to have come from a hybrid swarm.

^fIndicates 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 trout populations in the Kootenai River drainage. This chip allows us to simultaneously genotype up to 96 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 (in this report rainbow trout refers collectively to redband rainbow trout *Oncorhynchus mykiss gairdneri* and coastal rainbow trout *O. m. irideus*), westslope cutthroat (*O. clarkii lewisi*), and Yellowstone cutthroat trout (*O. c. bouvieri*) a single locus can, at best, distinguish only 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 (westslope markers), and 20 loci that distinguish Yellowstone cutthroat from westslope 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 rainbow trout at all rainbow markers and had no alleles characteristic of westslope cutthroat trout at the westslope markers or Yellowstone cutthroat trout at the Yellowstone markers, then it was considered to have come from a non-hybridized rainbow 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 rainbow and westslope cutthroat trout the data set contains information from 38 marker loci. Likewise, when evidence of hybridization was detected only between rainbow and Yellowstone cutthroat trout (no westslope cutthroat trout alleles at westslope markers, at least some rainbow markers polymorphic, and Yellowstone cutthroat trout alleles present at, at least, some Yellowstone markers) the data set contains information from 39 marker loci. When all three sets of markers were polymorphic, this generally indicates hybridization among all three taxa. In this situation, the westslope markers (19) provide information about westslope cutthroat 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 westslope cutthroat trout admixture using only the 19 westslope markers and the amount of Yellowstone cutthroat trout admixture using only the 20 Yellowstone markers. The amount of rainbow 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. 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 rainbow trout was given a value of zero and an allele characteristic of westslope or Yellowstone cutthroat trout a value of one. Thus, at a single marker locus the hybrid index for an individual could have a value of zero (only rainbow trout alleles present, homozygous), one (both rainbow and westslope or Yellowstone cutthroat trout alleles present, heterozygous), or two (only westslope or Yellowstone cutthroat trout alleles present, homozygous). These values summed over all marker loci analyzed yields an individual's hybrid index. Considering rainbow and westslope cutthroat trout, therefore, non-hybridized rainbow trout would have a hybrid index of zero, non-hybridized westslope cutthroat trout a hybrid index of 76, F_1 (first generation) hybrids a hybrid index of 38, and post F_1 hybrids could have values ranging from zero to 76. 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 marker loci. If the hybrid indices reasonably conformed to the expected random distribution, then the sample was considered to have come from a hybrid swarm.

The strongest evidence that a sample showing evidence of hybridization at the marker loci 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 genotypes at marker loci and the observed distribution of genotypes at marker loci and the observed distribution of sentences, the distribution of genotypes at marker loci and the observed 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 at the marker loci 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 another 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 nine loci that collectively based on allele frequency differences (distinguishing loci) can differentiate redband from coastal rainbow trout (Table 3). We verified this by analyzing samples

previously identified as being redband rainbow trout from Murray Springs State Trout Hatchery which are derived from fish collected above the falls in Callahan Creek (N=4) and the West Fork Yahk River (N=7), coastal rainbow trout from the Jocko River State Trout Hatchery (N=8), westslope cutthroat trout from the Washoe Park State Trout Hatchery (N=10), and Yellowstone cutthroat trout from the Yellowstone River State Trout Hatchery (N=5) and Slough Creek (N=5). We then used the data from the marker and distinguishing loci from these samples and the program STRUCTURE (Pritchard et al. 2000, 2007) to determine how well redband rainbow, coastal rainbow, westslope cutthroat, and Yellowstone cutthroat trout could be distinguished from each other. STRUCTURE does not consider an individual's sample of origin. In contrast, it allows one to vary the potential number of groups (K) from which individuals were collected so that the most likely number of groups can be ascertained. For the K groups it also estimates the proportion of each individual's genome (q) that was apparently derived from each group. In this analysis, we set K to four to correspond to the number of taxa.

The results indicated the four groups identified by STRUCTURE strongly corresponded to the four taxa. On the average, the redband rainbow trout had 98 (SD=0.06) percent of their genome attributed to their own group. Similarly, the coastal rainbow trout had an average of 96 (SD=0.06) percent of their genome assigned to their own group. The remainder was mainly assigned to the redband rainbow trout group. Finally, both cutthroat trout were identified as constituting distinct groups with well over 99 percent of each individual's genome being attributed to having originated from their respective group. Thus, we used STRUCTURE to examine whether or not the samples possessed evidence of hybridization between redband and coastal rainbow trout.

Samples were obtained from at least two locations in most tributaries to the Kootenai River and three locations in the river. We used the log likelihood G test of Goudet et al. (1996) available in GENEPOP version 4.0 (Rousset 2008) to test for allele frequency differences between samples collected from the same creek or river. Significance was determined using the Bonferroni correction for multiple tests (hence modified level) using the procedure proposed by Rice (1989). When no significant differences at the modified level existed between samples from the same stream or river, they were combined for further analysis. Conversely, when differences were detected between samples they were treated separately for analysis.

With the exception of the three samples collected from the Kootenai River, the samples were not a random sample of tributary and river reaches in the Kootenai River drainage in Montana below Libby Dam. Rather, professional judgment was used to target sections of stream and river reaches that were perceived to most likely be used by fluvial trout from the Kootenai River. This was done to attempt to resolve the uncertainty of whether fish collected from the Kootenai River constituted their own population(s) or originated from the tributaries. In order to address this issue, we proposed to use assignment analyses (e.g. Paetkau et al. 1995; Rannala and Mountain 1997) treating the Kootenai River samples as potentially being distinct from the tributaries as well as treating them as unknowns. The latter analysis would force all Kootenai River fish to be assigned to a tributary sample. If the Kootenai River fish tended to assign to their own sample with higher probabilities than when they were forced to assign to a tributary, then this would suggest the river contained its own population(s). In contrast, if most Kootenai River fish assigned to tributaries with higher probabilities than to their own sample, this would suggest the river mainly contained individuals originating from the tributaries.

An important assumption of assignment tests is that the samples mainly contained individuals from a single population. If this assumption is strongly violated, then meaningful interpretation of the results obtained from assignment tests is largely precluded. Thus, we examined whether or not this assumption appeared to be valid for our samples using three approaches. Samples containing individuals from two or more genetically divergent populations may contain a deficit of observed compared to expected heterozygotes based on random mating expectations (Hardy-Weinberg proportions). We used the Markov Chain method of Guo and

Thompson (1992) available in GENEPOP version 4.0 to test if observed genotypic proportions in the samples reasonably conformed to Hardy-Weinberg proportions. Since multiple tests were performed within a sample, significance was determined using the Bonferroni correction.

The Bonferroni correction is extremely conservative so we used two additional methods to assess the likelihood that a sample may contain individuals from two or more populations. In samples showing evidence of hybridization between rainbow and westslope cutthroat trout, a nonrandom distribution of westslope cutthroat trout alleles among individuals would suggest the sample may contain fish from populations with different amounts of admixture. Furthermore, we used the q values obtained from STRUCTURE to determine if the redband and coastal rainbow trout "alleles" were randomly distributed among the fish in the sample. In this analysis, q values were placed into bins corresponding to the presence of zero to eighteen coastal rainbow trout "alleles" in an individual. Thus, fish in the zero bin could potentially represent non-hybridized redband rainbow trout and those in the one bin potentially non-hybridized coastal rainbow trout. The distribution of binned q values in a sample was statistically compared to the expected binomial distribution based on the mean q for the sample. In samples containing a nonrandom distribution of westslope cutthroat trout alleles, those individuals with an unusually high proportion of westslope cutthroat trout alleles were eliminated from the latter analysis as their inclusion would reduce mean q when considering only redband and coastal rainbow trout potentially producing erroneous results.

The chip also contained 14 loci usually polymorphic in redband rainbow trout and 13 loci usually polymorphic in westslope cutthroat trout. With non-hybridized samples, data from these loci would allow an assessment of amounts of genetic variation within populations and divergence among populations.

Finally, the chip contained two mitochondrial DNA (mtDNA) loci that distinguish rainbow from cutthroat trout. Because mtDNA is maternally inherited, the data from these loci would allow a determination of whether an F_1 hybrid was produced from a rainbow or cutthroat trout female.

Results and Discussion

Yellowstone Markers

Among all the samples, no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers. Thus, these loci were invariant (monomorphic) for the same allele in all of the samples and with the exception of providing no evidence of hybridization with Yellowstone cutthroat trout they contained no information. These loci, therefore, were eliminated from the data set. Because of this, in the analysis of potential hybridization between redband and coastal rainbow trout K was set to three in STRUCTURE and the analysis was based only on the rainbow and westslope cutthroat trout markers and the distinguishing loci between redband and coastal rainbow trout.

Apparent Westslope Cutthroat Trout Polymorphism

In many samples, the westslope marker *OclWD_114315L_Garza* possessed the allele usually characteristic of rainbow trout at a frequency substantially higher than that observed at the other westslope markers. This suggests that this locus is usually polymorphic in westslope cutthroat trout in the drainage and it was not considered to be a marker in subsequent analyses.

Dunn Creek

Samples were collected from two reaches of Dunn Creek. Between the samples, evidence of genetic variation was detected at 73 loci and the allele frequencies significantly differed between them at 21 of these

loci. These differences remained significant at the modified level. The samples, therefore, were treated separately for further analysis.

Lower Dunn Creek Below Highway 37 Culvert 4392

In the sample from lower Dunn Creek, alleles characteristic of westslope cutthroat trout were detected at ten of the rainbow markers and at 12 of the westslope markers. The westslope cutthroat trout alleles were not randomly (X^2_5 =35.114, P<0.001) distributed among the fish in the sample. In contrast, two individuals possessed a substantially higher amount of admixture than the others (Figure 1A). When these two individuals were eliminated from the data, the westslope cutthroat trout alleles appeared to be randomly (X^2_2 =0.750, P>0.50) distributed among the remaining fish. These latter fish, therefore, appeared to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a predominant (0.993) rainbow trout genetic component. STRUCTURE indicated that these remaining fish predominantly possessed a coastal rainbow trout genetic contribution. A couple of individuals, however, appeared to also contain a substantial redband rainbow trout contribution (Figure 1B) resulting in a nonrandom (X^2_2 =13.720, P<0.01) distribution of q values. Considering all the data, with the exception of four fish this sample appears to have mainly contained a coastal rainbow trout genetic contribution as well as a relatively small redband rainbow and westslope cutthroat trout of some four fish this sample appears to have mainly contained a coastal rainbow trout contribution.

