# Appendix G
# HMA Genetic Reports

Genetic Analysis of the
Adobe Town, WY HMA 2017

E. Gus Cothran

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Department of Veterinary Integrative Bioscience
Texas A&M University
College Station, TX 77843-4458

The following is a report of the genetic analysis of the Adobe Town, WY HMA.

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 40 samples were received by Texas A&M University, Animal Genetics Lab on November 14, 2017. 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 *(TWY)*; 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 Adobe Town 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 Adobe Town herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the Adobe Town herd to a standard set of domestic breeds is shown in Figure 1.

Genetic Variants: A total of 90 variants were seen in the Adobe Town herd which is much higher than the average for domestic breeds and for feral horses. Of these, 25 had frequencies below 0.05. This percentage of variants at risk of future loss is above the average for feral horse populations and slightly above that for domestic breeds. Allelic diversity as represented by *Ae* is high for feral herds as is *MNA*.

Genetic Variation: Genetic variation, as indicated by heterozygosity, in the Adobe Town herd is well above the feral mean. *Ho* is very slightly higher than *He* but the difference is compatible with genetic equilibrium.

Genetic Similarity: Overall similarity of the Adobe Town herd to domestic breeds was somewhat high for feral herds. Highest mean genetic similarity of the Adobe Town herd was with Light Racing and Riding breeds, followed very closely by Old World Spanish breeds and by the North American breeds. As seen in Fig. 1, the Adobe Town herd fits most closely to the Morgan Horse but the tree is somewhat distorted and there is evidence for some Spanish relationship shown in the tree as well. The combined evidence suggests a mixed origin and the variability measures are consistent with this.

The Adobe Town herd was previously analyzed in 2003 (using blood typing methods) and in 2012 using DNA based analysis. Variability levels were relatively high in both previous sampling periods and the values of variability measures were quite similar. Sample size was small in 2003 and the blood typing variation values are not directly comparable but they do reflect very similar levels. Both previous data sets suggest mixed ancestry, primarily from North American derived breeds with some Spanish component.

**SUMMARY**

Genetic variability of this herd is high. The values related to allelic diversity in particular suggest a herd with highly mixed ancestry. This view is consistent with the similarity values seen and the heterozygosity measures. The herd ancestry likely includes some Spanish component based upon this data and the data from 2003 and 2012.

**RECOMMENDATIONS**

Current variability levels are good and this herd has shown no obvious change in diversity

levels since 2012. Re-sampling of the herd should be considered by 2022 to check for changes in

variation.  However, unless there is some serious change in population size resampling could be

delayed for two to three additional years.

**Table 1.** Allele frequencies of genetic variants observed in Adobe Town HMA 2017 feral horse herd.

| VHL20 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| I | J | K | L | M | N | O | P | Q | R | S |
| 0.336 | 0.013 | 0.125 | 0.063 | 0.174 | 0.063 | 0.050 | 0.100 | 0.063 | 0.013 | 0.000 |

| HTG4 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| I | J | K | L | M | N | O | P | Q | R |
| 0.000 | 0.000 | 0.188 | 0.087 | 0.563 | 0.025 | 0.025 | 0.112 | 0.000 | 0.000 |

| AHT4 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| H | I | J | K | L | M | N | O | P | Q | R |
| 0.325 | 0.137 | 0.112 | 0.063 | 0.013 | 0.112 | 0.013 | 0.225 | 0.000 | 0.000 | 0.000 |

| HMS7 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| I | J | K | L | M | N | O | P | Q | R |
| 0.000 | 0.150 | 0.000 | 0.187 | 0.200 | 0.200 | 0.175 | 0.025 | 0.063 | 0.000 |

| AHT5 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| I | J | K | L | M | N | O | P | Q | R |
| 0.000 | 0.387 | 0.125 | 0.075 | 0.013 | 0.250 | 0.150 | 0.000 | 0.000 | 0.000 |

| HMS6 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| I | J | K | L | M | N | O | P | Q | R |
| 0.000 | 0.000 | 0.000 | 0.112 | 0.125 | 0.075 | 0.150 | 0.538 | 0.000 | 0.000 |

| ASB2 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| B | I | J | K | L | M | N | O | P | Q | R |
| 0.000 | 0.050 | 0.000 | 0.250 | 0.000 | 0.175 | 0.163 | 0.075 | 0.000 | 0.262 | 0.025 |

| HTG10 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| H | I | J | K | L | M | N | O | P | Q | R | S | T |
| 0.000 | 0.063 | 0.000 | 0.013 | 0.200 | 0.125 | 0.336 | 0.062 | 0.013 | 0.038 | 0.150 | 0.000 | 0.000 |

| HMS3 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| H | I | J | K | L | M | N | O | P | Q | R | S |
| 0.000 | 0.112 | 0.000 | 0.000 | 0.000 | 0.162 | 0.025 | 0.275 | 0.213 | 0.038 | 0.175 | 0.000 |

| ASB17 | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
| 0.000 | 0.100 | 0.000 | 0.000 | 0.013 | 0.000 | 0.050 | 0.000 | 0.187 | 0.212 | 0.062 | 0.175 | 0.013 | 0.175 | 0.013 | 0.000 |

| ASB2 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V |
| 0.038 | 0.000 | 0.125 | 0.250 | 0.100 | 0.100 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.237 | 0.000 | 0.150 | 0.000 |

| LEX33 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| F | G | K | L | M | N | O | P | Q | R | S | T |
| 0.000 | 0.013 | 0.050 | 0.475 | 0.050 | 0.000 | 0.125 | 0.000 | 0.175 | 0.087 | 0.025 | 0.000 |

**Table 2.** Genetic variability measures.

| | N | Ho | He | Fis | Ae | TNV | MNA | Ra | %Ra |
|---|---|---|---|---|---|---|---|---|---|
| **Adobe Town, WY 2017** | **40** | **0.773** | **0.771** | **-0.003** | **4.68** | **90** | **7.5** | **25** | **0.278** |
| | | | | | | | | | |
| 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 Adobe Town, WY 2017 eral horse herd to major groups of domestic horses.

| | Mean $S$ | Std | Minimum | Maximum |
|---|---|---|---|---|
| Light Racing and Riding Breeds | **0.790** | **0.025** | **0.755** | **0.819** |
| Oriental and Arabian Breeds | **0.770** | **0.038** | **0.732** | **0.836** |
| Old World Iberian Breeds | **0.787** | **0.026** | **0.750** | **0.818** |
| New World Iberian Breeds | **0.778** | **0.032** | **0.731** | **0.805** |
| North American Gaited Breeds | **0.786** | **0.025** | **0.752** | **0.818** |
| Heavy Draft Breeds | **0.713** | **0.060** | **0.629** | **0.810** |
| True Pony Breeds | **0.717** | **0.044** | **0.654** | **0.774** |

**Figure 1.** Partial RML tree of genetic similarity to domestic horse breeds.
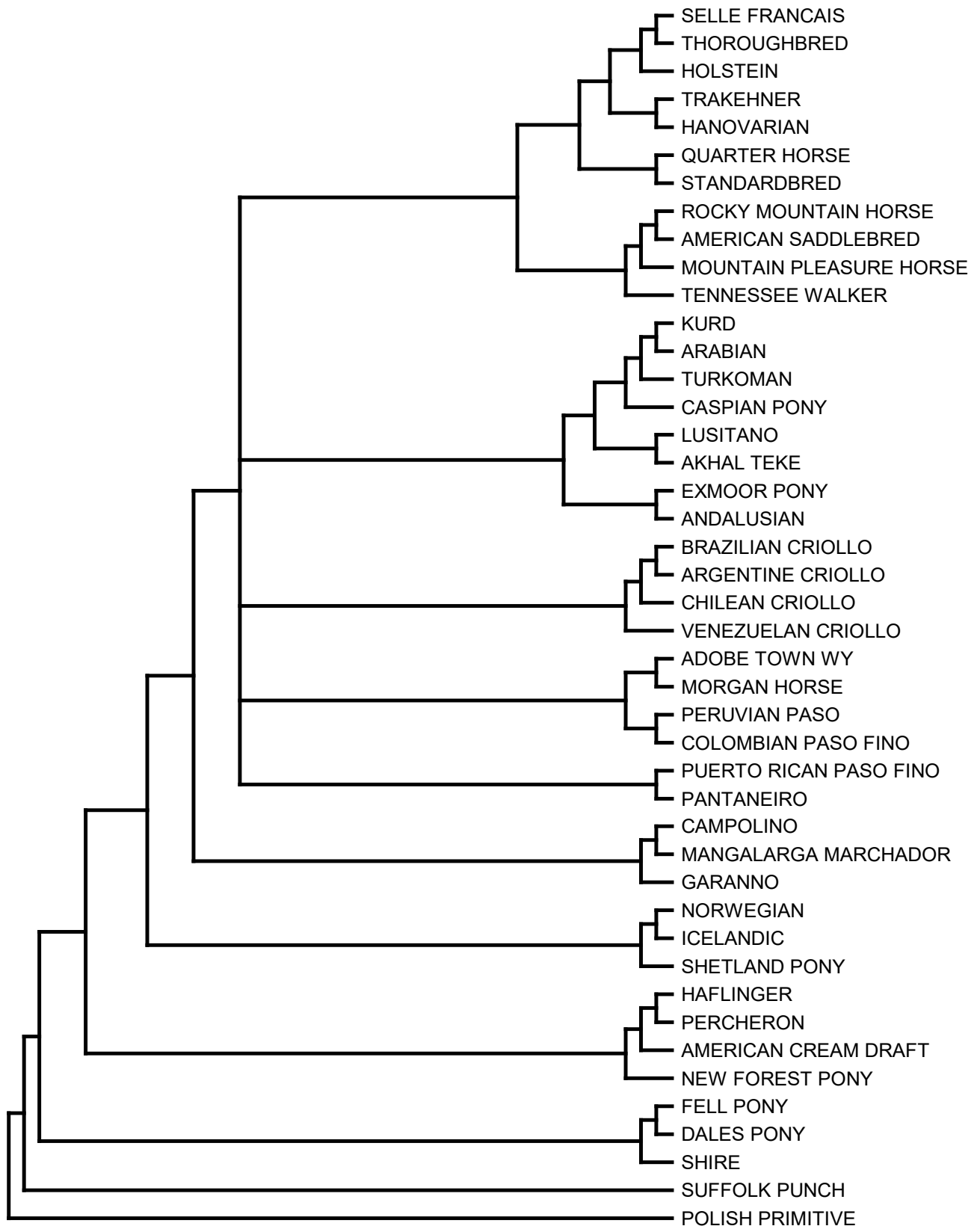
**Appendix 1**. DNA data for the Adobe Town, WY 2017 herd.

