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Jim,

We have completed the analysis of the high priority samples from the Beaverhead-Deerlodge National Forest.

The results are summarized below.

Sample Location	N	Collection Date	Legal Description	%RBT	%YSCT	%WSCT
Divide Creek South Fork	18	8/7/02	12S 14W 16BB	0.04	0.05	0.91
Divide Creek South Fork	25	8/7/02	12S 14W 9AD	0.04	0.01	0.95
Divide Creek South Fork (trib 1)	6	8/7/02	12S 14W 16BB	0.07	0.00	0.93
Divide Creek North Fork (upper)	25	8/13/02	12S 14W 5DB	0.00	0.06	0.94
Divide Creek North Fork (lower)	25	6/25/02	11S 14W 4DC	0.00	0.04	0.96
Hell Roaring Creek	14	7/15/02	14S01E 2Y C,C	0.00	0.91	0.09
Long Creek	4	8/19/02	12S 03W 31 D,B	0.02	0.17	0.81
Little Beaver Creek	25	8/14/02	15S 08W 17AS	0.01	0.09	0.89
Blair Lake Trib	10	7/17/02	14S 01E 36BC	0.00	0.77	0.23
West Creek	3	8/20/02	13S 04W 17BD	0.03	0.09	0.88
Clover Creek East Fork	15	8/7/02	13S 05W 04CB	0.00	0.08	0.92
Dad Creek	25	8/14/02	12S 12W 29AB	0.00	0.29	0.71

Sample numbers: *South Fork Divide Creek #3163 and #3164, South Fork Divide Creek tributary 1 #3165, North Fork Divide Creek (upper) #3166, North Fork Divide Creek (lower) #3167, Hell Roaring Creek #3169, Long Creek #3170, Little Beaver Creek #3171, Blair Lake tributary #3172, West Creek 3173, #East Fork Clover Creek #3174, Dad Creek #3175.*

Unfortunately, there were no samples that did not contain hybrids. In all cases, the hybridized individuals appeared to be post-F1 hybrids suggesting that hybridization has been an ongoing occurrence for some time.

The sample from Divide Creek Tributary 1 consists of six rather than seven samples because one tube did not contain a fin clip.

Brief Description of Methods:

Polymerase chain reaction (PCR) amplification of paired interspersed nuclear DNA elements (PINEs) was used to determine each fish's genetic characteristics at multiple regions of the nuclear DNA. This method produces DNA fragments that can be used to distinguish between various cutthroat trout subspecies (*Oncorhynchus clarki spp.*), rainbow trout (*O. mykiss*) and their hybrids,

and between bull trout (*Salvelinus confluentus*), brook trout (*S. fontinalis*), and their hybrids. The presence of a PINE marker is dominant to absence. First-generation (F_1) hybrids will have all the diagnostic markers characteristic of the two hybridizing species. Backcrossed individuals will possess some, but not all, markers characteristic of both parental species. The appearance of a marker indicates the individual is either heterozygous or homozygous for that marker, which precludes us from directly calculating allele frequencies.

Unless the distribution of markers dictates otherwise, we assume the samples conform to random mating expectations in order to estimate the average genetic contribution from each species. In these cases, we report the percent genetic contribution from each species present in the population. When hybridization is present in these situations, the population is considered a hybrid swarm. Regardless of the percent contribution from the non-native species, in hybrid swarms, all individuals are of hybrid origin, even those that appear "pure" at our diagnostic loci. It is not possible to rescue pure individuals from these populations, as they likely do not exist. Due to the random reshuffling of alleles during sexual reproduction, many individuals will appear pure for one or the other parental species due to the limited number of marker loci used. It has been shown that 6 markers are adequate to provide coarse classification of hybridization, but upwards of 70 markers are required to discriminate between pure individuals, if they exist, and backcrossed individuals in hybrid swarms (Boecklen and Howard 1997).

However, when the distribution of non-native markers appears to be non-random, it is not valid to report genetic contributions of the component species at the population level, as they do not come from a randomly mating population. It is likely that the individuals in these samples either come from populations where hybridization is recent or are from admixtures of populations. Samples can be analyzed at the individual level only. These samples are not considered to come from hybrid swarms and some pure individuals may exist. In these cases, we report the number of individuals with genotypes corresponding to each species and/or the types of hybrids detected and do not report genetic contribution percentages.

Literature Cited:

Boecklen WJ, and Howard DJ (1997) Genetic analysis of hybrid zones: numbers of markers and power of resolution. *Ecology* 78 (8) pp. 2611-2616.

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