In the sample, evidence of genetic variation at a level allowing a meaningful test for deviations of observed genotypic proportions from expected Hardy-Weinberg proportions existed at 35 loci. At three of these loci, there were significantly less heterozygotes than expected. These differences, however, were not significant at the modified level suggesting they most likely represented chance departures from homogeneity rather than actual deviations from expected random mating proportions. Thus, the only evidence that the sample may have contained individuals from more than one population is the nonrandom distribution of westslope cutthroat alleles and q values among the fish.

Upper Dunn Creek Above Highway 37 Culvert 4393

Alleles characteristic of westslope cutthroat trout were detected at all the rainbow and westslope markers in the sample from upper Dunn Creek. The westslope cutthroat trout alleles, however, were not randomly $(X_{10}^2=61.024, P<0.001)$ distributed among the fish in sample. Rather, the hybrid indices tended to divide the fish into two groups: those with a small amount of admixture and those with a substantial and highly variable amount of admixture (Figure 2). Regardless of whether the latter fish were included or excluded from the data, STRUCTURE did not allow us to investigate the relative contribution of redband and coastal rainbow trout to the sample. It recognized redband and coastal rainbow trout as constituting a single group and the upper Dunn Creek fish as another.

Evidence of genetic variation at a level allowing a meaningful test for deviations of observed genotypic proportions from expected Hardy-Weinberg proportions existed at 67 loci in the sample. At three of these loci, there were significantly less heterozygotes than expected. These differences, however, were not significant at the modified level. Thus, they most likely represented chance departures from homogeneity rather than actual deviations from expected random mating proportions. The highly nonrandom and variable distribution of hybrid indices, however, suggests the sample very likely contained individuals from more than one population and that it definitely did not come from a hybrid swarm.

Fisher River

Samples were collected from the Fisher River below Wolf Creek and between West Fisher Creek and Silver Butte Creek. Between the samples, evidence of genetic variation was detected at 56 loci. At six of these loci

the allele frequencies significantly differed between the samples and these differences remained significant at the modified level. These samples, therefore, were treated separately for further analysis.

Fisher River Below Wolf Creek 4394

Samples were collected from two reaches of the Fisher River below Wolf Creek. The allele frequencies significantly differed between the samples at two of the 40 polymorphic loci. At the modified level, however, these differences were not significant suggesting they more likely represent chance departures from homogeneity rather than evidence of genetic differences. Since there was no conclusive evidence of genetic differences between the samples, they were combined for subsequent analysis.

The rainbow marker *OmyRD_RAD_59515_Hoh* had the allele characteristic of westslope cutthroat trout at a frequency substantially higher than observed at the other rainbow markers. This suggests that this locus was polymorphic in the rainbow trout contributing to this sample and it was not considered to be a marker in the following analyses.

In the sample, alleles characteristic of westslope cutthroat trout were detected at seven of the rainbow markers and three of the westslope markers. The westslope cutthroat trout alleles appeared to be randomly $(X_2^2=2.528, P>0.10)$ distributed among the fish in the sample suggesting it came from a hybrid swarm between rainbow and westslope cutthroat trout with a minor (0.006) westslope cutthroat trout genetic component. In contrast to the hybrid indices, q values obtained from STRUCTURE did not appear to be randomly $(X_7^2=264.417, P<0.001)$ distributed among the individuals. Some individuals had a high redband rainbow trout genetic contribution and others a high coastal rainbow trout genetic contribution (Figure 3). The remaining individuals had a highly variable redband and coastal rainbow trout contribution ranging from predominantly redband to predominantly coastal rainbow trout genetic contribution and a small westslope cutthroat trout component. At the individual level, the former two components were highly variable among the fish indicating the sample did not come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout.

There is good evidence that this sample did not contain individuals from a single population. Among 32 comparisons to Hardy-Weinberg proportions three were statistically significant. These remained significant at the modified level and all involved a deficit of heterozygotes. Furthermore, the q values were highly variable among the fish ranging from essentially redband rainbow to essentially coastal rainbow trout.

Wolf Creek 4395

Wolf Creek was sampled in two locations. None of the allele frequencies significantly differed between the samples at the 66 polymorphic loci. Since there was no conclusive evidence of genetic differences between the samples, they were combined for further analysis.

Like the sample from the Fisher River below Wolf Creek, the rainbow marker *OmyRD_RAD_59515_Hoh* had the allele characteristic of westslope cutthroat trout at a frequency substantially higher than observed at the other rainbow markers. This lends further support to the interpretation that this locus was polymorphic in the rainbow trout contribution to the fish in this area. Thus, this locus was not considered to be a marker in the following analyses.

Alleles characteristic of westslope cutthroat trout were detected at 16 of the rainbow markers and 16 of the westslope markers in the sample. The westslope cutthroat trout alleles did not appear to be randomly $(X_4^2=36.553, P<0.001)$ distributed among the fish in the sample. This appeared to mainly be due to the presence of one fish in the sample with a hybrid index of 27 (Figure 4A). When this fish was removed from

the sample, the westslope cutthroat trout alleles appeared to be randomly ($X_2^2=0.2.942$, P>0.10) distributed among the remaining fish. These latter fish, therefore, appeared to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a predominant (0.992) rainbow trout genetic component. The q values obtained from STRUCTURE indicated they did not appear to be randomly ($X_7^2=230.131$, P<0.001) distributed among these remaining individuals. They were highly variable ranging from predominantly redband rainbow to coastal rainbow trout with many other fish having a substantial amount of admixture (Figure 4B). On the average, therefore, the fish in this sample had a substantial redband and coastal rainbow trout genetic contribution and with the exception of one fish a small westslope cutthroat trout component. At the individual level, the former two components were highly variable among the fish indicating the sample did not come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout.

This sample certainly did not appear to contain individuals from a single population. Among 40 comparisons to Hardy-Weinberg proportions three were statistically significant. These remained significant at the modified level and all involved a deficit of heterozygotes. This situation also pertained to the 35 comparisons performed when the individual with a substantial amount of admixture between rainbow and westslope cuthroat trout was removed from the data. Furthermore, the hybrid indices and q values among the fish did not conform to random binomial expectations.

Fisher River between West Fisher Creek and Silver Butte Creek 4396

Samples were collected from two reaches of the Fisher River between West Fisher Creek and Silver Butte Creek. Between the samples, 55 loci were polymorphic. None of the allele frequencies significantly differed between the samples at any of these loci. Since there was no compelling evidence of genetic differences between the samples, they were combined for subsequent analysis.

Like the samples from the Fisher River below Wolf Creek and Wolf Creek, the rainbow marker *OmyRD_RAD_59515_Hoh* had the allele characteristic of westslope cutthroat trout at a frequency substantially higher than observed at all but one of the other rainbow markers. The rainbow marker *OmyRD_RAD_30378_Hoh* also possessed the allele usually characteristic of westslope cutthroat trout at an unusually high frequency. Again, we interpreted these anomalies to indicate that these loci were polymorphic in the rainbow trout contribution to the fish in this area. Thus, these loci were not considered to be markers in subsequent analyses.

In the sample, alleles characteristic of westslope cutthroat trout were detected at 13 of the rainbow markers and 11 of the westslope markers. The westslope cutthroat trout alleles did not appear to be randomly $(X^2_4=13.836, P<0.05)$ distributed among the fish in the sample. This was due solely to one fish with a hybrid index of 17 (Figure 5A). When this fish was eliminated from the data, the westslope cutthroat trout alleles appeared to be randomly (X^2_2 =1.694, P>0.10) distributed among the remaining individuals. These latter fish, therefore, appeared to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a predominant (0.992) rainbow trout genetic component. When the fish with a substantial amount of admixture between rainbow and westslope cutthroat trout was eliminated from the sample, the q values obtained from STRUCTURE did not appear to be randomly ($X_{4}^{2}=75.313$, P<0.001) distributed among the remaining individuals. In contrast, significantly more fish possessed an apparently high redband rainbow trout contribution or were substantially admixed between redband and coastal rainbow trout than expected by chance (Figure 5B). On the average, therefore, the fish in this sample had a predominant redband rainbow trout genetic contribution, a small coastal rainbow trout contribution, and with the exception of one fish a minor westslope cutthroat trout component. At the individual level, the components were highly variable among the fish indicating the sample did not come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout.

There is good evidence that this sample did not contain individuals from a single population. Considering all the fish, 35 comparisons to Hardy-Weinberg proportions were performed and four of these were statistically significant. These remained significant at the modified level and all involved a deficit of heterozygotes. When the highly admixed fish between rainbow and westslope cutthroat trout was eliminated from the data, five of the 32 comparisons to Hardy-Weinberg proportions were significant. Again these differences remained significant at the modified level and all involved a deficit of heterozygotes. Furthermore, in the sample there was the one individual with a comparatively high amount of admixture between rainbow and westslope cutthroat trout and without this fish the q values were not randomly distributed among the other individuals.

Libby Creek

Samples were collected from Libby Creek below Big Cherry Creek and near Highway 2. Between the samples, evidence of genetic variation was detected at 47 loci. At nine of these loci the allele frequencies significantly differed between the samples and these differences remained significant at the modified level. These samples, therefore, were treated separately for further analysis.

Libby Creek Below Big Cherry Creek 4397

Libby Creek below Big Cherry Creek was sampled in two locations. None of the allele frequencies significantly differed between the samples at the 37 polymorphic loci. Since there was no conclusive evidence of genetic differences between the samples, they were combined for subsequent analysis.

