Genetic Analysis of three populations of the feral horses of the Piceance CO HMA

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09-30-03

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The following is a report of the genetic analysis of the Barcus Creek, 84 Mesa and Spring Creek herds of the Piceance CO HMA. All the populations plus other herds of the White River Recreation Area were previously analyzed in a report written in 1995. This report will be referred to for some analysis and a copy is provided here as well

METHODS

A total of 30 blood samples were received by the University of Kentucky on September 10, 2002. Seventeen genetic marker systems were analyzed. Seven systems were red blood cell alloantigen loci (the *A, C, D, K, P, Q* and *U* horse blood groups) tested by standard serological methods of aggulutination 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 Cholinesterase *(ES)*, Vitamin D Binding Protein *(GC)*, Glucose Phosphate Isomerase *(GPI)*, Alpha Hemoglobin *(HB)*, 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 *AHT4, AHT5, ASB2, ASB17, ASB23, HMS3, HMS6, HMS7, HTG4, HTG10, LEX33,* and *VHL20.* 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 and was calculated for biochemical loci and all marker systems *(Het)*; 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 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.

RESUTLS AND DISCUSSION

Variants present and allele frequencies for the blood group and biochemical markers 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 three Piceance feral horse populations. Also shown in Table 2 are values for earlier samples of these feral horse populations plus values from a representative group of domestic horse breeds. The breeds were selected to cover the range of variability measures is domestic horse populations. Mean values for feral herds (based upon data from 54 herds) and mean values for domestic breeds (based upon 118 domestic horse populations) also are shown.

Mean genetic similarity of the 2002 Piceance herds to domestic horse breed types are shown in Table 3. A dendrogram of similarity to domestic breeds was shown in the 1995 WRRA report. Comparison among herds in the WRRA also is taken from the 1995 report.

Genetic variants. It is difficult to compare variants between the herd in the early 1990s and now due to the small sample sizes. In general, the herds have similar numbers of variants and the actual variants present are mostly the same. The Barcus Creek herd now has a high proportion of the *Pi-R* marker that was not observed in 1992 or 1993. This may indicate that this

variant was in a stallion (possibly an immigrant) that has impacted this herd since then. The 84 Mesa herd also shows some differences in alleles that likely represent immigration into this herd from other areas (for example from Spring Creek). In general, the number of variants in these herds is low, however, if the entire WRRA is considered the number is about average for a feral population.

Genetic variation. In general, variation in these herds is low. For the Barcus Creek herd, variation appears to have increased somewhat over the past ten years. For the other two herds, *Ho* has decreased. However, sample sizes are small so it is possible that there has been no real change in variation. Different relative levels of variation in the different measures shows that sample size probably is a consideration in the values.

Genetic similarity. Genetic similarity levels are very low. This is due to the low variability of the herds. The WRRA populations tend to cluster within the Arabian type breed group. This also may be due to low variation and is not likely due to direct relationship.

The similarity among herds within the WRRA also is very low but all values are within a very tight range. All herds probably share a common ancestry but show evidence of some differentiation due to separation and small population size.

SUMMARY

Overall, little has changed since 1995. The 1995 gives a more comprehensive analysis of the entire area and should be consulted. Variation appears to be declining somewhat but it is difficult to fully evaluate variation without seeing what has happened in the other herds not sampled in 2002. The population subdivision exhibited in the WRRA is a good way to maintain variation in the long term. Allelic diversity appears to be as high or higher than 10 years ago which is likely due to the subdivision with limited migration among groups.

RECOMMENDATIONS

This herd area should be closely monitored. Variation levels are low overall and are below presumed critical levels for some herds. The subdivision should help maintain the variation now present but this is at a minimal level. Also, because all subpopulations appear to have a common origin, the subdivision with occasional migration will not completely eliminate the threat of inbreeding. This herd should be watched for possible evidence of inbreeding depression such as common physical defects or low reproduction. If such evidence is observed, importation of horses from another HMA should be considered. The Little Brookcliffs area would be a good source of horses.

