FWP Repository ID: 59601
FWP Reference #: 700126

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

May 21, 2013

Jim Dunnigan
Montana Fish, Wildlife & Parks
385 Fish Hatchery Road
Libby, Montana 59923

Jim;

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

Sample #	Water Name/Location/ Collection Date/ Collector	a N	b #Markers	c Taxa ID	d Power	e %	f # Fish
4453	**Howard Lake 48.098547 115.527256 6/15/2011 Jim Dunnigan**	**27**	**R19W18Y20**	**IRT X CRT X WCT**			
4454	**Howard Creek 48.11003 115.54206 8/23/2012 Jim Dunnigan**	**29**	**R19W18Y20**	**IRT X CRT X WCT**			
4455	**Libby Creek at Howard Creek Confluence 48.12043-12307 115.54556-54143 8/23/2012 Jim Dunnigan**	**27**	**R19W18Y20**	**IRT X CRT X WCT**			
4456	**Ruby Creek 48.51378-50243 115.96220-99710 7/24/2012 Jim Dunnigan**	**49 (74)**	**R19W19Y20**	**WCT**	**R99Y99**		

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

[b]Number of marker loci analyzed for rainbow *Oncorhynchus mykiss* (R), westslope cutthroat *O. clarkii lewisi* (W), and Yellowstone cutthroat trout *O. c. bouvieri* (Y).
[c]Taxa: 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.
[d]Power: the number corresponds to the percent chance we have to detect 0.5% introgression in a hybrid swarm (a random mating population in which 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.
[e]Indicates 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.
[f]Indicates the number of individuals with genetic characteristics corresponding to the taxa ID code column when the sample contains individuals from two or more genetically distinct groups.

Methods and Data Analysis

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

If a sample possessed alleles characteristic of only 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 had to possess alleles characteristic of westslope cutthroat trout. 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 genetically variable, 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 genetically variable (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.

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

In order to determine whether or not alleles at the marker loci were randomly distributed among the fish in a sample showing evidence of hybridization, we calculated a hybrid index for each fish in the sample. The hybrid index for an individual was calculated as follows. At each marker locus, an allele characteristic of 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.

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

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

Failure to detect evidence of hybridization 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.

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 different locations in the Libby Creek drainage. 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, they were combined for further analysis. Conversely, when differences were detected between samples they were treated separately for subsequent analysis.

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.

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 a 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.

The chip also contained 14 loci usually polymorphic in redband rainbow trout and 14 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.

Results and Discussion

Genetic Differences Among the Howard Lake, Howard Creek, and Libby Creek Samples

Between the Howard Lake and Howard Creek samples, 32 loci were polymorphic. The allele frequencies were statistically heterogeneous between the samples at 12 of these loci and the differences were significant at the modified level. Evidence of genetic variation was detected at 39 loci between the Howard Lake and Libby Creek samples. The allele frequencies were statistically heterogeneous between the samples at 14 of these loci and the differences remained significant at the modified level. Finally, 41 loci were polymorphic between the Howard Creek and Libby Creek samples. The allele frequencies were statistically heterogeneous between the samples at seven of these loci and the differences were significant at the modified level. Thus, there was good evidence that genetic differences exist among all the samples so they were treated separately for further analysis.

OclWD_114315L_Garza variation

In the samples from Howard Lake, Howard Creek, and Libby Creek, the allele usually characteristic of Yellowstone cutthroat or rainbow trout was detected at the westslope marker *OclWD_114315L_Garza* at a frequency substantially higher than what was observed at the other westslope markers analyzed; 0.183, 0.414, and 0.389, respectively. Since no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers analyzed in the samples, they were excluded from subsequent analyses Furthermore, this indicates the variation detected at *OclWD_114315L_Garza* could represent hybridization with westslope cutthroat trout or rainbow trout genetic variation. In this situation, we strongly favor the latter interpretation as this variation was detected at unusually high frequency in previous samples analyzed from Libby Creek collected just below Big Cherry Creek (#4397, col. 7/15/11, 48.36412-37875 115.52733-53240, N=30, 0.300), Big Cherry Creek (#4398, col. 7/20/11, 48.32720-35267 115.52835-52615, N=30, 0.148), and Libby Creek Near Highway 2 (#4399, col. 8/19/11, 48.22450-22482 115.48008-47783, N=30, 0.113). Thus, we did not consider this locus to be a westslope marker in the analysis of these samples.

