1999 Gather Genetics Summary

Genetic Marker Analysis of the
Feral Horse Herd from the McCullough Peaks
Herd Management Area, Wyoming, 1999

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The feral horses of the McCullough Peaks area of Wyoming were first genetically analyzed in 1992. A total of 47 horses were tested for genetic variation at seven blood group and the biochemical genetic systems. The analysis indicated that levels of genetic variation within the herd were relatively high. Periodic sampling and analysis of genetic variation at several year intervals provides an opportunity to observe trends in genetic variation, which could provide information about how management strategies are working. In this report, genetic variation in the McCullough Peaks herd is re-examined in samples from horses collected in 1999.

METHODS

A total of 41 blood samples were received at the Univ. Kentucky on October 26, 1999. 31 of the samples were labeled as being from Dry Creek Bench and 10 were from Whistle Creek. As in the 1992 study, seventeen genetic marker systems were analyzed. Seven systems were red blood cell alloantigen loci (the *A*, *C*, *K*, *P*, *Q* and *U* horse groups) tested by standard serological methods of agglutination and compliment mediated hemolysis. The other 10 systems were biochemical polymorphisms detected by electrophoretic techniques. These systems were Albumin (*ALB)*, Alpha-1-beta Glycoprotein (*A1B)*, serum Cholenesterase *(ES)*, Vitamin D Binding Protein *(GC)*, Glucose Phosphate Isomerase *(GPI)*, alpha Hemoglobin (*AHB)*, Phosphoglucomutase *(PGM)*, Phosphogluconate Dehydrogenase *(PGD)*, Protease Inhibitor *(PI)*, and Transferrin *(TRF)*. In addition to the above genetic systems, DNA was extracted from the blood samples and tested for variation at 12 equine microsatellite (mSat) systems. These were *AHT*4, *AHT*5, *ASB*2 *HMS*2, *HMS*3, *HMS*6, *HMS*7, *HTG*4, *HTG*6, *HTG*7, *HTG*10 and *VHL*20. These systems were tested using an automated DNA sequencer to separate Polymerase Chain Reaction (PCR) products.

A variety of genetic variability measures were calculated from the gene marker data. The measures were observed heterozygosity *(Ho)* which is the actual number of loci heterozygous per individual and is based upon biochemical loci only; expected heterozygosity *(He)* which is the predicted number of heterozygous loci based upon gene frequencies, it was calculated for biochemical and all marker systems *(TtlHe)*; effective number of alleles *(Ae)* which is a measure of marker system diversity; total number of variants *(TNV)*; and estimated inbreeding level *(Fis)* which is calculated as 1-*Ho/He*. These same measures were calculated for the mSat data.

Genetic markers also can provide some information about ancestry in some cases. Genetic resemblance to domestic horse breeds was calculated using a chord genetic distance measure which was then used in the construction of a genetic tree diagram (dendogram) by use of a restricted maximum likelihood (RML) procedure.

RESULTS AND DISCUSSION

Variants present and allele frequencies for the blood group and biochemical markers are given in Table 1. The observed variants were quite similar to those found in the 1992 sample with some minor differences. There were four variants found in 1992 that were not observed in the 1999 sample and seven variants found in the 1999 sample not seen in the 1992 horses. All differences were based upon only one or two copies of the variant in one sample that was not seen in the other. This can largely be explained by sampling error.

The comparison of the 1999 samples from Dry Creek Bench and Whistle Creek also showed only minor differences. The larger sample from the Dry Creek herd had 10 variants not found in Whistle Creek while this smaller sample had two variants not found in the Dry Creek sample. In all cases, the frequencies of the unshared variants were low, usually less than 0.05. For this reason, the two samples were pooled for all further analysis.

The allele frequencies of the variants found at the mSat systems are given in Table 2. There is no comparable mSat data from the 1992 collections.