In the sample, alleles characteristic of westslope cutthroat trout were detected at five of the rainbow markers and five of the westslope markers. The westslope cutthroat trout alleles appeared to be randomly (X^2_2 =4.072, P>0.10) distributed among the fish in the sample suggesting it came from a hybrid swarm between rainbow and westslope cutthroat trout with a minor (0.008) westslope cutthroat trout genetic component. In contrast to the hybrid indices, q values obtained from STRUCTURE did not appear to be randomly (X^2_5 =84.127, P<0.001) distributed among the individuals. They were highly variable ranging from predominantly coastal rainbow trout to highly admixed fish (Figure 6). On the average, therefore, the fish in this sample had a predominant coastal rainbow trout genetic contribution, a moderate redband rainbow trout contribution, and a minor westslope cutthroat trout component. At the individual level, the former two components were highly variable among the fish indicating the sample did not come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout.

There is some indication that this sample may have contained individuals from more than one population. We performed 32 comparisons to expected Hardy-Weinberg proportions in the sample. Although two of these comparisons indicated a statistically significant deficit of heterozygotes, these differences were not significant at the modified level. Thus, they most likely represented chance departures from homogeneity due to the number of comparisons performed. The q values, however, clearly indicated a nonrandom distribution for the contribution of redband and coastal rainbow trout to the fish in the sample.

Big Cherry Creek 4398

Samples were collected from two reaches of Big Cherry Creek. Between the samples, 37 loci were polymorphic. At two of these loci, there was a significant allele frequency difference between the samples. At the modified level, however, these differences were not significant indicating that they most likely represented chance departures from homogeneity rather than evidence of genetic differences between the samples. Since there was no compelling evidence of genetic differences between the samples, they were combined for further analysis.

Alleles characteristic of westslope cutthroat trout were detected at six of the rainbow markers and three of the westslope markers in the sample. The westslope cutthroat trout alleles appeared to be randomly (X^2_3 =2.562, P>0.10) distributed among the fish in the sample. Thus, this sample appeared to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a predominant (0.990) rainbow trout genetic component. In contrast, the q values obtained from STRUCTURE did not appear to be randomly (X^2_6 =81.157, P<0.001) distributed among the individuals. Most of the fish in the sample appeared to have a predominant coastal rainbow trout contribution but, a couple of individuals were substantially admixed with a major redband rainbow trout genetic component (Figure 7). On the average, therefore, the fish in this sample had a predominant coastal rainbow trout genetic contribution, a moderate redband rainbow trout contribution, and a minor westslope cutthroat trout component. At the individual level, the former two components were highly variable among the fish indicating the sample did not come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout.

The only indication that this sample may have contained individuals from more than one population comes from the nonrandom distribution of q values. We performed 33 comparisons to expected Hardy-Weinberg proportions in the sample and only one had a statistically significant deficit of heterozygotes. This difference, however, was not significant at the modified level suggesting it most likely represented a chance departure from homogeneity due to the number of comparisons performed. The q values, however, clearly indicated two individuals had a substantially higher redband rainbow trout contribution than the others.

Libby Creek Near Highway 2 4399

There was good evidence that the sample from Libby Creek collected near Highway 2 contained hybrids among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at seven of the rainbow markers and five of the westslope markers. The westslope cutthroat trout alleles appeared to be randomly (X^2_2 =2.260, P>0.10) distributed among the fish in the sample. This sample, therefore, appeared to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a predominant (0.992) rainbow trout genetic component. In contrast to the hybrid indices, q values obtained from STRUCTURE did not appear to be randomly (X^2_6 =81.157, P<0.001) distributed among the individuals. There was a broad range of values extending from mainly redband rainbow trout to highly admixed (Figure 8). On the average, therefore, the fish in this sample had a predominant redband rainbow trout genetic contribution, a substantial coastal rainbow trout contribution, and a minor westslope cutthroat trout component. At the individual level, the former two components were highly variable among the fish indicating the sample did not come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout.

A reason the sample did not appear to have come from a hybrid swarm is that it appeared to contain individuals from more than one population. Among the 32 comparisons for deviations from expected Hardy-Weinberg proportions, two loci contained a statistically significant deficit of heterozygotes. These differences were significant at the modified level. Furthermore, the q values were not randomly distributed among the fish in the sample indicating at the individual level a highly variable amount of admixture between redband and coastal rainbow trout.

Flower Creek

Samples were collected from three reaches of Flower Creek. Among the samples, evidence of genetic variation was detected at 73 loci. At 67 of these loci, the allele frequencies significantly differed among the samples and these differences remained significant at the modified level. These samples, therefore, were treated separately for further analysis.

Lower Flower Creek 4400

The sample from lower Flower Creek definitely contained evidence of hybridization among redband rainbow, coastal rainbow, and westslope cutthroat trout. Alleles characteristic of westslope cutthroat trout were detected at all of the rainbow markers and all of the westslope markers analyzed in the sample. The westslope cutthroat trout alleles, however, did not appear to be randomly (X^2_{10} =153.781, P<0.001) distributed among the fish in the sample. In contrast, the hybrid indices were highly variable among the fish ranging from little if any hybridization between rainbow and westslope cutthroat trout to a substantial amount of admixture between these fishes (Figure 9A). When the individuals with a hybrid index of nine or more were removed from the data, q values obtained from STRUCTURE also did not appear to be randomly (X^2_7 =38.795, P<0.001) distributed among the remaining individuals. Rather, they indicated a highly variable amount of admixture between redband and coastal rainbow trout in these fish (Figure 9B). Thus, the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout. Based on the distribution of hybrid indices and q values at the individual level, however, it is unlikely that any individual in the sample was non-hybridized. Overall, the fish in the sample had a substantial redband rainbow, coastal rainbow, and westslope cutthroat trout genetic contribution.

A reason the sample did not appear to have come from a hybrid swarm is that it may have contained individuals from more than one population. When all individuals in the sample were considered, there were 66 comparisons for deviations from expected Hardy-Weinberg proportions. At three of these loci, there was a statistically significant deficit of heterozygotes but, these differences were not significant at the modified level. Furthermore, when the fish with a hybrid index of nine or more were eliminated from the data, there were no significant deviations from expected Hardy-Weinberg proportions at the 23 polymorphic loci among the remaining individuals. The nonrandom distribution of westslope cutthroat trout alleles and of q values among the fish, however, suggests that at the individual level the amount of hybridization was extremely variable and that the fish very likely did not represent a single population.

Middle Flower Creek Near Baseball Fields 4401

There was substantial evidence that the sample from middle Flower Creek collected near the baseball fields contained hybrids among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at all of the rainbow markers and all of the westslope markers that were analyzed. The westslope cutthroat trout alleles, however, did not appear to be randomly ($X_{12}^2=325.930$, P<0.001) distributed among the fish in the sample. There was an excess of individuals with a hybrid index of zero and three had a comparatively high proportion of westslope cutthroat trout alleles (Figure 10A). When the latter three fish were eliminated from the sample, q values obtained from STRUCTURE also did not appear to be randomly (X_7^2 =19.748, P<0.01) distributed among the remaining individuals. Rather, there was a very broad range among the values suggesting at the individual level there was a highly variable amount of admixture between redband and coastal rainbow trout (Figure 10B). Thus, the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout. Based on the hybrid indices and distribution of q values, however, it is unlikely that any individual in the sample was non-hybridized. Overall, the fish in the sample had a substantial redband rainbow, coastal rainbow, and westslope cutthroat trout contribution but, the extent of admixture among these fishes was highly variable among individuals.

A likely reason the sample did not appear to have come from a hybrid swarm is that it almost certainly contained individuals from more than one population. When all individuals in the sample were considered, there were 72 comparisons for deviations from expected Hardy-Weinberg proportions. At 16 of these loci, there was a statistically significant deficit of heterozygotes and these differences were significant at the modified level. When the three fish with a relatively high westslope cutthroat trout genetic contribution were eliminated from the data, only 31 loci remained polymorphic. At one of these loci, however, there was a

significant deficit of heterozygotes. This difference remained significant at the modified level. Besides there being a deficit of heterozygotes at some loci, the nonrandom distribution of westslope cutthroat trout alleles and q values among these fish also suggests they did not represent a single population.

Upper Flower Creek Between the Reservoir and Lower Dam 4402

Compared to the majority of the other samples, the sample from upper Flower Creek contained an unusually high proportion of westslope cutthroat trout alleles. They were detected at all of the rainbow and westslope cutthroat trout markers analyzed. Furthermore, all individuals except one were definitely hybrids between rainbow and westslope cutthroat trout with a predominant westslope cutthroat trout genetic contribution (Figure 11A). The exception was an individual that may have been a non-hybridized westslope cutthroat trout alleles did not appear to be randomly (X_{12}^2 =119.289, P<0.001) distributed among the individuals in the sample.

Only two of the 54 comparisons in the sample to expected Hardy-Weinberg proportions were statistically significant. Both of these deviations involved a deficit of heterozygotes but, the differences were not significant at the modified level. Thus, the only indication the sample did not come from a single population was the nonrandom distribution of westslope cutthroat trout alleles among the fish in the sample.

Because of the high proportion of westslope cutthroat trout alleles in the sample, meaningful statistical analysis of q values obtained from structure was precluded. The results, however, indicated that both redband and coastal rainbow trout had a genetic contribution to the fish in the sample (Figure 11B). Overall, therefore, this sample mainly appeared to contain hybrids among westslope cutthroat, redband rainbow, and coastal rainbow trout with a predominant westslope cutthroat trout genetic contribution and one non-hybridized westslope cutthroat trout. The fish, however, clearly did not appear to constitute a hybrid swarm among these fishes.

Parmenter Creek

Samples were collected from Parmenter Creek around the Highway 2 bridge and above the bridge on Dome Mountain Road. Between the samples, evidence of genetic variation was detected at 52 loci. At three of these loci, the allele frequencies significantly differed between the samples and these differences remained significant at the modified level. These samples, therefore, were treated separately for further analysis.

