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Dave;

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

Sample #	Water Name/Location/ Collection Date/ Collector	a N	b #Markers	c Taxa ID	d Power	e %	f # Fish
4382	**South Fork Madison River 9/4/2012 Bruce Roberts**	**113**	**R39Y40**	**WCT X RBT** **WCT X RBT**		**W94.5 X R5.5**	**89** **24**

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

Methods and Data Analysis

We developed a 'chip' that allows us to simultaneously genotype up to 95 single nucleotide polymorphic loci (SNPs) obtained from the literature (Aguilar and Garza 2008; Finger et al. 2009; Harwood and Phillips 2011; Kalinowski et al. 2011; Amish et al. 2012) or via personal communication (Shawn Narum and Nathan Campbell, Columbia River Inter-Tribal Fish Commission, Hagerman, Idaho) in 96 trout using a Fluidigm EP1 Genotyping System. Each SNP locus has only two states (alleles). Thus, considering hybridization among rainbow (*Oncorhynchus mykiss*), 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), 20 loci that distinguish westslope cutthroat from rainbow and Yellowstone cutthroat trout (westslope markers), and 20 loci that distinguish Yellowstone cutthroat from westslope cutthroat and rainbow trout (Yellowstone markers, Table 1). We verified the diagnostic property of each marker by analyzing them in reference samples that had previously been determined to be non-hybridized westslope cutthroat, Yellowstone cutthroat, or rainbow trout by analysis of allozymes, paired interspersed nuclear elements (PINEs), a combination of insertion/deletion (indel loci) events and microsatellite loci, or two or all of these techniques (Table 2).

If a sample possessed alleles characteristic of only westslope cutthroat trout at all westslope markers and had no alleles characteristic of rainbow trout at the rainbow markers or Yellowstone cutthroat trout at the Yellowstone markers, then it was considered to have come from a non-hybridized westslope cutthroat trout population. Evidence for potential hybridization between rainbow and westslope cutthroat trout was generally considered to be present when three criteria were met. First, the sample had to contain alleles characteristic of rainbow trout at, at least, some of the rainbow markers. Next, at least some of the westslope markers also had to be genetically variable (polymorphic). Finally, no Yellowstone cutthroat trout alleles were detected at the Yellowstone markers. In this situation, the alleles at the rainbow markers shared between westslope cutthroat and Yellowstone cutthroat trout can confidently be assigned to having originated from westslope cutthroat trout and the alleles shared between rainbow and Yellowstone cutthroat trout at the westslope markers can confidently be assigned to having originated from rainbow trout. Thus, in terms of hybridization between westslope cutthroat and rainbow trout the data set contains information from 39 diagnostic loci. Likewise, when evidence of hybridization was detected only between westslope and Yellowstone cutthroat trout (no rainbow alleles at rainbow markers, at least some westslope markers polymorphic, and Yellowstone cutthroat trout alleles present at, at least, some Yellowstone markers) the data set contains information from 40 diagnostic loci. When all three sets of markers were polymorphic, this generally indicates hybridization among all three taxa. In this situation, the rainbow markers (19) provide information about rainbow trout hybridization and the Yellowstone markers (20) provide information about Yellowstone cutthroat trout hybridization.

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

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

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

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

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

The strongest evidence that a sample showing evidence of hybridization did not come from a hybrid swarm is failure of the observed distribution of hybrid indices to reasonably conform to the expected random distribution. The most likely reasons for this are that the population has only recently become hybridized or the sample contains individuals from two or more populations with different amounts of admixture. At times, the distribution of genotypes at marker loci and the observed distribution of hybrid indices can provide insight into which of these two factors appears mainly responsible for the nonrandom distribution of the alleles from the hybridizing taxa among individuals in the sample. At other times, the distribution of genotypes at marker loci and the observed distribution of hybrid indices may provide little or no insight into the cause of the nonrandom distribution of alleles among individuals. The latter situation is expected to be fairly common as the two factors usually responsible for the nonrandom distribution of alleles are not necessarily mutually exclusive. Regardless of the cause, when alleles at the marker loci do not appear to be randomly distributed among individuals in a sample, estimating the amount of admixture has little if any biological meaning and, therefore, is generally not reported.

Failure to detect evidence of hybridization in a sample does not necessarily mean the population is non-hybridized because there is always the possibility that we would not detect evidence of hybridization because of sampling error. When no evidence of hybridization was detected in a sample, we assessed the likelihood the population is non-hybridized by determining the chances of not detecting as little as a 0.5 percent genetic

contribution of a non-native taxon to a hybrid swarm. This is simply 0.995^{2NX} where N is the number of fish in the sample and X is the number of marker loci analyzed.

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

Results and Discussion

South Fork Madison River 4382

When the upper South Fork Madison River was first sampled (#1297, col. 10/13/98, N=10), allozyme analysis indicated it contained a hybrid swarm between westslope cutthroat and rainbow trout with a predominant (0.96) westslope cutthroat trout genetic component. By 2006, the genetic characteristics of the trout in the upper reach had markedly changed. This change was probably due to the invasion of individuals with a high proportion of rainbow trout alleles and hybrids with a high amount of admixture and subsequent reproduction of these fish (#3414, col. 7/10/06, N=26; #3918, col. 7/16/09, N=25; #4269, col. 8/30/11, N=55).

