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# Genetic studies of blood markers in Przewalski's horses

*ABSTRACT:* **Ninety-six Przewalski's horses (*Equus przewalskii*) were blood typed using systems of inherited blood variants known to be highly effective for parentage testing of domestic horses (*E. caballus*). Sixteen red cell antigenic factors detected using sera prepared by alloimmunization of domestic horses were shown to be inherited in six systems (*A, C, D, P, Q,* and *U*) and in the same patterns as domestic horses. Family data confirmed autosomal, codominant inheritance at five loci of serum protein variants (*Al, Tf, Xk, Pi,* and *Es*) and three loci of red cell proteins (*PGM, PHI,* and *Hb*). One serum protein locus (*Gc*) and two red cell protein loci (*PGD* and *CA*) appeared to be monomorphic. Despite the narrow genetic base and high inbreeding coefficients of captive Przewalski's horses, average heterozygosity calculated over 18 loci was estimated to be 0.320 ± 0.05, which was similar to that found in five breeds of domestic horses.**

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PRZEWALSKI'S HORSE (*Equus przewalskii*) is the only living species of wild horse. So-called wild horses found in several regions of the world, such as sparsely populated areas of the western United States, are feral domestic horses (*E. caballus*) and not true wild horses. The two species are visibly distinct and have a difference in chromosomal number[2] but are quite closely related as shown by studies of cranial and skeletal features[14], chromosomes[22], and blood type markers[7,10,18–20,23,26,30]. The interspecific cross yields fertile offspring[27].

Przewalski's horse probably had a range in prehistoric times throughout Europe and into Asia. It is thought to be extinct in the wild and the only remaining examples are about 600 animals in zoo collections (see cover). A studbook maintained by Volf[31] traces the pedigrees of all animals within about 11 generations to no more than 13 founder animals from Mongolia: 11 animals obtained at the turn of the century, one mare caught in 1947, and a domestic pony mare (*E. caballus*).

Separate breeding lines occur. The Munich line traces its ancestry back to nine of the wild-caught horses. The Prague line includes wild-caught horses, as well as the domestic horse. Munich line breeders have been reluctant to introduce descendents of the domestic mare into their stock and thus separate breeding lines have evolved[21].

The small number of founders and the isolation of animals in small zoo populations has led to high inbreeding coefficients. In North America the average inbreeding coefficient for the Munich line is 0.273 and for the Prague line, 0.142[21]. Continued inbreeding within small populations is thought to lead to homozygosity and narrow the base of genetic diversity, perhaps rendering the species unable to adapt to environmental changes. The cheetah may become the classic example of such a scenario[16,17]. Data showing increased juvenile mortality and decreased life-span in Przewalski's horses[3,4] provide reason for concern for the long-term survival of the remaining zoo populations of this species as well.

Genetic studies of Przewalski's horses may provide important information about breeding management. Knowledge of discrete, assayable genetic variants can be applied to assure accurate animal identification. Genetic variants also can be used to verify parentage, thus ensuring accuracy of the studbook record, the base used to calculate various genetic parameters such as inbreeding coefficients, linkage, drift, and amount of genetic variability in individuals or populations. Analysis of red blood cell surface antigens and electrophoretic/isoelectric focusing studies of blood proteins provide a means to examine genetic variation and to make variability and heterozygosity comparisons with other species. This study presents gene frequency data for 18 loci of Przewalski's horses

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and compares the polymorphisms and average heterozygosity with data similarly obtained for five breeds of domestic horses.

## Materials and Methods

Two 10-ml blood samples were submitted from each horse, an ACD anti-coagulant tube to use as a red cell source and a dry tube as a serum source. Blood samples were sent to the laboratory at ambient temperature, then refrigerated until tests were completed. A total of 96 Przewalski's horses was tested (48 females, 48 males), 70 of which were of the Munich line. None of the animals traced to the mare caught in 1947. Thirty-nine horses were tested more than once, some as many as 10 times, affording the opportunity to verify types and extend the markers detected as new techniques and systems were added.