| AID | VHL20 | HTG4 | AHT4 | HMS7 | AHT5 | HMS6 | ASB2 | HTG10 | HMS3 | ASB17 | ASB23 | LEX33 | LEX3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 102412 | IQ | MO | HM | LM | JK | MN | KM | IM | OP | MM | SU | KL | LO |
| 102413 | IM | MM | HO | OQ | JM | MO | KM | IN | IR | MP | GI | LQ | MN |
| 102414 | IK | MM | HM | LM | KN | LL | NQ | LN | IQ | MN | JK | OQ | FK |
| 102415 | IR | MM | JK | NN | JN | OP | IN | KL | MM | NR | LL | KK | LN |
| 102416 | IK | KK | HM | LN | JN | NP | MQ | NO | NO | MP | IS | LR | NP |
| 102417 | LP | MM | LM | MN | JJ | LP | KO | LN | IP | NR | JU | LQ | HK |
| 102418 | JM | KM | HO | JO | NN | NP | KM | MR | MQ | FM | JS | LL | LO |
| 102419 | MP | MM | HO | LO | JN | PP | MM | LN | PR | NP | GU | LL | HO |
| 102420 | KM | MM | MO | NQ | JO | MP | OR | LQ | MP | NN | IJ | LR | MM |
| 102421 | IM | MM | MO | LL | JJ | MP | OQ | LM | MO | MN | IS | LL | FM |
| 102422 | IK | LM | HH | MN | KL | MO | KO | MQ | PP | PS | JU | GL | FH |
| 102423 | KL | KM | HO | NO | JJ | OP | QQ | NN | OR | MP | LL | LL | MM |
| 102424 | IO | KL | KO | LQ | NO | PP | QQ | NR | PR | KN | SS | OS | LL |
| 102425 | KN | MM | KO | JL | JO | OP | MN | NN | MM | PR | UU | LQ | MO |
| 102426 | IQ | KP | HH | MN | NO | PP | KO | MN | OR | KN | IS | LR | PP |
| 102427 | IP | KO | HO | MO | JK | PP | KN | IQ | MR | MP | IS | LO | LM |
| 102428 | MN | MN | IO | OQ | JJ | NP | OQ | LR | MP | PR | JL | OQ | FO |
| 102429 | IQ | KM | HO | LL | JO | PP | KM | NN | MR | NR | GK | OS | LL |
| 102430 | IM | NP | IN | JO | JN | PP | KQ | NR | OP | NO | JS | LR | MM |
| 102431 | LM | LM | IK | MN | LO | PP | KQ | LL | OP | NR | IK | LQ | FL |
| 102432 | IM | MP | HK | JN | JK | OP | KM | LM | OO | OR | IK | LQ | FF |
| 102433 | IN | MM | HI | LN | JO | PP | NQ | MN | IP | FP | JS | LO | MM |
| 102434 | II | KM | HJ | JO | JN | LP | KN | NO | OR | KM | SU | LQ | KK |
| 102435 | IP | MP | HM | MN | JJ | PP | MQ | LM | OP | OR | KU | LL | HH |
| 102436 | II | MM | HM | NN | JO | PP | NN | MN | OP | NP | JS | LO | PP |
| 102437 | NQ | MM | HH | LM | JO | PP | KN | MN | II | FM | JS | LO | HH |
| 102438 | KM | KM | IO | MO | KO | LO | NR | LN | OP | MR | JK | LQ | MM |
| 102439 | MM | KP | IJ | JN | LN | OO | IQ | OO | MM | RR | IJ | LO | NN |
| 102440 | KQ | KL | HI | JL | JK | MP | KN | NR | OR | MN | LS | LL | HH |
| 102441 | IN | KL | JO | MO | KN | LL | II | IL | IO | NN | JU | LO | LL |
| 102442 | II | LM | HI | PP | JL | MP | KK | LR | OO | PP | JK | LL | LL |
| 102443 | KK | KM | IM | NO | JN | MM | KQ | NN | IN | FM | JL | LR | HH |
| 102444 | IP | MM | IO | JM | NN | LO | KN | PR | OR | OP | JK | QQ | LL |
| 102445 | MP | MP | HO | OO | NO | LP | QQ | RR | OR | OP | JS | LQ | KK |
| 102446 | LO | KM | HI | MQ | JN | PP | QQ | IL | MR | IM | JU | QR | NN |
| 102447 | LP | MM | JJ | MO | NN | MP | KQ | NR | OP | NQ | SU | LL | OO |
| 102448 | MO | MP | OO | LM | JJ | OP | MN | RR | OQ | FF | SS | KM | FF |
| 102449 | IO | MM | JJ | JJ | KL | NO | MQ | LN | OR | FF | IJ | LM | PP |
| 102450 | II | MP | HO | JJ | KL | NP | MQ | NO | PR | RR | JU | MM | PP |
| 102451 | IP | LP | HJ | LM | NO | PP | KM | NN | IP | KR | LS | QR | LL |

Genetic Analysis of the
Salt Wells Creek HMA, Manuel Gap subgroup, WY

E. Gus Cothran

April 11, 2011

Department of Veterinary Integrative Bioscience
Texas A&M University
College Station, TX 77843-4458

The following is a report of the genetic analysis of the Salt Wells Creek HMA, Marvel Gap, WY. The herd will be referred to as the Salt Wells Creek HMA MG in this report.

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 25 samples were received by Texas A&M University, Equine Genetics Lab on November 22, 2010. 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 Salt Wells Creek HMA MG 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 Salt Wells Creek HMA MG herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the Salt Wells Creek HMA MG herd to a standard set of domestic breeds is shown in Figure 1.

**Genetic Variants:** A total of 80 variants were seen in the Salt Wells Creek HMA MG herd which is above the mean for feral herds and just slightly below the mean for domestic breeds. Of these, 15 had frequencies below 0.05 which is a low percentage of variants at risk of future loss. Allelic diversity as represented by *Ae* is somewhat the average for feral herds while *MNA* is greater than the mean.

**Genetic Variation:** Observed heterozygosity in the Salt Wells Creek HMA MG herd is well above the feral mean while He is only slightly higher than average. *Ho* is a good bit higher

than *He.* Differences such as this can indicate a recent reduction in population size, within the past few generations, but this not possible to confirm by DNA data alone.

**Genetic Similarity:** Overall similarity of the Salt Wells Creek HMA MG herd to domestic breeds was about average for feral herds. Highest mean genetic similarity of the Salt Wells Creek HMA MG herd was with Light Racing and Riding breeds, followed closely by the Oriental and Arabian breeds. As seen in Fig. 1, however, the Salt Wells Creek HMA MG herd pairs with the Morgan Horse within a group of South American breeds. These results indicate a herd with mixed origins with no clear indication of primary breed type. As with most trees involving feral herds, the tree is somewhat distorted.

## SUMMARY

Genetic variability of this herd in general is high with exceptionally high *Ho*. Indeed, this was one of the highest *Ho* values seen for a feral herd. The rest of the estimates of variation were high but not exceptionally so and there was a low percentage of variation that is at risk. There is a possibility that this herd has seen a recent loss of population size which would explain the differences in *Ho* compared to *He* and would increase the risk to genetic diversity which would be considered slight based upon variation levels seen. Genetic similarity results suggest a herd with mixed ancestry.

## RECOMMENDATIONS

Current variability levels are high enough that no action is needed at this point but the herd should be monitored closely if it is known that the herd size has seen a recent decline. If there has been a recent population decline, variability levels could drop quickly over the next 5-10 years.

**Table 1.** Allele frequencies of genetic variants observed in Salt Wells Creek HMA MG feral horse herd.

**VHL20**

| I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.260 | 0.000 | 0.000 | 0.140 | 0.200 | 0.080 | 0.120 | 0.180 | 0.000 | 0.020 | 0.000 |

**HTG4**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.260 | 0.200 | 0.440 | 0.020 | 0.020 | 0.060 | 0.000 | 0.000 |

**AHT4**

| H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.160 | 0.260 | 0.220 | 0.000 | 0.000 | 0.080 | 0.020 | 0.260 | 0.000 | 0.000 | 0.000 |

**HMS7**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.280 | 0.000 | 0.220 | 0.160 | 0.200 | 0.080 | 0.040 | 0.020 | 0.000 |

**AHT5**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.020 | 0.360 | 0.120 | 0.000 | 0.140 | 0.240 | 0.120 | 0.000 | 0.000 | 0.000 |

**HMS6**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.020 | 0.180 | 0.280 | 0.100 | 0.120 | 0.300 | 0.000 | 0.000 |

**ASB2**

| B | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.060 | 0.000 | 0.220 | 0.000 | 0.020 | 0.420 | 0.100 | 0.000 | 0.160 | 0.020 |

**HTG10**

| H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.020 | 0.000 | 0.020 | 0.200 | 0.180 | 0.220 | 0.140 | 0.000 | 0.120 | 0.100 | 0.000 | 0.000 |

**HMS3**

| H | I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.140 | 0.000 | 0.000 | 0.000 | 0.160 | 0.080 | 0.380 | 0.100 | 0.020 | 0.120 | 0.000 |

**ASB17**

| D | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.120 | 0.060 | 0.000 | 0.000 | 0.000 | 0.120 | 0.000 | 0.180 | 0.060 | 0.100 | 0.080 | 0.000 | 0.280 | 0.000 | 0.000 |

**ASB23**

| G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.220 | 0.180 | 0.160 | 0.060 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.300 | 0.000 | 0.080 | 0.000 |

**LEX33**

| F | G | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.120 | 0.020 | 0.480 | 0.020 | 0.000 | 0.000 | 0.000 | 0.240 | 0.120 | 0.000 | 0.000 |

**Table 2.** Genetic variability measures.

| | N | Ho | He | Fis | Ae | TNV | MNA | Ra | %Ra |
|---|---|---|---|---|---|---|---|---|---|
| **MARVEL GAP WY** | 25 | 0.813 | 0.775 | -0.050 | 4.63 | 80 | 6.67 | 15 | 0.188 |
| | | | | | | | | | |
| 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 Salt Wells Creek HMA MG feral horse herd to major groups of domestic horses.

| | Mean S | Std | Minimum | Maximum |
|---|---|---|---|---|
| Light Racing and Riding Breeds | 0.776 | 0.019 | 0.756 | 0.806 |
| Oriental and Arabian Breeds | 0.772 | 0.038 | 0.714 | 0.816 |
| Old World Iberian Breeds | 0.755 | 0.018 | 0.733 | 0.781 |
| New World Iberian Breeds | 0.764 | 0.022 | 0.723 | 0.795 |
| North American Gaited Breeds | 0.761 | 0.027 | 0.717 | 0.787 |
| Heavy Draft Breeds | 0.733 | 0.046 | 0.689 | 0.804 |
| True Pony Breeds | 0.704 | 0.044 | 0.647 | 0.752 |