In order to prevent increased admixture in this reach two management actions have been taken. Below the reach, a three foot drop was blasted in bedrock to prevent further invasion. During September 2011, 242 trout were captured from the upper reach, marked, and placed in live cars in the stream. After genetic analysis (#4271), all individuals with greater than a 15% rainbow trout genetic contribution were removed and placed below the barrier. The remaining fish were released into the upper reach. The present sample represents a continuation of the latter effort.

In the sample, alleles characteristic of rainbow trout were detected at all of the rainbow markers and all of the westslope markers were polymorphic. No alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers. As expected from the previous samples, the allele frequencies were statistically heterogeneous (X^2_{37}=414.663, P<0.001) among the rainbow and westslope markers and the rainbow trout alleles did not appear to be randomly distributed (X^2_{12}=732.743, P<0.001) among the fish in the sample. Rather, there was a much more variable and wider range of hybrid indices than expected by chance (Figure 1). As in 2011, all individuals with greater than a 15% rainbow trout genetic contribution (hybrid index greater than 11, Table 3) were placed below the barrier and the remaining individuals with an average westslope cutthroat trout genetic contribution of 0.945 were released into the upper reach.

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Table1

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	
	Taxa and characteristic alleles	
	Rainbow	Westslope/Yellowstone
OmyRD_RAD_29252_Hoh	11	22
OmyRD_RAD_77157_Hoh	11	22
OmyRD_RAD_30378_Hoh	11	22
OclRD_P53T7R1_Har	11	22
OmyRD_RAD_30423_Hoh	11	22
OmyRD_RAD_59515_Hoh	11	22
OclRD_Thymo_320Kal	11	22
OmyRD_RAD_48301_Hoh	11	22
OmyRD_RAD_49759_Hoh	11	22
OclRD_P53T7R2_Har	11	22
OmyRD_URO_302May	11	22
OmyRD_RAD_20663_Hoh	11	22
OmyRD_RAD_51740_Hoh	11	22
OmyRD_RAD_22111_Hoh	11	22
OmyRD_RAD_55820_Hoh	11	22
OmyRD_RAD_5666_Hoh	11	22
OmyRD_F5_136May	11	22
OmyRD_RAD_42014_Hoh	11	22
OmyRD_RAD_54584_Hoh	11	22
	Westslope Markers	
	Taxa and characteristic alleles	
	Westslope	Rainbow/Yellowstone
OclRD_CLK3W5_Har	11	22
OclWD_CLK3W1_Har	11	22
OclWD101119_Garza	11	22
OmyWD_RAD_76689_Hoh	11	22
OclWD_114315L _Garza	11	22
OclWD_Tnsf_387Kal	11	22
OmyWD_RAD_55391_Hoh	11	22
OclWD_P53_307Kal	11	22
OclWD111312_Garza	11	22
OclWD_107031L _Garza	11	22
OclWD_PrLcW1_Har	11	22
OmyWD_RAD_54516_Hoh	11	22
OclWD_105075L_Garza	11	22
OmyWD_RAD_52968_Hoh	11	22
OclWD114336_Garza	11	22
OclWD103713_Garza	11	22
OclWD107074_Garza	11	22
OclWD109651_Garza	11	22
OclWD_129170L _Garza	11	22
OclWD_ppie_32NC	11	22

Table 1-continued

	Yellowstone Markers	
	Taxa and characteristic alleles	
	Yellowstone	Westslope/Rainbow
OclYD_CLK3Y1_Har	11	22
OclYGD100974_Garza	11	22
OclYGD110571_Garza	11	22
OclYSD117432_Garza	11	22
OclYGD127236_Garza	11	22
OclYGD112820_Garza	11	22
OclYGD104216_Garza	11	22
OclYGD113600_Garza	11	22
OclYSD129870_Garza	11	22
OclYGD104569_Garza	11	22
OclYGD117286_Garza	11	22
OclYGD117370_Garza	11	22
OclYSD107607_Garza	11	22
OclYGD106457_Garza	11	22
OclYSD106367_Garza	11	22
OclYGD107031_Garza	11	22
OclYGD106419_Garza	11	22
OclYSD123205_Garza	11	22
OclYGD109525_Garza	11	22
OclYSD113109_Garza	11	22

Table 2

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

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

Table 3

Fish identification number and hybrid index for trout collected from the South Fork Madison River.

