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# Transferrin and Hemoglobin Polymorphism in Feral Horses (*Equus caballus*)

## Abstract

The North American feral horse (*Equus caballus*) is quite abundant (45,000-50,000) yet little is known about their origins, the genetic composition of this population, or their relationships to domestic breeds. To study these relationships we examined protein variations in hemoglobin and the plasma enzyme transferrin among feral and domestic horses to assess their relationship to one another. Eighty-six feral horses from six genetically isolated herds represented (1) Pryor Mountain, MT, (2) Challis, ID, (3) Modoc, CA, (4) Powell Mt., NV, (5) Devil's Lake, CA, and Kiger Plateau, OR. Nine domestic horses described as Barbs, from Porterville, CA were also examined. Plasma transferrins were separated by polyacrylamide gel electrophoresis; whole blood was subjected to isoelectric focusing to identify hemoglobin polymorphisms. Four different transferrin variants were identified and the Pryor and Challis horses differed significantly ($P < 0.02$) from the entire population in transferrin frequencies. Inter-herd variation was less among the five far western herds than between themselves and the Pryor and Challis horses. Four hemoglobin alleles were identified, and only the Pryor horses failed to conform to Hardy-Weinberg equilibrium. The Porterville Barbs were indistinguishable from the feral horses on the basis of transferrin and hemoglobin polymorphism. It appears the Pryor horses, and to a lesser degree the Challis horses, have different transferrin and/or hemoglobin polymorphisms from the five other herds. In general, they show less diversity among these two parameters. The causes for this lack of diversity are not known but may be the result of a population bottleneck and genetic drift, or substantially different origins from the other herds.

## Introduction

In spite of a relatively large population, relatively little is known about either the origin or biology of the North American feral horse (*Equus caballus*). These horses, estimated at 45,000 to 50,000 in number, represent a myriad of breeds and it has become increasingly apparent that feral horses are not a homogenous population. There appear to be significant differences between feral horses and their domestic counterparts, and between different herds of feral horses with respect to anatomy (Hall 1972), size (Hall 1972, Denhardt 1948), color patterns (Hall 1972, Feist 1971), behavior (Boyd 1979, Miller 1979, Nelson 1980, Klingel 1979), adrenal physiology (Kirkpatrick *et al.* 1977) and reproductive biology (Kirkpatrick and Turner 1986). However, the aforementioned differences between various populations of feral horses and between feral horses and domestic horses are the interpretations of endocrinologists, behaviorists, and reproductive physiologists. A shift to genetic studies is a logical next approach to learning more about the diversity of feral horses.

The examination of serum proteins has been used successfully to distinguish various breeds of domestic horses (Gahne 1966, Kaminski 1965, 1969, 1970, Helms and Allen 1971, Allen and Dalton 1975) as well as for various captive populations of Przewalskii's Horse (Podlizchouk and Kaminski 1971), mules, donkeys and zebras (Blake *et al.* 1981, Osterhoff 1966) and Icelandic ponies (Hesselhot 1966). Kaminski (1978) has shown differences in transferrin phenotypes which are striking and significant for different horse breeds and populations.

Kilmartin and Clegg (1967) and Clegg (1970) demonstrated four distinct heritable alpha-chain sequences. Isoelectric focusing techniques have been used by Ryder *et al.* (1979) to identify hemoglobin polymorphisms and their frequencies in *E. przewalskii*.

Since no previous studies of feral horse genetics have been reported, it was of interest to examine this aspect of their biology. Consequently, allelic variations and frequencies at the transferrin locus, and of hemoglobin were examined among six genetically isolated feral horse herds in the western United States and a herd of domestic horses described as Barbs.

