Genetic Analysis of the West Douglas HMA, CO

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The following is a report of the genetic analysis of the West Douglas HMA, CO.

A few general comments about the genetic variability analysis based upon DNA microsatellites compared to blood typing. The DNA systems are more variable than blood typing systems, thus variation levels will be higher. Variation at microsatellite loci is strongly influenced by allelic diversity and changes in variation will be seen in allelic measures more quickly that at heterozygosity, which is why more allelic diversity measures are calculated. For mean values, there are a greater proportion of rare domestic breeds included in the estimates than for blood typing so relative values for the measures are lower compared to the feral horse values. As well, feral values are relatively higher because the majority of herds tested are of mixed ancestry which results in a relatively greater increase in heterozygosity values based upon the microsatellite data. There are no specific variants related to breed type so similarity is based upon the total data set.

METHODS

A total of 35 samples were received by Texas A&M University, Equine Genetics Lab on October 30, 2006. DNA was extracted from the samples and tested for variation at 12 equine microsatellite (mSat) systems. These were *AHT*4, *AHT*5 *ASB*2, *ASB*17, *ASB*23, *HMS*3, *HMS*6, *HMS*7, *HTG*4, *HTG*10, *LEX*33, 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; expected heterozygosity *(He)*, which is the predicted number of heterozygous loci based upon gene frequencies; effective number of alleles *(Ae)* which is a measure of marker system diversity; total number of variants *(TNV)*; mean number of alleles per locus (*MNA*); the

number of rare alleles observed which are alleles that occur with a frequency of 0.05 or less (*RA*); the percent of rare alleles (*%RA*); and estimated inbreeding level *(Fis)* which is calculated as 1-*Ho/He*.

Genetic markers also can provide information about ancestry in some cases. Genetic resemblance to domestic horse breeds was calculated using Rogers' genetic similarity coefficient, *S.* This resemblance was summarized by use of a restricted maximum likelihood (RML) procedure.

RESULTS AND DISCUSSION

Variants present and allele frequencies are given in Table 1. No variants were observed which have not been seen in horse breeds. Table 2 gives the values for the genetic variability measures of the West Douglas horse herd. Also shown in Table 2 are values from a representative group of domestic horse breeds. The breeds were selected to cover the range of variability measures in domestic horse populations. Mean values for feral herds (based upon data from 126 herds) and mean values for domestic breeds (based upon 80 domestic horse populations) also are shown.

Mean genetic similarity of the West Douglas herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the West Douglas herd to a standard set of domestic breeds is shown in Figure 1.

Genetic Variants: A total of 55 variants were seen in the West Douglas herd which is below the mean for feral herds and well below the mean for domestic breeds. Of these, 5 had frequencies below 0.05. This is not a high percentage of variants at risk of future loss. Allelic diversity as represented by *Ae* also is below the average for feral herds as is *MNA.*

Genetic Variation: Genetic variation, as indicated by heterozygosity, in the West Douglas herd is well below the feral mean but well above the critical level. *Ho* is slightly lower than *He* but not enough to indicate a significant level of inbreeding. The West Douglas herd was previously tested in 2002 and heterozygosity at that time, based upon blood typing data, was far below the feral mean and below the critical level. DNA data collected at that time showed *Ho* at 0.753; *He*=0.712, *Ae*= 3.57, *TNV* =70; *MNA* =5.83; RA= 20; and %Ra= 0.286. This indicates that variability has declined since 2002 when it was already low.

Genetic Similarity: Overall similarity of the West Douglas herd to domestic breeds was relatively low. This could be due to the low allelic diversity. Highest mean genetic similarity of the West Douglas herd was with North American Gaited breeds, followed very closely by the Light Racing and Riding breeds. As seen in Fig. 1, the West Douglas herd fits most closely to the Chilean Criollo and other South American Criollo horses, which indicates the most likely ancestry, is from the Spanish breeds. As with most trees involving feral herds, the tree is somewhat distorted. Blood typing results indicated closest relationship with the North American breeds. It is likely that the ancestry is from the North American breeds based upon all information.

SUMMARY

Genetic variability of this herd is low and has declined since 2002. Allelic diversity is particularly low which is indicative of variation loss based upon microsatellite data. The low variation would appear to be due to small population size. Ancestry appears to be primarily from North American breeds probably representing ranch stock.