Measures of genetic variation for the blood group and biochemical genetic systems along with comparative information from a range of domestic breeds and other feral populations are given in Table 3. Means for all domestic breeds and feral herds examined at the Univ. of Kentucky also are shown in Table 3. Observed and expected heterozygosity (*Ho* and *He)* values for biochemical systems of the McCullough Peaks herd are well above the mean values for both domestic breeds and feral populations. Values this high are probably indicative of mixed origins for the herd. The positive *Fis* value indicates a slight deficit of *Ho* compared to *He* but the difference is not statistically significant. However, there was a significant deficit of *He* compared to *Ho* at the *GC* system. A common cause of positive *Fis* (*He* higher than *Ho)* values is the mixing of different populations into a single sample. As this sample represented a mixing of the Dry Creek and Whistle Creek, variation at individual systems also was examined for each of these localities separately. The *Ho* deficit at *GC* was present at both localities. Also, a significant excess of *He* to *Ho* was observed at the *ALB* system in the Whistle Creek sample. *ALB* is tightly linked to *CG, so* these systems are not independent. Deficits of *Ho* at the *ALB* system also were present in the Dry Creek sample and the pooled sample but the values were not statistically significant. It is not apparent what the reason for the significant deficits in observed heterozygosity is but it does not appear to be due to mixed sampling. It also should not be due to the probable mixed origins of the herd because the sampling bias of mixed sampling would be observed in just a couple of generations of interbreeding.

Total heterozygosity *(TtlHe*) from both biochemical and blood group systems also was higher in the McCullough Peaks herd than the average of either domestic breeds or feral

populations. Genetic diversity as represented by allelic variation was relatively high as well, as shown by the *Ae* and *TNV* values. *Ae* was higher than the mean for both domestic breeds and feral herds. However, the McCullough Peaks *TNV* value was less than the mean observed for domestic breeds. It was almost 10 variants higher than the mean for feral herds. Feral herds will generally be expected to have fewer variants than a domestic breed simply because of differences in total population size. The relatively high *TNV* is consistent with a mixed origin of the McCullough Peaks herd. If you compare both the 1992 and 1999 samples, the *TNV* values are essentially the same and the combined *TNV* for both sample periods is 66.

The genetic variability measures for the microsatellite systems are shown in Table 4. Only six feral herds (including the McCullough Peaks herd) have been examined using mSats and far fewer domestic breeds, as well. Therefore, only a mean value for all populations is given. As with the blood group and biochemical systems, genetic variation of the McCullough Peaks herd based on mSats was relatively high. With the mSat systems, there was a slight excess of *Ho* relative to *He* but it was not statistically significant. In general, the mSat data shows the same pattern as the blood group and biochemical genetic systems. Variability levels are higher with mSats but that is the only major difference.

The variability data show that there has been no significant change in genetic variation within the McCullough Peaks herd over the past seven years. This is not an unexpected result as seven years is a fairly short time for an animal as long lived as horses, approximately one generation. Many of the horses sampled in 1999 would represent essentially the same genes as those sampled in 1992. With no real change in variation, there is no change in recommendations for this herd. There is sufficient variation present in this herd that, as long as there is no drastic reduction in population size, there should be no real genetic concerns. Recent population size

has been sufficient to maintain relatively high variation for a feral horse population. Continued maintenance of population size should allow genetic variation to be maintained at a healthy level for several generations.

Genetic relationship of the McCullough Peaks herd to domestic breeds are shown in Figure 1. Data from both the 1992 and 1999 sample were included in the analysis. As expected, the two samples showed greater resemblance to each other than to any breed. As had been observed in 1992, the McCullough Peaks herd clustered within the heavy draft horse breed group. Its position on the branch with the Exmoor Pony on the periphery of this cluster suggests that the McCullough Peaks herd is of mixed origins with a strong draft horse influence. It is not possible to say what the exact mixture is; however, the presence of the *TF-F1* variant suggest Quarter Horse may be one of the breeds.

Table 1. Gene frequencies of variants observed at the biochemical and blood group genetic marker systems in the 1999 sample of the McCullough Peaks, WY herd.

