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Dear Valerie,

A quick, informal note. Here are a few reports so you can get an idea of what can be done. Also, here is the original proposal I submitted to the BLM for doing this type of work.

The key points are the maintenance of genetic variation in the herd and minimizing inbreeding. The key to the above is effective population (Ne) size. The more data that can be obtained the better I can estimate Ne and the more information I can provide about the current genetic status of your herd. Also, we currently know very little about the interaction between population dynamics and genetics of feral horses. This is a good chance to learn something.

Ces

STRATEGIES FOR GENETIC MANAGEMENT OF FERAL HORSE POPULATIONS ON PUBLIC LANDS IN THE UNITED STATES

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The following document is a proposal from the Univerversity of Kentucky Equine Blood Typing Research Laboratory for research support to study the genetics of feral horses on public lands in the United States. The objectives of the proposed research are twofold. First, to assess levels of genetic variability in a variety of feral herds under potentially different selective regimes and populational conditions. These populations will be compared to domestic breeds in order to develop an understanding of how domestication and human selection influence genetic variation in the horse as opposed to natural selection. Second, there are many questions regarding the origins of the feral horses of North America. These questions can only be answered by an in depth genetic analysis of feral horses. A comprehensive study of this sort will require the testing of a large number of breeds from all parts of the world. This work will contribute not only to an understanding of the genetic origins of the feral populations of the United States but also to a better understanding of the genetic relationships among modern horse breeds and the evolution of horses under domestication. The data obtained in this study can be of value in the development of management strategies for the feral horse herds.

BACKGROUND

Since the passage of the Wild and Free-Roaming Horse and Burro Act in 1971, the Bureau of Land Management has been charged with managing wild horse populations on public lands. The management policy must often obtain a balance between preserving the horse herds and maintaining the often delicate ecosystems in which the horses live. To maintain this balance, horse population sizes must be kept at levels low enough to prevent the herds from damaging the public lands. Since there are few natural predators of horses this means periodic removal of horses. However, population sizes small enough to prevent ecological damage may pose problems to the long term health of the horse herds. Modern tools of genetic analysis can be important in determining management strategies that can both keep population sizes low and maintain the long term health of herds.

The concept of Minimum Viable Populations (MVP) has been a central issue in conservation biology since the formal inception of the discipline (see Soule and Wilcox, 1980 and Soule, 1986). Basically, the MVP is the minimum number of breeding individuals that must be maintained for a population to survive a given period of time (in the absence of unavoidable catastrophes). The major concern for small populations is loss of genetic variability through genetic drift and/or inbreeding. Loss of genetic variability can lead to lowered overall health or vigor of the population and, for the long term, loss of adaptability. Genetic drift is the loss of variation due to sampling errors in the union of gametes at fertilizations. The rate of loss of genetic variation due to genetic drift is $1/4Ne$ where Ne is the effective population size (essentially the number of individuals that contribute to the next generation). The loss of genetic variation by inbreeding is due to the increased likelihood of an offspring inheriting the same gene from each parent because their genomes share a common ancestor.

In random mating populations, such as ones found in most mammalian species, inbreeding considerations alone require that population numbers should not be less than fifty individuals (Franklin, 1980). In the long term, without intensive management, genetic variability can only be maintained if population sizes are an order of magnitude greater.

These estimates are based upon rare or endangered species where there is little or no possibility of the indroduction of new individuals. The situation for wild horses on public lands is somewhat different, although there are additional considerations. For example, it may be desirable to maintain the particular phenotype that is common to the area.

MANAGEMENT PROPOSAL

Among the problems involved in the management of the wild horse herds is the question of what exactly is the resource protected. The legend of the wild horses of the American West is that they are descendents of horses lost by the early Spanish explorers and settlers around 400 years ago. On the other extreme, many believe that the wild horses are simply derived from horses that escaped from ranches within the last century. The truth is probably somewhere in between these extremes. Genetic marker analysis can help to determine the origins of the wild populations.

Because genetic markers are inherited characteristics, markers that are shared by two populations or taxons are indicators of common ancestry. Genetic markers have been used to access genetic relationships among organisms since the mid - 1960's and a wide variety of statistical methods for analyzing genetic relationships have been developed. To test for possible ancestral relationships of the feral populations, data from the feral herds will be compared to data from as many breeds as possible. At the present time, data for approximately 40 breeds is available for analysis at the University of Ken-

tucky and additional breeds are being added regularly.