Standard immunological procedures involving hemagglutination and complement mediated hemolysis[28,29] were used to detect red cell antigens. Reagents were produced by alloimmunization in *E. caballus*, followed by extensive testing and absorption of the immune sera using red cells of domestic horses until each reagent behaved as monospecific. Samples were tested with as many as 40 reagents of different specificities that recognize factors for domestic horses at seven blood group loci. Not all animals were tested for all the factors. The factors summarized in this report are as follows (listed as the system in capital letters, followed by lower case letters for each factor of the system): Aabcdefg, Ca, Dabcdefghikno, Ka, Pabc, Qa, and Ua. As many as 14 additional reagents were used which have not yet been assigned international nomenclature. Alleles appeared to be similar to those encountered in *E. caballus*; assignment was based on reaction patterns and analysis of family data. Nomenclature used was in accord with that agreed upon for *E. caballus* by the International Society for Animal Blood Group Research (ISABR).

Standard methods of starch[6,25] and polyacrylamide[13] gel electrophoresis were used to identify inherited variants at the following loci: albumin (*Al*), transferrin (*Tf*), esterase (*Es*), *Xk*, *Gc*, protease inhibitor (*Pi*), 6-phosphogluconate dehydrogenase (*PGD*), phosphoglucomutase (*PGM*), phosphohexose isomerase (*PHI*), catalase (*Cat*), carbonic anhydrase (*CA*) and acid phosphatase (*AP*). Polyacrylamide gel isoelectric focusing was used for the detection of $\alpha$-hemoglobin variants (*Hb*)[8].

When gel variants appeared to be indistinguishable from those of domestic horses, nomenclature used was that agreed upon by ISABR members for *E. caballus*. Variants designated with square brackets are those for which terminology has not yet been standardized for *E. caballus*. *Tf*-[*E*] is found infrequently in some breeds of domestic horses. Under both starch and polyacrylamide gel conditions, the *Tf*-[*E*] variant migrates just slightly cathodally to *Tf-D* and differs from other *Tf* variants in that the two bands characteristic of transferrins are of approximately equal staining intensity, whereas other variants have distinct major and minor bands. This variant corresponds to that called *Tf*-[$E_2$] by others[10,20].

Electrophoretically assayed variants that seem to be unique to *E. przewalskii* are identified by braces; e.g., *Tf*-{*D**}; *Tf*-{*I*}; *Es*-{*P*}; *Pi*-{*P*}; *Xk*-{*P*}. *Tf*-{*D**} corresponds to *Tf*-$E_1$ of others[10,20]. *Tf*-{*I*} may be unique to Przewalski's horses. Under conditions of our gels it seems to correspond in position to an infrequently seen variant of domestic horses designated *Tf*-[$H_2$], but is lighter staining. *Es*-{*P*} is an allele apparently unique to Przewalski's horses, best distinguished with isoelectric focusing[11], but also seen with polyacrylamide electrophoresis. *Pi*-{*P*} is a fast migrating type of protease inhibitor that probably corresponds to *Pi-D* in another study[20] but was not subdivided under our conditions. The other *Pi* variant observed in these samples is apparently the same as *Pi*-$S_1$ in domestic horses. It has been agreed by ISABR members that the *Pa* locus will now be designated *Xk* and variants will be designated by the *Xk* terminology. *Xk-F* and *Xk-K* appear to correspond to those variants found in domestic horses; *Xk*-{*P*} under polyacrylamide gel conditions is slightly faster than the slow variant (*Xk-S*) seen in domestic horses.