SYSTEM	Variant								
TRF	*D*	*F1*	*F2*	*G*	*H2*	*O*	*R*		
	.354	.012	.195	.012	.244	.134	.049		
A1B	*F*	*K*							
	.037	.963							
ALB	*A*	*B*							
	.488	.512							
PGD	*F*	*S*							
	.610	.390							
ES	*F*	*G*	*I*	*L*	*R*				
	.122	.549	.268	.049	.012				
GC	*F*	*S*							
	.768	.232							
PGM	*F*	*S*							
	.061	.939							
GPI	*F*	*I*							
	.085	.915							
AHB	*BI*	*BII*							
	.571	.427							
PI	*F*	*I*	*L*	*L2*	*N*	*S*	*T*	*U*	
	.012	.341	.195	.073	.024	.049	.085	.220	
A SYS	*adf*	*b*	*bc*	-					
	.511	.060	.308	.121					
C SYS	*a*	-							
	.348	.652							
D SYS	*ad*	*d*	*dk*	*dgh*	*de*	*deo*	*bc*	*cg*	*cegi*
	.119	.178	.150	.038	.054	.286	.050	.097	.028
K SYS	*a*	-							
	.038	.962							
P SYS	*ac*	*ad*	*b*	-					
	.501	.102	.051	.346					
Q SYS	*abc*	*ac*	*b*	*c*	-				
	.103	.017	.135	.240	.505				
U SYS	a	-							
	.276	.724							

Table 2. Variants observed and their frequency in the McCullough Peaks feral horse herd for DNA microsatellite systems.

System	Variant						
VHL20	*I*	*J*	*L*	*M*	*O*	*P*	*R*
	.275	.125	.013	.025	.424	.038	.100
HTG4	*K*	*L*	*M*	*O*	*P*		
	.037	.061	.573	.122	.207		
AHT4	*H*	*I*	*J*	*K*	*N*	*O*	
	.110	.024	.317	.110	.110	.329	
HMS7	*J*	*L*	*M*	*N*	*O*	*P*	
	.012	.622	.110	.195	.024	.037	
HTG6	*G*	*I*	*J*	*M*	*O*		
	.024	.049	.073	.037	.817		
AHT5	*J*	*K*	*L*	*M*	*N*	*O*	*Q*
	.237	.150	.275	.063	.162	.038	.075
HMS6	*L*	*M*	*O*	*P*			
	.110	.220	.146	.524			
ASB2	*I*	*K*	*M*	*N*	*O*	*Q*	*R*
	.300	.100	.200	.137	.150	.038	.075
HTG10	*I*	*K*	*L*	*M*	*O*	*Q*	*R*
	.038	.087	.038	.386	.300	.013	.138
HTG7	*K*	*M*	*N*	*O*			
	.353	.183	.220	.244			
HMS3	*I*	*M*	*N*	*O*	*P*	*R*	
	.024	.146	.220	.049	.500	.061	
HMS2	*J*	*K*	*M*	*N*	*Q*		
	.125	.175	.375	.300	.025		