**Figure 1.** Partial RML tree of genetic similarity to domestic horse breeds.
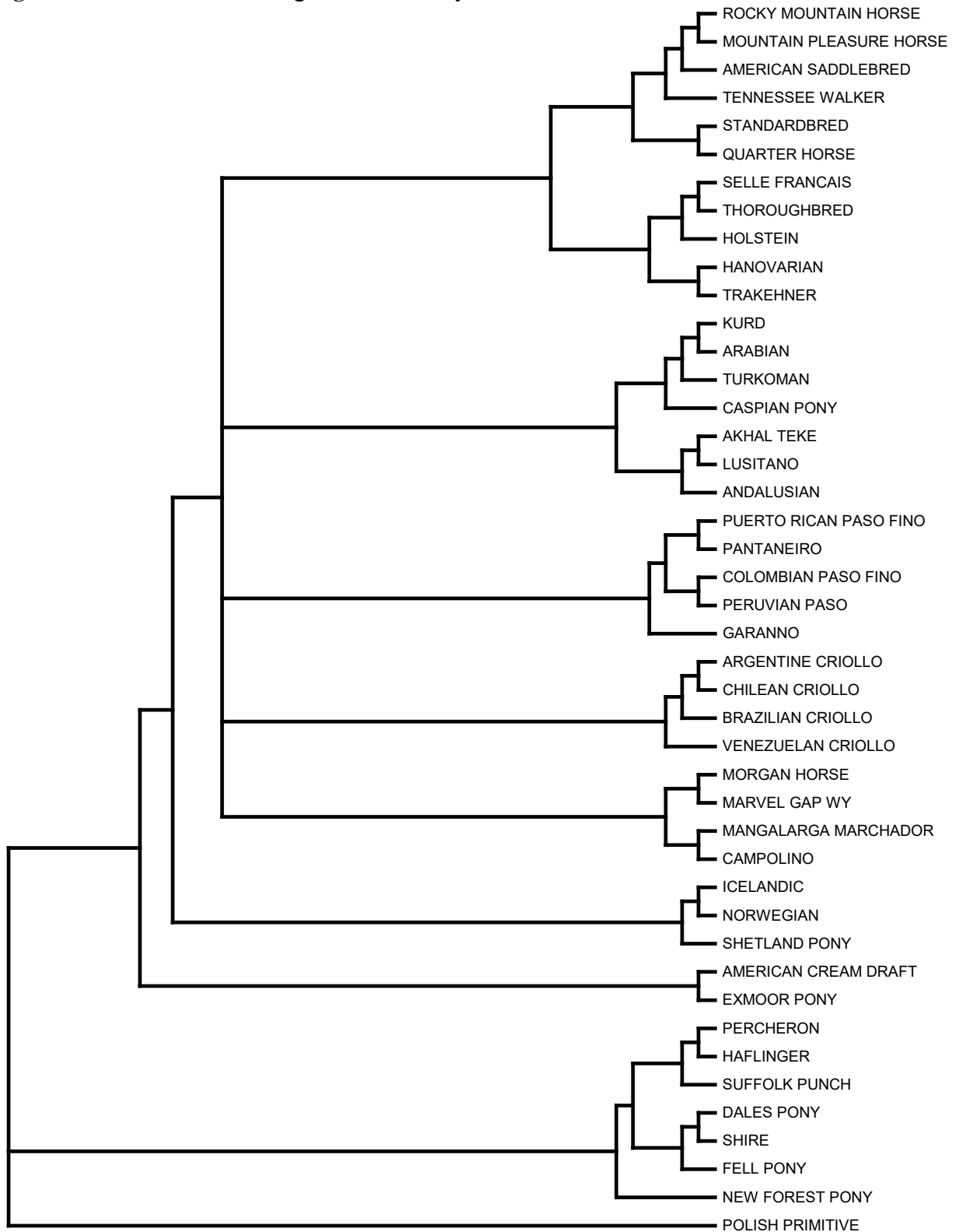
**Appendix 1**. DNA data for the Salt Wells Creek HMA, Marvel Gap Subgroup, WY herd.

| AID | VHL20 | HTG4 | AHT4 | HMS7 | AHT5 | HMS6 | ASB2 | HTG10 | HMS3 | ASB17 | ASB23 | LEX33 | LEX3 |
|-----|-------|------|------|------|------|------|------|-------|------|-------|-------|-------|------|
| 48679 | MM | KP | JM | NN | JN | KP | NR | LL | MR | FM | IJ | LL | FM |
| 48680 | OO | KL | IJ | JJ | JM | LP | NN | LO | IP | RR | IK | GL | HO |
| 48681 | MM | KK | MO | NN | NN | MP | KN | LM | OR | FO | JK | LR | FM |
| 48682 | IP | LM | JO | LL | JJ | MN | KO | MM | MN | NR | IL | GL | HM |
| 48683 | MO | LM | IJ | JJ | JM | LP | NQ | LO | IP | PR | KL | GQ | NO |
| 48684 | LN | MM | HO | LM | JO | MM | QQ | IN | NO | MR | SS | LQ | MM |
| 48685 | IL | MN | IM | NO | JN | MP | KN | NR | MM | KK | KS | LL | FN |
| 48686 | IP | LM | JO | JL | JM | LP | NN | LN | IP | PR | IS | LQ | HO |
| 48687 | MP | MM | JO | LM | KO | MM | KN | MO | MN | NR | JS | GQ | HM |
| 48688 | IN | KL | IJ | MO | JN | MP | KK | LQ | NO | OR | KU | LQ | MM |
| 48689 | PP | LO | MN | MM | KN | LN | NO | KM | OQ | GR | II | LQ | FF |
| 48690 | IL | KM | IO | LN | JO | PP | IK | NQ | IP | FO | JJ | KL | OO |
| 48691 | IP | KM | HI | JL | JM | NO | KO | MQ | OO | FK | IU | LM | LL |
| 48692 | IP | KM | IO | LN | JN | NO | MQ | MN | OO | FG | JK | QR | FF |
| 48693 | OP | KM | IO | JM | KM | LP | KN | LO | OP | MR | KS | LQ | OO |
| 48694 | II | MP | HI | JN | JN | LP | NQ | MR | OO | FG | SU | LL | LL |
| 48695 | IL | MM | HI | JM | JN | MM | NQ | NN | OO | OR | SS | LQ | NN |
| 48696 | LP | KM | HJ | JP | KK | MP | KN | NQ | MO | MP | LU | LL | HH |
| 48697 | II | MM | JO | JL | JO | LO | IN | OR | IM | MM | IJ | LR | NN |
| 48698 | LL | LL | HO | JP | JO | PP | KN | NQ | OR | MP | SS | GR | OO |
| 48699 | MN | KM | HO | NQ | MN | LO | NO | QR | OR | KK | IK | LQ | OO |
| 48700 | IO | KL | IJ | JJ | KM | OP | NQ | LO | IO | MR | IJ | LQ | HH |
| 48701 | MN | LP | IO | LO | JJ | MO | NQ | LN | IR | KN | IS | LQ | LL |
| 48702 | MO | KM | HI | NO | IN | LM | NO | NR | OR | OR | JS | LR | FF |
| 48703 | MR | MM | JO | LM | NO | MN | IN | MO | MO | MR | SS | GR | NN |

Genetic Analysis of the
Salt Wells Creek HMA, Miller Mountain Trap, WY

E. Gus Cothran

April 11, 2011

Department of Veterinary Integrative Bioscience
Texas A&M University
College Station, TX 77843-4458

The following is a report of the genetic analysis of the Salt Wells Creek HMA, Miller Mountain Trap, WY. The herd will be referred to as the Salt Wells Creek HMA MMT in this report.

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 25 samples were received by Texas A&M University, Equine Genetics Lab on November 22, 2010. 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 Salt Wells Creek HMA MMT 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 Salt Wells Creek HMA MMT herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the Salt Wells Creek HMA MMT herd to a standard set of domestic breeds is shown in Figure 1.

**Genetic Variants:** A total of 86 variants were seen in the Salt Wells Creek HMA MMT herd which is above the mean for feral herds as well as the mean for domestic breeds. Of these, 21 had frequencies below 0.05 which is just above the average percentage of variants at risk of future loss. Allelic diversity as represented by *Ae* is well above the average for feral herds while *MNA* is greater than the mean.

**Genetic Variation:** Observed heterozygosity in the Salt Wells Creek HMA MMT herd is above the feral mean while *He* is a good deal higher than average. *Ho* is a lower than *He*. Differences such as this can indicate population subdivision as the variation measures appear high for inbreeding to be a likely explanation for the positive *Fis* value.

**Genetic Similarity:** Overall similarity of the Salt Wells Creek HMA MMT herd to domestic breeds was just above average for feral herds. Highest mean genetic similarity of the Salt Wells Creek HMA MMT herd was with Light Racing and Riding breeds, followed by the Oriental and Arabian breeds with the North American Gaited breeds almost as close. As seen in Fig. 1, however, the Salt Wells Creek HMA MMT herd clusters within a group of "cold blood" horse breeds. These results indicate a herd with mixed origins with no clear indication of primary breed type.

## SUMMARY

Genetic variability of this herd in general is on the high side but some of the diversity may be related to unrecognized population subdivision. Even if this is true, the *Ho* values indicated good levels of genetic variation. Genetic similarity results suggest a herd with mixed ancestry.

## RECOMMENDATIONS

Current variability levels are high enough that no action is needed at this point.

**Table 1.** Allele frequencies of genetic variants observed in Salt Wells Creek HMA, Miller Mountain subgroup feral horse herd.

**VHL20**

| I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.167 | 0.015 | 0.000 | 0.030 | 0.167 | 0.167 | 0.045 | 0.197 | 0.076 | 0.136 | 0.000 |

**HTG4**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.121 | 0.182 | 0.545 | 0.015 | 0.061 | 0.076 | 0.000 | 0.000 |

**AHT4**

| H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.242 | 0.030 | 0.152 | 0.106 | 0.091 | 0.015 | 0.000 | 0.242 | 0.121 | 0.000 | 0.000 |

**HMS7**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.121 | 0.152 | 0.318 | 0.076 | 0.227 | 0.106 | 0.000 | 0.000 | 0.000 |

**AHT5**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.303 | 0.136 | 0.061 | 0.197 | 0.167 | 0.136 | 0.000 | 0.000 | 0.000 |

**HMS6**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.061 | 0.136 | 0.197 | 0.015 | 0.212 | 0.379 | 0.000 | 0.000 |

**ASB2**

| B | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.000 | 0.258 | 0.000 | 0.167 | 0.061 | 0.061 | 0.152 | 0.182 | 0.121 |

**HTG10**

| H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.045 | 0.000 | 0.000 | 0.182 | 0.303 | 0.015 | 0.258 | 0.000 | 0.000 | 0.182 | 0.015 | 0.000 |

**HMS3**

| H | I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.061 | 0.000 | 0.000 | 0.000 | 0.106 | 0.303 | 0.015 | 0.288 | 0.197 | 0.030 | 0.000 |

**ASB17**

| D | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.091 | 0.000 | 0.000 | 0.061 | 0.030 | 0.106 | 0.000 | 0.136 | 0.333 | 0.015 | 0.076 | 0.045 | 0.091 | 0.000 | 0.000 | 0.000 | 0.000 | 0.015 |

**ASB23**

| G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.045 | 0.303 | 0.258 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.364 | 0.000 | 0.030 | 0.000 |

**LEX33**

| F | G | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.076 | 0.076 | 0.091 | 0.000 | 0.000 | 0.045 | 0.015 | 0.606 | 0.076 | 0.000 | 0.015 |

**Table 2.** Genetic variability measures.

| | N | Ho | He | Fis | Ae | TNV | MNA | Ra | %Ra |
|---|---|---|---|---|---|---|---|---|---|
| **Miller Mountain WY** | 25 | 0.730 | 0.765 | 0.045 | 4.62 | 86 | 7.17 | 21 | 0.244 |
| | | | | | | | | | |
| 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 Salt Wells Creek HMA MMT feral horse herd to major groups of domestic horses.

| | Mean S | Std | Minimum | Maximum |
|---|---|---|---|---|
| Light Racing and Riding Breeds | 0.790 | 0.032 | 0.748 | 0.836 |
| Oriental and Arabian Breeds | 0.773 | 0.043 | 0.719 | 0.826 |
| Old World Iberian Breeds | 0.755 | 0.007 | 0.746 | 0.762 |
| New World Iberian Breeds | 0.759 | 0.031 | 0.718 | 0.816 |
| North American Gaited Breeds | 0.770 | 0.036 | 0.719 | 0.804 |
| Heavy Draft Breeds | 0.751 | 0.025 | 0.719 | 0.784 |
| True Pony Breeds | 0.719 | 0.061 | 0.624 | 0.771 |