Howard Lake 4453

Howard Lake has been extensively stocked with rainbow trout from 1928 to the present (Jim Dunnigan, Montana Fish, Wildlife & Parks, personal communication). From 1928-1972, 3,300 to 20,000 rainbow trout of undesignated origin were annually stocked into the lake. Beginning in 1974 and continuing through 2001, 1000-3000 Arlee rainbow trout were stocked annually. From 2002 through 2009, about 3000 triploid

Kamloops redband rainbow trout were annually stocked into the lake. In 2010 until the present, 3000 Gerrard triploid redband rainbow trout have been stocked annually. Natural reproduction does occur in two of the inlet streams to the lake (Jim Dunnigan, personal communication) so it is certainly possible despite the reliance on stocking sterile triploids since 2002 the lake may still contain a fair proportion of fertile diploid individuals.

Alleles characteristic of westslope cutthroat trout were detected at two of the rainbow markers but, at none of the westslope markers analyzed in the sample from Howard Lake. This could indicate a small amount of hybridization or it could simply be rainbow trout genetic variation. In this situation, we tend to favor the former interpretation as hybridization with westslope cutthroat trout has been detected in numerous other samples collected throughout the Libby Creek drainage. Although the allele frequencies were statistically heterogeneous (X^2_{36}=59.548, P<0.001) among the rainbow and westslope markers, the westslope cutthroat trout alleles appeared to be randomly distributed (X^2_1=3.809, P>0.05) among the fish in the sample. This sample, therefore, appears to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a major (0.998) rainbow trout genetic contribution. In contrast, the q values obtained from STRUCTURE did not appear to be randomly (X^2_9=31.627, P<0.001) distributed among the individuals. Most of the fish in the sample appeared to have intermediate q values encompassing a broad range but, one fish had a value suggesting it may be a redband rainbow trout (Figure 1). The former fish very well may constitute progeny of diploids and the latter a stocked triploid. Overall, therefore, the fish in this sample appeared to have a substantial redband and coastal rainbow trout genetic contribution and a minor westslope cutthroat trout component. At the individual level, however, 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 and that the lake may predominantly contain fertile diploids and a small proportion of stocked triploid redband rainbow trout.

There is reasonable evidence that this sample contained individuals from genetically different populations. Out of 27 meaningful comparisons of observed to expected random mating genotypic proportions, two were significantly different. These comparisons remained significant at the modified level and both involved a deficit of heterozygotes. Furthermore, there was a nonrandom distribution of q values with many fish appearing to possess a substantial redband and coastal rainbow trout genetic contribution but, one individual appeared to be non-hybridized redband trout suggesting it may represent a stocked triploid.

Howard Creek 4454

In the sample from Howard Creek, alleles characteristic of westslope cutthroat trout were detected at five of the rainbow markers and two of the westslope markers that were analyzed. Although the allele frequencies were statistically heterogeneous (X^2_{36}=69.474, P<0.001) among the rainbow and westslope markers, the westslope cutthroat trout alleles appeared to be randomly distributed (X^2_2=2.361, P>0.10) among the fish in the sample. This sample , therefore, appears to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a major (0.994) rainbow trout genetic contribution. In contrast, the q values obtained from STRUCTURE did not appear to be randomly (X^2_4=73.005, P<0.001) distributed among the individuals in the sample. Most of the fish in the sample possessed q values characteristic of redband rainbow trout or a small to moderate amount of hybridization with coastal rainbow trout (Figure 2). There were three fish, however, with a q value indicating substantial hybridization between coastal and redband rainbow trout. The latter three fish do not appear to be solely responsible for the nonrandom distribution of redband and coastal rainbow trout genetic material among the fish as when they are removed from the data the distribution of q values is still nonrandom (X^2_2=14.508, P<0.001). Overall, therefore, the fish in this sample mainly appeared to have a substantial to moderate redband rainbow trout genetic contribution and a minor westslope cutthroat trout component. At the individual level, however, 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 and three individuals appeared to have an unusually large coastal rainbow trout genetic contribution.