Lower Parmenter Creek Around Highway 2 Bridge 4403

This sample appeared to contain hybrids among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at nine of the rainbow markers and three of the westslope markers. The westslope cutthroat trout alleles appeared to be randomly $(X^2_4=2.881, P>0.50)$ distributed among the fish in the sample indicating all of them contained on the average a small (0.024) westslope cutthroat trout genetic contribution. In contrast to the westslope cutthroat trout alleles, the q values obtained from STRUCTURE did not appear to be randomly $(X^2_7=17.107, P<0.05)$ distributed among the individuals. Rather, there was a broad range of values suggesting at the individual level a highly variable but substantial amount of admixture between redband and coastal rainbow trout (Figure 12) among the fish. Overall, therefore, the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout but, all fish appeared to be hybrids among these taxa with a substantial redband and coastal rainbow trout genetic contribution and a small westslope cutthroat trout component.

The only suggestion that this sample may have contained individuals from more than one population was the nonrandom distribution of q values. The westslope cutthroat trout alleles appeared to be randomly distributed

among the fish in the sample and none of the 31 comparisons of observed to expected Hardy-Weinberg genotypic proportions were statistically significant.

Upper Parmenter Creek Above Dome Mountain Road Bridge 4404

The sample from upper Parmenter Creek collected above the bridge on Dome Mountain Road appeared to contain hybrids among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at ten of the rainbow markers and ten of the westslope markers. The westslope cutthroat trout alleles did not appear to be randomly ($X_6^2=19.831$, P<0.01) distributed among the fish in the sample mainly because a few individuals had a relatively high westslope cutthroat trout genetic contribution (Figure 13A). When the individuals with a hybrid index of five or more were eliminated from the data, the westslope cutthroat alleles appeared to be randomly ($X_{3}^{2}=1.434$, P>0.10) distributed among the remaining fish. These latter fish, therefore, appeared to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a predominant (0.984) rainbow trout genetic component. The q values obtained from STRUCTURE excluding the individuals with a hybrid index of five or more also did not appear to be randomly (X_4^2 =14.446, P<0.01) distributed among the remaining fish. This was mainly due to an excess of fish with a high coastal rainbow trout genetic contribution and one individual with a substantial amount of admixture between redband and coastal rainbow trout (Figure 13B). Thus, the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout. Overall, essentially all of the fish in the sample appeared to be hybrids that on the average mainly contained a coastal rainbow trout genetic component and a relatively small redband rainbow and westslope cutthroat trout genetic contribution.

A likely reason the sample did not appear to have come from a hybrid swarm is that it almost certainly contained individuals from more than one population. When all individuals in the sample were considered, there were 35 comparisons of observed to expected Hardy-Weinberg genotypic proportions. There was a statistically significant deviation between these distributions at four of these loci. These deviations were significant at the modified level and all involved a deficit of heterozygotes. Similar results were obtained when the fish with a hybrid index of five or more were removed from the data. At four of 34 loci, there was a statistically significant difference between observed and expected Hardy-Weinberg genotypic distributions. These were the same four loci that showed significant deviations from expected random mating proportions in the former analyses. Again these differences were significant at the modified level and all involved a deficit of heterozygotes. The nonrandom distribution of westslope cutthroat trout alleles and q values among the fish in the sample further suggest it did not contain individuals from a single population.

Pipe Creek 4405

Samples were collected from Pipe Creek above and below the river road bridge. Between the samples, 60 loci were polymorphic. There were no statistically significant allele frequency differences between the samples at any of these loci. Since there was no evidence of genetic differences between the samples, they were combined for further analysis.

This sample appeared to contain hybrids among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at 15 of the rainbow markers and 15 of the westslope markers. The westslope cutthroat trout alleles did not appear to be randomly $(X_{5}^{2}=34.558, P<0.001)$ distributed among the fish in the sample. This appeared to mainly be due to the presence of two fish, one with a hybrid index of 16 and the other with a hybrid index of 17 (Figure 14A). When these fish were removed from the sample, the westslope cutthroat trout alleles appeared to be randomly $(X_{2}^{2}=1.361, P>0.10)$ distributed among the remaining individuals suggesting they came from a hybrid swarm between rainbow and westslope cutthroat trout with a small (0.009) westslope cutthroat trout genetic contribution. When the two individuals with an unusually high amount of admixture with westslope cutthroat

trout were eliminated from the data, the q values obtained from STRUCTURE did not appear to be randomly (X^2_5 =53.896, P<0.001) distributed among the remaining fish in the sample. Rather, there was an extremely broad range of values with one fish having a predominant redband rainbow trout genetic contribution but, most fish appeared have a predominant coastal rainbow trout genetic component (Figure 14B). Overall, therefore, the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout but, all fish appeared to be hybrids. On the average, the hybrids had a substantial coastal rainbow trout genetic contribution and a relatively small redband rainbow and westslope cutthroat trout but, a few exceptions to this situation.

The only indications that this sample may have contained individuals from more than one population were the nonrandom distribution of westslope cutthroat trout alleles and of q values among the fish. When all individuals were considered, there were 40 comparisons of observed to expected Hardy-Weinberg genotypic proportions. Although six of these comparisons were statistically significant, they were not at the modified level. Thus, these apparent deviations most likely represented chance departures from homogeneity due to the number of comparisons performed. This situation also pertained when the two individuals with a substantial westslope cutthroat trout genetic component were removed from the data. In this situation, there were 29 comparisons with three being significant but, not at the modified level.

Bobtail Creek 4406

Samples were collected from Bobtail Creek near the river road bridge and above the upper bridge. Between the samples, 43 loci were polymorphic. At four of these loci, there was a significant allele frequency difference between the samples. At the modified level, however, these differences were not significant indicating that they most likely represented chance departures from homogeneity rather than evidence of genetic differences between the samples. Since there was no compelling evidence of genetic differences between the samples, they were combined for further analysis.

In the sample, the rainbow marker *OmyRD_RAD_30378_Hoh* possessed the allele usually characteristic of westslope cuthroat trout at an unusually high frequency compared to the other rainbow markers. This situation was also observed in the sample collected from the Fisher River between West Fisher Creek and Silver Butte Creek. Thus, we interpreted this anomaly to most likely indicate that this locus was polymorphic in the rainbow trout genetic contribution to the fish in the sample. This locus, therefore, was not considered to be a marker in subsequent analyses. We also were unable to obtain reliable data from the westslope marker *OclWD101119_Garza*. This locus, therefore, was also not used in the data analyses.

This sample contained good evidence of hybridization among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at five of the rainbow markers and eight of the westslope markers. The westslope cutthroat trout alleles appeared to be randomly (X_3^2 =2.744, P>0.10) distributed among the fish in the sample. Thus, these fish appeared to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a major rainbow trout (0.988) genetic contribution. In contrast to the westslope cutthroat trout alleles, the q values obtained from STRUCTURE did not appear to be randomly (X_6^2 =163.584, P<0.001) distributed among the individuals. Rather, there was broad range of values spanning from highly admixed to predominantly coastal rainbow trout (Figure 15) among the fish. Overall, therefore, the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout but, all fish appeared to be hybrids. On the average, the fish tended to have a predominant coastal rainbow trout, a substantial redband rainbow trout, and a minor westslope cutthroat trout genetic contribution. At the individual level, however, the former two components were highly variable.

There were two indications that the fish in the sample may not have come from a single population. First, among the 34 comparisons of observed to expected Hardy-Weinberg genotypic proportions two were statistically significant. These remained significant at the modified level and both deviations were due to a deficit of heterozygotes. Second, the q values were not randomly distributed among the fish in the sample.

Cedar Creek 4407

Samples were collected from Cedar Creek above the Highway 2 bridge and further upstream. Between the samples, 31 loci were polymorphic. At one of these loci, there was a significant allele frequency difference between the samples. At the modified level, however, this difference was not significant indicating that it most likely represented a chance departure from homogeneity rather than evidence of a genetic difference between the samples. Since there was no compelling evidence of genetic differences between the samples, they were combined for further analysis.

The rainbow marker *OclRD_Thymo_320Kal* possessed the allele usually characteristic of westslope cutthroat trout at an unusually high frequency compared to the other rainbow markers analyzed in the sample. We interpreted this anomaly to most likely indicate that this locus was polymorphic in the rainbow trout genetic contribution to the fish in the sample. This locus, therefore, was not considered to be a marker in subsequent analyses. We also were unable to obtain reliable data from the westslope marker *OclWD101119_Garza*. This locus, therefore, was also not used in the data analyses.

This sample appeared to contain hybrids among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at two of the rainbow markers and two of the westslope markers. The westslope cutthroat trout alleles appeared to be randomly $(X_2^2=0.270, P>0.50)$ distributed among the fish in the sample. These fish, therefore, appeared to have come from a hybrid swarm between rainbow and westslope cutthroat trout alleles, the q values obtained from STRUCTURE did not appear to be randomly $(X_2^2=58.918, P<0.001)$ distributed among the individuals. Rather, there was a fairly broad distribution of values ranging from predominantly redband rainbow trout to highly admixed (Figure 16). At the individual level, therefore, there appeared to be a highly variable amount of admixture between redband and coastal rainbow trout among the fish. Overall, the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout but, all fish appeared to be hybrids. On the average, the fish tended to have a predominant redband rainbow trout, a small coastal rainbow trout, and a minor westslope cutthroat trout genetic contribution. At the individual level, however, the former two components were highly variable.

There were two indications that the fish in the sample may not have come from a single population. First, among the 31 comparisons of observed to expected Hardy-Weinberg genotypic proportions two were statistically significant. These remained significant at the modified level and both deviations were due to a deficit of heterozygotes. Second, the q values were not randomly distributed among the fish in the sample.

Quartz Creek

Samples were collected from Quartz Creek from the 601 bridge to the PIT station and from Kimberlins to the 601 bridge. Between the samples, evidence of genetic variation was detected at 71 loci and the allele frequencies significantly differed between them at four of these loci. These differences remained significant at the modified level. The samples, therefore, were treated separately for further analysis.

Lower Quartz Creek From 601 Bridge to PIT Station 4408

We were unable to obtain reliable data from the westslope marker *OclWD101119_Garza* in the sample. This locus, therefore, was not used in the data analyses.