**Figure 1.** Partial RML tree of genetic similarity to domestic horse breeds.
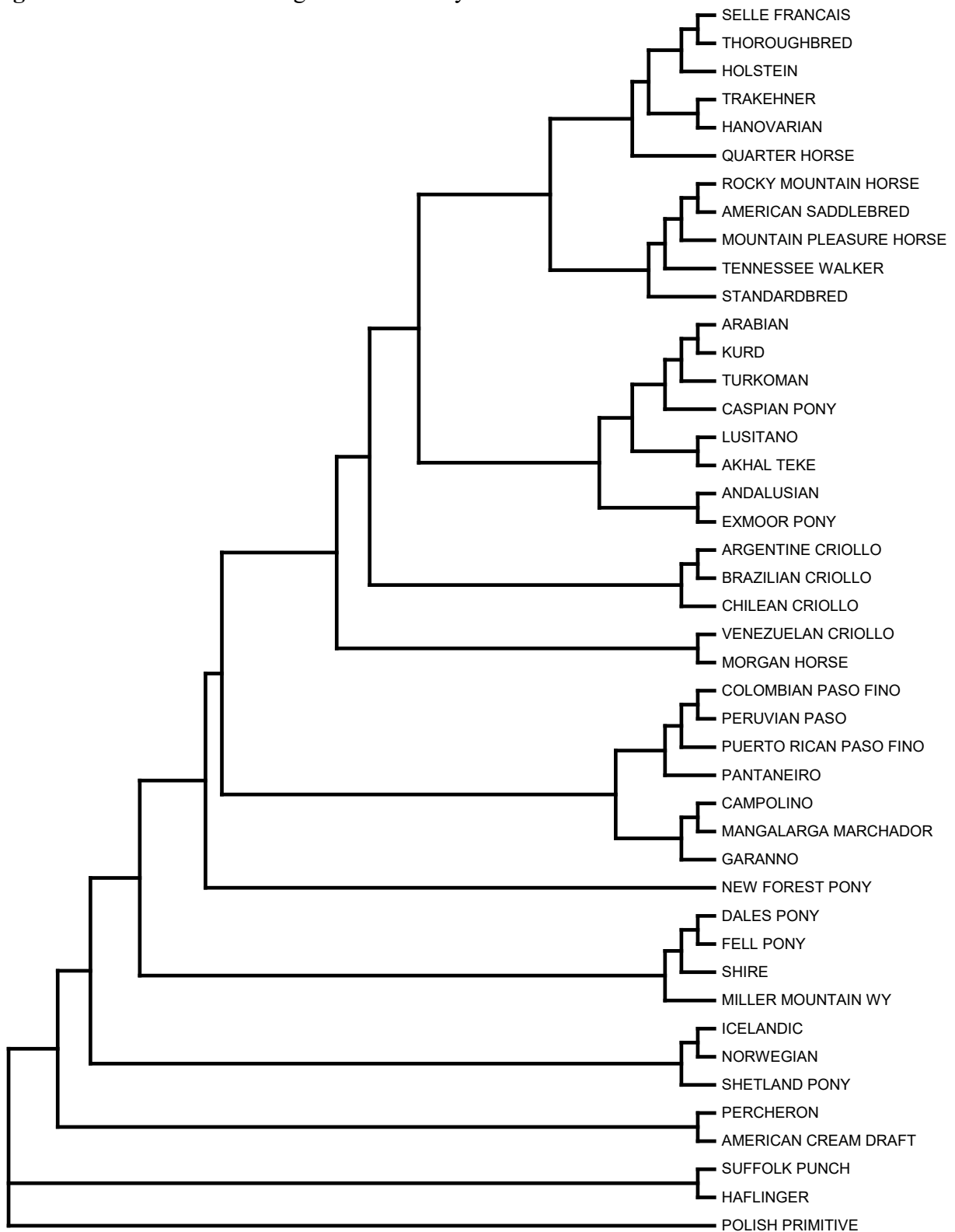
**Appendix 1**. DNA data for the Salt Wells Creek HMA, Miller Mountain subgroup, WY herd.

| AID | VHL20 | HTG4 | AHT4 | HMS7 | AHT5 | HMS6 | ASB2 | HTG10 | HMS3 | ASB17 | ASB23 | LEX33 | LEX3 |
|-----|-------|------|------|------|------|------|------|-------|------|-------|-------|-------|------|
| 48654 | PR | LL | HO | JO | NN | LP | KK | IL | PP | IP | IS | QQ | FF |
| 48655 | PP | KL | HJ | LL | JN | MO | PR | MO | MN | MP | JJ | QQ | PP |
| 48656 | PQ | LM | HO | JN | JK | LM | QR | MO | MN | NR | JS | OQ | FF |
| 48657 | PR | MM | HP | KL | JM | KP | KM | MM | QQ | KN | KS | QQ | IP |
| 48658 | NN | MM | HO | LN | NN | MM | MN | OR | IP | JP | JK | QQ | LP |
| 48659 | NN | NP | LO | KN | JJ | MO | NR | OR | PP | NQ | KK | KP | KM |
| 48660 | MR | OO | JK | LL | JJ | MM | NR | OO | NQ | FN | KK | QQ | FL |
| 48661 | IM | KM | HO | JM | KO | OP | NP | RR | NN | KM | JS | KR | FF |
| 48662 | MP | MM | HP | KL | MM | OO | KP | MO | QQ | KM | SS | GQ | PP |
| 48663 | NR | MM | JO | MO | JN | LP | MQ | IL | PP | NQ | IS | QR | LM |
| 48664 | MR | LP | LM | LL | KM | PP | PQ | LR | NP | MN | JJ | LQ | FF |
| 48665 | II | MP | LO | KM | JL | OP | MP | MN | NP | JM | JU | LQ | FN |
| 48666 | II | MM | JO | LN | JJ | NP | MM | RR | IP | NN | JS | OQ | KP |
| 48667 | NP | LM | HK | NO | NO | MP | KM | IR | IP | II | JS | QQ | FM |
| 48668 | JM | LM | HP | LL | MM | KO | KP | MO | MQ | KN | KS | QQ | IP |
| 48669 | RR | MM | HJ | LM | KN | KP | KM | LM | NN | FF | KS | GT | HL |
| 48670 | LO | KO | HO | JN | LO | PP | MR | OS | QR | NN | JS | QR | LL |
| 48671 | LP | MM | JJ | MO | JJ | PP | MQ | MM | MN | QR | JJ | QR | FP |
| 48672 | NN | MM | JO | LN | JJ | MM | OQ | OR | PP | NN | JK | KQ | LL |
| 48673 | IM | KM | HO | JN | JL | MP | MR | MR | IR | NN | JS | OR | LP |
| 48674 | IP | MM | HP | KO | JO | LO | KP | LM | MQ | MR | SS | QQ | PP |
| 48675 | IM | MM | OP | KL | MM | LP | KQ | MM | NQ | KM | KS | LQ | FF |
| 48676 | OP | MO | II | NN | JK | OP | KK | LL | OP | PP | KS | LQ | PP |
| 48677 | NQ | KL | JO | JO | JN | MP | OO | LR | MP | FN | KS | GQ | FF |
| 48678 | NN | KP | LO | KN | LN | LO | QQ | OO | PP | NN | JS | KQ | LL |

Genetic Analysis of the
Great Divide Basin HMA, WY0002

E. Gus Cothran

May 15, 2012

Department of Veterinary Integrative Bioscience
Texas A&M University
College Station, TX 77843-4458

The following is a report of the genetic analysis of the Great Divide Basin HMA, WY0002.

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 60 samples were received by Texas A&M University, Equine Genetics Lab on November 18, 2011. 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 Great Divide Basin HMA 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 Great Divide Basin HMA herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the Great Divide Basin HMA herd to a standard set of domestic breeds is shown in Figure 1.

**Genetic Variants:** A total of 93 variants were seen in the Great Divide Basin HMA herd. This is only three less than the highest number observed for a feral herd and well above the mean for domestic breeds. Of these, 31 had frequencies below 0.05 which is a high percentage of variants at risk of future loss. Allelic diversity as represented by *Ae* and *MNA* is quite high as well.

**Genetic Variation:** Observed heterozygosity in the Great Divide Basin HMA herd is well above the feral mean as is *He*. *Ho* is almost identical to *He* indicating genetic equilibrium.

**Genetic Similarity:** Overall similarity of the Great Divide Basin HMA herd to domestic

breeds was about average for feral herds. Highest mean genetic similarity of the Great Divide Basin HMA herd was with Old World Iberian breeds followed closely by Light Racing and Riding breeds and New World Iberian breeds. As seen in Fig. 1, however, the Great Divide Basin HMA herd clusters with the Shetland Pony and outside any major group. These results indicate a herd with mixed origins with no clear indication of primary breed type. As with most trees involving feral herds, the tree is somewhat distorted.

## SUMMARY

Genetic variability of this herd in general is high but understanding the diversity of this herd is somewhat complicated. This herd was previously sampled in 2003. At that time the sample consisted of two subdivisions of the herd area labeled North and South. Genetic variability levels of both groups were relatively high but not quite as high as seen here. Much of the high variability was attributed to mixing of the two groups and that would fit the herd now. However, the herds did not appear to be a single population but maintained some independence. This may not be the case now as the almost zero *Fis* is what would be expected for a single interbreeding group and not a subdivided population. The high percentage of variation that is at risk also is consistent with a formally subdivided population now interbreeding. Genetic similarity results suggest a herd with mixed ancestry.

## RECOMMENDATIONS

Current variability levels are high enough that no action is needed, however, if population size drops below 150 breeding age animals, diversity levels can change quickly.

**Table 1.** Allele frequencies of genetic variants observed in Great Divide Basin HMA feral horse herd.

| VHL20 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| I | J | K | L | M | N | O | P | Q | R | S |
| 0.350 | 0.000 | 0.000 | 0.083 | 0.275 | 0.167 | 0.033 | 0.017 | 0.025 | 0.050 | 0.000 |

| HTG4 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| I | J | K | L | M | N | O | P | Q | R |
| 0.000 | 0.000 | 0.092 | 0.192 | 0.483 | 0.000 | 0.233 | 0.000 | 0.000 | 0.000 |

| AHT4 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| H | I | J | K | L | M | N | O | P | Q | R |
| 0.117 | 0.033 | 0.250 | 0.159 | 0.133 | 0.008 | 0.158 | 0.125 | 0.017 | 0.000 | 0.000 |

| HMS7 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| I | J | K | L | M | N | O | P | Q | R |
| 0.000 | 0.008 | 0.008 | 0.283 | 0.108 | 0.359 | 0.217 | 0.017 | 0.000 | 0.000 |

| AHT5 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| I | J | K | L | M | N | O | P | Q | R |
| 0.033 | 0.292 | 0.017 | 0.008 | 0.175 | 0.317 | 0.158 | 0.000 | 0.000 | 0.000 |

| HMS6 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| I | J | K | L | M | N | O | P | Q | R |
| 0.000 | 0.000 | 0.183 | 0.225 | 0.075 | 0.142 | 0.158 | 0.217 | 0.000 | 0.000 |

| ASB2 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| B | I | J | K | L | M | N | O | P | Q | R |
| 0.000 | 0.068 | 0.000 | 0.060 | 0.000 | 0.256 | 0.171 | 0.103 | 0.000 | 0.308 | 0.034 |

| HTG10 | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H | I | J | K | L | M | N | O | P | Q | R | S | T |
| 0.000 | 0.025 | 0.000 | 0.050 | 0.108 | 0.225 | 0.025 | 0.258 | 0.008 | 0.008 | 0.293 | 0.000 | 0.000 |

| HMS3 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| H | I | J | K | L | M | N | O | P | Q | R | S |
| 0.000 | 0.108 | 0.000 | 0.000 | 0.000 | 0.225 | 0.083 | 0.125 | 0.293 | 0.083 | 0.083 | 0.000 |

| ASB17 | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
| 0.000 | 0.050 | 0.125 | 0.008 | 0.000 | 0.008 | 0.033 | 0.000 | 0.267 | 0.243 | 0.042 | 0.025 | 0.008 | 0.125 | 0.058 | 0.008 |

| ASB23 | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V |
| 0.083 | 0.025 | 0.100 | 0.117 | 0.233 | 0.158 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.167 | 0.000 | 0.117 | 0.000 |

| LEX33 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| F | G | K | L | M | N | O | P | Q | R | S | T |
| 0.000 | 0.000 | 0.192 | 0.208 | 0.008 | 0.008 | 0.243 | 0.008 | 0.150 | 0.183 | 0.000 | 0.000 |