Deviations of observed from expected random mating genotypic proportions and the nonrandom distribution of q values both suggest the sample may have contained individuals from two or more genetically divergent populations. Out of 29 meaningful comparisons to expected Hardy-Weinberg proportions, five were significant. These differences remained significant at the modified level and four of them involved a deficit of heterozygotes suggesting there was a tendency for there to be a deficit of heterozygotes among the fish.

Libby Creek Above and Below Confluence with Howard Creek 4455

Alleles characteristic of westslope cutthroat trout were detected at six of the rainbow markers and three of the westslope markers analyzed in the sample from Libby Creek. Although the allele frequencies were statistically heterogeneous (X^2_{36}=54.867, P<0.001) among the rainbow and westslope markers, the westslope cutthroat trout alleles appeared to be randomly distributed (X^2_1=1.541, P>0.05) among the fish in the sample. This sample, therefore, appears to have come from a hybrid swarm between rainbow and westslope cutthroat trout with a major (0.950) rainbow trout genetic contribution. In contrast, the q values obtained from STRUCTURE did not appear to be randomly (X^2_3=15.021, P<0.01) distributed among the individuals. With the exception of one fish, all of the individuals in the sample possessed q values characteristic of redband rainbow trout or a small to moderate amount of hybridization with coastal rainbow trout (Figure 3). The exceptional fish contained a substantial redband and coastal rainbow trout genetic component. This fish did not appear to be solely responsible for the nonrandom distribution of redband and coastal rainbow trout genetic material among the fish as when it was eliminated from the data the distribution of q values was still nonrandom (X^2_2=10.236, P<0.001). Overall, therefore, the fish in this sample mainly appeared to have a substantial to moderate redband rainbow trout genetic contribution and a minor westslope cutthroat trout component. At the individual level, however, 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 and one individual appeared to have an unusually large coastal rainbow trout genetic contribution.

There is some indication that this sample may have contained individuals from genetically different populations. Out of 29 meaningful comparisons of observed to expected random mating genotypic proportions, three were significantly different. These comparisons remained significant at the modified level and two involved a deficit of heterozygotes. Furthermore, there was a nonrandom distribution of q values and one fish had an unusually high coastal rainbow trout genetic contribution.

Possible Migrants from Howard Lake into Howard and Libby Creek

The primary reason for sampling Howard Lake, Howard Creek, and Libby Creek at the confluence with Howard Creek was to determine if there was any evidence of trout dispersing from the lake into the creeks. In order to examine this, we used the migrant analysis of Rannala and Mountain (1997) available in GENECLASS2 (Piry et al. 2004). In order to allow for the possibility of fish dispersing into the sample locations from reaches further downstream in the Libby Creek drainage, in this analysis we included the samples collected from Big Cherry Creek, Libby Creek below Big Cherry Creek, and Libby Creek near Highway 2. With low levels of genetic divergence between samples, the possibility of spuriously identifying individuals as possible migrants increases. Thus, in order to account for this we estimated levels of genetic divergence (F_{ST}) between all possible pairs of samples using the procedure of Weir and Cockerham (1984) available in GENEPOP version 4.0.

Among all the samples, only ten percent of the individuals were identified as being potential first generation migrants (Table 4). Over half of these possible migrants involved the Big Cherry Creek, Libby Creek below