## Methods and Materials

Whole blood and plasma samples were collected from 86 feral horses from six different and reproductively isolated herds. The herds included Pryor Mountain, Montana (N = 8), Kiger Plateau, Oregon (N = 21), Challis, Idaho (N = 9), Modoc, CA (N = 23), Powell Mountain (Inyo National

Forest, Nevada) (N = 8), and Devil's Lake, CA (N = 17) (Figure 1). Whole blood and plasma were also collected from nine domestic horses belonging to the Wild Horse Research Farm in Porterville, CA. These latter horses were said to be Barbs, a breed originally indigenous to North Africa. Blood was collected by venipuncture from horses gathered by roundup, and stored frozen as plasma or whole blood. It was impossible to separate horses by harem band and herd location was the only characterization possible.


Figure 1. Geographic location of study herds.

Transferrins were separated from other blood proteins by vertical polyacrylamide slab-gel electrophoresis as described by Maizel (1971). Stacking and resolving gels were 2.5 percent and 7.5 percent polyacrylamide respectively. Samples were prepared for electrophoresis by placing 3 microliter plasma aliquots in 3 microliters of Tris-chloride buffer/glycerol, pH 6.7, and one microliter of 0.25 percent bromphenol blue. Five microliter samples of this mixture were subjected to electrophoresis.

Electrophoresis was carried out on an LKB Multiphor 2117 electrophoresis unit. The buffer system was Tris-glycine, pH 8.3, as described by Davis (1964). Pre-electrophoresis, a condition necessary for concentrating the protein sample, was carried out for ten minutes at 50mA and 250 V. The remainder of the run was carried out at 200 V and 10°C for four hours. Proteins were stained with 0.025 percent Coomasie Blue R-250 and 5 percent acetic acid. Gels were stored in 3.5 percent acetic acid and photographed against a light table.

Hemoglobin polymorphism was examined by isoelectric focusing as described by Ryder (1979). The isoelectric focusing was carried out in 1.5 mm, 5.8 percent polyacrylamide horizontal slab-gels on an LKB Multiphor unit. Three ml of pH 6-8 ampholine (LKB) was incorporated in each gel. Blood samples were prepared by placing 20 microliters of red cell hemolysate in 50 microliters of 0.1 percent KCN. The hemolysate-KCN mixture was absorbed in 5 x 8 mm Whatman No. 3 filter paper and placed directly on the gel on the cathode side. The anode buffer was 1 m $H_3PO_4$ and the cathode buffer was 1 m NaOH. Isoelectric focusing was carried out at 25 mA and 400 V, at 10°C, for 30 minutes, after which the filter paper was removed. The focusing was continued at 900 V for three hours. Gels were fixed in 12.5 percent trichloroacetic acid.

Data were treated statistically by chi-square analysis. Differences were considered significant at the $P < 0.05$ level of confidence.

## Results

Gel electrophoresis yielded four transferrin phenotypes among the 86 feral and nine domestic horses. These four alleles, depicted in Figure 2, were identified as Jf1, Ms1, Lj1, and La1 according to the classification scheme of Kaminski (1978). Table 1 gives the frequencies for each allele.

Chi-square analysis of observed allele frequencies did not, for the most part, violate those expected in a Hardy-Weinberg distribution. Allele frequencies for both the Pryor Mountain horses and the Challis horses differed significantly ($P < 0.02$) from the expected allele frequencies for the entire population examined. Chi-square analysis of allele frequencies between individual herds revealed significant differences in 14 of 21 comparisons (Table 2). Nine of those significant differences (or 64% of the total significant differences) were found between the Pryor Mountain horses (5) and other herds and the Challis horses (4) and other herds.

