13 July 2004

Jim Brammer Beaverhead Deerlodge National Forest 420 Barrett Street Dillon, MT 59725

Jim,

We have completed the analysis of the first shipment of "low" priority samples from the Beaverhead-Deerlodge National Forest, Red Rocks drainage.

The results are summarized in Tables 1 and 2.

I have provided the proportion genetic contribution of westslope cutthroat, Yellowstone cutthroat, and rainbow trout assuming random mating populations for all samples except the Bear Creek Diversion. This sample contained an individual that appears to be an F1 hybrid between westslope cutthroat and rainbow trout. As such, the assumptions required to calculate allele frequencies are known to be violated. In addition to the F1, two of the remaining five individuals also exhibited evidence of hybridization with rainbow trout. The remaining three individuals had westslope markers only. However, we are unable to assess their true status.

In cases where we did not detect introgression, I have presented the probability that we would have detected 1% or more admixture if it existed. Please contact me if you have any further questions, I'd be happy to discuss the results with you.

Paul Spruell Wild Trout and Salmon Genetics Lab Division of Biological Sciences University of Montana Missoula MT 59812 (406) 243-6749

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 present 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.

Table 1. Sample Location Information

						UTM	
				Sample			
Sample #	Stream	Collection Date	Reach	Size	TRS	begin	end
24.02	Price Creek, West	07/04/0000		0	14S, 4W,	4000400	0400044
3182	FORK	07/31/2002	0	8	31AC	4936130	0409914
3183			2	2			
3184			3	6	150 111 22		
3185	Price Creek	07/30/2002		19	BC	4936176	0410708
3186	THE OFER	01/00/2002	4	11	DO	4000170	0410700
3187			5	8			
3188	Price Creek (trib 2)	07/30/2002	0	8		/033210	0/10612
0100		01/00/2002	NA	Ŭ	100, 411, 00/1	4000210	0410012
3189	Cottonwood Creek	07/10/2002	10/1	12			
0100	Oblighwood Oreck	01/10/2002		12	16S. 11W.		
3190			8	6	6DC	4925306	0342026
3191			9	6			
					14S, 4W,		
3192	Price Creek	07/31/2002		11	19DD	4937877	410355
3193			2	5			
3194			3	6			
	Price Creek, West						
3195	Fork	07/31/2002		21	15S, 4W, 6BD	4934365	0409589
			4	21			
3196	Price Creek	07/30/2002		12	15S, 4W, 5CB	4934129	0410885
3197			6	2			
3198			7	9			
3199			8	1			
		/ /		-	8S, 14W,		
3200	Painter Creek (trib 1)	09/10/2002		3	14DD	5000277	0321130
	above barrier		3	3			
2201	Daintar Crook (trib 1)	00/27/2002		10	8S, 14W,	1009597	0221021
3201		06/27/2002		12	2400	4990007	0321931
3202	below barrier		1	6			
3203			2	6	00 1414/		
3204	Painter Creek (trib 2)	09/03/2002		25	03, 14W, 14CD	5000789	0319836
3204		03/03/2002	1	11	1400	5000703	0313030
3205			י ר	1/			
3200			2	14	16S 11W		
3207	Cottonwood Creek	07/10/2002		3	5CB	4925650	342758
			7	3			
					16S. 11W.		
3208	Cottonwood Creek	07/10/2002		8	3BC	4926238	346354
3209			3	2			
3210			4	6			
					15S, 8W,		
3211	Sawmill Creek	08/14/2002		25	27AC	4928580	376074
	above barrier		10	25			
					15S, 7W,		
3212	Shineberger Creek	08/14/2002		25	21CC	4929293	383250
3213			1	18			
3214			2	7			