Big Cherry Creek, Libby Creek near Highway 2, and Howard Lake samples. The amount of divergence among these samples, however, was relatively small (range 0.000-0.053, mean=.036, Table 5). Thus, we can not exclude to possibility that some, if not all, of these fish may spuriously have been identified as migrants. In the Howard Creek sample, two individuals were identified as possible migrants from Howard Lake (Table 4). Likewise, one individual in the Libby Creek sample collected at the confluence with Howard Creek was identified as a potential migrant from Howard Lake (Table 4). We suspect that these fish may actually be migrants for two reasons. First, there was a moderate amount of genetic divergence between the creek and lake samples reducing the likelihood of spurious identification (Table 5). Furthermore, all three of these individuals had a high amount of admixture with coastal rainbow trout which was unusual compared to the other fish in the samples but, was a general characteristic of the fish in Howard Lake. There was also some evidence of fish dispersing from Howard Creek and Libby Creek at the confluence with Howard Creek further downstream as collectively three fish from these samples were identified as potential migrants in the Libby Creek near Highway 2 sample (Table 4). All three of these fish had a very high redband rainbow trout genetic contribution which was unusual compared to the other fish in the Libby Creek near Highway 2 sample but, generally is characteristic of the fish collected from Howard Creek and Libby Creek at the confluence with Howard Creek. There was also a moderate to large amount of genetic divergence between the two upstream and the downstream samples reducing the likelihood of erroneous migrant detection (Table 5).

Ruby Creek 4456

No alleles characteristic of rainbow trout were detected at the rainbow markers, no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers, and only alleles characteristic of westslope cutthroat trout were detected at the westslope markers analyzed in the sample from Ruby Creek. Thus, there was no evidence of hybridization with either rainbow or Yellowstone cutthroat trout in the sample. These results are similar to those obtained from a previous allozyme (#692, col. 8/25/92, T32N R34W S29, N=25) analysis of trout collected from Ruby Creek. With the combined sample size of 74, and 2162 rainbow trout and 2460 Yellowstone cutthroat trout diagnostic alleles examined we have much better than a 99 percent chance of detecting as little as a 0.5 percent rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was a non-hybridized westslope cutthroat trout population. Ruby Creek, therefore, almost certainly contains non-hybridized westslope cutthroat trout.

In the sample, only one locus was polymorphic and only one copy of the variant allele was detected at the locus. Thus, meaningful comparison of observed to expected random mating genotypic proportions was precluded.

Robb Leary

Sally Painter

Angela Lodmell

Literature Cited

Amish, S. J., P. A. Hohenlohe, S. Painter, R. F. Leary, C. Muhlfeld, F. W. Allendorf, and G. Luikart. 2012. RAD sequencing yields a high success rate for westslope cutthroat and rainbow trout species-diagnostic SNP assays. Molecular Ecology Resources 12:653-660.

Brunelli, J. P., G. H. Thorgaard, R. F. Leary, and J. L. Dunnigan. 2008. Single-nucleotide polymorphisms associated with allozyme differences between inland and coastal rainbow trout. Transactions of the American Fisheries Society 137:1292-1298.

Campbell, N. R., S. J. Amish, V. L. Pritchard, K. S. McKelvey, M. K. Young, M. K. Schwartz, J. C. Garza, G. Luikart, and S. R. Narum. 2012. Development and evaluation of 200 novel SNP assays for population genetic studies of westslope cutthroat trout and genetic identification of related taxa. Molecular Ecology Resources 12:942-949.

Campbell, N. R., K. Overturf, and S. R. Narum. 2009. Characterization of 22 novel single nucleotide polymorphism markers in steelhead and rainbow trout. Molecular Ecology Resources 9:318-322.

Finger, J. A., M. R. Stephens, N. W. Clipperton, and B. May. 2009. Six diagnostic single nucleotide polymorphism markers for detecting introgression between cutthroat and rainbow trouts. Molecular Ecology Resources 9:759-763.

Goudet, J., M. Raymond, T. deMeeus, and F. Rousset. 1996. Testing differentiation in diploid populations. Genetics 144:1933-1940.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372.

Harwood, A. S., and R. B. Phillips. 2011. A suite of twelve single nucleotide polymorphism markers for detecting introgression between cutthroat and rainbow trout. Molecular Ecology Resources 11:382-385.

Kalinowski, S. T., B. J. Novak, D. P. Drinan, R. deM Jennings, and N. V. Vu. 2011. Diagnostic single nucleotide polymorphisms identifying westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) and rainbow trout (*Oncorhynchus mykiss*). Molecular Ecology Resources 11:389-393.

Piry, S., A. Alapetite, J.-M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity 95: 536-539.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Pritchard, J. K., W. Wen, and D. Faulsh. 2007. Documentation for *structure* software: version 2.2. http://pritch.bsd.uchicago.edu/software.