Breed data for domestic horses were taken from Bowling and Clark[5]. For determining gene frequencies of Przewalski's horses, all 96 animals were considered members of one breeding group. Blood group frequencies were calculated as follows: for single factor systems *C*, *P*, *Q*, and *U*, assuming Hardy-Weinberg equilibrium, the frequency of the

**Table I. Inheritance of blood factors in Przewalski's horses shown by distribution of phenotypes in offspring from various matings**

| Mating* | Phenotypes of offspring | | | |
|---|---|---|---|---|
| *A* system ($N$ = 44) | Aa | Aa/Ace | Ace | |
| Aa × Aa | 2 | 0 | 0 | |
| × Aa/Ace | 14 | 13 | 0 | |
| Aa/Ace × Aa/Ace | 4 | 6 | 3 | |
| × Ace | 0 | 1 | 1 | |
| *C* system ($N$ = 45) | Ca | -/- | | |
| Ca × Ca | 15 | 4 | | |
| × -/- | 18 | 7 | | |
| -/- × -/- | 0 | 1 | | |
| *D* system ($N$ = 44) | Dadn | Dadn/Dcg | Dcg | Dcg/dn |
| Dadn × Dadn/Dcg | 0 | 2 | 0 | 0 |
| × Dadn/Ddn | 1 | 0 | 0 | 0 |
| Dadn/cg × Dadn/Dcg | 2 | 6 | 4 | 0 |
| × Dcg | 0 | 13 | 11 | 0 |
| × Dcg/Ddn | 0 | 1 | 1 | 0 |
| Dcg × Dcg | 0 | 0 | 2 | 0 |
| × Dcg/Ddn | 0 | 0 | 0 | 2 |
| *P* system ($N$ = 31) | Pac | -/- | | |
| Pac × Pac | 1 | 0 | | |
| × -/- | 7 | 9 | | |
| -/- × -/- | 0 | 12 | | |
| *Q* system ($N$ = 44) | Qa | -/- | | |
| Qa × Qa | 14 | 6 | | |
| × -/- | 5 | 10 | | |
| -/- × -/- | 0 | 9 | | |
| *U* system ($N$ = 44) | Ua | -/- | | |
| Ua × Ua | 3 | 1 | | |
| × -/- | 13 | 11 | | |
| -/- × -/- | 0 | 16 | | |

* $N$ = number of matings for which sire, dam, and offspring were blood typed

null allele was taken as the square-root of the frequency of the null class and the frequency of the named factor was 1 minus the frequency of the null allele; for *A* and *D* by direct counting, assuming no null allele and no ambiguous phenotypes. Allelic frequencies for protein polymorphisms were determined by direct counting from phenotypes, with the addition of family data for *Es* to define animals heterozygous for the null allele. The computer program of Dowling and Moore[9] was used to calculate Nei's measures of normalized genetic identity (*I*) and standard genetic distance (*D*) between two populations[15]. Average heterozygosity also was calculated with this program.

## Results and Discussion

Family data showing the inheritance of red cell antigenic markers are shown in Table I. As in domestic horses, factors detected by reagents behaved as autosomal (data not shown) codominants. In the *A* and *D* systems some alleles were determined by a combination of two or three factors (e.g., *Ace*, *Dadn*). Undetectable alleles acting as recessives to those specificities detected by reagents were present at the *C*, *P*, *Q*, and *U* loci, as in domestic horses. At the *A* locus, both $A^{adf}$ and $A^{adg}$ were found (as well as $A^{ce}$) but too few families were tested with reagents Ad, Af, and Ag to include in the family data or determine gene frequencies. If reagents had been made using red cells from Przewalski's horses for hetero- or alloimmunization, it is possible that additional variants might have been detected.

Family data for markers detected by electrophoresis and isoelectric focusing are shown in Tables II and III. Markers are inherited as autosomal (data not shown), codominant alleles, as in domestic horses.

Analysis of genetic data for parents and offspring confirmed the studbook record of parentage for all animals in this study. Gene frequency data are given for six loci of red cell antigens in Table IV and for eight other loci of polymorphisms in Table V. Loci that were monomorphic are not included in the tables, but variants found and numbers tested are as follows: $Ka^-$ (92); $PGD^F$ (76); $Gc^F$ (60); $CA^I$ (28).