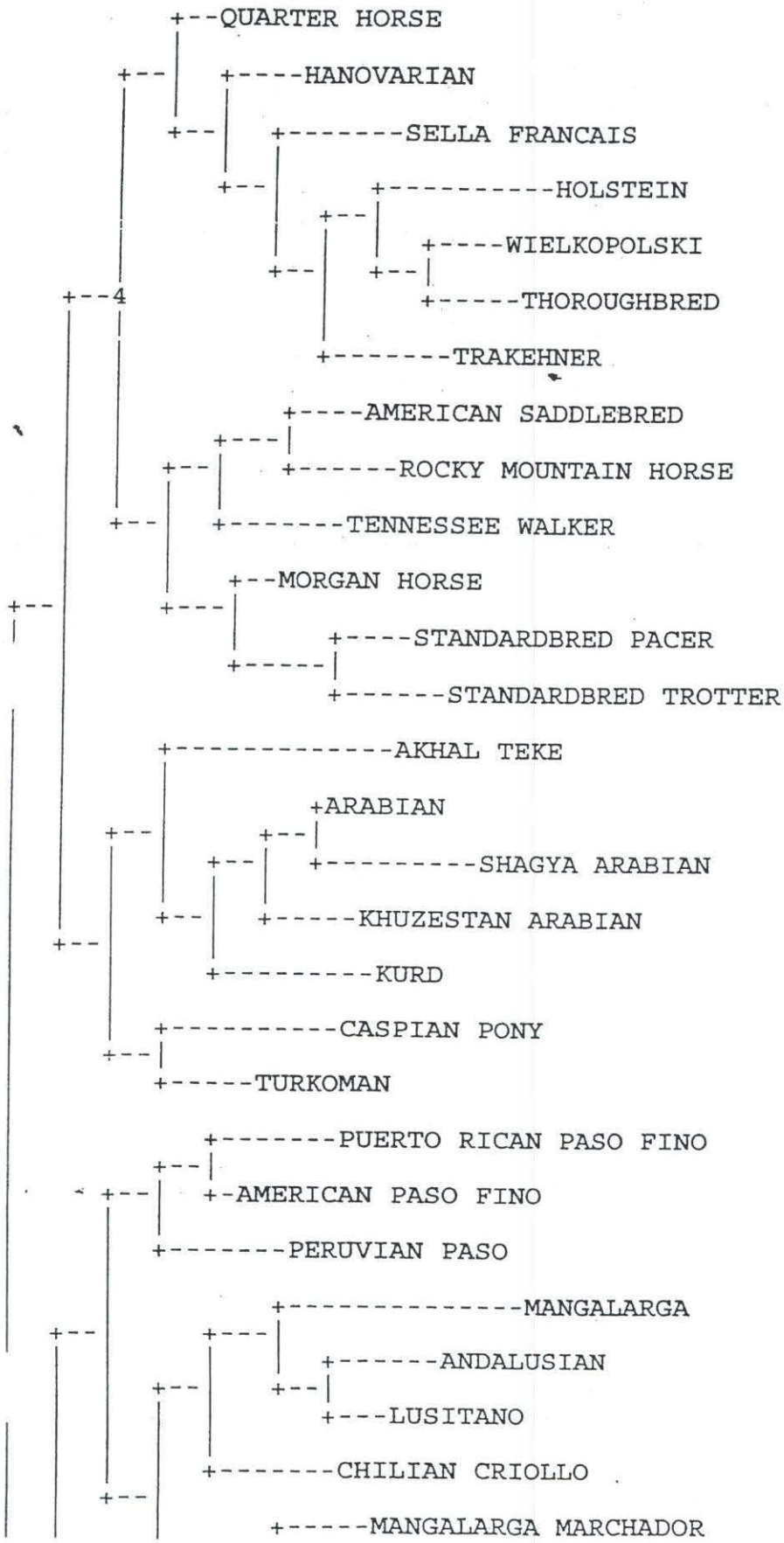
Table 3. Genetic variability measures for the McCullough Peaks herd, selected domestic breeds, selected feral herds, and the mean values from 114 domestic breeds and 46 feral populations. *Ho, He* and *Fis* are derived from biochemical genetic systems only. The remaining measures were calculated from both biochemical genetic and blood group systems.

DOMESTIC BREED	*N*	*Ho*	*He*	*Fis*	*Tt1He*	*Ae*	*TNV*
AMERICAN SADDLEBRED	259	0.404	0.409	0.013	0.435	2.625	96
ARABIAN	117	0.307	0.327	0.061	0.376	2.132	67
EXMOOR PONY	101	0.451	0.467	0.033	0.472	2.523	65
FRIESIAN	314	0.307	0.306	-0.003	0.348	1.901	54
LIPIZZANER	98	0.335	0.324	-0.034	0.366	2.396	69
LUSITANO	41	0.393	0.325	-0.207	0.371	2.184	65
MORGAN HORSE	22	0.395	0.399	0.009	0.407	2.407	61
QUARTER HORSE	168	0.396	0.393	-0.007	0.440	2.653	87
SHETLAND PONY	25	0.368	0.407	0.095	0.452	2.595	71
SUFFOLK PUNCH	122	0.438	0.432	-0.013	0.465	2.459	65
THOROUGHBRED	265	0.294	0.288	-0.019	0.325	2.009	64
MEAN	114	0.370	0.363	-0.015	0.413	2.395	65.2
FERAL HERD							
MCCULLOUGH PEAKS, WY 1999	41	0.405	0.431	0.060	0.469	2.536	62
MCCULLOUGH PEAKS, WY 1992	46	0.391	0.394	0.008	0.438	2.487	61
15 MILE HERD AREA, WY	47	0.379	0.365	-0.038	0.411	2.286	59
GREEN MOUNTAIN, WY	40	0.380	0.339	-0.120	0.420	2.469	56
MUSKRAT BASIN, WY	38	0.374	0.352	-0.061	0.395	2.372	57
CHLORIDE HERD, UT	82	0.246	0.243	-0.014	0.290	1.686	40
GARFIELD FLATS, NV	99	0.429	0.427	-0.006	0.439	2.234	58
PAINTED ROCK, AZ	41	0.288	0.261	-0.104	0.326	1.703	39
PRYOR MOUNTAIN, MT	100	0.421	0.424	0.007	0.460	2.484	71
SHAKLEFORD BANKS, NC	89	0.365	0.365	0.000	0.434	2.563	67
MEAN	46	0.359	0.349	-0.018	0.379	2.188	52.3