Rannala, B., and J. L. Mountain. 1997. Detecting immigration using multilocus genotypes. Proceedings National Academy of Sciences 94:9197-9221.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103-106.

Stephens, M. R., N. W. Clipperton, and B. May. 2009. Subspecies-informative SNP assays for evaluating introgression between native golden trout and introduced rainbow trout. Molecular Ecology Resources 9:339-343.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 28:1358-1370.

Table 1

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

Rainbow Markers			Reference
	Taxa and characteristic alleles		
Locus	Rainbow	Westslope/Yellowstone	
OmyRD_RAD_29252_Hoh	11	22	Amish et al. 2012
OmyRD_RAD_77157_Hoh	11	22	Amish et al. 2012
OmyRD_RAD_30378_Hoh	11	22	Amish et al. 2012
OclRD_P53T7R1_Har	11	22	Harwood and Phillips 2011
OmyRD_RAD_30423_Hoh	11	22	Amish et al. 2012
OmyRD_RAD_59515_Hoh	11	22	Amish et al. 2012
OclRD_Thymo_320Kal	11	22	Kalinowski et al. 2011
OmyRD_RAD_48301_Hoh	11	22	Amish et al. 2012
OmyRD_RAD_49759_Hoh	11	22	Amish et al. 2012
OclRD_P53T7R2_Har	11	22	Harwood and Phillips 2011
OmyRD_URO_302May	11	22	Finger et al. 2009
OmyRD_RAD_20663_Hoh	22	11	Amish et al. 2012
OmyRD_RAD_51740_Hoh	11	22	Amish et al. 2012
OmyRD_RAD_22111_Hoh	22	11	Amish et al. 2012
OmyRD_RAD_55820_Hoh	22	11	Amish et al. 2012
OmyRD_RAD_5666_Hoh	11	22	Amish et al. 2012
OmyRD_F5_136May	22	11	Finger et al. 2009
OmyRD_RAD_42014_Hoh	22	11	Amish et al. 2012
OmyRD_RAD_54584_Hoh	22	11	Amish et al. 2012

Westslope Markers			
	Taxa and characteristic alleles		
Locus	Westslope	Rainbow/Yellowstone	
OclWD_CLK3W1_Har	22	11	Harwood and Phillips 2011
OclWD101119_Garza	22	11	Campbell et al. 2012
OmyWD_RAD_76689_Hoh	22	11	Amish et al. 2012
OclWD_114315L _Garza	22	11	Campbell et al. 2012
OclWD_Tnsf_387Kal	22	11	Kalinowski et al. 2011
OmyWD_RAD_55391_Hoh	22	11	Amish et al. 2012
OclWD_P53_307Kal	22	11	Kalinowski et al. 2011
OclWD111312_Garza	22	11	Campbell et al. 2012
OclWD_107031L _Garza	22	11	Campbell et al. 2012
OclWD_PrLcW1_Har	22	11	Harwood and Phillips 2011
OmyWD_RAD_54516_Hoh	22	11	Amish et al. 2012
OclWD_105075L_Garza	22	11	Campbell et al. 2012
OmyWD_RAD_52968_Hoh	22	11	Amish et al. 2012
OclWD114336_Garza	11	22	Campbell et al. 2012
OclWD103713_Garza	22	11	Campbell et al. 2012
OclWD107074_Garza	22	11	Campbell et al. 2012
OclWD109651_Garza	22	11	Campbell et al. 2012
OclWD_129170L _Garza	11	22	Campbell et al. 2012
OclWD_ppie_32NC	11	22	Campbell et al. 2012

Table 1-continued

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

Table 2

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

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

Table 3

SNP loci that differentiate redband and coastal rainbow trout.

Locus	Taxa and predominant alleles		Reference
	Redband	Coastal	
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

Table 4

Results of migrant analysis using samples from Big Cherry Creek, Libby Creek below Big Cherry Creek (Libby BBC), Libby Creek near Highway 2 (Libby-2), Libby Creek at Howard Creek confluence (Libby-How), Howard Creek (Howard C) and Howard Lake (Howard L). Numbers in bold indicate potential first generation migrants.