Many alleles of Przewalski's horse appeared to be shared with *E. caballus*, and the most common variants at each locus were often the most common found in domestic horses as well (e.g., *Aa*, *Ca*, *Xk-K*, *PGD-F*, *PHI-I*, and *Gc-F*). An exception was the higher frequency in Przewalski's horses of

**Table II. Inheritance of electrophoretically detected serum protein variants in Przewalski's horses shown by distribution of phenotypes in offspring from various matings**

| Mating† | Phenotypes of offspring | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Albumin (Al) (*N* = 44) | | | | A | | AB | | B | |
| AB × B | | | | 0 | | 4 | | 5 | |
| B × B | | | | 0 | | 0 | | 35 | |
| Transferrin (Tf) (*N* = 45) | {D*} | [E] | $F_2$ | {D*}[E] | {D*}$F_2$ | [E]$F_2$ | {D*I} | [E]{I} | $F_2${I} |
| [E] × [E]$F_2$ | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $F_2$ × [E]$F_2$ | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| × $F_2$ | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| {D*}[E] × {D*}$F_2$ | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| × {D*I} | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| × [E]{I} | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |
| {D*}$F_2$ × [E]$F_2$ | 0 | 0 | 0 | 1 | 3 | 3 | 0 | 0 | 0 |
| × {D*I} | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| × [E]{I} | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| × $F_2${I} | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 |
| [E]$F_2$ × [E]$F_2$ | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| × $F_2${I} | 0 | 0 | 5 | 0 | 0 | 7 | 0 | 0 | 2 |
| {D*I} × {D*I} | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| [E]{I} × [E]{I} | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Xk* (*N* = 42) | FK | | | F{P} | | K | | K{P} | {P} |
| F × K | 2 | | | 0 | | 0 | | 0 | 0 |
| FK × F{P} | 4 | | | 0 | | 0 | | 1 | 0 |
| × K | 9 | | | 0 | | 9 | | 0 | 0 |
| × K{P} | 1 | | | 1 | | 0 | | 1 | 0 |
| K × K | 0 | | | 0 | | 12 | | 0 | 0 |
| × K{P} | 0 | | | 0 | | 1 | | 1 | 0 |
| Esterase (Es) (*N* = 41) | {P} | | | I | H | I{P} | | H{P} | HI |
| {P} × {P} | 1 | | | 0 | 0 | 0 | | 0 | 0 |
| × I{P} | 1 | | | 0 | 0 | 1 | | 0 | 0 |
| × HI | 0 | | | 0 | 0 | 1 | | 0 | 0 |
| × I | 0 | | | 0 | 0 | 2 | | 0 | 0 |
| I{P} × I{P} | 4 | | | 0 | 0 | 4 | | 0 | 0 |
| × H{P} | 0 | | | 0 | 0 | 1 | | 0 | 0 |
| × {P}** | 5 | | | 0 | 0 | 1 | | 0 | 0 |
| × HI | 0 | | | 1 | 0 | 0 | | 0 | 0 |
| × I** | 9** | | | 0 | 0 | 10 | | 0 | 0 |
| **May have Es-O | | | | | | | | | |
| Protease inhibitor (Pi) (*N* = 41) | | | | {P} | | {P}S | | S | |
| {P} × {P} | | | | 21 | | 0 | | 0 | |
| × {P}S | | | | 10 | | 4 | | 0 | |
| × S | | | | 0 | | 1 | | 0 | |
| {P}S × {P}S | | | | 2 | | 1 | | 1 | |
| × S | | | | 0 | | 0 | | 1 | |

† *N* = number of matings for which sire, dam, and offspring have been blood typed. Square brackets [ ] are used to designate variants found in both *E. przewalskii* and *E. caballus* for which a standard nomenclature has not been determined. Braces { } are used to designate variants apparently unique to *E. przewalskii*