RECOMMENDATIONS

Current variability levels are low enough that this herd should be monitored for possible inbreeding effects such as reduced fertility or deformities. The addition of a small number of young mares from another herd area in Colorado would help restore variation and prevent inbreeding problem. Four animals would be sufficient to get a good start.

Table 1. Allele frequencies of genetic variants observed in the West Douglas HMA feral horse herd.

VHL20																	
I	J	K	L	M	N	O	P	Q	R	S							
0.000	0.000	0.000	0.029	0.329	0.286	0.000	0.271	0.086	0.000	0.000							
HTG4																	
I	J	K	L	M	N	O	P	Q	R								
0.000	0.000	0.457	0.200	0.043	0.057	0.000	0.243	0.000	0.000								
AHT4																	
H	I	J	K	L	M	N	O	P	Q	R							
0.214	0.014	0.143	0.057	0.000	0.000	0.000	0.571	0.000	0.000	0.000							
HMS7																	
I	J	K	L	M	N	O	P	Q	R								
0.000	0.000	0.000	0.157	0.200	0.286	0.357	0.000	0.000	0.000								
AHT5																	
I	J	K	L	M	N	O	P	Q	R								
0.000	0.000	0.114	0.186	0.157	0.486	0.057	0.000	0.000	0.000								
HMS6																	
I	J	K	L	M	N	O	P	Q	R								
0.000	0.000	0.000	0.414	0.200	0.100	0.000	0.286	0.000	0.000								
ASB2																	
B	I	J	K	L	M	N	O	P	Q	R							
0.000	0.000	0.000	0.200	0.000	0.343	0.157	0.000	0.286	0.014	0.000							
HTG10																	
H	I	J	K	L	M	N	O	P	Q	R	S	T					
0.000	0.000	0.000	0.171	0.000	0.586	0.014	0.086	0.000	0.000	0.143	0.000	0.000					
HMS3																	
H	I	J	K	L	M	N	O	P	Q	R	S						
0.000	0.114	0.000	0.000	0.000	0.329	0.100	0.000	0.371	0.086	0.000	0.000						
ASB17																	
D	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
0.000	0.229	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.114	0.000	0.000	0.286	0.000	0.000	0.000	0.171
ASB23																	
G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V		
0.000	0.000	0.000	0.057	0.357	0.443	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.143	0.000		
LEX33																	
F	G	K	L	M	N	O	P	Q	R	S	T						
0.000	0.000	0.329	0.357	0.000	0.000	0.000	0.000	0.000	0.314	0.000	0.000						