Table 1. Allele frequencies of genetic variants observed in the BARCUS CREEK CO feral horse herd.

System	Allele	Frequency
Trf	D	.610
	F2	.056
	H2	.056
	O	.056
	R	.222
A1B	F	.333
	K	.667
Es	G	.111
	I	.667
	L	.222
Al	A	.500
	B	.500
Gc	F	1.000
PGD	F	.889
	S	.111
PGM	S	1.000
GPI	I	1.000
Hb	BI	.667
	BII	.333
Pi	H	.056
	L	.167
	R	.277
	S	.278
	U	.222
A	adf	.625
	b	.111
	-	.264
J	a	.528
	-	.472
D	dk	.266
	dghm	.111
	deo	.222
	dek	.067
	bcm	.056
	cgm	.278
K	a	.057
	-	.943
P	ac	.059
	ad	.059
	-	.882
Q	abc	.118
	c	.411
	-	.471
U	a	.118
	-	.882

Table 1. Allele frequencies of genetic variants observed in the 84 MESA CO feral horse herd.

System	Allele	Frequency
Trf	D	.458
	F2	.250
	H2	.167
	R	.125
A1B	K	1.000
Es	F	.125
	I	.667
	L	.125
	S	.083
Al	A	.417
	B	.583
Gc	F	.958
	S	.042
PGD	F	.917
	S	.083
PGM	F	.083
	S	.917
GPI	I	1.000
Hb	AII	.042
	BI	.666
	BII	.292
Pi	F	.042
	G	.042
	H	.083
	I	.083
	L	.042
	L2	.125
	P	.125
	S	.250
	T	.041
	U	.125
	E	.042
A	adf	.518
	b	.042
	c	.125
	e	.042
	-	.273
C	a	.500
	-	.500
D	ad	.021
	d	.068
	dk	.409
	dghm	.146
	de	.021
	deo	.083
	dek	.043
	bcm	.042
	cgm	.167
K	-	1.000
P	ac	.182
	ad	.182
	b	.090
	-	.546

Q	abc	.043
	c	.457
	-	.500
U	a	.423
	-	.577

Table 1. Allele frequencies of genetic variants observed in the SPRING CREEK WRRA CO feral horse herd.

System	Allele	Frequency
Trf	F2	.500
	H2	.278
	O	.111
	R	.111
A1B	K	.778
	S	.222
Es	G	.500
	I	.500
Al	A	.111
	B	.889
Gc	F	.944
	S	.056
PGD	F	.889
	S	.111
PGM	S	1.000
GPI	I	1.000
Hb	BI	.889
	BII	.111
Pi	I	.556
	P	.444
A	adf	.666
	-	.334
C	a	1.000
D	ad	.276
	d	.003
	dghm	.111
	deo	.277
	bcm	.333
K	-	1.000
P	ac	.029
	ad	.029
	-	.942
Q	abc	.057
	b	.366
	-	.577
U	a	.184
	-	.816

Table 2. Measures of genetic variation.

Herd	*N*	*Ho*	*He*	*Het*	*Fis*	*TNV*	*Ae*
Barcus Creek 2002	12	0.383	0.341	0.373	-0.123	45	2.123
Barcus Creek 1992&93	37	0.311	0.348	0.364	0.107	56	1.972
84 Mesa 2002	9	0.289	0.341	0.410	0.152	58	2.469
84 Mesa 1993	18	0.340	0.383	0.349	0.112	54	2.046
Spring Creek 2002	9	0.289	0.268	0.282	-0.076	37	1.755
Sqring Creek 1994	5	0.300	0.248	0.265	-0.210	30	1.664
Feral Horse Mean	54	0.360	0.351	0.385	-0.035	53.50	2.218
Standard Deviation		0.051	0.053	0.067	0.118	12.50	0.339
Domestic Horse Mean	118	0.371	0.365	0.414	-0.014	65.40	2.358
Standard Deviation		0.049	0.043	0.035	0.065	11.10	0.253

Table 3. Rogers' genetic similarity of the Piceance CO feral horse herd to major groups of domestic horses. bc=Barcus Creek, 84=84 Mesa, sp= Spring Creek.