	Sample					
Sample	Big Cherry	Libby BBC	Libby-2	Libby-How	Howard C	Howard L
Big Cherry	26		**1**			**1**
Libby BBC	**2**	28	**2**			
Libby-2	**1**	**2**	23			
Libby-How			**2**	26	**1**	
Howard C			**1**		26	
Howard L			**1**	**1**	**2**	26

Table 5

F_{ST} between samples collected from Big Cherry Creek, Libby Creek below Big Cherry Creek (Libby BBC), Libby Creek near Highway 2 (Libby-2), Libby Creek at Howard Creek confluence (Libby-How), Howard Creek (Howard C), and Howard Lake (Howard L).

	Sample				
Sample	Big Cherry	Libby BBC	Libby-2	Libby-How	Howard C
Libby BBC	0.000				
Libby-2	0.030	0.044			
Libby-How	0.156	0.182	0.053		
Howard C	0.107	0.129	0.030	0.044	
Howard L	0.052	0.053	0.037	0.109	0.085

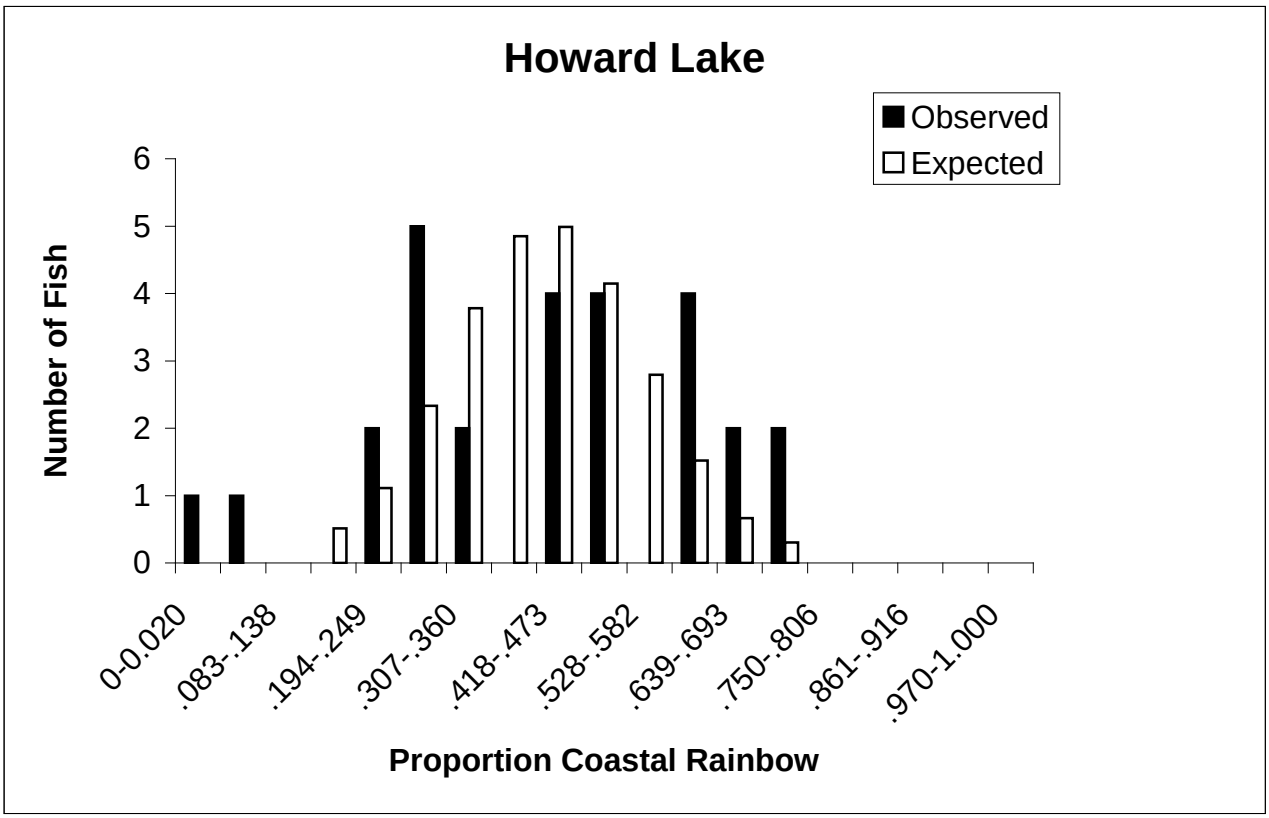