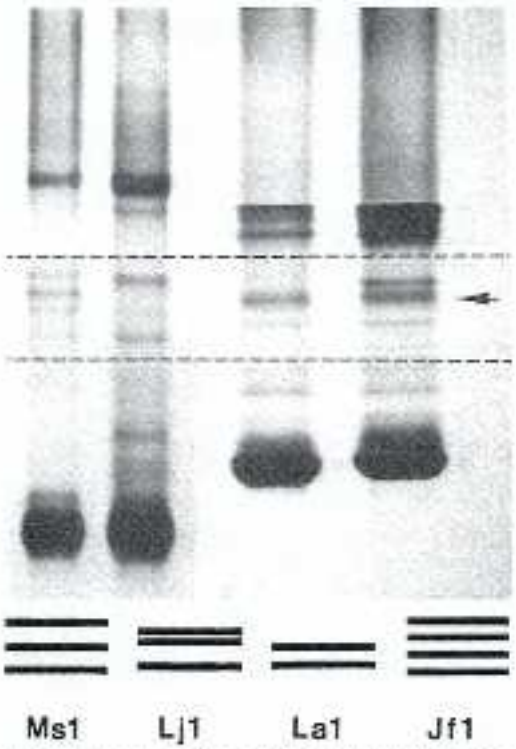


Figure 2. Banding patterns for the four transferrin polymorphisms. The four different forms of the enzyme transferrin are indicated by the protein bands between the dashed lines.

Particular alleles were also examined for uniqueness to populations. The Lj1 allele was found in only two populations, the Pryor Mountain and Powell Mountain herds. In the Pryor Mountain herd it represented three of the eight horses examined (37.5%), whereas in the Powell Mountain herd it was found in only one of the eight horses. The Ms1 allele was most common, being displayed in all herds except the Challis group. In the other six herds this allele presented at a higher frequency than any other. In the Challis herd only the Jf1 allele was evident; this herd was the only one to demonstrate a single allele type. Several herds (Kiger, Modoc, Powell Mountain and Devil's Lake) contained horses possessing allele types unique to them within each population.

Isoelectric focusing of hemoglobin resulted in either two or four bands. The band or bands at the pH 8 side of the gel represent "slow" alpha hemoglobin, with $_{60}$lysine, while the band or bands at the pH 6 side represent "fast" alpha-hemoglobin, with $_{60}$glutamine. In turn, both the fast and slow hemoglobin variants have either $_{24}$tyr or $_{24}$phe, both of which have different isoelectric points. The genes controlling the hemoglobin variants are labeled BI ($a_f^{24\ tyr}$——$a_s^{24\ tyr}$) and BII ($a_f^{24\ phe}$——$a_s^{24\ phe}$). The hemoglobin genotypes for the 95 horses in this study are shown in Table 3. The BI and BII forms appear in approximately equal numbers and the frequencies for these genes for all herds, collectively, do not differ significantly ($P > 0.05$) from those expected in a Hardy-Weinberg distribution. Among individual herds, only the Pryor Mountain horses differed significantly ($P < 0.02$) from a Hardy-Weinberg distribution.

TABLE 1. Transferrin phenotype frequencies among one domestic and six feral populations of horses

| Herd Location | N | Phenotype Jf1 | Ms1 | Lj1 | La1 | $X^2$ | P |
|---|---|---|---|---|---|---|---|
| Pryor Mountain, MT | 8 | 0 | 5 | 3 | 0 | 23.82 | <0.02 |
| Kiger Plateau, OR | 21 | 8 | 12 | 0 | 1 | 2.60 | >0.30 |
| Challis, ID | 9 | 9 | 0 | 0 | 0 | 24.71 | <0.02 |
| Modoc, CA | 23 | 1 | 18 | 0 | 4 | 7.31 | >0.05 |
| Powell Mt., NV | 8 | 0 | 7 | 0 | 1 | 5.35 | >0.30 |
| Devil's Lake, CA | 17 | 7 | 9 | 1 | 0 | 5.35 | >0.30 |
| Porterville, CA | 9 | 0 | 5 | 0 | 4 | 3.71 | >0.30 |