This sample contained good evidence of hybridization among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at 13 of the rainbow markers and 11 of the westslope markers. The westslope cutthroat trout alleles did not appear to be randomly (X_6^2 =56.023, P<0.001) distributed among the fish in the sample. The nonrandom distribution, however, appeared to mainly be due to one fish with a hybrid index of eight and another with a hybrid index of 16 (Figure 17A). When these two fish were eliminated from the data, the westslope cutthroat alleles appeared to be randomly (X_{4}^{2} =4.042, P>0.10) distributed among the remaining fish suggesting that these individuals all were hybrids between rainbow and westslope cutthroat trout with a minor (0.008) westslope cutthroat trout genetic component. When the two individuals with a relatively high westslope cutthroat trout genetic component were removed from the data, the q values obtained from STRUCTURE did not appear to be randomly (X^2 = 39.216, P<0.001) distributed among the individuals. Rather, there was a broad range of values spanning from predominantly redband rainbow trout through highly admixed to predominantly coastal rainbow trout (Figure 17B). At the individual level, therefore, there was a highly variable amount of admixture between redband and coastal rainbow trout among the fish. Thus, considering all the data the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout but, all fish appeared to be hybrids. On the average, the fish tended to have a substantial coastal and redband rainbow trout genetic component and a minor westslope cutthroat trout genetic contribution. At the individual level, however, the components were highly variable among the fish.

The only indications that this sample may have contained individuals from more than one population were the nonrandom distribution of westslope cutthroat trout alleles and of q values among the fish. When all individuals were considered, there were 31 comparisons of observed to expected Hardy-Weinberg genotypic proportions. Although three of these comparisons were statistically significant, they were not at the modified level. Thus, these apparent deviations most likely represented chance departures from homogeneity due to the number of comparisons performed. This situation also pertained when the two individuals with a comparatively high westslope cutthroat trout genetic component were removed from the data. In this situation, there were 22 comparisons with two being significant but, not at the modified level.

Upper Quartz From Kimberlins to 601 Bridge 4409

We were unable to obtain reliable data from the westslope marker *OclWD101119_Garza* in the sample. This locus, therefore, was not used in the data analyses.

There was substantial evidence that the sample from upper Quartz Creek collected from Kimberlins to the 601 bridge contained hybrids among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at all of the rainbow and westslope markers. The westslope cutthroat trout alleles, however, did not appear to be randomly (X^2_{g} =186.311, P<0.001) distributed among the fish in the sample. This was mainly due to the presence of individuals in the sample with a hybrid index of seven or more (Figure 18A). When these fish were eliminated from the data, the westslope cutthroat alleles appeared to be randomly (X^2_{g} =0.439, P>0.50) distributed among the remaining fish. These latter individuals, therefore, appeared to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a predominant rainbow trout (0.992) genetic contribution. When the individuals with a relatively high westslope cutthroat trout genetic component were removed from the data, the q values obtained from STRUCTURE did not appear to be randomly (X^2_{g} =29.370, P<0.01) distributed among the individuals. Rather, there was a broad distribution of values ranging from predominantly redband rainbow trout to highly admixed (Figure 18B). At the individual level, therefore, there was a highly variable

amount of admixture between redband and coastal rainbow trout among the fish. Thus, considering all the data the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout but, all fish appeared to be hybrids. On the average, the fish tended to have a substantial coastal and redband rainbow trout genetic component and a minor westslope cutthroat trout genetic contribution. At the individual level, however, the components were highly variable among the fish and some had a substantial westslope cutthroat trout genetic component.

The only indications that this sample may have contained individuals from more than one population were the nonrandom distribution of westslope cutthroat trout alleles and of q values among the fish. When all individuals were considered, there were 61 comparisons of observed to expected Hardy-Weinberg genotypic proportions. Although four of these comparisons were statistically significant, they were not at the modified level. Thus, these apparent deviations most likely represented chance departures from homogeneity due to the number of comparisons performed. This situation also pertained when the five individuals with an unusually high westslope cutthroat trout genetic component were removed from the data. In this situation, there were 25 comparisons with one being significant but, not at the modified level.

Obrien Creek

Samples were collected from Obrien Creek near the waterworks and at the Lynx Creek bridge. Between the samples, evidence of genetic variation was detected at 74 loci and the allele frequencies significantly differed between them at two of these loci. These differences remained significant at the modified level. The samples, therefore, were treated separately for further analysis.

Lower Obrien Creek near Waterworks 4410

This sample contained good evidence of hybridization among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at all of the rainbow markers and all of the westslope markers that were analyzed. The westslope cutthroat trout alleles did not appear to be randomly ($X_{10}^2=229.094$, P<0.001) distributed among the fish in the sample. The nonrandom distribution was due to a broad range of hybrid indices among the fish which spanned from a minor to substantial westslope cutthroat trout genetic component (Figure 19A). Thus, the amount of admixture between rainbow and westslope cutthroat trout was highly variable among the fish in the sample. When the four fish with a hybrid index of nine or more were eliminated from the data, the q values obtained from STRUCTURE did not appear to be randomly (X^2 ₇=30.696, P<0.001) distributed among the remaining individuals. In contrast, there was a broad distribution of values ranging from predominantly redband rainbow trout to highly admixed with coastal rainbow trout (Figure 19B). At the individual level, therefore, there was also a highly variable amount of admixture between redband and coastal rainbow trout among the fish. Thus, considering all the data the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout but, all fish appeared to be hybrids. On the average, the fish tended to have a predominant redband rainbow trout genetic component and a substantial coastal rainbow and westslope cutthroat trout genetic contribution. At the individual level, however, the components were highly variable among the fish.

The nonrandom distribution of westslope cutthroat trout alleles and of q values among the fish both suggest the sample may have contained individuals from more than one population. When all individuals were considered, there were 65 comparisons of observed to expected Hardy-Weinberg genotypic proportions. Although three of these comparisons were statistically significant, they were not at the modified level. Thus, these apparent deviations most likely represented chance departures from homogeneity due to the number of comparisons performed. This situation also pertained when the four individuals with a relatively high westslope cutthroat trout genetic component were removed from the data. In this situation, there were 30 comparisons and none of them were significant.

Upper Obrien Creek at Lynx Creek Bridge 4411

There was substantial evidence that the sample from upper Obrien Creek collected at the Lynx Creek bridge contained hybrids among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at all of the rainbow markers and all of the westslope markers that were analyzed. The westslope cutthroat trout alleles, however, did not appear to be randomly ($X_{11}^2=70.131$, P<0.001) distributed among the fish in the sample. The nonrandom distribution was due to a broad range of hybrid indices among the fish which spanned from a minor to substantial westslope cutthroat trout genetic component (Figure 20A). The amount of admixture between rainbow and westslope cutthroat trout, therefore, was highly variable among the fish in the sample. When the individuals with a relatively high westslope cutthroat trout genetic component (hybrid index of 15 or more) were removed from the data, the q values obtained from STRUCTURE appeared to be randomly ($X_{6}^{2}=9.765$, P>0.10) distributed among the remaining individuals. This may be more of a statistical artifact than biological reality as this comparison was statistically extremely weak because of the small sample size. Furthermore, there was a broad distribution of values ranging from predominantly redband rainbow trout to highly admixed (Figure 20B). Thus, we are hesitant to conclude that the redband and coastal rainbow trout alleles actually are randomly distributed among these few fish. Considering all the data, the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout. On the average, the fish tended to have a predominant redband rainbow trout genetic component but, also a substantial coastal rainbow and westslope cutthroat trout genetic contribution. At the individual level, however, the components were highly variable among the fish.

The only suggestion that this sample may have contained individuals from more than one population was the nonrandom distribution of westslope cutthroat trout alleles among the fish. When all individuals were considered, there were 72 comparisons of observed to expected Hardy-Weinberg genotypic proportions. Although two of these comparisons were statistically significant, they were not at the modified level. Thus, these apparent deviations most likely represented chance departures from homogeneity due to the number of comparisons performed. This situation also pertained when the individuals with a hybrid index of 15 or more were removed from the data. In this case, there were 28 comparisons and none of them were significant.

Lake Creek Below Falls 4412

Samples were collected from Lake Creek below the falls near the Highway 2 bridge and just below the bypass. In the samples, one fish clearly appeared to be a nonhybridized westslope cutthroat trout. When this fish was removed from the data, evidence of genetic variation was detected at 51 loci between the samples. The allele frequencies were statistically homogeneous between the samples at all of these loci. Excluding the westslope cutthroat trout, since there was no evidence of genetic differences between the samples they were combined for subsequent analysis.

We were unable to obtain reliable data from the rainbow marker *OmyRD_RAD_29252_Hoh* in the sample. This locus, therefore, was not used in the data analyses. Furthermore, the westslope marker *OclWD101119_Garza* possessed the allele usually characteristic of westslope cutthroat trout at an unusually high frequency compared to the other westslope markers analyzed in the sample. We interpreted this anomaly to most likely indicate that this locus was polymorphic in the westslope cutthroat trout genetic contribution to the fish in the sample. This locus, therefore, was not considered to be a marker in subsequent analyses.

Without the westslope cutthroat trout, this sample contained good evidence of hybridization among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at 13 of the rainbow markers and eight of the westslope markers that were analyzed. The westslope cutthroat trout alleles did not appear to be randomly ($X_2^2=12.352$, P<0.01)

distributed among the fish in the sample. The nonrandom distribution, however, appeared to mainly be due to one fish with a hybrid index of 19 (Figure 21A). When this fish was eliminated from the data, the westslope cutthroat alleles appeared to be randomly ($X_2^2=2.047$, P>0.10) distributed among the remaining fish suggesting that all these individuals were hybrids between rainbow and westslope cutthroat trout with a minor (0.006) westslope cutthroat trout genetic component. When the fish with a hybrid index of 19 was eliminated from the data, the q values obtained from STRUCTURE did not appear to be randomly $(X^{2}_{7}=287.956, P<0.001)$ distributed among the remaining individuals. In contrast, there was a broad distribution of values ranging from predominantly redband rainbow trout to highly admixed with coastal rainbow trout to predominantly coastal rainbow trout (Figure 21B). At the individual level, therefore, there was a highly variable amount of admixture between redband and coastal rainbow trout among the fish but, most contained a predominant coastal rainbow trout genetic contribution. Thus, considering all the data the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout but, all fish appeared to be hybrids. On the average, the fish tended to have a predominant coastal rainbow trout genetic component, a substantial redband rainbow trout component, and a minor westslope cutthroat trout genetic contribution. At the individual level, however, the components were highly variable among the fish and one appeared to be a nonhybridized westslope cutthroat trout.