Figure 1. Observed and expected random distribution of the proportion of the genome per individual derived from coastal rainbow trout in a sample showing evidence of hybridization between redband and coastal rainbow trout collected from Howard Lake. Note the observed distribution significantly differs from the expected random distribution indicating that the sample did not come from a hybrid swarm between redband and coastal rainbow trout.

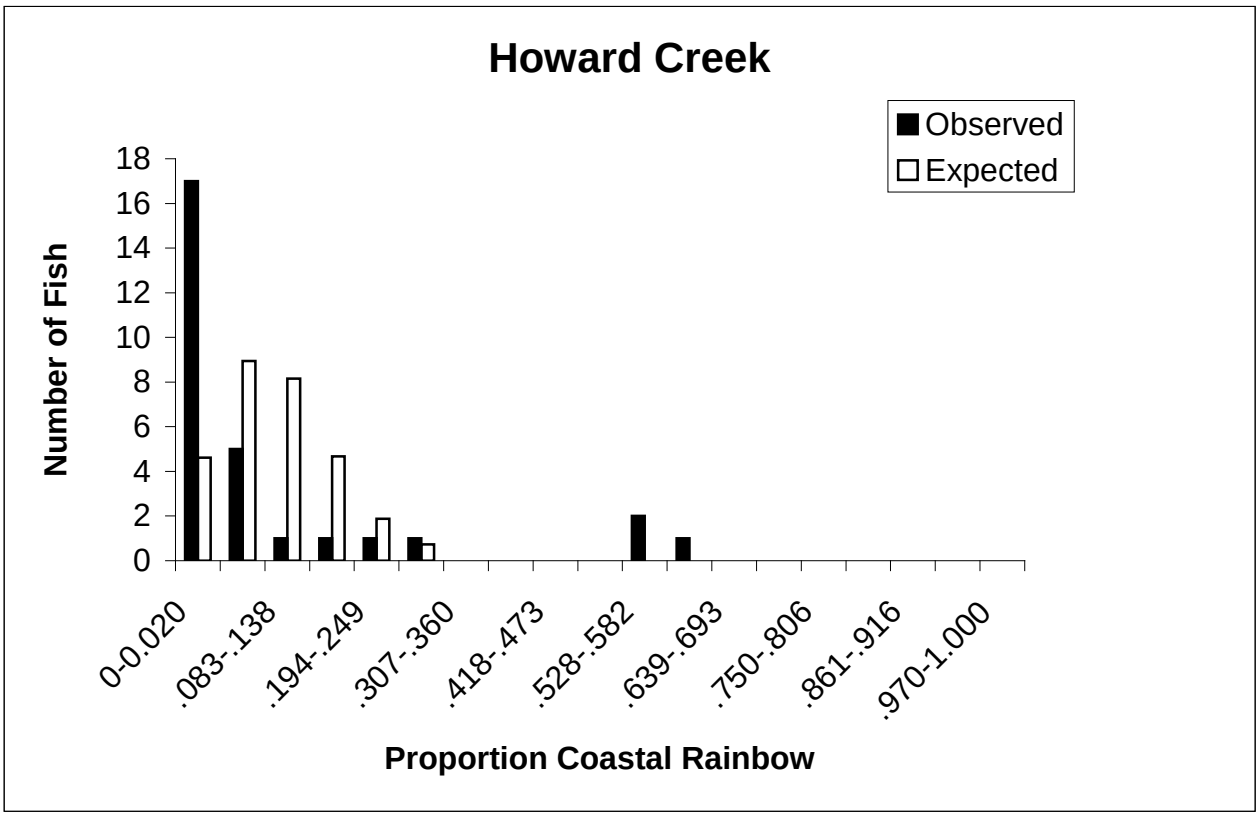

Figure 2. Observed and expected random distribution of the proportion of the genome per individual derived from coastal rainbow trout in a sample showing evidence of hybridization between redband and coastal rainbow trout collected from Howard Creek. Note the observed distribution significantly differs from the expected random distribution indicating that the sample did not come from a hybrid swarm between redband and coastal rainbow trout.