					8S, 13W,		
3215	Brown's Creek	08/27/2002		25	29CD	4996993	0324922
3216			1	8			
3217			2	17			
					15S, 8W,		
3218	Sawmill Creek	08/14/2002		25	23DC	4929476	377373
3219			1	2			
3220			8	9			
3221			9	14			
					8S, 14W,		
3222	Painter Creek	08/28/2002		25	25AD	4997813	0322388
3223			3	6			
3224			4	11			
 3225			5	8			
					16S, 11W,		
 3226	Bear Creek diversion	07/08/2002	NA	6	2CA	4925695	0348247
					15S, 10W,		
3227	Deadman Creek	07/15/2002		25	18DD	4922055	0351936
			18	25			
2220	Deer Greek			•	16S, 11W,	4004000	0045705
3220	Bear Creek			o	9AA	4924962	0345765
			4	8	160 11W		
3000	Bear Creek			13	165, 11W, 2CB	1025181	0347648
3229	Deal Cleek		2	0	200	4920401	0347040
3230			2	0			
3231		tel. encodere	3 **	4			
 3232		ink smudge		1	168 101		
3033	Deadman Creek	07/17/02		25	14CB	1921035	03526/1
2224	Deauman Creek	01/11/02	16	2 . 9	1400	+324033	0002041
0204 2025			10	0			
ა∠ა ວ		interneting	۱ <i>۱</i> **	10			
3236		ink smuage		2			

		% ge	enetic contribution		Pr to detect 1%		
Sample #	Stream	WSCT	YSCT	RBT	YSCT	RBT	
	Price Creek, West						
3182	Fork	100			0.47	0.62	
3183		100			0.15	0.21	
3184		100			0.38	0.52	
3185	Price Creek	100			0.78	0.9	
3186		100			0.59	0.73	
3187		100			0.47	0.62	
3188	Price Creek (trib 2)	100			0.47	0.62	
3189	Cottonwood Creek	0.961	0.033	0.006			
3190		0.954	0.046				
3191		0.956	0.022	0.022			
3192	Price Creek	100			0.59	0.73	
3193		100			0.33	0.45	
3194		100			0.38	0.52	
0101	Price Creek, West	100			0.00	0.02	
3195	Fork	100			0.82	0.92	
3196	Price Creek	0.978	0.022				
3197		0.927	0.073				
3198		0.981	0.019				
3199		100			0.08	0.11	
3200	Painter Creek (trib 1)	100			0.00	0.11	
5200	ahove barrier	100			0.21	0.5	
2201	Painter Crook (trib 1)	100			0.62	0.77	
3201	Failler Creek (thb T)	100			0.02	0.77	
3202	below barrier	100			0.30	0.52	
3203	Deinter Creek (trib 2)	100			0.30	0.32	
3204	Painter Creek (trib 2)	100			0.87	0.45	
3205		100			0.59	0.73	
3206		100			0.68	0.82	
3207	Cottonwood Creek	0.974		0.026	0.21		
	0.11	0.000		0.040	0.47		
3208	Cottonwood Creek	0.982		0.018	0.47		
3209		0.958		0.042	0.15		
3210		0.988		0.012	0.38		
3211	Sawmill Creek	0.947	0.047	0.006			
	above barrier						
3212	Shineberger Creek	0.941	0.050	0.009			
3213		0.921	0.071	0.008			
3214		0.97	0.019	0.011			
3215	Brown's Creek	100			0.98	0.98	
3216		100			0.47	0.62	
3217		100			0.75	0.87	
3218	Sawmill Creek	0.937	0.060	0.003			
3219		0.927	0.073			0.21	
3220		0.880	0.120			0.66	
3221		0.967	0.028	0.005			

Table 2. Sample Genetic Information

3222	Painter Creek	100			0.87	0.95
3223		100			0.38	0.52
3224		100			0.59	0.73
3225		100			0.47	0.62
3226	Bear Creek diversion	**	**	**	**	**
3227	Deadman Creek	0.971		0.029	0.87	
3228	Bear Creek	0.991		0.009	0.47	
3229	Bear Creek	0.878	0.082	0.04		
3230		0.886	0.098	0.016		
3231		0.929	0.038	0.033		
3232		0.464	0.286	0.25		
3233	Deadman Creek	0.926	0.021	0.053		
3234		0.981		0.019	0.47	
3235		0.946	0.026	0.054		
3236		0.718	0.073	0.209		