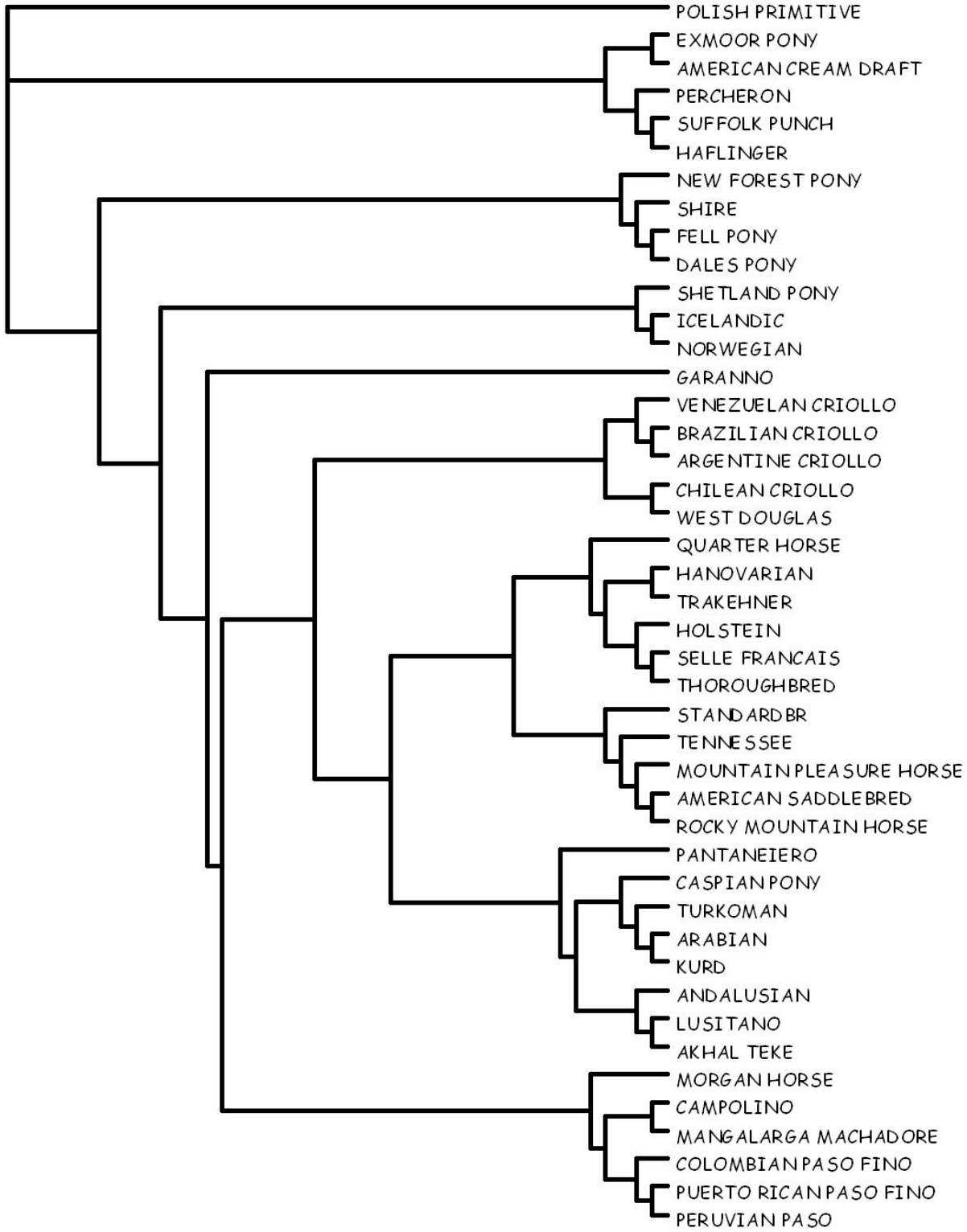
Table 2. Genetic variability measures.

	N	*Ho*	*He*	*Fis*	*Ae*	*TNV*	*MNA*	*Ra*	*%Ra*
WEST DOUGLAS CO	**35**	**0.686**	**0.691**	**0.008**	**3.33**	**55**	**4.58**	**5**	**0.091**
Cleveland Bay	47	0.610	0.627	0.027	2.934	59	4.92	16	0.271
American Saddlebred	576	0.740	0.745	0.007	4.25	102	8.50	42	0.412
Andalusian	52	0.722	0.753	0.041	4.259	79	6.58	21	0.266
Arabian	47	0.660	0.727	0.092	3.814	86	7.17	30	0.349
Exmoor Pony	98	0.535	0.627	0.146	2.871	66	5.50	21	0.318
Friesian	304	0.545	0.539	-0.011	2.561	70	5.83	28	0.400
Irish Draught	135	0.802	0.799	-0.003	5.194	102	8.50	28	0.275
Morgan Horse	64	0.715	0.746	0.041	4.192	92	7.67	33	0.359
Suffolk Punch	57	0.683	0.711	0.038	3.878	71	5.92	13	0.183
Tennessee Walker	60	0.666	0.693	0.038	3.662	87	7.25	34	0.391
Thoroughbred	1195	0.734	0.726	-0.011	3.918	69	5.75	18	0.261
Feral Horse Mean	126	0.716	0.710	-0.012	3.866	72.68	6.06	16.96	0.222
Standard Deviation		0.056	0.059	0.071	0.657	13.02	1.09	7.98	0.088
Minimum		0.496	0.489	-0.284	2.148	37	3.08	0	0
Maximum		0.815	0.798	0.133	5.253	96	8.00	33	0.400
Domestic Horse Mean	80	0.710	0.720	0.012	4.012	80.88	6.74	23.79	0.283
Standard Deviation		0.078	0.071	0.086	0.735	16.79	1.40	10.11	0.082
Minimum		0.347	0.394	-0.312	1.779	26	2.17	0	0
Maximum		0.822	0.799	0.211	5.30	119	9.92	55	0.462

Table 3. Rogers' genetic similarity of the West Douglas HMA feral horse herd to major groups of domestic horses.