		Mean *S*	Std	Minimum	Maximum
Light Racing and Riding Breeds	bc	0.806	0.013	0.787	0.834
	84	0.825	0.023	0.786	0.857
	sp	0.754	0.021	0.717	0.788
Oriental and Arabian Breeds	bc	0.805	0.019	0.770	0.838
	84	0.842	0.027	0.800	0.881
	sp	0.758	0.028	0.696	0.798
Old World Iberian Breeds	bc	0.812	0.027	0.756	0.845
	84	0.850	0.026	0.798	0.876
	sp	0.753	0.024	0.705	0.787
New World Iberian Breeds	bc	0.812	0.021	0.783	0.838
	84	0.853	0.029	0.797	0.887
	sp	0.759	0.022	0.717	0.780
North American Gaited Breeds	bc	0.801	0.027	0.753	0.841
	84	0.843	0.028	0.795	0.875
	sp	0.747	0.030	0.699	0.785
Heavy Draft Breeds	bc	0.747	0.034	0.689	0.790
	84	0.814	0.035	0.736	0.853
	sp	0.721	0.037	0.648	0.761
True Pony Breeds	bc	0.774	0.029	0.739	0.820
	84	0.802	0.030	0.766	0.867
	sp	0.734	0.031	0.676	0.775

Appendix 1. Blood group and biochemical data for individual horses of the Piceance, CO feral horse herd.