**Table III. Inheritance of electrophoretically detected variants of red cell proteins in Przewalski's horses shown by distribution of phenotypes in offspring from various matings**

| Mating | Phenotypes of offspring | | |
|---|---|---|---|
| Phosphoglucomutase (PGM) (*N* = 17) | F | FS | S |
| F × F | 1 | 0 | 0 |
| × FS | 6 | 4 | 0 |
| FS × FS | 1 | 3 | 2 |
| Phosphohexose isomerase (PHI) (*N* = 13) | I | IS | S |
| I × I | 2 | 0 | 0 |
| × IS | 4 | 1 | 0 |
| × S | 0 | 1 | 0 |
| IS × IS | 1 | 3 | 1 |
| α-Hemoglobin (Hb) (*N* = 18) | I | I/II | II |
| I × I/II | 2 | 2 | 0 |
| × II | 0 | 5 | 0 |
| I/II × I/II | 0 | 1 | 0 |
| × II | 0 | 1 | 6 |
| II × II | 0 | 0 | 1 |

*PGM-F* compared to *PGM-S*. Four loci (*Tf*, *Xk*, *Es*, and *Pi*) had alleles apparently unique to Przewalski's horses.

We are not aware of published gene frequencies for red cell antigens of Przewalski's horses, but the specificities are similar to those reported previously. Factor Qa has not been previously reported for European zoo animals[7,18,26]. In our study Qa was found only in Munich line animals. Other apparent exceptions seen in red cell antigen data comparisons, particularly in the *A* and *D* systems, can be explained as being due to a more extensive battery of reagents in the present report.

Polymorphisms of serum and red cell proteins are similar to those published previously for Przewalski's horses with a few exceptions that are mostly due to nomenclature differences. We were unable to confirm previous reports of polymorphism for *CA*[10] or *AP*[7], but have tested relatively few samples for these loci.

We noted polymorphism for *Cat* (Cat-M = 21 animals; Cat-F = 7 animals), but by Hardy-Weinberg testing this distribution of phenotypes and lack of Cat-S types does not fit the domestic horse model in which the M phenotype is the heterozygote for *F* and *S* alleles. The population data suggest that the Cat-M type of Przewalski's horses be interpreted as homozygous for a slow allele, and the F type as heterozygous for a slow and a fast allele. In this model, the homozygous fast type has not yet been seen by us. Thus, one or both *Cat* alleles of *E. przewalskii* may be different from those of *E. caballus*. We did not include the *Cat* locus in the comparisons of Table VII.

Family data and gene frequencies for four serum (plasma) protein loci were reported by Putt and Whitehouse[20] using 130 animals, which was largely a non-overlapping set of individuals with ours. Their technique of isoelectric focusing for diagnosis of *Tf*, *Pr* (*Pi*), *Pa* (*Xk*) and *Es* allowed the discrimination of more variants at *Pi* and *Tf* than reported in this study. In their population, *Tf*-{*I*} and *Tf*-{*D**} were in higher frequency than in ours. In our population, the fastest migrating *Xk* variant (*Xk-F*) was at a higher frequency than the slow one (*Xk*-{*P*}), but in their study the frequencies were in a reverse relationship. Whether the same variants were being detected by the two techniques remains to be determined.

**Table IV. Gene frequencies for red cell antigens of Przewalski's horses**

| Locus | *N** | Allele | Freq |
|---|---|---|---|
| *A* | 91 | *a* | 0.63 |
| | | *ce* | 0.37 |
| *C*† | 89 | *a* | 0.46 |
| *D* | 92 | *cg* | 0.72 |
| | | *adn* | 0.25 |
| | | *dn* | 0.03 |
| *P*† | 90 | *a* | 0.24 |
| *Q*† | 90 | *a* | 0.18 |
| *U*† | 90 | *a* | 0.18 |

* *N* = number of horses tested for the specificities
† The frequency of the alternative allele (a "negative allele" for which no antiserum is reactive) at these loci is 1 minus the value given here