There are several potential difficulties that must be considered in an analysis of the possible genetic origins of feral horse populations. First, all horses are related, at least in terms of sharing a common ancestor. Thus, most genetic variants found in horses are likely to be present in any breed. In addition, most modern horse breeds are a mixture of horses from a variety of origins and few breeds have bloodlines that are "pure", at least in terms of the last 200 years. Next, most wild populations probably are derived from a small number of founders or have experienced a period of small population size. The loss of alleles through genetic drift is greatest with small populations. Thus, allele frequencies in current feral populations may be quite different from those of the ancestral breed. As well, most genetic variants that are unique markers of a breed or a place of origin tend to be rare. Rare alleles are the most likely to be lost through genetic drift or inbreeding (Berg, 1986). Finally, it must be kept in mind that measures of genetic similarity are simply measures of resemblance and do not necessarily indicate genetic relatedness; although, often relatedness can be inferred. Despite these potential problems, preliminary results (see below) indicate that genetic analyses can provide valuable information about the ancestry of feral horses.

The major difficulty confronting managers of feral horse populations is balancing population size with herd health and viability. Horses are exotic species to this continent and the environments that feral horses occur in are often fragile ones. According to Coblentz (1990), exotic organisms are frequently the most pervasive influence affecting biodiversity in many ecosystems and may cause many extinctions or serious alterations to the physical environment. Genetic marker analysis can be used as a management tool for maintenance of small populations.

In terms of genetic management of a small population, effective population size (Ne) is the most important consideration. As mentioned earlier, Ne is operationally the number of individuals that contribute to the next generation. Ne can be estimated by the formula $\frac{4NmNf}{Nm+Nf}$ where Nm = the number of breeding males and Nf = the number of breeding females. The social structure of horses is such that Nf greatly exceeds Nm. If we assume that there are 3 reproducing females to every breeding stallion, a total of 68 successfully reproducing individuals would be required to maintain an effective population size of approximately 50. Considering immature individuals, bachelor stallions and mares that fail to produce a surviving foal, the census population number required for an effective population size of 50 would easily exceed 100 individuals.

The above estimate is based upon the assumption that the dominant stallion of a harem group is the sire of all or nearly all offspring produced by that harem group. Recent evidence reported by Bowling and Touchberry (1990) for feral horses indicate that up to one third of the offspring of a harem band are not sired by the dominant harem stallion. If this finding is true for all herds, the ratio of reproducing females to males is reduced and Ne will be higher. Only by genetic marker typing can the necessary parentage verification analyses be performed to determine how many individuals are actually part of the successfully reproducing population. Thus, genetic typing will provide information for the most accurate estimate of effective population size.

Genetic marker analysis also is useful for determining the current genetic status of a herd. At the Univ. of Kentucky we currently test for 18 polymorphic genetic marker systems. These data can be used to calculate a number of measures of gentic variability including level of polymorphism,

individual heterozygosity and effective number of alleles. Additionally, genetic marker data can be used to estimate inbreeding levels.

The goal of genetic management is to maintain genic variability. Based upon data from most breeds and some feral herds, horses naturally have high levels of genetic variation, both in terms of the number of identified allelic variants and individual heterozygosity. The natural social system of horses (population subdivision into harem bands) is conducive to maintaining high levels of genetic variation. However, small population size will have a greater influence on levels of genetic variation than will population structure. Low levels of heterozygosity in a feral population would be an indication of inbreeding and/or genetic drift.

One of the best ways to preserve genetic variability in small populations is to artificially subdivide the populations into smaller breeding units (Chesser et. al., 1980). Loss of genic variation will occur in the subpopulations through genetic drift and inbreeding; however, because the loss is random, different variants will be lost in the different subpopulations. Individuals must be exchanged among subpopulations before fitness declines due to inbreeding depression. Choices of which individuals are placed in the subdivisions and which individuals are exchanged among subdivisions can be based upon genetic marker analysis or simply by random choices.

The above scheme was formulated for rare and endangered species and for most feral horse populations is unnecessary. Only if there are unique populations that should remain pure would such a plan be necessary. With the exception of unique herds, the feral horse populations are already subdivided among the various tracts of public land managed by the BLM. Exchange of horses of the various herds also is a viable strategy for maintaining genetic variation within feral populations. However, this again raises the question of what is the resource being managed. The effort should be directed at

maintaining those herds with unique characteristics such as old Spanish origins rather than those of mixed and recent origin. Genetic marker typing should be used in concert with external morphological characteristics in making these decisions.