		Biochemical Systems												Blood Group Systems											
Accno.	Loc	TF		A1B		ES		AL		GC		PGD		PGM		GPI		HB	PI	A		C	D		K	P	Q	U
Barcus Creek																												
02-10662	bc17	D	D	F	K	L	L	A	B	F	F	F	F	S	S	I	I	B1B2	L S	ab-d-	-	a	--cde-g---		m-o	-	a-	--c	-
02-10663	bc17	D	O	K	K	I	I	A	B	F	F	F	F	S	S	I	I	B1B2	H L	a--d-	-	a	-bcde----k		m--	-	--	--c	-
02-10666	bc17	F2R		K	K	I	I	A	B	F	F	F	F	S	S	I	I	B1B1	R S	a--d-	-	a	---d--gh	k	m--	-	--	--c	-
02-10677	bc17	D	D	F	K	I	I	A	B	F	F	F	F	S	S	I	I	B1B1	R R	-----	-	a	--cde-g---		m-o	a	--	abc	-
02-10678	bc17	D	D	F	F	I	I	B	B	F	F	F	S	S	S	I	I	B1B1	L U	ab-d-	-	-	--cde-g---		m-o	-	a-	--c	-
02-10679	bc17	D	D	K	K	L	L	A	A	F	F	F	F	S	S	I	I	B1B2	R U	a--d-	-	-	---de---	k	--o	-	--	--c	-
02-10680	bc17	D	R	F	F	I	I	A	B	F	F	F	F	S	S	I	I	B2B2	S U	a--d-	-	a	---d----	k	---	-	--	abc	a
02-10681	bc17	R	R	K	K	I	I	A	B	F	F	F	S	S	S	I	I	B1B2	R U	a--d-	-	a	--cd--g-	k	m--	-	--	---	-
02-10682	bc17	D	H2	K	K	G	G	A	B	F	F	F	F	S	S	I	I	B1B1	S S	a--d-	-	a	--cd--gh	-	m--	-	--	---	a
84 Mesa																												
02-10664	8418	D	F2	K	K	F	F	A	B	F	F	F	F	F	S	I	I	B1B2	I I	a-cd-	-	a	---d--gh--		m--	-	-b	--c	a
02-10665	8418	D	F2	K	K	I	I	B	B	F	F	F	F	S	S	I	I	B1B1	F G	a--de	-	a	a--de-----		--o	-	--	--c	a
02-10667	8418	D	F2	K	K	I	I	A	B	F	F	F	F	S	S	I	I	B1B2	T U	ab-d-	-	a	--cd--gh	-	m--	-	--	--c	-
02-10668	8418	H2R		K	K	I	I	A	B	F	F	F	F	S	S	I	I	B1B2	E L2	a-cd-	-	a	---d----	k	---	-	a-	--c	a
02-10669	8418	F2H2		K	K	F	L	A	B	F	F	F	F	S	S	I	I	B1B2	L2S	a--d-	-	-	-bcd----	k	m--	-	a-	---	a
02-10670	8418	D	D	K	K	I	L	A	B	F	F	F	S	F	S	I	I	B1B2	S U	-----	-	-	---d----	k	---	-	--	abc	a
02-10671	8418	F2R		K	K	I	L	B	B	F	F	F	F	S	S	I	I	B1B2	H L2	a-cd-	-	a	---de---	k	---	-	a-	--c	a
02-10672	8418	D	F2	K	K	I	I	A	B	F	S	F	F	S	S	I	I	B1B1	S U	a--d-	-	a	---de-gh	-	m-o	-	-b	---	a
02-10673	8418	D	H2	K	K	I	I	A	B	F	F	F	F	S	S	I	I	B1B1	L P	a--d-	-	a	--cd--g-	k	m--	-	a-	--c	a
02-10674	8418	H2R		K	K	I	S	A	B	F	F	F	F	S	S	I	I	B1B1	H S	-----	-	a	---d----	k	---	-	a-	--c	-
02-10675	8418	D	D	K	K	I	I	B	B	F	F	F	F	S	S	I	I	A2B1	P S	a--d-	-	a	--c---g-	-	m--	-	a-	--c	-
02-10676	8418	D	D	K	K	I	S	A	A	F	F	F	S	S	S	I	I	B1B2	P S	a--d-	-	-	---d----	k	---	-	a-	---	-
Spring Creek																												
02-10683	sc19	F2H2		K	S	I	I	B	B	F	F	F	F	S	S	I	I	B1B1	I I	a--d-	-	a	abcd----	-	m--	-	--	-b-	-
02-10684	sc19	F2R		K	K	I	I	B	B	F	F	F	F	S	S	I	I	B1B2	P P	a--d-	-	a	a--d--gh	-	m--	-	--	---	-
02-10685	sc19	F2O		K	K	G	G	A	B	F	S	F	F	S	S	I	I	B1B1	I P	a--d-	-	a	-bcde---	-	m-o	-	--	abc	-
02-10686	sc19	F2H2		K	K	G	I	B	B	F	F	F	F	S	S	I	I	B1B1	I I	a--d-	-	a	a--de---	-	--o	-	--	---	a
02-10687	sc19	F2H2		K	K	G	I	B	B	F	F	S	S	S	S	I	I	B1B1	I I	a--d-	-	a	a--d----	-	---	-	--	-b-	-
02-10688	sc19	F2H2		K	S	G	G	A	B	F	F	F	F	S	S	I	I	B1B1	I P	a--d-	-	a	---de---	-	--o	-	a-	-b-	a
02-10689	sc19	H2O		K	S	G	I	B	B	F	F	F	F	S	S	I	I	B1B1	I P	a--d-	-	a	-bcde---	-	m-o	-	--	-b-	a
02-10690	sc19	F2R		K	K	G	I	B	B	F	F	F	F	S	S	I	I	B1B2	P P	a--d-	-	a	-bcd--gh	-	m--	-	--	---	-
02-10691	sc19	F2F2		K	S	G	I	B	B	F	F	F	F	S	S	I	I	B1B1	I P	-----	-	a	-bc-----	-	m--	-	--	-b-	-

Appendix 2. DNA data for the Cedar Ridge Trap UT feral horse herd.