**Table V. Gene frequencies for blood protein polymorphisms in Przewalski's horses**

| Locus | *N** | Marker | Gene freq |
|---|---|---|---|
| *Al* | 96 | A | 0.09 |
| | | B | 0.91 |
| *Tf* | 96 | {D*} | 0.20 |
| | | [E] | 0.40 |
| | | $F_2$ | 0.30 |
| | | {I} | 0.10 |
| *Xk* | 96 | F | 0.24 |
| | | K | 0.69 |
| | | {P} | 0.07 |
| *Es* | 95 | H | 0.05 |
| | | I | 0.29 |
| | | {P} | 0.59 |
| | | O | 0.07 |
| *Pi* | 68 | {P} | 0.82 |
| | | S | 0.18 |
| *PGM* | 46 | F | 0.77 |
| | | S | 0.23 |
| *PHI* | 36 | I | 0.77 |
| | | S | 0.23 |
| *Hb* | 45 | BI | 0.46 |
| | | BII | 0.54 |

* *N* = number of horses tested. Square brackets [ ] indicate variants found in both *E. caballus* and *E. przewalskii* for which an internationally uniform nomenclature has not been established. Braces { } indicate variants apparently unique to *E. przewalskii*

**Table VI. Number of blood type variants per locus with a gene frequency equal to or greater than 0.001 in *E. przewalskii* and five breeds of *E. caballus***

| | Breeds of *E. caballus** | | | | | *E. przewalskii* |
|---|---|---|---|---|---|---|
| | TB | AR | ST | MH | QH | PZ |
| Locus | | | | | | |
| RBC antigenic alleles (33)† | | | | | | |
| *A* | 5 | 5 | 6 | 8 | 6 | 2 |
| *C* | 2 | 2 | 2 | 2 | 2 | 2 |
| *D* | 8 | 8 | 11 | 11 | 12 | 3 |
| *K* | 2 | 2 | 2 | 2 | 2 | 1 |
| *P* | 3 | 3 | 3 | 3 | 3 | 2 |
| *Q* | 2 | 2 | 2 | 2 | 2 | 2 |
| *U* | 2 | 2 | 2 | 2 | 2 | 2 |
| RBC total: | 24 | 24 | 28 | 30 | 29 | 14 |
| Serum proteins (37) | | | | | | |
| *Al* | 2 | 2 | 3 | 3 | 2 | 2 |
| *Tf* | 6 | 5 | 7 | 9 | 10 | 4 |
| *Es* | 3 | 4 | 5 | 7 | 7 | 4 |
| *Xk* | 2 | 2 | 3 | 3 | 3 | 3 |
| *Pi* | 8 | 9 | 8 | 8 | 8 | 2 |
| *Gc* | 2 | 2 | 2 | 2 | 2 | 1 |
| Protein total: | 23 | 24 | 28 | 32 | 32 | 16 |
| RBC proteins (15) | | | | | | |
| *PGD* | 2 | 3 | 2 | 2 | 3 | 1 |
| *PGM* | 2 | 2 | 2 | 2 | 2 | 2 |
| *PHI* | 1 | 2 | 2 | 3 | 3 | 2 |
| *CA* | 2 | 2 | 3 | 2 | 3 | 1 |
| *Hb* | 2 | 2 | 3 | 2 | 3 | 2 |
| RBC protein total: | 9 | 11 | 12 | 11 | 14 | 8 |
| Combined RBC & protein total (85) | 56 | 59 | 68 | 73 | 75 | 38 |

* Breeds are: TB = Thoroughbred, AR = Arabian, ST = Standardbred, MH = Morgan horse, QH = Quarter horse
† Numbers in parentheses are total numbers of variants detectable in *E. caballus* with the methods applied

With the exception of *Pi*, the gene frequency data show the loci to be in Hardy-Weinberg equilibrium, despite small population size, inbreeding, and the presence of breeding line traditions that have maintained the Munich line separate. For *Pi* the data showed an excess of *Pi-S* homozygotes and a deficiency for *Pi-FS* ($\chi^2 = 12.46$; $P < 0.01$). It is possible that this effect was due to breeding line differences, although both variants exist in both lines. The *Pi-FS* type might occasionally have been scored in error as *Pi-F*, but since most animals were typed several times, and types carefully rechecked if they did not agree with the previous record, this explanation is not compelling. Perhaps application of the isoelectric focusing technique[20], which discriminated five *Pi* alleles for Przewalski's horses, might resolve the apparent conflict.