	Mean *S*	Std	Minimum	Maximum
Light Racing and Riding Breeds	0.699	0.020	0.669	0.723
Oriental and Arabian Breeds	0.685	0.036	0.650	0.731
Old World Iberian Breeds	0.673	0.023	0.653	0.699
New World Iberian Breeds	0.662	0.021	0.636	0.691
North American Gaited Breeds	0.701	0.016	0.671	0.713
Heavy Draft Breeds	0.657	0.048	0.579	0.710
True Pony Breeds	0.657	0.022	0.623	0.684

Figure 1. Partial RML tree of genetic similarity to domestic horse breeds.



Appendix 1. DNA data for the West Douglas HMA, CO herd.

AID	VHL20	HTG4	AHT4	HMS7	AHT5	HMS6	ASB2	HTG10	HMS3	ASB17	ASB23	LEX33	LEX3
15622	PP	KK	OO	LN	LN	LP	KM	OO	MN	NR	LL	LL	LL
15623	MM	KL	OO	LO	LN	LP	NP	KK	IM	NR	LL	LL	LL
15624	MN	PP	JO	MO	MN	NN	MP	MR	NP	FO	KU	RR	LL
15625	NP	KK	H	LO	NN	MP	MM	MM	PP	FO	KL	KL	LL
15626	MM	KL	JO	MO	MN	MN	MQ	MR	NP	FR	LL	KR	MM
15627	MM	KK	HO	NN	LM	LM	NP	KK	IP	VV	KL	KR	LL
15628	MM	KK	HO	LO	KL	LL	NP	KM	MP	NR	KL	KL	LL
15629	NP	KK	OO	LN	KM	LP	KK	MM	MN	FN	KL	KL	LL
15630	MQ	LP	HO	NO	LN	LM	PP	MM	IP	FV	LU	KL	LL
15631	NP	KL	OO	NN	NN	LM	KK	MO	IP	RV	KU	KL	LL
15632	MN	LP	HO	MO	NN	MP	KM	RR	IN	RR	KU	KR	LL
15633	MP	KL	HJ	MO	KN	MN	MP	RR	MP	OR	KL	KL	MM
15634	NP	MP	HO	NO	MN	LM	MM	MM	IP	OR	JK	LR	LL
15635	MN	KK	OO	LN	LM	LP	PP	KM	MM	NR	KL	LL	LL
15636	PQ	KL	OO	NN	NN	LP	KP	MO	MP	FN	LL	LR	LL
15637	NN	KM	OO	LN	LL	LL	MP	KM	MP	RR	LU	KR	LL
15638	NP	KL	OO	NN	KN	LP	NP	MM	PP	FR	LL	KL	LL
15639	NP	PP	HI	OO	LN	LM	MM	MM	PQ	NN	KU	LR	LL
15640	MP	KN	KO	MN	KN	LP	NP	KM	MQ	RV	KL	KK	LL
15641	MP	KL	JO	OO	KL	LP	KP	MM	MM	NN	KL	KK	IL
15642	PQ	KP	JJ	LM	NN	LP	KK	MM	PP	OV	KL	KL	FL
15643	MN	KN	KO	MO	NO	LP	NP	KM	MQ	NV	KK	KR	LL
15644	NQ	KK	OO	LN	MN	MP	KP	MO	MM	FR	KL	LR	LL
15645	MN	KK	KO	MO	NO	LP	NP	KM	IQ	FF	JL	KR	LL
15646	MM	LP	HJ	MO	NN	LN	MM	NR	NP	FO	JL	RR	LL
15647	MP	KN	OO	LO	KO	LP	KP	MM	MQ	FN	KL	LR	LL
15648	LP	MP	HJ	MN	MN	MP	MM	KM	IM	OV	JK	KR	LL
15649	NP	PP	OO	OO	LM	LN	MM	MR	NP	OV	KU	KR	LL
15650	NP	KK	OO	NN	MM	LP	NP	MM	MM	FR	KL	LL	LL
15651	NN	LL	HJ	MO	NN	LN	KM	RR	MP	RR	KU	RR	LL
15652	MN	NP	HK	MO	KO	LP	MM	MO	PQ	FF	LL	KR	LL
15653	PP	PP	HO	OO	LN	LL	MN	MM	PP	NV	U	KL	LL
15654	MQ	KL	OO	NO	LN	MP	NP	KM	MM	FR	KL	LL	LL
15655	NQ	KL	JO	LM	NN	LM	KM	MM	PP	RV	LL	LR	FI
15656	LM	PP	HO	MO	NN	MP	MN	MM	MP	NV	KK	KR	LL