PRELIMINARY RESULTS

The wild horse or mustang has an important place in the heritage of the American West. In recognition of the mustang as a "symbol of the historic and pioneer spirit of the West" the U.S. government in 1971 enacted the Wild and Free-Roaming Horse and Burro Act. This act, in part, states that the wild horse herds shall be managed "in a manner that is designed to achieve and maintain a thriving natural ecological balance on the public lands." When areas are found to be overpopulated, the Act provides for wild horses to be captured and removed for private maintenance. As pointed out earlier, a significant part of effective management of isolated herds is an understanding of the genetic makeup of the herds, with an additional question of interest being "What is the origin of these wild horses?" There is no question that the original wild horses of North America were descended from horses brought by early Spanish explorers and settlers. How much of this Spanish ancestry is retained in current mustang populations is unresolved. In this study, I report the results of genetic analysis of seven samples of horses of feral origin.

The first sample will be referred to as Mustangs (or pooled mustangs). All horses in this sample (n=156) were in private ownership and were either wild caught or descended from wild caught horses. These horses were from a variety of different bloodlines and geographic origins. In future analyses, when sufficient samples are obtained, this group will be divided into distinct bloodlines or groups with similar geographic origins. Sample two (n=110), the

Kiger herd, is from west-central Oregon. All horses in this sample were wild caught in October 1989. Sample three (n=50) represents the Florida Cracker horse. The Spanish introduced horses into Florida by the early 1600s. The Cracker represents descendents of feral Seminole Indian horses from the swamps of southern Florida. About half of this sample was in private ownership while the rest are maintained in state preserves. Sample four consists of two samples of horses, wild caught in the Cerbat Mountains of northern Arizona, captured 18 years apart (n=14 and n=8 for the 1972 and 1990 samples, respectively). The horses of the Cerbats are believed to have been isolated for over 100 years in an extremely arid habitat at an elevation of about 2100m. The feral population size in 1972 was in excess of 70 individuals. By 1990 the population size in the same area was estimated to be 21. Sample 5 (n=76) was from the Cruce ranch on the Mexico-Arizona border. The herd was feral when sampled. It was said to have come from Mexican stock with no introductions of new stock since the 1880s. However, there also was some information that suggested that there may have been introduction of Quarter Horses into the herd. Sample six (n=14) was wild caught in the Pryor Mountains of southern Montana. I have no information about the history of this population at this time. Sample seven represents horses classified as American Spanish Barbs (n=64). These horses are similar to sample one in that they are of diverse feral origin. They are considered to represent, conformationally, the Barb type; however, little is actually known of their ancestry. All were in private ownership.

Genetic analyses were based upon 17 polymorphic genetic loci (7 red cell antigen systems and 10 biochemical polymorphisms). The systems examined were the A, C, D, K, P, Q, and U blood groups and the Al, Es, Gc, Hb, PGD, PGM, GPI, Pi, Tf, and AlB systems. Standard equine blood typing methodologies were employed and a total of 125 variants were recognized.

Levels of genetic variability within the feral horse populations were comparable to those of domestic horse breeds with the exception of the Cerbat sample (Table 1). Variability was measured as the effective number of alleles (i.e., the average number of alleles per locus that contribute to heterozygosity) and effective heterozygosity (expected heterozygosity based upon Hardy-Weinberg principles). The Mustang, Kiger, and Pryor samples had levels of variation above the median for domestic breeds. For the Mustang sample this was not surprising due to the diverse origins of the horses. For the Kiger and Pryor samples, the high variability was largely due to evenness in frequency of the variants rather than to the actual diversity of variants observed.

The level of variation in the Cerbat samples was greatly reduced compared to most breeds. Only 36 different variants were observed for the 17 loci in the 1972 sample and this was reduced to 25 by 1990. Individuals of the 1990 sample were virtually identical genetically, especially at the blood group loci. One interesting observation: all individuals of the 1990 Cerbat sample were heterozygous at the Tf locus and 5 of the total 25 variants were Tf alleles. This may be an indication of selection acting upon this chromosome region.

Genetic similarity (Rogers' 1972 coefficient, S) of the feral samples to domestic breeds is shown in Table 2. The breeds in Table 2 are grouped into draft, pony, hotblood, and Spanish groups. Highest average S for all feral samples except the Cruce and Cerbat samples was with the Spanish breeds. The Cruce and Cerbat herds were most similar to the hotblood group. Highest individual S values for the feral samples were with either hotblood or Spanish breeds. It should be noted that several of the hotblood breeds have a significant contribution from Spanish breeds in their ancestry, and that these breeds tended to have the highest similarity, among the hotblood group, to the

feral horses.

What is most clear from the genetic similarity analyses is that determining the ancestry of feral horses is not a simple matter. A better understanding of the ancestry will require a more complete understanding of which genetic markers are most diagnostic of particular breed lineages. In relation to the presumed Spanish ancestry of the feral horses, there are several markers that are considered indicative of Spanish ancestry. These are the Tf-F3, Tf-J, Pi-W, Dcfgkm and Ddekl variants.