A likely reason the sample did not appear to have come from a hybrid swarm is that it almost certainly contained individuals from more than one population. When all individuals in the sample were considered, there were 66 comparisons of observed to expected Hardy-Weinberg genotypic proportions. There was a statistically significant deviation between these distributions at 19 of these loci. These deviations were significant at the modified level and all but one involved a deficit of heterozygotes. Similar results were obtained when the westslope cutthroat trout and the fish with a hybrid index of 19 were removed from the data. At four of 29 loci, there was a statistically significant difference between observed and expected Hardy-Weinberg genotypic distributions. These differences were also significant at the modified level and three involved a deficit of heterozygotes. The nonrandom distribution of hybrid indices and q values among the fish in the sample further suggest it did not contain individuals from a single population.

Lake Creek Above Falls

Samples were collected from Lake Creek above the falls below the Lake Creek Road bridge and above the chase cutoff bridge. Between the samples, evidence of genetic variation was detected at 73 loci and the allele frequencies significantly differed between them at 47 of these loci. These differences remained significant at the modified level. The samples, therefore, were treated separately for further analysis.

Lower Lake Creek Above Falls at Lake Creek Road bridge 4413

There was substantial evidence that the sample from Lake Creek collected above the falls at the Lake Creek bridge contained hybrids between coastal rainbow and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at all of the rainbow markers and all of the westslope markers that were analyzed. The westslope cutthroat trout alleles, however, did not appear to be randomly $(X^2_{g}=105.240, P<0.001)$ distributed among the fish in the sample. The nonrandom distribution, however, was due to three fish with a hybrid index of 22 or more (Figure 22A). When these fish were eliminated from the data, the westslope cutthroat trout alleles appeared to be randomly $(X^2_{g}=0.214, P>0.50)$ distributed among the remaining fish. These latter individuals, therefore, appeared to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a predominant rainbow trout (0.993) genetic component. When the individuals with a hybrid index of 22 or more were removed from the data, the q values obtained from STRUCTURE appeared to be randomly $(X^2_{I}=0.773, P>0.10)$ distributed among the individuals. All of these individuals appeared to contain only a coastal rainbow trout genetic component (Figure 22B). Thus, this sample appeared to mainly contain fish from a hybrid swarm between coastal rainbow and westslope

cutthroat trout with a predominant (0.993) coastal rainbow trout genetic contribution and three fish with a much higher westslope cutthroat trout genetic component.

As discussed above, the sample almost certainly contained individuals from more than one genetically divergent population. Considering all the fish, there were 66 comparisons of observed to expected Hardy-Weinberg genotypic proportions. There was a statistically significant deviation between these distributions at 16 of these loci and these deviations were significant at the modified level. Furthermore, all the significant differences involved a deficit of heterozygotes and the westslope cutthroat trout alleles were far from randomly distributed among the fish. In contrast to these results, when the three fish with a hybrid index of 22 or more were eliminated from the data the remaining individuals appeared to have come from a single hybrid swarm. Among these fish, there were 24 comparisons of observed to expected Hardy-Weinberg genotypic proportions. At one of these loci, the observed distribution significantly differed from the expected distribution. This difference, however, was not significant at the modified level suggesting it most likely represents a chance departure from homogeneity. The westslope cutthroat trout alleles also appeared to be randomly distributed among these fish.

Upper Lake Above Falls Above Chase Cutoff Bridge 4414

Compared to the majority of the other samples, the sample from Lake Creek above the falls collected above the chase cutoff bridge contained an unusually high proportion of westslope cutthroat trout alleles. They were detected at all of the rainbow and westslope cutthroat trout markers analyzed. Furthermore, two of the fish appeared to be non-hybridized westslope cutthroat trout, two appeared to be hybrids between westslope cutthroat and rainbow trout with a substantial westslope cutthroat trout genetic component, and one a hybrid between redband and coastal rainbow trout with a substantial genetic contribution from both fishes (Figure 23). Although the small sample size precluded any meaningful comparisons to expected Hardy-Weinberg proportions, the sample clearly did not contain individuals from a single population.

Callahan Creek Below Falls 4415

Samples were collected from two reaches of Callahan Creek below the falls. Between the samples, 29 loci were polymorphic. At one of these loci, there was a significant allele frequency difference between the samples. At the modified level, however, this difference is not significant indicating that it most likely represents a chance departure from homogeneity rather than evidence of a genetic difference between the samples. Since there was no compelling evidence of genetic differences between the samples, they were combined for further analysis.

In the sample, the rainbow markers *OmyRD_RAD_30378_Hoh* and *OmyRD_RAD_59515_Hoh* possessed the allele usually characteristic of westslope cutthroat trout at an unusually high frequency compared to the other rainbow markers. At *OmyRD_RAD_30378*, this situation was also observed in the sample collected from the Fisher River between West Fisher Creek and Silver Butte Creek and in the sample from Bobtail Creek. At *OmyRD_RAD_59515_Hoh*, this situation was also observed in the samples collected from the Fisher River between West Fisher Creek and Silver Butte Creek, the Fisher River below Wolf Creek, and Wolf Creek. Thus, we interpreted these anomalies to most likely indicate that these loci were polymorphic in the rainbow trout genetic contribution to the fish in the sample. These loci, therefore, were not considered to be markers in subsequent analyses.

No evidence of hybridization with westslope cutthroat trout was detected in the sample collected from Callahan Creek below the falls. Only alleles characteristic of rainbow trout were detected at the rainbow markers and no alleles characteristic of westslope cutthroat trout were detected at the westslope markers. STRUCTURE indicated the fish in the sample mainly possessed a redband rainbow trout genetic

contribution. There were, however, some individuals that were clearly hybrids between redband and coastal rainbow trout with a substantial amount of admixture (Figure 24).

Although the sample contained clear evidence of hybridization between redband and coastal rainbow trout it did not appear to have come from a hybrid swarm between these fishes. The q values were not randomly $(X^2_4=56.362, P<0.001)$ distributed among the fish. Furthermore, among the 28 comparisons of observed to expected Hardy-Weinberg genotypic proportions four were statistically significant. These differences remained significant at the modified level and three of them involved a deficit of heterozygotes. Thus, this sample appears to have contained individuals from more than one population.

Star Creek Below Falls 4416

Only one reach of Star Creek below the falls was sampled. In the sample, the rainbow marker *OmyRD_RAD_30378_Hoh* possessed the allele usually characteristic of westslope cutthroat trout at an unusually high frequency compared to the other rainbow markers. At *OmyRD_RAD_30378*, this situation was also observed in the samples collected from the Fisher River between West Fisher Creek and Silver Butte Creek, Bobtail Creek, and Callahan Creek. We, therefore, interpreted this anomaly to most likely indicate that this locus was polymorphic in the rainbow trout genetic contribution to the fish in the sample. This locus, therefore, was not considered to be a marker in subsequent analyses.

No evidence of hybridization with westslope cutthroat trout was detected in the sample collected from Star Creek below the falls. Only alleles characteristic of rainbow trout were detected at the rainbow markers and no alleles characteristic of westslope cutthroat trout were detected at the westslope markers. STRUCTURE indicated that some of the fish in the sample may have been non-hybridized coastal rainbow trout (Figure 25). There were, however, some individuals that were clearly hybrids between redband and coastal rainbow trout with a highly variable amount of admixture (Figure 25). The q values, therefore, were not randomly $(X_6^2=40.255, P<0.001)$ distributed among the fish in the sample suggesting it did not come from a hybrid swarm.

There was good evidence that this sample did not contain individuals from a single population. Out of 28 comparisons of observed to expected Hardy-Weinberg genotypic proportions, two were statistically significant. These differences were significant at the modified level and both involved a deficit of heterozygotes. Furthermore, the q values were highly variable and not randomly distributed among the fish in the sample.

Yaak River Below Falls

Samples were collected from two reaches of the Yaak River below the falls. Between the samples, evidence of genetic variation was detected at 67 loci and the allele frequencies significantly differed between them at four of these loci. These differences remained significant at the modified level. The samples, therefore, were treated separately for further analysis.

Lower Yaak River Below Falls 4417

In the sample, the rainbow markers *OmyRD_RAD_30378_Hoh* and *OmyRD_RAD_59515_Hoh* possessed the allele usually characteristic of westslope cutthroat trout at an unusually high frequency compared to the other rainbow markers. At *OmyRD_RAD_30378*, this situation was also observed in the samples collected from the Fisher River between West Fisher Creek and Silver Butte Creek, Bobtail Creek, Callahan Creek, and Star Creek below the falls. At *OmyRD_RAD_59515_Hoh*, this situation was also observed in the samples collected from the Fisher River between West Fisher Creek and Silver Butte Creek, the Fisher River below Wolf Creek, wolf Creek, and Callahan Creek. Thus, we interpreted these anomalies to most likely indicate

that these loci were polymorphic in the rainbow trout genetic contribution to the fish in the sample. These loci, therefore, were not considered to be markers in subsequent analyses.

No evidence of hybridization with westslope cutthroat trout was detected in the sample. Only alleles characteristic of rainbow trout were detected at the rainbow markers and no alleles characteristic of westslope cutthroat trout were detected at the westslope markers that were analyzed. STRUCTURE indicated the q values were highly variable among the fish in the sample ranging from predominantly redband rainbow trout to individuals with a substantial amount of admixture between redband and coastal rainbow trout (Figure 26). The q values, therefore, were not randomly ($X_6^2=27.424$, P<0.001) distributed among the fish in the sample.

The only suggestion that this sample may have contained individuals from more than one population was the nonrandom distribution of q values among the fish. There were 26 comparisons of observed to expected Hardy-Weinberg genotypic proportions. Only one of these was significant but, it did not remain significant at the modified level. Thus, this difference most likely represented a chance departure from homogeneity due to the number of comparisons performed.