**Table 2.** Genetic variability measures.

|  | N | Ho | He | Fis | Ae | TNV | MNA | Ra | %Ra |
|---|---|---|---|---|---|---|---|---|---|
| **GREAT DIVIDE BASIN WY** | **60** | **0.785** | **0.787** | **0.003** | **4.929** | **93** | **7.75** | **31** | **0.33** |
|  |  |  |  |  |  |  |  |  |  |
| 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 Great Divide Basin HMA feral horse herd to major groups of domestic horses.

|  | Mean S | Std | Minimum | Maximum |
|---|---|---|---|---|
| Light Racing and Riding Breeds | 0.780 | 0.037 | 0.727 | 0.827 |
| Oriental and Arabian Breeds | 0.777 | 0.042 | 0.718 | 0.836 |
| Old World Iberian Breeds | 0.785 | 0.022 | 0.761 | 0.820 |
| New World Iberian Breeds | 0.779 | 0.030 | 0.729 | 0.807 |
| North American Gaited Breeds | 0.771 | 0.026 | 0.738 | 0.802 |
| Heavy Draft Breeds | 0.734 | 0.069 | 0.638 | 0.846 |
| True Pony Breeds | 0.747 | 0.028 | 0.713 | 0.777 |

**Figure 1.** Partial RML tree of genetic similarity to domestic horse breeds.
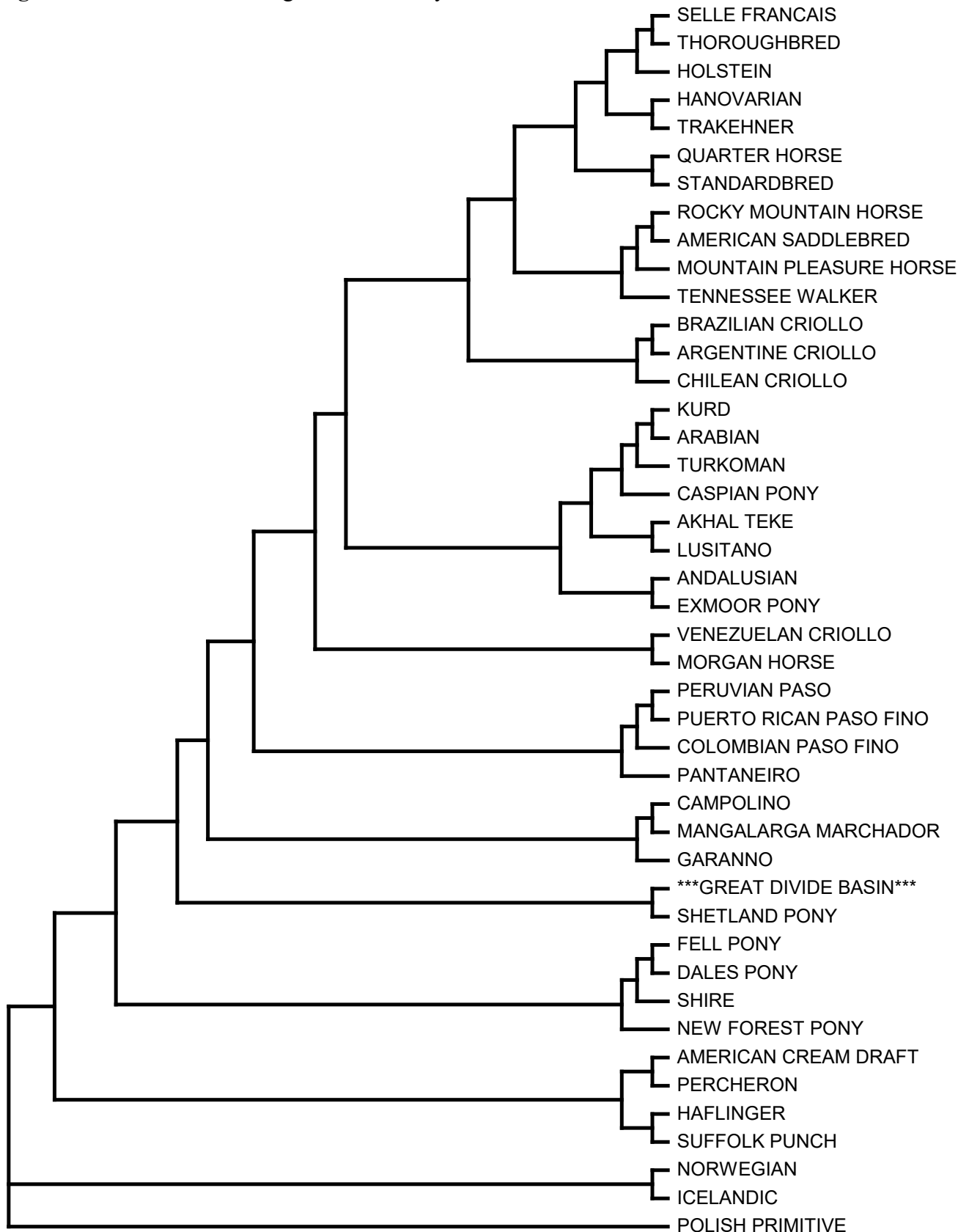
**Appendix 1**. DNA data for the Great Divide Basin HMA, WY herd.

| AID | VHL20 | HTG4 | AHT4 | HMS7 | AHT5 | HMS6 | ASB2 | HTG10 | HMS3 | ASB17 | ASB23 | LEX3 | LEX33 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 56831 | IP | KM | HJ | LL | MO | PP | NQ | MR | IO | FR | KL | LM | KL |
| 56832 | IM | LM | KL | NO | JN | LN | OQ | LN | IP | RR | LU | FN | RR |
| 56833 | LM | MM | JM | LL | MN | LN | MN | MO | OP | FG | KS | NP | RR |
| 56834 | II | LO | JK | LL | IJ | KN | KM | MO | PR | RS | HS | NP | OR |
| 56835 | IM | OO | JJ | NN | JN | OO | QQ | MR | IO | FM | UU | FN | KL |
| 56836 | IM | MO | JN | NO | MN | OO | RR | QR | MO | GH | IL | HH | LO |
| 56837 | IN | MM | NO | NO | NO | KL | MQ | MO | MP | NN | IK | FM | LR |
| 56838 | IO | OO | JN | NN | JL | KO | NQ | MR | IN | FG | JU | MN | KL |
| 56839 | IN | OO | NN | LN | NN | KL | QQ | LR | IO | MN | II | FM | LL |
| 56840 | MM | LM | JO | NO | MN | LN | QQ | KM | MP | MT | UU | MQ | LO |
| 56841 | IN | MM | JJ | NO | JO | KL | NO | MO | IM | MN | GK | MP | RR |
| 56842 | NO | OO | OP | OO | JM | OP | QQ | KM | NP | MN | LS | FK | KR |
| 56843 | MN | LL | HL | LN | MM | MP | IN | MP | PQ | MR | LL | KM | KK |
| 56844 | MN | LL | JN | LN | JJ | MP | IN | LN | PR | NN | GU | KN | OQ |
| 56845 | IM | LM | HK | LN | JN | LN | MN | OR | MN | MM | JK | LN | OR |
| 56846 | IR | MM | HL | LM | OO | LN | MN | OR | MP | MM | GK | FP | OQ |
| 56847 | MM | MM | JJ | NP | JO | LM | IM | OO | PP | NR | GK | FP | QQ |
| 56848 | MN | MO | JN | NO | MN | LL | MR | MR | MN | GM | SS | NN | OQ |
| 56849 | IL | MM | JK | NO | JM | MM | II | MN | NR | MM | IJ | KM | LL |
| 56850 | NN | KM | HK | LN | JM | OO | CO | MR | OQ | GO | JU | MN | LQ |
| 56851 | II | MM | JO | LM | NO | NP | KM | OO | MP | GN | GK | FN | KR |
| 56852 | MM | MM | HL | NO | NN | KP | CM | OR | IM | MN | JU | MN | KL |
| 56853 | IL | KL | HL | LO | IN | KP | KM | MO | OP | NS | JS | KQ | KR |
| 56854 | II | MO | OO | LP | NO | LO | QQ | MO | QR | NS | LS | EN | LL |
| 56855 | IR | KM | JJ | LL | MO | KP | NO | RR | IO | FN | KS | KM | KQ |
| 56856 | MQ | MO | HO | JL | NN | NP | NN | OR | NP | RS | IK | MN | OR |
| 56857 | NN | LM | OO | MO | JN | NP | QQ | KK | PP | MM | UU | MQ | OQ |
| 56858 | MM | MM | KL | LN | NO | LP | MN | OR | MM | NN | JK | FN | QR |
| 56859 | IL | LM | HO | NO | JJ | KP | OQ | OR | MP | NN | IK | FN | OQ |
| 56860 | IM | KM | NN | LN | JJ | OO | MM | LR | OO | GM | LL | NQ | OQ |
| 56861 | II | LO | HP | LL | JN | LO | QQ | MO | NQ | GS | JS | LN | OQ |
| 56862 | IR | KM | JL | OO | JN | NP | MM | OR | IP | NR | IK | HN | KO |
| 56863 | MN | MM | IJ | NN | JJ | NO | QQ | RR | MP | JR | SU | KN | KL |
| 56864 | IR | LO | KL | LN | MN | KM | MO | LM | QR | MM | KS | FM | KO |
| 56865 | IM | MM | JK | MO | NO | LP | MQ | OO | MO | KN | KK | FP | KL |
| 56866 | MN | MO | KO | LO | JM | LM | NN | KM | MM | MN | LS | MM | KO |
| 56867 | MQ | LM | HL | LN | JN | NN | NN | OO | MP | MN | GJ | LL | OQ |
| 56868 | IM | MO | NN | OO | NO | KP | MN | RR | IO | NP | LS | MM | MR |
| 56869 | NN | KK | KK | LM | JJ | LO | OQ | MO | IP | NO | KL | NN | LO |
| 56870 | IN | MO | NO | OO | MO | KK | KQ | LR | PQ | NS | GI | FF | LR |
| 56871 | IP | MO | LN | MN | KM | KO | MQ | IM | OP | FG | KL | FF | LL |
| 56872 | IL | KL | NN | MN | JN | KP | MQ | LM | PR | MO | LL | NN | KO |
| 56873 | MR | LM | IL | LN | JO | LO | IM | MR | MQ | MR | KS | NN | KQ |
| 56874 | IM | LM | KL | MN | NN | KN | QR | LR | PR | KO | KS | NN | KO |
| 56875 | NQ | MO | JK | LN | MM | MO | KM | MR | MQ | MQ | SU | FF | LR |
| 56876 | MM | LM | KL | LN | NO | PP | NO | LR | NP | GP | JK | NN | KO |
| 56877 | IM | LM | HH | LN | IO | LL | IM | RR | MP | MR | GH | PP | NQ |
| 56878 | IL | LL | KK | LN | NN | KL | MN | LO | NP | GM | JK | NN | OO |
| 56879 | IM | MO | OO | MN | JN | NP | MO | IR | NR | OR | JL | NN | LP |
| 56880 | LR | LM | LO | LM | JO | LP | MM | MR | MM | NR | IL | KK | QR |
| 56881 | IN | MO | LN | NO | MN | KP | IQ | IL | MQ | KS | IK | LL | OR |
| 56882 | IL | KM | JK | KN | IN | KL | KQ | OO | IQ | MN | HS | MM | KL |
| 56883 | LO | MO | JJ | LO | JK | NP | NQ | KR | OP | GN | KL | LL | KO |
| 56884 | IM | MM | KL | NO | NN | KO | KQ | MR | PR | KM | IS | MM | OO |
| 56885 | NO | OO | JN | MN | JM | LO | OQ | MM | MP | MP | LS | NN | LO |
| 56886 | IM | MO | JN | NO | NO | LL | QQ | LO | OR | GN | JK | QQ | LQ |
| 56887 | IL | LM | HJ | LN | JO | KP | CM | OO | MP | MR | KK | FF | KO |
| 56888 | IM | KM | JN | NO | JM | PP | MO | OR | MP | GN | GS | NN | KR |
| 56889 | IN | MO | IK | MN | JM | LM | OQ | RR | MP | MR | GU | FF | RR |
| 56890 | IM | MO | IJ | MN | JN | KN | MQ | LO | IP | GM | JK | QQ | OQ |

Genetic Analysis of the
White Mountain HMA, WY

E. Gus Cothran

April 30, 2012

Department of Veterinary Integrative Bioscience
Texas A&M University
College Station, TX 77843-4458

The following is a report of the genetic analysis of the White Mountain HMA, WY.