Table 4. Genetic variation measures from microsatellite systems for the 1999 sample of the McCullough Peaks herd, selected domestic breeds, selected feral herds, and the mean values from 56 domestic and feral populations.

BREED/HERD	*N*	*Ho*	*He*	*Fis*	*Ae*	*TNV*
AMERICAN SADDLEBRED	89	0.713	0.685	-0.041	3.664	87
ARABIAN	11	0.636	0.592	-0.074	2.762	53
EXMOOR PONY	72	0.577	0.603	0.044	2.725	62
FRESIAN	199	0.452	0.442	-0.024	2.013	59
LIPPIZANNER	150	0.686	0.698	0.018	3.419	89
LUSITANO	54	0.682	0.719	0.052	4.129	75
MORGAN HORSE	22	0.749	0.710	-0.054	3.822	74
QUARTER HORSE	16	0.714	0.727	0.018	3.967	69
SHETLAND PONY	35	0.675	0.661	-0.022	3.166	71
SUFFOLK PUNCH	21	0.642	0.728	0.119	4.113	69
THOROUGHBRED	94	0.679	0.698	0.027	3.664	73
feral						
MCCULLOUGH PEAKS, WY	41	0.717	0.674	-0.063	3.447	69
GARFIELD FLATS, NV	100	0.714	0.707	-0.010	3.697	74
GRANITE RANGE, NV	50	0.719	0.725	0.008	3.951	82
PAINTED ROCK, AZ	11	0.447	0.404	-0.106	1.958	33
PRYOR MOUNTAINS, MT	91	0.685	0.718	0.046	3.865	80
SHAKELFORD BANKS, NC	52	0.610	0.614	0.007	3.082	58
MEAN	56	0.675	0.667	-0.015	3.488	66.3

Figure 1. Restricted Maximum Liklihood dendrogram of genetic distance of the McCullough Peaks feral horse herd and selected domestic horse breeds.