Upper Yaak River Below Falls 4418

In the sample, the rainbow marker *OmyRD_RAD_30378_Hoh* possessed the allele usually characteristic of westslope cutthroat trout at an unusually high frequency compared to the other rainbow markers. At *OmyRD_RAD_30378*, this situation was also observed in the samples collected from the Fisher River between West Fisher Creek and Silver Butte Creek, Bobtail Creek, Callahan Creek, Star Creek below the falls, and lower Yaak River below the falls. We, therefore, interpreted this anomaly to most likely indicate that this locus was polymorphic in the rainbow trout genetic contribution to the fish in the sample. This locus, therefore, was not considered to be a marker in the following analyses.

A single fish in the sample clearly appeared to be a nonhybridized westslope cutthroat trout. When this individual was removed from the data, only alleles characteristic of rainbow trout were detected at the rainbow markers and no alleles characteristic of westslope cutthroat trout were detected at the westslope markers that were analyzed. Thus, there was no evidence of hybridization with westslope cutthroat trout among the remaining fish. The q values from STRUCTURE were randomly (X^2_3 =4.269, P>0.10) distributed among the latter individuals indicating that they came from a hybrid swarm between redband and coastal rainbow trout with a predominant (0.948) redband rainbow trout genetic component (Figure 27). The conclusion this group of fish represented a single hybrid swarm is strengthened by the observation that only two out of 25 comparisons of observed to expected Hardy-Weinberg genotypic proportions were statistically significant. These differences, however, were not significant at the modified level suggesting they most likely represented chance departures from homogeneity due to the number of comparisons performed rather than actual deviations from expected random mating genotypic proportions. The sample, therefore, appeared to be a mixture of mainly individuals from a hybrid swarm between redband and coastal rainbow trout and one nonhybridized westslope cutthroat trout.

Yaak River Above Falls 4419

Samples were collected from two reaches of the Yaak River above the falls. Between the samples, 57 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.

There was good evidence that the sample from the Yaak River above the falls contained hybrids among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at 14 of the rainbow markers and at 13 of the westslope markers that

were analyzed. The westslope cutthroat trout alleles, however, did not appear to be randomly (X^2_3 =36.403, P<0.001) distributed among the fish in the sample. The nonrandom distribution, however, appeared to be solely due to the presence of three fish that were definitely hybrids between rainbow and westslope cutthroat trout with a highly variable amount of admixture (Figure 28A). All of the other fish in the sample appeared to be non-hybridized rainbow trout. The q values from STRUCTURE indicated these fish were hybrids between redband and coastal rainbow trout with a major redband rainbow trout genetic contribution. The q values, however, were not randomly (X^2_2 =8.575, P<0.05) distributed among these fish (Figure 28B). Thus, these latter fish did not appear to have come from a hybrid swarm between redband and coastal rainbow trout.

The only indications that this sample did not contain individuals from only one population were the presence of the hybrids between rainbow and westslope cutthroat trout and the nonrandom distribution of q values when these fish were removed from the data. Out of the 24 comparisons of observed to expected Hardy-Weinberg genotypic proportions only two were statistically significant. At the modified level, however, these differences were not significant. Thus, they most likely represented chance departures from homogeneity due to the number of comparisons performed rather than actual deviations from expected random mating genotypic proportions.

Kootenai River at Libby Dam 4420

In the sample, the rainbow marker *OmyRD_RAD_30378_Hoh* possessed the allele usually characteristic of westslope cutthroat trout at an unusually high frequency compared to the other rainbow markers. At *OmyRD_RAD_30378*, this situation was also observed in the samples collected from the Fisher River between West Fisher Creek and Silver Butte Creek, Bobtail Creek, Callahan Creek, Star Creek below the falls, and lower and upper Yaak River below the falls. We, therefore, interpreted this anomaly to most likely indicate that this locus was polymorphic in the rainbow trout genetic contribution to the fish in the sample. This locus, therefore, was not considered to be a marker in the following analyses.

Alleles characteristic of westslope cutthroat trout were detected at eight of the rainbow markers and at ten of the westslope markers that were analyzed in the sample. Thus, there was good evidence of hybridization between rainbow and westslope cutthroat trout. The westslope cutthroat trout alleles, however, did not appear to be randomly (X_{3}^{2} =25.249, P<0.001) distributed among the fish in the sample. The nonrandom distribution appeared to mainly involve only two fish one with a hybrid index of four and the other with a hybrid index of ten (Figure 29A). When these two individuals were eliminated from the data, the westslope cutthroat trout alleles appeared to be randomly (X_{2}^{2} =4.005, P>0.10) distributed among the remaining fish. This latter group, therefore, appeared to constitute a hybrid swarm between rainbow and westslope cutthroat trout with a predominant (0.995) rainbow trout genetic component. The q values among the latter fish obtained from STRUCTURE, however, indicated they did not represent a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout. Compared to random expectations, there were significantly (X_{4}^{2} =60.280, P<0.001) more fish with a substantial amount of admixture or a substantial coastal rainbow trout genetic contribution than expected by chance.

The nonrandom distribution of westslope cutthroat trout alleles and of q values among the fish provide the only indications the sample may have contained individuals from more than one population. When all individuals were considered, there were 34 comparisons of observed to expected Hardy-Weinberg genotypic proportions. Although two of these comparisons were statistically significant, they were not at the modified level. Thus, these apparent deviations most likely represented chance departures from homogeneity due to the number of comparisons performed. This situation also pertained when the two individuals with an unusually high westslope cutthroat trout genetic component were removed from the data. In this situation, there were 31 comparisons and three were significant. These differences, however, were not significant at the modified level again suggesting they most likely represented chance departures from homogeneity due to the

number of comparisons performed rather than actual deviations from expected random mating genotypic proportions.

Kootenai River-Flower Creek to Pipe Creek 4421

There was good evidence that the sample from the Kootenai River collected between Flower Creek and Pipe Creek contained hybrids among redband rainbow, coastal rainbow, and westslope cutthroat trout. In the sample, alleles characteristic of westslope cutthroat trout were detected at eight of the rainbow markers and at six of the westslope markers that were analyzed. The westslope cutthroat trout alleles appeared to be randomly ($X_{3}^{2}=0.850$, P>0.50) distributed among the fish in the sample. Thus, this sample appeared to represent a hybrid swarm between rainbow and westslope cutthroat trout with a predominant (0.989) rainbow trout genetic component. The q values from STRUCTURE had an extremely broad distribution and, therefore, were not randomly ($\dot{X}_{6}^{2}=91.703$, P<0.001) distributed among the fish in the sample. They ranged from individuals appearing to contain mainly a redband rainbow trout contribution to highly admixed individuals to fish with a predominant coastal rainbow trout genetic contribution (Figure 30). Most of the fish fell into the latter category. Thus, considering all the data the sample did not appear to have come from a hybrid swarm among redband rainbow, coastal rainbow, and westslope cutthroat trout but, all fish appeared to be hybrids. On the average, the fish tended to have a predominant coastal rainbow trout genetic component, a substantial redband rainbow trout contribution, and a minor westslope cutthroat trout genetic contribution. At the individual level, however, the former two components were highly variable among the fish.

The only suggestion that this sample may have contained individuals from more than one population was the nonrandom distribution of q values among the fish. There were 32 comparisons of observed to expected Hardy-Weinberg genotypic proportions. Only one of these was significant but, it did not remain significant at the modified level. Thus, this difference most likely represented a chance departure from homogeneity due to the number of comparisons performed rather than an actual deviation from expected random mating genotypic proportions.

Kootenai River-Troy 4422

Alleles characteristic of westslope cutthroat trout were detected at 18 of the rainbow markers and at all of the westslope markers that were analyzed in the sample collected from the Kootenai River near Troy. Thus, there was good evidence of hybridization between rainbow and westslope cutthroat trout. The westslope cutthroat trout alleles, however, did not appear to be randomly (X^2_0 =1019.010, P<0.001) distributed among the fish in the sample. The nonrandom distribution appeared to mainly involve the three fish with a hybrid index of 37 or more (Figure 31A). When these three individuals were eliminated from the data, the westslope cutthroat trout alleles appeared to be randomly (X^2_2 =5.508, P>0.05) distributed among the remaining fish. This latter group, therefore, appeared to constitute a hybrid swarm between rainbow and westslope cutthroat trout with a predominant (0.995) rainbow trout genetic component. The q values obtained from STRUCTURE, however, did not appear to be randomly (X^2_7 =195.823, P<0.001) distributed among the latter fish. Compared to random expectations, there was a very broad range of q values among the fish. They ranged from individuals appearing to contain mainly a redband rainbow trout contribution to highly admixed individuals to fish with a predominant coastal rainbow trout genetic contribution (Figure 31B) with most of the fish falling into the latter category. On the average, the fish tended to have a predominant coastal rainbow trout genetic component, a substantial redband rainbow trout contribution, and a minor westslope cutthroat trout genetic contribution. At the individual level, however, the components were highly variable among the fish.

There was good evidence that this sample did not contain individuals from a single population. Considering all the fish, there were 68 comparisons of observed to expected Hardy-Weinberg genotypic proportions. Of these, three were statistically significant. These differences were significant at the modified level and all involved a deficit of heterozygotes. This situation also pertained when the three fish with a high amount of admixture between rainbow and westslope cutthroat trout were removed from the data. In this situation, there were 29 comparisons of observed to expected Hardy-Weinberg genotypic proportions. Of these, two were statistically significant and these differences remained significant at the modified level. Both of these deviations involved a deficit of heterozygotes. Besides there being a deficit of heterozygotes at some loci, additional evidence the sample did not contain individuals from a single population is provided by the nonrandom distribution of westslope cutthroat trout alleles and q values among the fish

Unreliability of Assignment Test

Among the samples, data were missing from at least one at the following loci: *OmyRD_RAD_29252_Hoh*, *OclWD101119_Garza*, *Omg_CHIT_80_May*, *Oclvar102483_Garza*, *Oclvar_pnpo_56NC*, and *Oclvar_cin_90NC*. These loci, therefore, were not used in the following analysis.