The Tf-J and Pi-W variants were not observed in any of the feral horses. The Tf-F3, Dcfgkm and Ddekl variants were all seen in the Mustang sample. This strongly suggests Spanish ancestry for at least some bloodlines of Mustangs. The Kiger herd had the Ddekl allele but at a very low frequency. The high genetic variation in this herd could be indicative of mixed ancestry. However, in contrast this herd is conformationally quite uniform and very Spanish. The Florida Cracker had both the Ddekl and Tf-F3 alleles at low frequency. This sample was fairly uniform genetically, possibly due to past inbreeding. The Barb sample did not have any of the listed Spanish variants but did have an undescribed Tf variant that we have only observed in two Spanish breeds from South America. Again, these Barb horses are considered to be Spanish based upon conformation. The Cruce herd had a relatively high frequency of the Ddekl allele (0.078) but none of the other Spanish markers. The pattern of variation within this herd was consistent with the population being closed or having had very few introductions. There was no clear evidence of recent Quarter Horse introductions. The Pryor herd did not carry any of the Spanish markers but did have high overall association with the Spanish breeds (Table 2). More samples are needed for this herd. The Cerbat herd was most notable for the extremely low variability. Due to this reduced variation, ancestral relationships can not be clearly deduced. None of the Spanish

markers were observed; however, the 1972 Cerbat sample did carry a new variant in the Q blood group system that, based upon recent work with the Peruvian Paso, may have come from Spanish horses.

Preliminary work does indicate that at least some of the horses of feral origin have Spanish ancestry. The results also suggest that other non-Spanish breeds could have played a part in the makeup of these populations. More work is needed to understand the associations among the markers and the breeds. It also is necessary to sample additional breeds, especially New World breeds of Spanish descent, and more feral horses to better understand the genetic origins of the wild horses of North America.

TABLE 1. GENETIC VARIABILITY IN DOMESTIC HORSE BREEDS AND HORSES OF FERAL ORIGIN.

BREED	EFFECTIVE NUMBER OF ALLELES	EFFECTIVE HETEROZYGOSITY
THOROUGHBRED	1.468	.319
STANDARDBRED-PACER	1.722	.419
STANDARDBRED-TROTTER	1.682	.405
PERUVIAN PASO	1.807	.447
AMERICAN SADDLEBRED	1.760	.432
AKHAL-TEKE	1.615	.381
ANDALUSIAN	1.778	.438
LUSITANO	1.741	.426
CHILIAN CRIOLLO	1.778	.438
CAMPOLINA	1.807	.447
PUERTO RICAN PASO FINO	1.739	.425
HACKNEY PONY	1.694	.410
GOTLAND	1.678	.404
APPALOOSA	1.771	.435
MORGAN HORSE	1.687	.407
AMERICAN PASO FINO	1.785	.440
QUARTER HORSE	1.781	.439
MOUNTAIN PLEASURE HORSE	1.743	.427
ROCKY MOUNTAIN HORSE	1.761	.432
MINIATURE HORSE	1.952	.488
TENNESSEE WALKER	1.670	.401
ARABIAN	1.646	.393
BELGIAN HALFBLOOD	1.884	.469
SHETLAND PONY	1.728	.421
HAFLINGER	1.838	.456
CREAM DRAFT	1.827	.452
BELGIAN DRAFT	1.823	.452
BRETON	1.491	.329
PERCHERON	1.754	.430
SHIRE	1.648	.393
SUFFOLK	1.859	.462
CLYDESDALE	1.557	.358

FERAL POPULATIONS	EFFECTIVE NUMBER OF ALLELES	EFFECTIVE HETEROZYGOSITY
MUSTANGS (pooled)	1.802	.445
FLORIDA CRACKER	1.734	.423
AMERICAN BARB	1.791	.442
KIGER HERD	1.878	.467
CRUCE HERD	1.679	.405
PRYOR MOUNTAINS	1.864	.463
CERBAT 1972	1.510	.338
CERBAT 1990	1.147	.129

TABLE 2. ROGERS' GENETIC SIMILARITY OF FERAL HORSES TO DOMESTIC HORSE BREEDS.