Fish Number	Hybrid Index	Fish Number	Hybrid Index	Fish Number	Hybrid Index
1	0	26	2	51	7
2	5	27	4	52	5
3	6	28	5	53	4
4	1	29	3	54	6
5	5	30	3	55	26
6	0	31	4	56	2
7	27	32	3	57	3
8	7	33	5	58	5
9	8	34	5	59	22
10	2	35	3	60	3
11	5	36	10	61	2
12	4	37	0	62	0
13	37	38	3	63	5
14	2	39	9	64	8
15	2	40	3	65	21
16	5	41	3	66	9
17	6	42	7	67	5
18	3	43	4	68	5
19	12	44	6	69	5
20	6	45	26	70	4
21	12	46	5	71	1
22	0	47	9	72	5
23	4	48	6	73	2
24	2	49	29	74	1
25	5	50	13	75	6

Table 3-continued

Fish Number	Hybrid Index	Fish Number	Hybrid Index
76	2	101	28
77	7	102	25
78	7	103	5
79	11	104	27
80	3	105	4
81	23	106	19
82	21	107	14
83	2	108	22
84	3	109	4
85	1	110	8
86	0	111	4
87	8	112	2
88	4	113	28
89	24		
90	14		
91	24		
92	2		
93	1		
94	3		
95	4		
96	5		
97	22		
98	6		
99	18		
100	1		

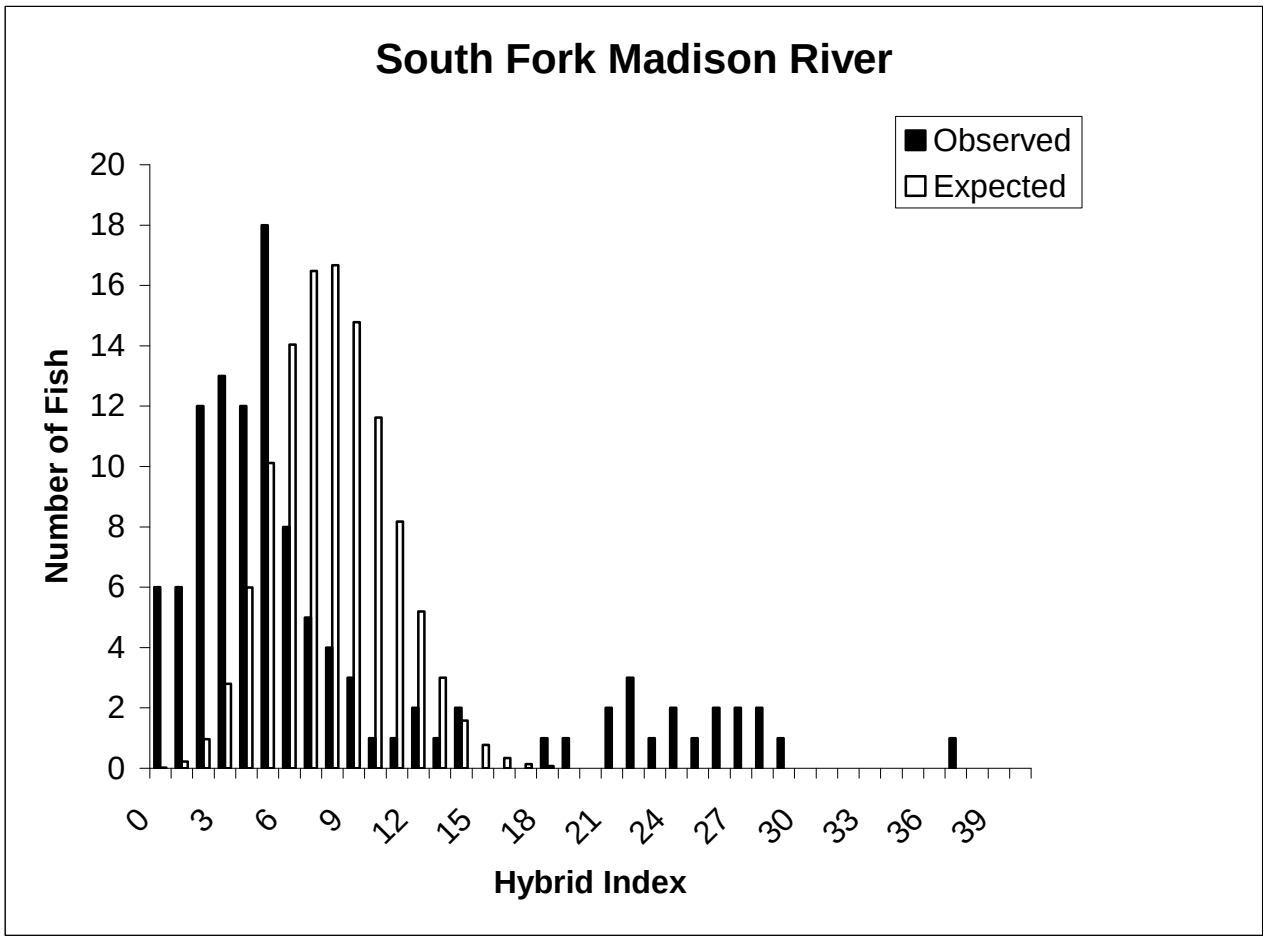

Figure 1. Observed and expected random distribution of hybrid indices among the trout collected from the upper reach of the South Fork Madison River showing evidence of hybridization between westslope cutthroat and rainbow trout. Note the observed distribution significantly ($P<0.001$) differs from the expected distribution. All fish with a hybrid index of greater than 11 were placed downstream of the reach below a fish passage barrier. The other fish were released back into the reach.