TABLE 2. Comparison of observed transferrin allele frequencies between herds

| Herd Location | N | Compared To | $X^2$ | P |
|---|---|---|---|---|
| Pryor Mountain | 8 | — | — | — |
| | 21 | Kiger Plateau | 11.30 | <0.02 |
| | 9 | Challis | 19.64 | <0.01 |
| | 23 | Modoc | 21.28 | <0.01 |
| | 8 | Powell Mt. | 4.33 | >0.10 |
| | 17 | Devil's Lake | 6.18 | <0.05 |
| | 9 | Porterville | 7.00 | <0.05 |
| Kiger Plateau | 21 | — | — | — |
| | 9 | Challis | 9.53 | <0.01 |
| | 23 | Modoc | 7.75 | <0.05 |
| | 8 | Powell Mt. | 4.34 | >0.10 |
| | 17 | Devil's Lake | 1.81 | >0.50 |
| | 9 | Porterville | 9.36 | <0.01 |
| Challis | 9 | — | — | — |
| | 23 | Modoc | 28.07 | <0.01 |
| | 8 | Powell Mt. | 16.99 | <0.01 |
| | 17 | Devil's Lake | 8.59 | <0.02 |
| | 9 | Porterville | 19.06 | <0.01 |
| Modoc | 23 | — | — | — |
| | 8 | Powell Mt. | 0.62 | >0.70 |
| | 17 | Devil's Lake | 11.82 | <0.01 |
| | 9 | Porterville | 2.73 | >0.20 |
| Powell Mt. | 8 | — | — | — |
| | 17 | Devil's Lake | 6.89 | >0.05 |
| | 9 | Porterville | 2.28 | >0.10 |
| Devil's Lake | 17 | — | — | — |
| | 9 | Porterville | 11.90 | <0.01 |

TABLE 3. Hemoglobin Genotypes by Herd

| | | Genotype | | | |
|---|---|---|---|---|---|
| Herd Location | N | BI/BI | BI/BII | BII/BII | P |
| Pryor Mountain | 8 | 5 | 2 | 1 | <0.02 |
| Kiger Plateau | 21 | 3 | 9 | 9 | >0.10 |
| Challis | 9 | 2 | 5 | 2 | >0.95 |
| Modoc | 23 | 8 | 10 | 5 | >0.50 |
| Powell Mountain | 8 | 1 | 5 | 2 | >0.50 |
| Devil's Lake | 17 | 3 | 8 | 6 | >0.50 |
| Porterville | 9 | 1 | 6 | 2 | >0.50 |
| Total | 95 | 23 | 45 | 27 | >0.70 |

(P = probability that genotype frequency differs from a normal Mendelian distribution by chance alone)

## Discussion

There are four major points of interest which emerge from this study of feral horse genetics. First, of ten known transferrin variants in equids (Chung and McKenzie 1985) only four were identified among the 95 horses examined in this study. In a similar study of 162 feral donkeys Blake *et al.* (1981) found all ten variants present. The ten variants are controlled by codominant autosomal alleles (Gahne 1966) and impart no known selective advantages. Considering the probable diverse origins of the present day feral horse herds, it might be expected that a larger number of variants would exist.

◀A second significant feature of this study is the possibility that one or more of the feral horse herds investigated possesses significant genetic differences from the others. The hypothesis tested was that the transferrin loci in all six feral herds and the domestic herd were in equilibrium. This hypothesis was rejected for both the Pryor herd ($X^2 = 23.82$, $P < 0.02$) and the Challis herd ($X^2 = 24.71$, $P < 0.02$). Additionally, the hypothesis that hemoglobin polymorphism conformed to a Hardy-Weinberg distribution among the 95 horses collectively and among each herd was tested. The hypothesis that the hemoglobin polymorphisms conformed to Hardy-Weinberg equilibrium among the entire 95 horses was not rejected ($X^2 = 0.59$, $P > 0.70$) but equilibrium among the Pryor horses was rejected ($X^2 = 6.0$, $P < 0.02$). This suggests that these two herds, the Pryor and Challis populations (1) have experienced a great deal of inbreeding over the years, (2) have different origins, or (3) have passed through significant genetic bottlenecks sometime in the past. The Pryor Mountain herd is well isolated by the Bighorn Basin of Wyoming and the Pryor Mountains of eastern Montana. Similarly, the Challis horses are separated from other herds by the Salmon River to the north, and mountains to the east, south and west. The remaining four feral herds, however, extend across a region—western Nevada north across northern California and into central Oregon—in which migration could easily have occurred. There are no formidable barriers across their collective ranges (Figure 1). Consequently, these data suggest a mixing of genes among the four westernmost herds and a genetic isolation among the Pryor and Challis herds, which is then reflected in differences in transferrin and hemoglobin polymorphisms. It has been presumed that the largest single source of feral horses was the stock turned loose during the Great Depression. During the almost 60 years since that time, or 10 to 12 generations of horses, it is purely speculative whether sufficient migration could have occurred to account for the homogeneity of the four western herds.