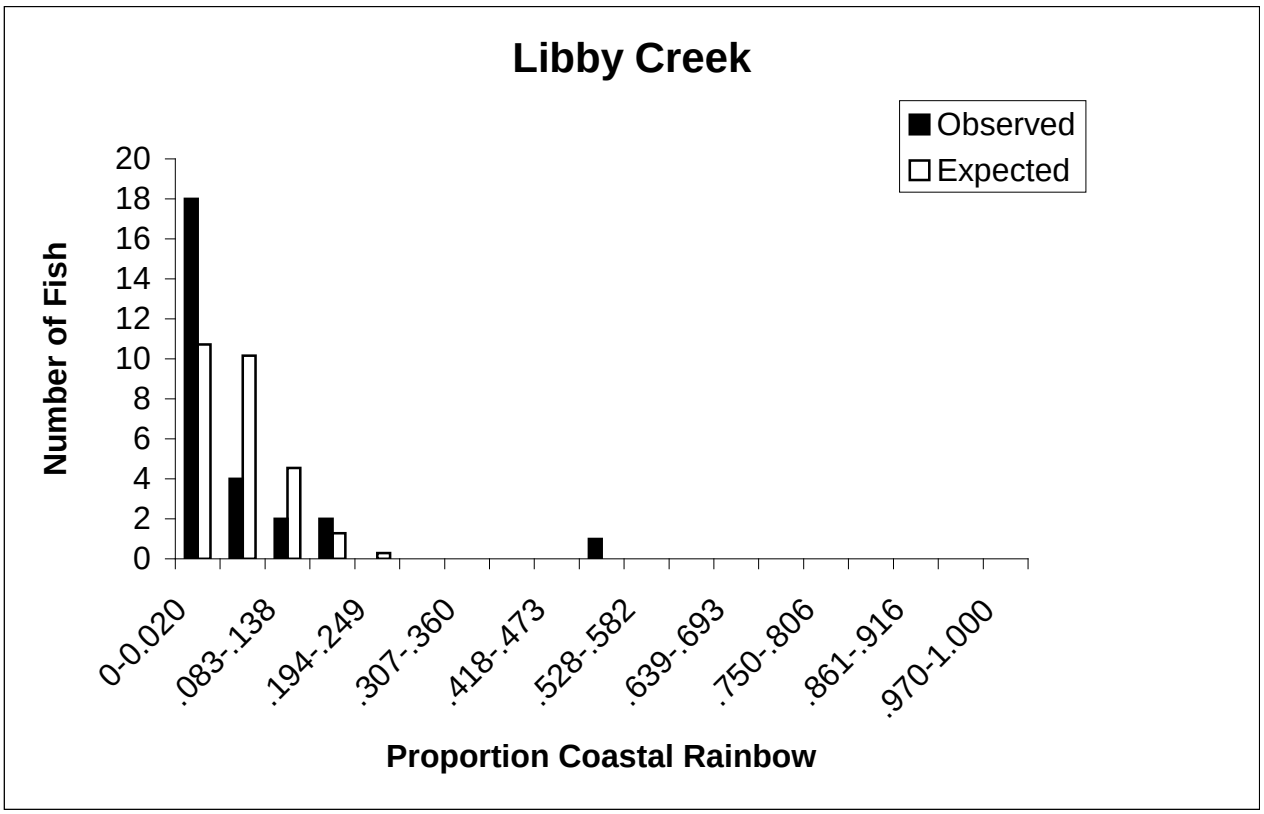

Figure 3. Observed and expected random distribution of the proportion of the genome per individual derived from coastal rainbow trout in a sample showing evidence of hybridization between redband and coastal rainbow trout collected from Libby Creek at the confluence with Howard Creek. Note the observed distribution significantly differs from the expected random distribution indicating that the sample did not come from a hybrid swarm between redband and coastal rainbow trout.