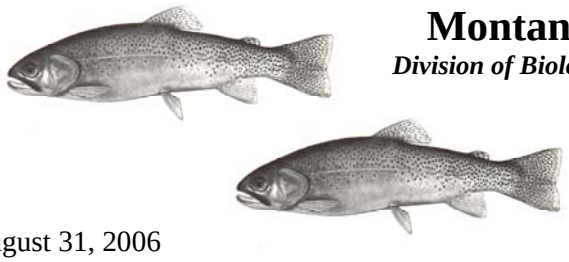

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Clint:

A technique examining insertion/deletion (indel) events has been used to analyze DNA extracted from fin clips taken from individuals in the following trout samples from the North Fork Flathead River drainage:

Summary of results.

Sample #	Water Name/Location/Collection Date/ Collector	N [a]	# markers [b]	Taxa ID [c]	Power (%) [d]	% WCT [e]	Individuals [f]
3304	**AK Creek**	5	R7Y1	WCT	R51Y10	100	
	8/2/2006 Clint Muhlfeld						
3305	**Cutthroat Creek**	30	R7Y1	WCT	R98Y44	100	
	8/1/2006 Clint Muhlfeld						
3307	**Foisey Creek**	62	R7Y1	WCT	R99Y71	100	
	8/2/2006 Clint Muhlfeld						

[a]Number of fish successfully analyzed. If combined with a previous sample, the number in parentheses indicates the combined sample size.
[b]Number of markers analyzed that are diagnostic for the non-native taxa (R=rainbow trout *Oncorhynchus mykiss*, W=westslope cutthroat trout *O. clarki lewisi*, Y=Yellowstone cutthroat trout *O. c. bouvieri*).
[c]Codes: 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. However, it must be noted that we cannot definitively 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 Power %). Taxa codes separated by "x" indicate hybridization between those taxa.
[d]Number corresponds to the percent chance we have to detect 1% hybridization given the number of individuals successfully analyzed and the number of diagnostic markers used. For example, with 25 individuals we have a 97% chance to detect as little as 1% hybridization with rainbow trout but, only a 40% chance to detect as little as 1% hybridization with Yellowstone cutthroat trout into what once was a westslope cutthroat trout population. Not reported when hybridization is detected.
[e]Indicates the genetic contribution of the hybridizing taxa in the order listed under c. This number is usually reported only if the sample appears to have come from a non-hybridized population or a hybrid swarm. The latter is a random mating population in which species markers are randomly distributed among individuals and essentially all individuals are of hybrid origin.
[f]Indicates number of individuals with genetic characteristics corresponding to the taxa ID code column when the sample can be analyzed on the individual level. This occurs when marker alleles are not randomly distributed among individuals and hybridization appears to be recent and/or if the sample appears to consist of a mixture of populations and hybrids and non-hybrids can be reliably distinguished.

Methods and Data Analysis

A technique developed by Ostberg and Rodriguez (2004) uses short synthetically made segments of DNA called primers, in pairs, to detect areas of DNA in trout that have undergone insertion/deletion (indel) events. During the polymerase chain reaction (PCR), the primers bind to specific areas of the organismal DNA and many copies of the DNA between the primers are made using dye labeled nucleotides. The indel events have resulted in length differences (alleles) in the region of DNA copied between the primers that characterize different trout taxa. These length differences have been found to be useful for the analysis of hybridization (e.g. Ostberg et al. 2004) and after PCR are separated from each other using capillary electrophoresis and visualized using an Applied Biosystems 3130x1 Genetic Analyzer. The alleles are labeled by the primers used and the number of nucleotides in the copied region. After electrophoresis, the alleles detected in an individual are determined by comparison to synthetic fragments of DNA of known length and alleles from previously analyzed individuals.

We used seven pairs of indel primers that distinguish westslope, *Oncorhynchus clarki lewisi*, and Yellowstone cutthroat trout, *O. c. bouvieri*, from rainbow trout, *O. mykiss* (Table 1). Unfortunately, only one of these seven primer pairs distinguishes westslope from Yellowstone cutthroat trout (Table 1) which greatly hinders our ability to conclusively detect hybridization between these fishes.

Primer pairs that produce alleles that distinguish different taxa are commonly termed diagnostic or marker loci because the alleles detected at them can be used to help determine whether a sample came from a non-hybridized population of one of the taxa or a population in which hybridization between taxa has or is occurring. Individuals from a non-hybridized population will possess alleles characteristic of only that taxon. In contrast, since half the DNA of first generation hybrids (F_1) comes from each of the parental taxa F_1 individuals will possess alleles characteristic of the two parental taxa at all diagnostic loci examined. In later generation hybrids (post F_1), the amount and particular regions of DNA acquired from the parental taxa will vary among individuals. Thus, the particular alleles detected in post F_1 hybrids will be highly variable among diagnostic loci within and among individuals.

An important aspect of indel alleles is that they are codominant. That is, when two different alleles at a diagnostic locus exist within an individual (heterozygote) both are readily detectable. Thus, the proportion of alleles from different taxa (proportion of admixture) in a sample can be directly determined by averaging the allele frequencies observed over all diagnostic loci analyzed.