As stated previously, a critical assumption of assignment tests is that genotypes in the samples reasonably conform to expected random mating proportions. Or in other words, that the genetic characteristics of the populations are well determined. This assumption does not appear to be valid for practically all of our samples. Among them, there were numerous cases of loci demonstrating a significant deficit of heterozygotes compared to expected Hardy-Weinberg proportions. Furthermore, in many samples the distribution of westslope cutthroat trout alleles and q values were not randomly distributed among the fish. Thus, many of our samples clearly contained individuals from more than one genetically divergent population and the genetic characteristics of individual populations are basically unknown. In this situation, assignment tests will very likely produce spurious and meaningless results.

This certainly appears to be the case. When all samples were treated as 'populations', the assignment test of Rannala and Mountain (1997) available in GENECLASS2 (Piry et al. 2004) only assigned 46 percent of the individuals to their sample of origin. Many of these 'correct' assignments, however, were questionable as they often involved certainties of less than 0.50.

When we considered the results of this analysis examining only the fish with a hybrid index between rainbow and westslope cutthroat trout of seven or more, it is clear that many individuals are being erroneously assigned to samples. Out of 70 such fish, only 50 percent were assigned to their sample of origin (Table 4). Among all the samples, only those from upper Dunn Creek, middle and upper Flower Creek, upper Obrien Creek, and upper Lake Creek above the falls had greater than a 0.150 westslope cutthroat trout genetic component. Of the 35 mis-assigned individuals, all but three were placed into one of these samples (Table 4). Thus, there was a tendency for the assignment test to place individuals with a substantial westslope cutthroat trout genetic contribution regardless of where they were sampled back to their sample of origin or into another sample with a relatively high westslope cutthroat trout genetic contribution. Many of the latter were obviously spurious as they involved assignment of fish collected above an upstream passage barrier to samples below the barrier (Table 4). For example, two fish collected from upper Dunn Creek were identified as having originated from upper Obrien Creek and one from upper Lake Creek above the falls.

Although we cannot use the data to reliably assess the potential origin of the trout in the Kootenai River, they are not completely without merit. First, they clearly indicate that hybridization and subsequent introgression between native redband and introduced coastal rainbow trout is prevalent within the drainage. There was evidence of this in all of the samples in which we were able to reliably examine this except lower Lake Creek above the falls. Second, the data clearly indicate that during the summer many stream and river reaches contain fish from two or more populations. Thus, there appears to be extensive movement, at least at some

time of the year, of fish within the drainage. Whether this results in extensive gene flow among populations, however, we cannot determine from the available data. Finally, most samples had individuals that were slightly hybridized with westslope cutthroat trout. Whether this represents a small amount of historic introgression between native redband rainbow and westslope cutthroat trout in the drainage or is the result of the introduction of coastal rainbow trout we also cannot determine from the data. Overall, the results suggest few, if any, nonhybridized redband rainbow trout exist in the region of the Kootenai River drainage from which the samples were obtained.

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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).

	Reference		
	Taxa and ch	aracteristic 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
OcIRD_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 cha	aracteristic alleles	
Locus	Westslope	Rainbow/Yellowstone	
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

	Yellowstone Markers		Reference
Locus	Taxa and cha		
	Yellowstone	Westslope/Rainbow	
OclYD_CLK3Y1_Har	22	11	Harwood and Phillips 201
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 1-continued

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 rainbow trout, CRT=coastal rainbow trout. N=sample size.

Sample	Таха	N	Location
Washaa Dark Stata Trout			
	WOT	10	Angoanda Montona
Flatchery	WCT	12	Anaconda, Montana
Big Foot Creek	WOT	2	Opper Kootenal 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 Hatcherv	CRT	7	Arlee, Montana
Arlee Rainbow		•	

SNP loci that differentiate redband and coastal rainbow trout.

Taxa and predominant alleles									
Locus	Redband	Coastal	Reference						
FLU_Omg_LDHB2_76100Brun	11	22	Brunelli et al. 2008						
Omg_CRB_2677_117_May	22	11	Stephens et al. 2009						
Omg_RAPD_167_May	11	22	Stephens et al. 2009						
Omyvar_104519_624_Gar	11	22	Campbell et al. 2012						
Omyvar_112208_328_Gar	22	11	Campbell et al. 2012						
Omyvar_101832_195_Gar	11	22	Campbell et al. 2012						
Omyvar_130720_100_Gar	11	22	Campbell et al. 2012						
Omyvar_127645_308_Gar	11	22	Campbell et al. 2012						
Omyvar_Ogo4_212_NC	22	11	Campbell et al. 2009						

Results of assignment test using all individuals and treating all samples as 'populations' obtained for the subset of individuals with a hybrid index between rainbow and westslope cutthroat trout of seven or more. Assigned=sample assigned to. Sample=sample of origin. Bold numbers represent individuals assigned to sample of origin. Samples highlighted in red represent those in which there was over a 0.150 westslope cutthroat trout genetic component. Samples highlighted in green represent those collected above barriers precluding upstream migration.

						As	signed					
Sample	Upper	Lower	Middle	Upper		Lower	Upper	Lower	Upper	Lake Ab	ove Falls	Yaak
	Dunn	Flower	Flower	Flower	Pipe	Quartz	Quartz	Obrien	Obrien	Lower	Upper	Above Falls
Lower Dunn			1							1		-
Upper Dunn	4								2		1	
Wolf									1			
Fisher-Silver Butte			1									
Lower Flower		5							1			
Middle Flower				1							2	
Upper Flower				11								
Upper Parmenter					1							
Pipe	1								1			
Lower Quartz	1					1						
Upper Quartz	1		2				1		1			
Lower Obrien								1	2		1	
Upper Obrien									8		1	
Lake Below Falls			1	1								
Lake Above Falls												
Lower										2	1	
Upper				3							1	
Yaak Below Falls												
Upper				1								
Yaak Above Falls									1			1
Kootenai-Dam							1				3	
Kootenai-Troy												



В

A



Figure 1. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from lower Dunn Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from lower Dunn Creek. The individuals with hybrid indices of eight and 18 were not included in this analysis.



Figure 2. Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from upper Dunn Creek.



Figure 3. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from the Fisher River below Wolf Creek.





Figure 4. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from Wolf Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from Wolf Creek. The individual with a hybrid index of 27 was not included in this analysis.

40





Figure 5. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from the Fisher River between West Fisher Creek and Silver Butte Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from the Fisher River between West Fisher Creek and Silver Butte Creek. The individual with a hybrid index of 17 was not included in this analysis.



Figure 6. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from Libby Creek below Big Cherry Creek.



Figure 7. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from Big Cherry Creek.



Figure 8. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from Libby Creek near Highway 2.



A



Figure 9. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from lower Flower Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from lower Flower Creek. The individuals with hybrid indices of nine or more were not included in this analysis.



A



Figure 10. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from middle Flower Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from middle Flower Creek. The individuals with hybrid indices of 40 or more were not included in this analysis.

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Figure 11. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from upper Flower Creek. B) The proportion of westslope cutthroat (Wct), coastal rainbow (Coast), and redband rainbow trout (Red) alleles in individuals' estimated using STRUCTURE indicating hybridization among these fishes in the sample from upper Flower Creek.



Figure 12. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from lower Parmenter Creek.



В



Figure 13. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from upper Parmenter Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from upper Parmenter Creek. The individuals with a hybrid index of five or more were not included in this analysis.

Α





Figure 14. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from Pipe Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from Pipe Creek. The individuals with hybrid indices of 16 and 17 were not included in this analysis.

Α



Figure 15. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from Bobtail Creek.



Figure 16. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from Cedar Creek.







Figure 17. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from lower Quartz Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from lower Quartz Creek. The individuals with hybrid indices of eight and 16 were not included in this analysis.





Figure 18. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from upper Quartz Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from upper Quartz Creek. The individuals with hybrid indices of seven or more were not included in this analysis.

A





Figure 19. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from lower Obrien Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from lower Obrien Creek. The individuals with hybrid indices of nine or more and were not included in this analysis.

A





Figure 20. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from upper Obrien Creek. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from upper Obrien Creek. The individuals with hybrid indices of 15 or more were not included in this analysis.

Α





Figure 21. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from Lake Creek below the falls when the nonhybridized westslope cutthroat trout was removed from the data. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from Lake Creek below the falls. The nonhybridized westslope cutthroat trout and the individual with a hybrid index of 19 were not included in this analysis.

A





Figure 22. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from the lower reach of Lake Creek above the falls. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from the lower reach of Lake Creek above the falls. The individuals with a hybrid index of 22 or more were not included in this analysis.

Α



Figure 23. The proportion of westslope cutthroat (Wct), coastal rainbow(Coast), and redband rainbow trout (Red) alleles in individuals' estimated using STRUCTURE in the fishes in the sample from upper Lake Creek above the falls.



Figure 24. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from Callahan Creek collected below the falls.



Figure 25. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from Star Creek collected below the falls.



Figure 26. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from the lower reach of the Yaak River below the falls.



Figure 27. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from the upper reach of the Yaak River below the falls. This analysis did not include the one fish in the sample that appeared to be a non-hybridized westslope cutthroat trout.



A



Figure 28. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from the Yaak River above the falls. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from the Yaak River above the falls. The individuals with a hybrid index greater than zero were not included in this analysis.

Α



B



Figure 29. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from the Kootenai River below Libby Dam. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from the Kootenai River below Libby Dam. The individuals with a hybrid index of four and ten were not included in this analysis.



Figure 30. Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from the Kootenai River collected between Flower Creek and Pipe Creek.







Figure 31. A) Observed and expected random distribution of hybrid indices indicating hybridization between rainbow and westslope cutthroat trout in the sample from the Kootenai River collected near Troy. B) Observed and expected random distribution of the proportion of coastal rainbow trout alleles in individuals' estimated using STRUCTURE indicating hybridization between redband and coastal rainbow trout in the sample from the Kootenai River collected near Troy. The individuals with a hybrid index of 37 or more were not included in this analysis.