	MUSTANGS pooled	FLORIDA CRACKER	AMERICAN BARB	KIGER HERD	CRUCE HERD	PRYOR HERD	CERBAT 1972	CERBAT 1990
CREAM DRAFT	.777	.739	.766	.763	.727	.764	.658	.554
BELGIAN DRAFT	.852	.770	.797	.829	.804	.794	.747	.679
BRETON	.748	.763	.714	.744	.701	.731	.664	.619
PERCHERON	.805	.758	.739	.780	.769	.767	.712	.624
SHIRE	.811	.795	.765	.798	.764	.763	.681	.688
SUFFOLK	.782	.739	.770	.755	.726	.784	.696	.580
CLYDESDALE	.763	.773	.721	.763	.726	.724	.673	.699
HAFLINGER	.827	.759	.749	.802	.783	.803	.773	.631
DRAFT HORSE MEAN	.796	.762	.753	.779	.750	.766	.700	.634
HACKNEY PONY	.818	.770	.766	.775	.788	.766	.740	.686
GOTLAND	.828	.784	.797	.783	.751	.820	.705	.573
MINIATURE HORSE	.844	.802	.823	.826	.778	.816	.699	.622
SHETLAND PONY	.830	.786	.781	.797	.766	.807	.713	.608
PONY MEAN	.830	.786	.792	.795	.771	.802	.714	.622
THOROUGHBRED	.806	.708	.756	.809	.773	.792	.710	.641
STDBRD. PACER	.814	.740	.751	.788	.782	.811	.735	.647
STDBRD. TROTTER	.817	.730	.756	.813	.794	.754	.725	.660
AM. SADDLEBRED	.894	.774	.805	.871	.888	.807	.774	.677
AKHAL-TEKE	.828	.777	.769	.819	.778	.751	.744	.731
APPALOOSA	.876	.776	.815	.875	.824	.824	.729	.660
ORGAN HORSE	.882	.768	.785	.868	.852	.843	.783	.687
QUARTER HORSE	.910	.777	.822	.874	.853	.872	.778	.669
MOUNTAIN PLEASURE	.908	.799	.832	.869	.851	.839	.792	.675
ROCKY MOUNTAIN	.875	.774	.823	.838	.830	.796	.770	.653
TENNESSEE WALKER	.844	.789	.791	.831	.816	.796	.769	.675
ARABIAN	.877	.759	.777	.873	.868	.820	.765	.661
BELGIAN HALFBLOOD	.870	.763	.781	.865	.836	.832	.756	.658
HOT BLOOD MEAN	.862	.764	.787	.846	.827	.811	.756	.669
ANDALUSIAN	.839	.761	.777	.829	.787	.782	.726	.610
LUSITANO	.863	.789	.801	.851	.799	.774	.743	.633
CHILIAN CRIOLLO	.878	.841	.827	.848	.814	.813	.732	.654
CAMPOLINA	.899	.843	.843	.885	.830	.837	.765	.657
P.R. PASO FINO	.864	.775	.781	.875	.823	.836	.728	.637
PERUVIAN PASO	.864	.804	.803	.852	.815	.848	.720	.618
AM. PASO FINO	.889	.822	.817	.875	.830	.838	.743	.670
SPANISH MEAN	.871	.805	.807	.859	.814	.818	.737	.640

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BUDGET

Current costs for genetic marker typing for 18 systems of the University of Kentucky Equine Blood Typing Research Laboratory is $28.50 per horse. This cost includes supplies and personnel expenses. A detailed accounting of these expenses can be supplied on request. The systems typed are as follows: Red cell antigen loci - the A, C, D, K, P, Q, and U blood group loci. Biochemical systems - Albumin (Al), a-1-B glycoprotein (AlB), serum esterase (Es), vitamin D binding protein (Gc), glucosephosephate isomerase (GPI), alpha hemoglobin (Hb), mannosephosphate isomerase (MPI), phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM), protease inhibitor system (Pi), and serum transferrin (Tf).

Additional costs include blood collection supplies, if needed. These supplies include clot and anticoagulate vacutainer tubes, a vacutainer needle, and styrofoam mailing container. The cost per supply kit is $5.00.

Computer analysis time. This is personnel time not included in laboratory testing costs, including that of the principle investigator, set at $1.50 per sample.

Finally, in order to analyze the relationship of feral populations to extant horse breeds, it is necessary to type as many individuals of as many breeds as possible. The costs for this portion of the study include all of the above expenses as well as shipping costs for samples from outside the USA. These samples must, for the most part, be voluntarily supplied by individual owners and therefore, we are not able to charge the owners for this typing if we have any hope of obtaining the samples. An additional cost of $5.00 per sample tested is therefore added to the testing cost to help defray this considerable expense of the proposed work.

The total cost to carry on the proposed study and provide management advice is thus $40.00 per sample tested. This fee will be reduced to $35.00

if the laboratory does not have to supply blood collection supplies. The total cost includes all consultant activity that might be required by the management unit from the principle investigation.