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 60 samples were received by Texas A&M University, Equine Genetics Lab on September 7, 2011. 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 White Mountain HMA 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 White Mountain HMA herd to domestic horse breed types

are shown in Table 3. A dendrogram of relationship of the White Mountain HMA herd to a

standard set of domestic breeds is shown in Figure 1.

**Genetic Variants:** A total of 91 variants were seen in the White Mountain HMA herd

which is far above the means for feral herds and domestic breeds. Of these, 34 had frequencies

below 0.05 which is a high percentage of variants at risk of future loss. Allelic diversity as

represented by *Ae* and *MNA* also have values much greater than the mean values.

**Genetic Variation:** Observed heterozygosity in the White Mountain HMA herd is slightly

below the feral mean while *He* is only slightly higher than average. *Ho* is lower than *He* but not

by a significant margin. The herd is very close to genetic equilibrium.

**Genetic Similarity:** Overall similarity of the White Mountain HMA herd to domestic breeds was about average for feral herds. Highest mean genetic similarity of the White Mountain HMA herd was with North American Gaited breeds, followed closely by the Light Racing and Riding breeds. As seen in Fig. 1, however, the White Mountain HMA herd clusters within a group Iberian horse breeds. These results indicate a herd with mixed origins with no clear indication of primary breed type. It is possible that there is some Spanish blood based upon the clustering but this is not strong evidence in this case. As with most trees involving feral herds, the tree is somewhat distorted.

**SUMMARY**

Genetic variability of this herd in general is on the high side but there is a high percentage of variation that is at risk and individual heterozygosity is below average by a small amount. The patterns seen here are very similar to what was seen in 2000 based upon blood typing. It was suggested then that there may be some gene flow into the population and that is consistent with the current data. The very high allelic diversity but high proportion of alleles at low frequency is just what would be expected if there was a small influx of horses into the herd at different times. It is possible there is introgression from the two neighboring HMAs with Salt Wells being the more probable based upon level of differentiation. Genetic similarity results suggest a herd with mixed ancestry.

**RECOMMENDATIONS**

Current variability levels are high enough that no action is needed at this point but the herd should be monitored closely due to the high proportion of rare alleles. This is especially true if it is known that the herd size has seen a recent decline.

**Table 1.** Allele frequencies of genetic variants observed in White Mountain HMA feral horse herd.

**VHL20**

| I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.167 | 0.000 | 0.000 | 0.083 | 0.191 | 0.317 | 0.050 | 0.000 | 0.017 | 0.175 | 0.000 |

**HTG4**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.025 | 0.125 | 0.700 | 0.017 | 0.033 | 0.100 | 0.000 | 0.000 |

**AHT4**

| H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.375 | 0.042 | 0.283 | 0.000 | 0.125 | 0.008 | 0.000 | 0.159 | 0.008 | 0.000 | 0.000 |

**HMS7**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.141 | 0.117 | 0.341 | 0.067 | 0.250 | 0.067 | 0.000 | 0.017 | 0.000 |

**AHT5**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.017 | 0.283 | 0.017 | 0.050 | 0.050 | 0.150 | 0.433 | 0.000 | 0.000 | 0.000 |

**HMS6**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.067 | 0.025 | 0.108 | 0.008 | 0.050 | 0.742 | 0.000 | 0.000 |

**ASB2**

| B | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.050 | 0.283 | 0.000 | 0.075 | 0.000 | 0.108 | 0.125 | 0.008 | 0.017 | 0.242 | 0.092 |

**HTG10**

| H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.025 | 0.000 | 0.017 | 0.017 | 0.292 | 0.117 | 0.125 | 0.000 | 0.000 | 0.382 | 0.025 | 0.000 |

**HMS3**

| H | I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.108 | 0.000 | 0.000 | 0.000 | 0.083 | 0.142 | 0.033 | 0.525 | 0.092 | 0.017 | 0.000 |

**ASB17**

| D | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.108 | 0.000 | 0.000 | 0.000 | 0.000 | 0.191 | 0.000 | 0.117 | 0.050 | 0.067 | 0.025 | 0.017 | 0.200 | 0.217 | 0.008 |

**ASB2**

| G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.025 | 0.000 | 0.125 | 0.067 | 0.358 | 0.250 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.100 | 0.000 | 0.075 | 0.000 |

**LEX33**

| F | G | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.017 | 0.083 | 0.350 | 0.125 | 0.000 | 0.092 | 0.108 | 0.133 | 0.017 | 0.025 | 0.050 |

**Table 2.** Genetic variability measures.

| | *N* | *Ho* | *He* | *Fis* | *Ae* | *TNV* | *MNA* | *Ra* | *%Ra* |
|---|---|---|---|---|---|---|---|---|---|
| **WHITE MOUNTAIN WY** | **60** | **0.701** | **0.715** | **0.020** | **4.06** | **91** | **7.58** | **34** | **0.374** |
| | | | | | | | | | |
| 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 White Mountain HMA feral horse herd to major groups of domestic horses.

| | Mean *S* | Std | Minimum | Maximum |
|---|---|---|---|---|
| Light Racing and Riding Breeds | 0.755 | 0.025 | 0.715 | 0.784 |
| Oriental and Arabian Breeds | 0.739 | 0.039 | 0.680 | 0.798 |
| Old World Iberian Breeds | 0.749 | 0.031 | 0.716 | 0.786 |
| New World Iberian Breeds | 0.746 | 0.036 | 0.700 | 0.789 |
| North American Gaited Breeds | 0.762 | 0.035 | 0.723 | 0.806 |
| Heavy Draft Breeds | 0.684 | 0.056 | 0.591 | 0.743 |
| True Pony Breeds | 0.720 | 0.026 | 0.695 | 0.759 |

**Figure 1.** Partial RML tree of genetic similarity to domestic horse breeds.
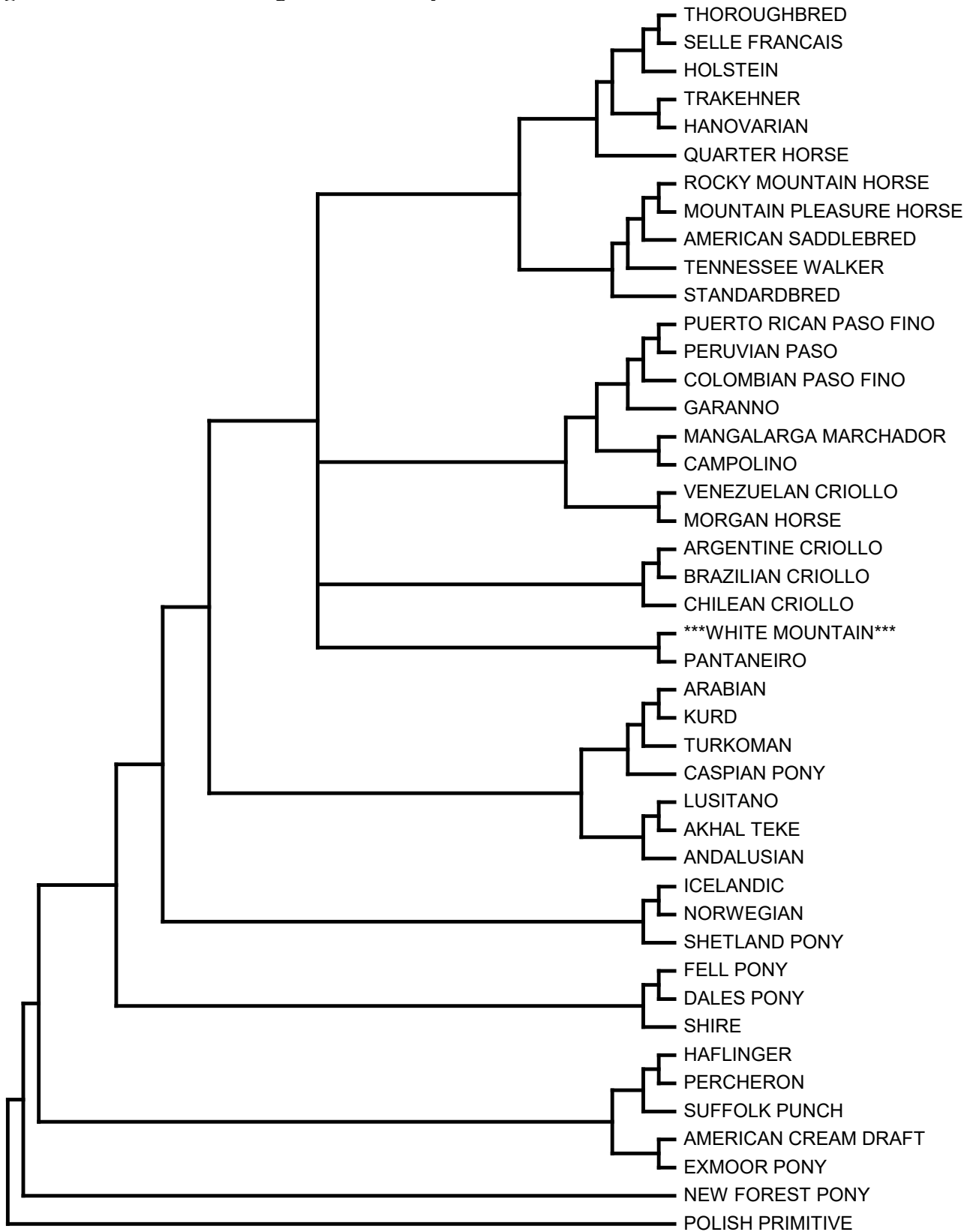
**Appendix 1**. DNA data for the White Mountain HMA, WY herd.