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|  |     |  +---|
|  |     |  |   +--CAMPOLINA
|  |     +--|
|  |        |   +---------ARGENTINE CRIOLLO
|  |        +---|
|  |            +--------BRAZILIAN CRIOLLO
|  |        +----WELSH PONY
|  |        |
|  |     +--|  +-----------------NORWEGIAN FJORD
|  |     |  |  |
|  |     |  +--|  +-------------HACKNEY PONY
--|--|     |    |  |
|  |     |     +--|          +--------------SHETLAND PONY
|  |     |        |  +-------|
|  |     |        +--|       +MINIATURE HORSE
|  |     |           |
|  |     |           +----------------ICELANDIC HORSE
|  |  +--|     +---------------------EXMOOR PONY
|  |  |  |  +--|
|  |  |  |  |  |        +--------MCCULLOUGH PEAKS 1992
|  |  |  |  |  +-------|
|  |  |  |  |           +--MCCULLOUGH PEAKS 1999
|  |  |  |  |
|  |  |  |  |                    +----------SUFFOLK PUNCH
|  |  |  |  |                 +--|
|  |  |  |  |              +--|  +---------AMERICAN CREAM DRAFT
|  |  |  |  |              |  |
|  |  |  +--|           +--|  +-------------NORIKER
|  |  |     |           |  |
|  |  |     |           |  |  +-----POLISH HEAVY HORSE
|  |  |     |        +--|  +--|
|  +--|     |        |  |     +--------BRETON
|     |     |     +--|  |
|     |     |     |  |  +----------BELGIAN DRAFT
|     |     |  +--|  |
|     |     |  |  |  +----PERCHERON
|     |     |  |  |
|     |     +--|  +----------HAFLINGER
|     |        |
|     |        |      +-------------DALES PONY
|     |        +------|
|     |               |      +-------------CLYDESDALE
|     |               +-------|
|     |                       +-------SHIRE
|     |
|     +-----GARRANO
|
+-------------------------------------------------------------PRZEWALSKI
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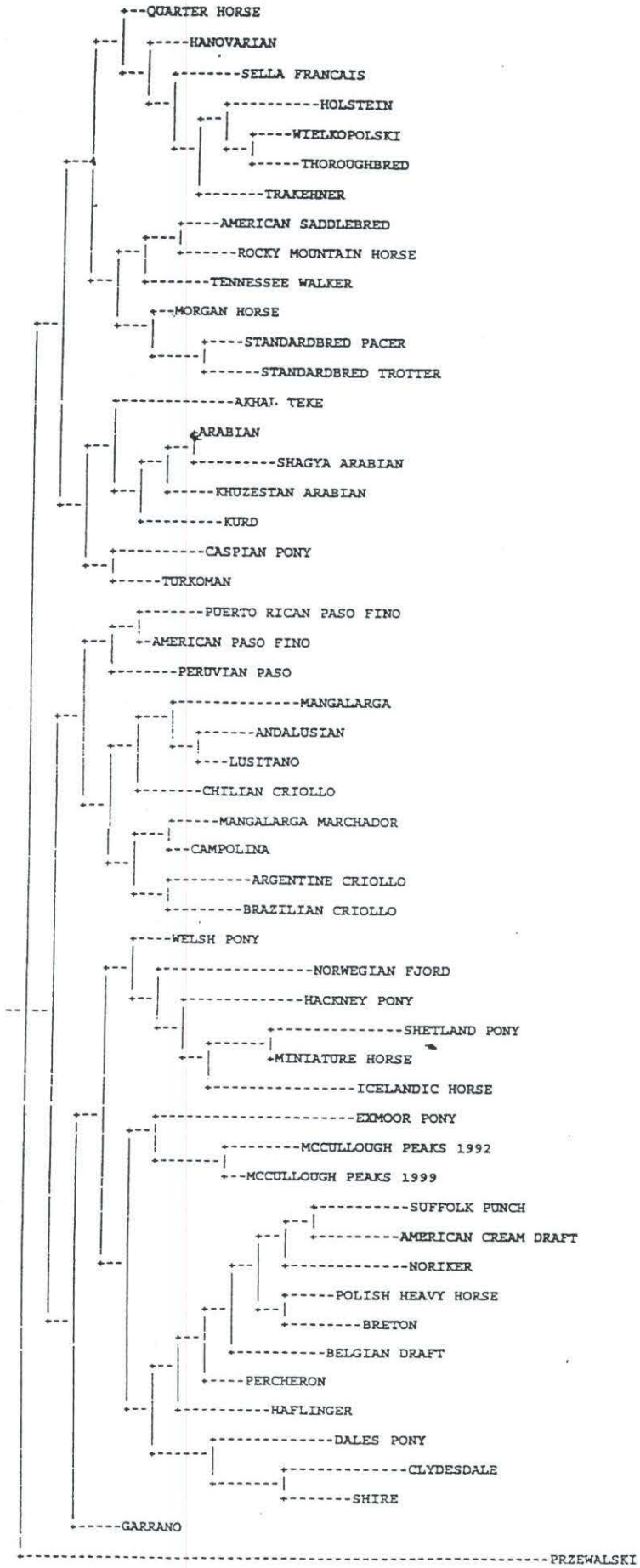
Figure 1. Restricted Maximum Liklihood dendrogram of genetic distance of the McCullough Peaks feral horse herd and selected domestic horse breeds.