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

A common attribute of hybrid swarms is that the allele frequencies will tend to be similar among diagnostic loci because the presence of the alleles at such loci in the population 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 appeared to be statistically homogeneous among the diagnostic loci using contingency table chi-square.

In order to determine whether or not the alleles at the diagnostic loci were randomly distributed among the fish in samples 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 diagnostic locus, the allele characteristic of the native taxon was given a value of zero and the 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 for native allele), one (both native and non-native alleles present, heterozygous), or two (only non-

native alleles present, homozygous for non-native alleles). 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 be characterized by a hybrid index of zero, non-hybridized rainbow trout by a hybrid index of 14, F_1 hybrids by a hybrid index of seven, and post F_1 hybrids could have values from zero to 14. The distribution of hybrid indices among the fish in a sample was statistically compared to the expected Poisson distribution based on the proportion of admixture in the sample estimated from the allele frequencies at the diagnostic loci. If the allele frequencies were statistically homogeneous among the diagnostic loci and the observed distribution of hybrid indices conformed to the expected Poisson distribution, then the sample was considered to have come from a hybrid swarm.

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

The strongest support that a sample showing evidence of hybridization did not come from a hybrid swarm is failure of the observed distribution of hybrid indices to conform to the expected Poisson 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 proportions of admixture. At times, the observed distribution of hybrid indices can provide insight into which of these two factors appears mainly responsible for the non-random distribution of the alleles from the hybridizing taxa among individuals in the population. At other times, the observed distribution of hybrid indices may provide little or no insight into the cause of the non-random distribution. The latter situation is expected to be fairly common as the two factors are not necessarily mutually exclusive. Regardless of the cause, when alleles at the diagnostic loci are not randomly distributed among individuals in a sample, estimating the proportion of admixture has little if any biological meaning and, therefore, is generally not calculated.

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 one percent genetic contribution of a non-native taxon to a hybrid swarm. This is simply 0.99^{2NX} where N is the number of fish in the sample and X is the number of marker loci analyzed.

Results and Discussion

AK Creek 3304

Indel alleles characteristic of only westslope cutthroat trout were detected in the sample from AK Creek. With a sample size of five fish, we have a 95% chance of detecting only as little as a 4.5% rainbow trout or a 31% Yellowstone cutthroat trout genetic contribution to a hybrid swam. Thus, there is a good possibility that the AK Creek population may be slightly hybridized with rainbow trout, Yellowstone cutthroat, or both but, evidence of this was not detected due to sampling error. Given this uncertainty, the conservative approach is to consider the AK Creek population non-hybridized westslope cutthroat trout unless future data indicate otherwise.

Cutthroat Creek 3305

Indel alleles characteristic of only westslope cutthroat trout were detected in the sample from Cutthroat Creek. With the sample size of 30, we have a 98.2% chance of detecting as little as a one percent rainbow trout genetic contribution to a hybrid swarm, but a 95% chance of detecting only as little as a five percent Yellowstone cutthroat trout genetic contribution. The Cutthroat Creek population, therefore, is almost certainly not hybridized with rainbow trout but, there is a reasonable chance it could be slightly hybridized with Yellowstone cutthroat trout and this was not detected because of sampling error. We suspect the latter is unlikely, however, because the presence of Yellowstone cutthroat trout alleles in North Fork Flathead River drainage populations is uncommon (e.g. Hitt et al. 2003; Boyer 2006). Thus, unless future data indicate otherwise the Cutthroat Creek population should be considered to be non-hybridized westslope cutthroat trout.

Foisey Creek 3307

Fish were collected from upper (N=32) and lower (N=30) Foisey Creek. Since indel alleles characteristic of only westslope cutthroat trout were detected in both samples, they were combined for further analysis. With the combined sample size of 62, we have better than a 99% chance of detecting as little as a one percent rainbow trout genetic contribution to a hybrid swarm but, only a 95% chance of detecting as little as a 2.5% Yellowstone cutthroat trout genetic contribution to a hybrid swarm. The Foisey Creek population, therefore, is almost certainly not hybridized with rainbow trout but, there is a reasonable chance it may be slightly hybridized with Yellowstone cutthroat trout and this was not detected because of sampling error. We suspect the latter is unlikely, however, because the presence of Yellowstone cutthroat trout alleles in North Fork Flathead River drainage populations is uncommon (e.g. Hitt et al. 2003; Boyer 2006). Thus, unless future data indicate otherwise the Foisey Creek population should be considered to be non-hybridized westslope cutthroat trout.

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John Powell

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Table 1: Fragment lengths of diagnostic insertion or deletion alleles for westslope cutthroat, Yellowstone cutthroat, and rainbow trout (adapted from Ostberg and Rodriguez 2004).

Marker	Yellowstone	Westslope	Rainbow
OCC34	225	225	215
OCC35	230	230	200
OCC36	325	325	275
			285
OCC37	270	270	260
OCC38	175	175	150
OCC42	190	190	160
OM55	180	220	200