| AID | VHL20 | HTG4 | AHT4 | HMS7 | AHT5 | HMS6 | ASB2 | HTG10 | HMS3 | ASB17 | ASB23 | LEX33 | LEX3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 55250 | IN | LM | JO | JN | JJ | MP | IM | MR | PQ | SS | KU | LL | FP |
| 55251 | NR | MM | IJ | JK | NO | PP | IM | MN | PP | KR | II | OP | FF |
| 55252 | IO | LM | HH | JL | OO | MP | MQ | NR | MP | KK | KK | OP | FL |
| 55253 | IM | LM | HJ | JN | JN | MP | IM | RR | PP | KS | SU | LL | FF |
| 55254 | NR | MM | HJ | JL | OO | PP | IR | MR | PP | KR | IS | LT | FH |
| 55255 | NN | MM | LO | KL | JJ | PP | II | MR | NN | OR | KL | MT | FF |
| 55256 | IM | MN | HJ | LO | NO | NP | KN | MR | MP | OP | IJ | KR | HP |
| 55257 | IN | MM | HJ | LN | NO | PP | IK | RR | PQ | QR | KL | MS | HL |
| 55258 | MN | MM | HH | LM | JO | PP | IR | MR | NP | FR | KK | LL | HP |
| 55259 | LL | MP | HH | JN | JJ | OP | IK | MR | PQ | FM | KU | LQ | KP |
| 55260 | IM | LM | JL | NN | NO | MP | IR | MR | PP | FS | KK | KP | FF |
| 55261 | MN | MP | HJ | LN | OO | PP | IN | MR | NP | FK | KL | MP | HP |
| 55262 | MR | MM | JO | KM | JO | PP | IM | MN | PP | MR | LL | KL | FP |
| 55263 | IO | LP | OO | LO | IO | PP | IK | MN | IP | KS | IK | PQ | FN |
| 55264 | NR | MM | HJ | LN | OO | PP | IQ | MM | NP | SS | KU | PT | FH |
| 55265 | NN | MM | HI | KL | NO | MP | QQ | MR | PQ | MN | KL | KL | FF |
| 55266 | NR | MM | LO | JN | NO | MP | IQ | NR | NP | KS | JS | LL | FF |
| 55267 | MR | MM | JJ | KN | JJ | PP | IP | MR | NP | MS | IK | PQ | FP |
| 55268 | MN | KP | HI | JK | NO | MP | IM | OR | PP | KR | LL | LM | FP |
| 55269 | RR | MM | HO | JL | NO | PP | MQ | RR | IP | KK | KL | LT | FP |
| 55270 | MM | MM | JJ | LL | NN | PP | QQ | MR | PP | SS | JK | LL | FH |
| 55271 | LN | MM | HJ | LO | JN | PP | BI | MO | MP | FR | LU | KQ | FO |
| 55272 | NR | LL | HM | LN | JM | OP | QQ | OR | NQ | QS | KL | LL | FP |
| 55273 | MN | MP | LO | LM | LO | PP | QR | MO | IN | MR | KS | QQ | FF |
| 55274 | LM | MM | HO | LN | JL | KK | NQ | MO | OQ | NS | KS | LO | FH |
| 55275 | MN | MM | HH | LL | OO | PP | IN | RR | PQ | KO | KL | OS | FF |
| 55276 | IN | MM | HH | LO | JM | KO | BQ | IO | IN | MM | GK | KL | FN |
| 55277 | LL | LL | HO | NO | LO | KP | KR | MO | OO | MO | KK | OR | KN |
| 55278 | IM | MM | JJ | JN | OO | MP | IR | MR | PP | FR | KK | LP | FF |
| 55279 | IN | KN | HJ | MO | JO | MP | NQ | KR | IN | MO | KS | LQ | FP |
| 55280 | IR | MM | JO | KL | NO | MP | MM | NO | PP | KM | KL | KQ | PP |
| 55281 | MN | MO | HL | LL | JJ | KP | BI | OR | IP | SS | IK | GM | FF |
| 55282 | IO | MP | HH | LN | JJ | LO | NN | KR | PR | NT | LS | LO | MM |
| 55283 | NO | MP | OP | LL | JO | LO | IN | IR | OR | NR | JL | LO | MM |
| 55284 | LR | LL | JL | NO | MO | PP | BQ | OR | IP | MS | LS | QQ | II |
| 55285 | NR | MP | HJ | LO | JO | PP | IQ | NN | II | RS | IK | LM | FF |
| 55286 | IR | KM | JO | KN | NO | PP | MQ | NO | PP | KM | LL | KQ | PP |
| 55287 | IL | MM | LO | NN | MM | KP | KN | MM | NP | FF | IS | MO | HP |
| 55288 | MR | MP | HH | KN | NO | PP | IM | NR | PP | RS | LU | LP | FF |
| 55289 | II | OO | HH | QQ | KO | PP | QQ | LS | IN | OR | IL | LQ | PP |
| 55290 | OR | MM | HL | JN | JO | PP | IR | RR | PP | RR | LS | LM | FF |
| 55291 | IN | MM | HJ | JL | OO | PP | PQ | MR | PP | KO | KL | KM | FF |
| 55292 | IR | MM | JJ | LL | NO | MP | MQ | NO | NP | FM | KL | KM | PP |
| 55293 | OR | PP | HO | MN | MN | LM | KO | MM | MM | NR | LU | LQ | MM |
| 55294 | MN | MM | HO | LN | JO | PP | II | RR | NQ | KR | LL | OS | FF |
| 55295 | LQ | LM | LO | LN | LL | KO | BK | MS | MM | KM | JL | LQ | FF |
| 55296 | MN | MM | LO | LM | JO | PP | NR | NR | PP | RS | IK | LP | FF |
| 55297 | NR | MM | HJ | JN | OO | PP | QQ | NR | NP | NR | II | PP | FF |
| 55298 | RR | MM | JL | KN | JO | PP | IQ | MO | PQ | FK | KU | MT | PP |
| 55299 | MN | MM | IO | KL | OO | PP | IR | MR | PP | FK | IK | LO | FF |
| 55300 | NR | MM | HL | JN | JO | PP | IR | RR | PP | FR | KS | LL | FF |
| 55301 | LM | MM | HJ | LN | JJ | KP | BM | IO | MP | RS | KL | GM | PP |
| 55302 | MQ | LM | HH | MN | KL | PP | KN | LS | MP | PS | JK | LQ | FF |
| 55303 | MN | MM | JL | KM | JO | PP | QQ | NR | PQ | FS | KK | LT | FF |
| 55304 | NN | MP | HH | LL | IJ | PP | II | MO | IN | RS | GU | LL | LL |
| 55305 | NN | MM | JJ | JK | JO | PP | NQ | MR | PP | KS | JK | LM | FF |
| 55306 | MN | MM | JJ | LL | OO | PP | NN | MR | PQ | OS | JL | MM | PP |
| 55307 | IN | LM | HI | KL | NO | MP | IN | MR | MP | KK | IK | OP | FF |
| 55308 | NN | MM | HJ | JN | JO | PP | IQ | MR | PP | KR | KS | LL | FF |
| 55309 | IN | LO | LL | JL | JO | PP | QR | MR | II | PS | GL | LQ | PP |

Genetic Analysis of the
Little Colorado HMA, WY

E. Gus Cothran

May 7, 2012

Department of Veterinary Integrative Bioscience
Texas A&M University
College Station, TX 77843-4458

The following is a report of the genetic analysis of the Little Colorado HMA, WY.

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 45 samples were received by Texas A&M University, Equine Genetics Lab on September 7, 2011. 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 Little Colorado HMA 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 Little Colorado HMA herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the Little Colorado HMA herd to a standard set of domestic breeds is shown in Figure 1.

**Genetic Variants:** A total of 80 variants were seen in the Little Colorado HMA herd which is above the mean for feral herds and right at the mean for domestic breeds. Of these, 15 had frequencies below 0.05 which is a low percentage of variants at risk of future loss. Allelic diversity as represented by *Ae* and *MNA* are both greater than the mean but not by a large margin.

**Genetic Variation:** Observed heterozygosity in the Little Colorado HMA herd is well above the feral mean as is *He*. *Ho* is slightly lower than *He*. The difference is not significant and the herd appears to be in genetic equilibrium.

**Genetic Similarity:** Overall similarity of the Little Colorado HMA herd to domestic breeds was a little above average for feral herds. Highest mean genetic similarity of the Little Colorado HMA herd was with Light Racing and Riding breeds, followed by the North American Gaited breeds. As seen in Fig. 1, however, the Little Colorado HMA herd clusters within the group of Nordic horse breeds. This same relationship was seen in the sample from this herd collected in 2007. This does not likely represent true relationship but instead is an artifact of a herd with mixed origins with changes in the genetic basis of the herd over time. The herd most likely is derived from riding or ranch stock of North American origin but possibly some draft blood which could be the cause of the placement in the tree. As with most trees involving feral herds, the tree is somewhat distorted. In comparison to other feral herds from Wyoming (Fig. 2), there is no herd that is closely similar.

## SUMMARY

Genetic variability of this herd in general is on the high side but compared to 2007 there has been a loss of observed heterozygosity. Every other measure of genetic variation was essentially the same with what differences there were attributable to sampling variation. The change in $Ho$ is accompanied by a change in $Fis$ from a negative to a positive value. This could be an indication in a loss of population size which has resulted in greater inbreeding (positive $Fis$). However, this would not be expected in a period of only 5 years. Genetic similarity results suggest a herd with mixed ancestry.

## RECOMMENDATIONS

Current variability levels are high enough that no action is needed at this point but the herd should be monitored closely due to the observed change in in $Ho$. This is especially true if it is known that the herd size has seen a recent major reduction in size.

**Table 1.** Allele frequencies of genetic variants observed in Little Colorado HMA feral horse herd.

**VHL20**

| I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.144 | 0.000 | 0.000 | 0.033 | 0.278 | 0.256 | 0.100 | 0.089 | 0.022 | 0.078 | 0.000 |

**HTG4**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.100 | 0.078 | 0.189 | 0.000 | 0.244 | 0.389 | 0.000 | 0.000 |

**AHT4**

| H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.200 | 0.056 | 0.300 | 0.011 | 0.044 | 0.000 | 0.000 | 0.345 | 0.044 | 0.000 | 0.000 |

**HMS7**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.167 | 0.000 | 0.300 | 0.067 | 0.455 | 0.000 | 0.000 | 0.011 | 0.000 |

**AHT5**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.222 | 0.100 | 0.044 | 0.233 | 0.223 | 0.178 | 0.000 | 0.000 | 0.000 |

**HMS6**

| I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.000 | 0.078 | 0.178 | 0.044 | 0.178 | 0.522 | 0.000 | 0.000 |

**ASB2**

| B | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.089 | 0.000 | 0.078 | 0.000 | 0.089 | 0.356 | 0.244 | 0.033 | 0.111 | 0.000 |

**HTG10**

| H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.167 | 0.000 | 0.078 | 0.078 | 0.267 | 0.000 | 0.256 | 0.011 | 0.000 | 0.143 | 0.000 | 0.000 |

**HMS3**

| H | I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.167 | 0.000 | 0.000 | 0.000 | 0.244 | 0.233 | 0.056 | 0.211 | 0.000 | 0.089 | 0.000 |

**ASB17**

| D | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.067 | 0.111 | 0.000 | 0.056 | 0.000 | 0.067 | 0.000 | 0.078 | 0.167 | 0.044 | 0.011 | 0.000 | 0.288 | 0.100 | 0.011 |

**ASB2**

| G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.000 | 0.167 | 0.044 | 0.189 | 0.112 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.244 | 0.000 | 0.244 | 0.000 |

**LEX33**

| F | G | K | L | M | N | O | P | Q | R | S | T |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.000 | 0.067 | 0.322 | 0.311 | 0.000 | 0.000 | 0.111 | 0.000 | 0.122 | 0.056 | 0.011 | 0.000 |

**Table 2.** Genetic variability measures.

| | N | Ho | He | Fis | Ae | TNV | MNA | Ra | %Ra |
|---|---|---|---|---|---|---|---|---|---|
| **LITTLE COLORADE WY** | **45** | **0.761** | **0.768** | **0.009** | **4.54** | **80** | **6.67** | **15** | **0.188** |
| | | | | | | | | | |
| 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 Little Colorado HMA feral horse herd to major groups of domestic horses.