A second interpretation of the differences in polymorphism is that the eastern, and particularly the Montana horses had a very different origin than that of the western herds. Early journals document the existence of the Pryor horses as early as 1810. Little is known about the breed-type from which the present feral horse herds originated, but this must be considered as a possibility for genetic differences, or a lack thereof, among different populations. A third possibility is that of inbreeding. At the time of this study the Pryor herd had approximately 125 animals; Challis, 110; Kiger, 251; Modoc, 491; Powell Mountain, 240, and Devil's Lake, 136. The four western herds have historically been considerably larger than the Pryor or Challis herds. Anecdotal evidence indicates that the Pryor population may have been as low as 10-15 animals by about 1900; after two hard winters in 1977-78 and 1978-79, the Pryor herd was reduced to about 80 horses and less than 20 breeding stallions. Thus, the differences demonstrated by this study may merely reflect genetic drift, caused by a historical "bottleneck" and a degree of inbreeding in the small Pryor and Challis populations and a good mixing of genes in the other four. If inbreeding is suggested, it is noteworthy that neither the Pryor nor the Challis herds suffer from a decline in foaling rates, which are greater than 40 percent and consistent with most other western herds (Kirkpatrick and Turner 1986).

It is important to exercise some caution in interpreting these results, since only a small percentage of total herd populations were sampled. Of the 125 Pryor horses, only 8, or 6.4 percent, were sampled. Similar percentages of horses from other ranges were sampled, with the largest group (Modoc) still representing only 4.6 percent of the total population on that range. While statistical differences are evident, care must be taken in attributing these differences to entire populations.

Finally, the examination of transferrin and hemoglobin polymorphisms among the domestic Porterville Barbs failed to provide a unique genetic marker with which to distinguish these horses. In fact, the similarity of the transferrin polymorphisms between the Porterville horses and those of the four western herds, and particularly those of the Modoc range ($X^2 = 2.73$, $P > 0.20$) suggests a common origin; only five of the 14 significant differences among the 21 inter-herd comparisons were found among the four western feral herds and the Porterville herd. Also, with the exception of the Pryor Mountain herd, the hemoglobin polymorphisms for the other six herds were indistinguishable in form and frequency from those found in domestic breeds (Kilmartin and Clegg 1967) and *Equus przewalskii* (Ryder *et al.* 1979). The possibility that other genetic markers exist which might distinguish the Barbs of Porterville cannot be ruled out by this limited study.

The determination of effective population sizes in feral horse herds would be desirable (Berg 1986). Currently there are no data available inferring genetically effective population sizes in the particular herds studied, or for that matter in other North American feral horse herds. Availability of appropriate estimates, derived from a larger view of enzyme polymorphisms, could permit a modeling approach with which to examine the potential for genetic divergence in feral horse herds.

This pilot study has demonstrated significant differences in transferrin and hemoglobin polymorphisms between certain feral horse herds, and a similarity of those same parameters between a domestic herd of Barb horses and feral horses of Nevada, California, and Oregon. The recent work of George and Ryder (1986) which examined restriction endonuclease maps of mitochondrial DNA among domestic and exotic equids provides a much more powerful tool for examining the genetics of feral equids and directs the next logical step in this study.

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