		Microsatellite Loci											
		VHL20	HTG44	AHTT54	HMS77	AHTT55	HMS66	AS2	HTG10	HMS33	ASB17	ASB23	LEX33	LEX33
ID														
Barcus Creek														
02-10662	bc62	MN	MM	KO	LL	MM	OO	NO	PS	IO	KP	GJ	LO	KK
02-10663	bc62	II	KM	OO	LN	JN	MP	KM	MN	MP	FO	JL	KL	LL
02-10666	bc62	MP	LM	HJ	LN	KM	PP	NO	LS	IQ	KP	JK	MQ	FK
02-10677	bc62	MM	MM	KO	JJ	KN	OP	NN	OS	PQ	KR	KV	LL	LL
02-10678	bc62	MM	MM	KO	LL	MM	OP	NO	LS	IO	MR	GJ	KM	FL
02-10679	bc62	MN	MM	KO	JJ	KN	PP	NN	LO	PQ	PR	JV	LQ	LO
02-10680	bc62	MN	MM	IK	LM	NN	PP	NN	OS	PQ	NP	JK	LQ	LO
02-10681	bc62	MN	MM	KO	JJ	MN	OP	NN	MO	OQ	MP	KV	LM	FO
02-10682	bc62	IR	MM	JO	LO	JO	MP	MO	OO	NO	NN	JU	KR	FL
84 Mesa														
02-10664	ms63	MR	MO	HJ	NN	JN	PP	MQ	MO	OP	FN	JK	OR	KK
02-10665	ms63	IL	MN	OO	LO	NO	LP	IM	MR	QR	MN	GU	QR	MM
02-10667	ms63	IP	MM	HJ	LN	KO	PP	KN	OO	IP	IO	JJ	QQ	FK
02-10668	ms63	II	MP	OO	JL	JM	PP	MO	KR	II	FR	LL	OQ	LM
02-10669	ms63	IM	MM	IO	JJ	JM	OP	NO	KO	IP	FP	GL	OQ	LL
02-10670	ms63	IM	LO	JN	MN	NN	PP	KK	OO	MQ	OP	JU	LM	KL
02-10671	ms63	IM	MP	OO	JL	JM	PP	MN	KN	IM	FR	GK	KO	LM
02-10672	ms63	IM	MP	JO	LO	IL	PP	OQ	MO	PQ	NP	UU	PR	FP
02-10673	ms63	IN	MM	OO	LN	KK	OP	NQ	LO	MQ	IN	KS	LR	HK
02-10674	ms63	MP	MM	JO	MN	NN	LP	NN	OS	OP	OR	GS	KM	KM
02-10675	ms63	IR	MM	JO	MN	KK	PP	NQ	LL	MP	IM	KS	LO	HO
02-10676	ms63	IN	KL	JO	MN	JN	MO	NN	IR	PP	KM	JJ	LL	KP
Spring Creek														
02-10683	sc64	NO	LN	HO	LN	JM	MM	IN	LL	NP	NR	KS	GO	HL
02-10684	sc64	IN	MP	JO	LN	JN	MO	NQ	LR	NO	NR	LU	LQ	HP
02-10685	sc64	MN	NP	HJ	LN	LM	MP	IN	LL	PR	NR	KK	KQ	LL
02-10686	sc64	IQ	LM	HK	NO	NN	MP	IN	LL	PR	NR	KS	KQ	LL
02-10687	sc64	NO	LM	KO	LN	LM	KP	MN	LL	NP	RR	LS	OQ	HH
02-10688	sc64	II	MN	HH	OO	MN	LM	NN	LL	MN	NR	LS	GQ	MM
02-10689	sc64	IQ	LM	HO	NO	JM	MM	NN	LL	NP	NR	KS	GQ	LM
02-10690	sc64	IO	LP	JO	LN	NN	KO	NN	LR	OP	NN	LU	OQ	HH
02-10691	sc64	NR	LM	HJ	LN	MM	MO	IN	LL	NP	NN	KS	GQ	LL