| | Mean S | Std | Minimum | Maximum |
|---|---|---|---|---|
| Light Racing and Riding Breeds | 0.786 | 0.027 | 0.749 | 0.820 |
| Oriental and Arabian Breeds | 0.753 | 0.034 | 0.712 | 0.800 |
| Old World Iberian Breeds | 0.760 | 0.021 | 0.739 | 0.793 |
| New World Iberian Breeds | 0.747 | 0.015 | 0.728 | 0.769 |
| North American Gaited Breeds | 0.770 | 0.019 | 0.747 | 0.793 |
| Heavy Draft Breeds | 0.714 | 0.048 | 0.670 | 0.799 |
| True Pony Breeds | 0.717 | 0.044 | 0.647 | 0.765 |

**Figure 1.** Partial RML tree of genetic similarity to domestic horse breeds.
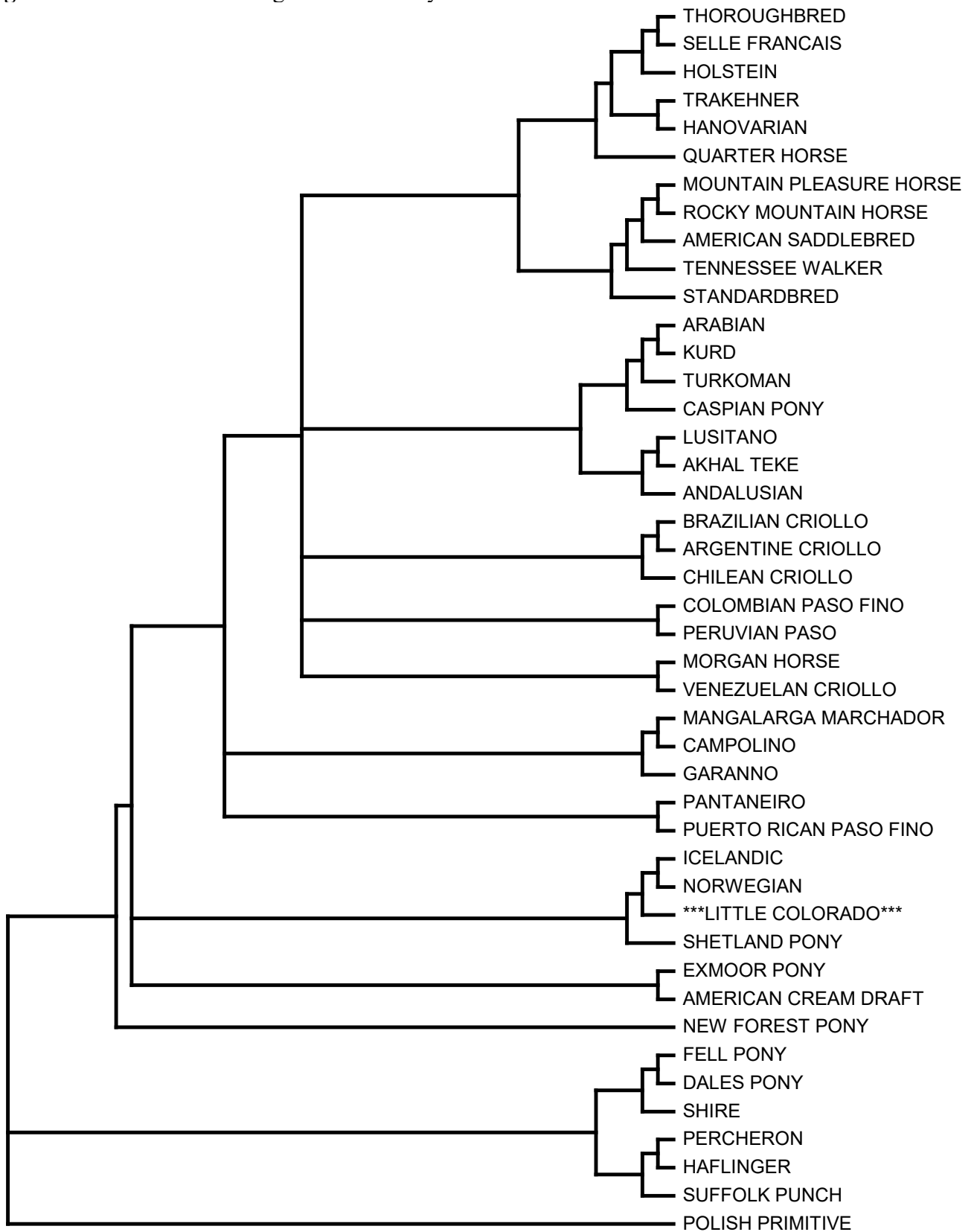
**Appendix 1**. DNA data for the Little Colorado HMA, WY herd.

| AID | VHL20 | HTG4 | AHT4 | HMS7 | AHT5 | HMS6 | ASB2 | HTG10 | HMS3 | ASB17 | ASB23 | LEX33 | LEX3 |
|-----|-------|------|------|------|------|------|------|-------|------|-------|-------|-------|------|
| 55205 | IR | KP | HJ | LM | MN | PP | MN | KR | MN | MR | IK | GQ | NN |
| 55206 | LL | PP | IP | JL | MO | MP | OQ | MM | OP | OR | SU | OR | LL |
| 55207 | IM | KP | OP | LN | MO | LO | IK | OR | IN | MS | LU | OO | HH |
| 55208 | MR | MO | HO | JL | JN | PP | KO | MO | II | FR | KU | KK | MM |
| 55209 | MM | PP | JO | JL | LO | MP | IQ | OO | MN | NR | IU | KL | MM |
| 55210 | LP | PP | LO | NN | JO | LP | MN | KR | IO | SS | LU | KL | LL |
| 55211 | MM | OP | JJ | JN | JN | LM | NQ | MO | MP | GI | SS | KL | LL |
| 55212 | MQ | LO | LL | LN | MO | PP | NN | IK | NR | RS | JS | GK | LL |
| 55213 | NO | MO | HP | LN | MO | PP | MN | IK | IN | RT | JS | KQ | LL |
| 55214 | MR | KO | JJ | JL | MM | PP | IN | KM | MM | FR | KU | KQ | KK |
| 55215 | NN | MO | HJ | LL | MN | OP | KN | LO | NN | GR | LU | KR | NN |
| 55216 | NP | MO | HO | JL | MN | LO | MN | LR | NP | NR | LU | KQ | KK |
| 55217 | NN | LM | HJ | LQ | MN | MO | NN | IO | MR | KR | II | GO | PP |
| 55218 | IP | LO | IO | NN | KO | MP | OQ | IR | MP | KN | SU | LQ | KK |
| 55219 | NP | OP | HJ | JN | KO | MP | MM | LM | PR | NR | KS | KL | NN |
| 55220 | MN | KP | JO | LL | MN | PP | NN | KM | MN | FO | IK | KQ | NN |
| 55221 | MN | OP | HO | LN | JK | LO | NP | LO | MM | KN | IU | LL | FF |
| 55222 | MN | PP | JO | NN | JK | OP | NP | MR | MM | KN | IU | LL | KK |
| 55223 | MN | KP | JJ | MN | LM | PP | KO | MM | MN | RR | KK | KL | MM |
| 55224 | OO | PP | HO | NN | JO | PP | NO | RR | IO | IS | LS | LO | NN |
| 55225 | NN | MO | HJ | LL | MO | OP | NN | MM | NN | GR | II | GK | LL |
| 55226 | IP | KL | HO | JN | JK | MO | KQ | RR | PP | NS | SU | LO | LL |
| 55227 | II | KL | IO | NN | JK | PP | KQ | IR | PP | NO | LU | LO | LL |
| 55228 | NN | MO | JP | LN | MN | OP | NO | MM | NR | OR | IU | KK | LL |
| 55229 | OO | MO | KO | NN | JJ | PP | MO | KM | IR | IR | JS | KL | NN |
| 55230 | MP | OP | IO | LN | JO | LN | OO | IO | PP | MR | IU | LL | MN |
| 55231 | MO | PP | HO | JN | JO | LP | NN | IO | MM | FG | KS | LO | MP |
| 55232 | MM | PP | JJ | JL | JN | PP | NO | MO | MM | NR | SU | KL | MP |
| 55233 | IM | OP | HO | MN | NN | PP | OO | IM | NP | GR | KK | KL | HN |
| 55234 | NR | KP | HJ | JN | LN | MP | OO | MR | NN | GR | KK | LL | MP |
| 55235 | MN | OP | OO | NN | MN | OP | NO | LO | NR | IR | SU | KL | LM |
| 55236 | IR | MP | IO | MN | KN | PP | MQ | IP | IM | NP | KK | LS | FM |
| 55237 | MM | OO | JO | LN | LN | MM | IQ | IO | PR | IR | IS | KR | LM |
| 55238 | IN | MP | HO | LN | MN | NO | NO | MO | NO | GS | KS | KR | HN |
| 55239 | MN | MM | JO | LL | JN | MO | NO | IO | IM | MN | IL | GK | LN |
| 55240 | NP | MP | JJ | NN | JJ | MO | II | LO | PR | NR | LS | KR | FL |
| 55241 | MM | OO | JO | JN | JN | PP | IQ | MO | IP | MR | SS | KL | LM |
| 55242 | IO | MO | JO | NN | OO | PP | NQ | IO | PP | KM | UU | KQ | KN |
| 55243 | OP | LM | JO | NN | MO | MN | NO | IM | MP | NN | SU | KL | KN |
| 55244 | II | LM | HO | JN | JK | MP | NO | II | II | FG | LU | QQ | NN |
| 55245 | NO | OP | HO | LN | JK | OO | OP | LR | MN | KN | JU | LO | FK |
| 55246 | MR | KP | JO | JM | MN | MP | KO | MO | IN | FG | IK | GL | HM |
| 55247 | NR | PP | HJ | LM | MN | MP | NO | MM | MP | GR | IK | LQ | HN |
| 55248 | MN | MP | OO | JL | MO | NO | IN | OO | IO | MS | SS | KO | HO |
| 55249 | IQ | PP | LO | NN | JM | PP | NN | OO | IN | RS | LS | KQ | LM |

**Figure 2.** Dendrogram of genetic similarity of Wyoming herds.

```
                                         SIMILARITY

   .60      .64      .68      .72      .76      .80      .84      .88      .92      .96     1.00
   +----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+


              ******************************************************************************************** CEDAR RIDGE TRAP
              *
              *                    ************************************************************************ HAPPY CREEK SPRINGS
              *                    *
              *                    *         ****************************************************** COOPER CREEK
              *                    *    ******
              *                    *    *    ************************************************** MCCULLOUGH PEAKS
              *                    *    *  *
              *                    *    *  *               ************************************** ADOBE TOWN
              *                    *  ***        *******
              *                  *** * *    ********   ************************************** SALT WELLS EAST
              *                  * * * *    *      *
              *                  * * * * ***   ************************************** DIVIDE BASIN
              *                  * * ** * *
   ***              * * ** **** ******************************************** SALT WELLS WEST
   * *              * * **   *
   * *              * * **    ************************************************** LITTLE COLORADO
   * *              * * **
   * *              * * **         ********************************************** EAGLES NEST
   * *        ******* ******************
   * *        *      *  *  *          *    ************************************** LOST CREEK
   * *        *      *  *  *      *********
   * *        *      *  *  *          ************************************** STEWART CREEK
   * *        *      *  *
   * *    ****  *  *  *      ************************************** MUSKRAT BASIN
   * *    *  *  *  *  ***************************
   * *    *  *  *      ************************************** DISHPAN BUTTE
   * *    *  *  *
   * *********  *  ************************************************************* ANTELOPE HILLS
   *       *  *
   *       *  ************************************************************** WHITE MOUNTAIN
   *       *
   *       ************************************************************** CONANT CREEK
   *
   ************************************************************************* COLD SPRINGS TRAP


   +----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+----+
   .60      .64      .68      .72      .76      .80      .84      .88      .92      .96     1.00
```