The number of variants detectable at each locus in Przewalski's horses is compared with the number of variants detectable in five breeds of domestic horses (Thoroughbred, Arabian, Standardbred, Morgan horse and Quarter horse) in Table VI. These breeds can be classified as "riding type," not draft horses or ponies. Thoroughbreds and Standardbreds are primarily bred as sport horses (racing); the others are primarily bred for show ring competition and pleasure riding. Fewer alleles were recognized in the Przewalski's horse samples than those of the domestic horses, noticeably for the *A*, *D*, *Tf*, and *Pi* loci for which 10 or more alleles each have been described in domestic horses.

Measures of normalized genetic identity (*I*) and standard genetic distance (*D*) between two populations[15] for five breeds of domestic horses and Przewalski's horse were calculated over 18 loci: *A*, *C*, *D*, *K*, *P*, *Q*, *U*, *Al*, *Tf*, *Xk*, *Es*, *Pi*, *Gc*, *PGD*, *PHI*, *PGM*, *CA*, and *Hb*. Average heterozygosity ($J_x$) for each breed also was calculated. The results are shown in the matrix of Table VII.

The distance measurements showed the domestic horses clustered rather closely at a value of about 0.9 or greater; the relationship of Przewalski's horse to any of the domestic breeds was about 0.7 or slightly greater. These values might be interpreted to indicate evolutionary divergence at the level of subspecies rather than full species[1], which is consistent with other measures of divergence (e.g., skeletal, chromosomal, reproductive performance of hybrids).

Average heterozygosity for Przewalski's horses calculated over 18 loci was estimated to be $0.320 \pm 0.050$. This number was slightly higher than that of Thoroughbreds and lower than that of Arabians, Standardbreds, Morgan horses, and Quarter horses, but taking standard error into consideration, the differences were not significant. In light of the narrow genetic base and high inbreeding coefficients of captive Przewalski's horses, this high level of heterozygosity was unexpected. It might be argued that the heterozygosity is an artifact of combining the horses into a single population, ignoring breeding lines. However, with the exception of one locus, the sample tested was determined to be in Hardy-Weinberg equilibrium, so this explanation is not persuasive. The heterozygosity might be due to contamination of the stock with domestic horses. The restricted range of variants found in Przewalski's horses, compared with domestic horses, suggests that the extent of contamination is minimal and not likely to be more widespread than the one known cross. One is led to speculate what relationship the Przewalski's horse data may have to the recent report[12] of a great excess of genetic variation in 10 inbred strains of mice compared to expectation.

ADDENDUM: In the course of testing more animals, a third *Pi* marker has been found that may explain the deviation from Hardy-Weinberg equilibrium observed with the previous data. In addition, animals descending from the 1947 wild-caught mare have now been typed and found to possess markers *Tf-D*, *Tf-F*$_3$ and *CA-F* as well as markers found in the Prague and Munich lines.

**Table VII. Normalized genetic identity, *I*, standard genetic distance, *D*, and average heterozygosity, $J_x$*, for *E. przewalskii* and five breeds of *E. caballus* at 18 loci†**

| | Breeds of *E. caballus* | | | | | *E. przewalskii* |
|---|---|---|---|---|---|---|
| | TB | AR | ST | MH | QH | PZ |
| TB | 0.313 ± 0.062 | 0.096 ± 0.051 | 0.110 ± 0.041 | 0.081 ± 0.038 | 0.084 ± 0.053 | 0.367 ± 0.132 |
| AR | 0.909 | 0.330 ± 0.059 | 0.075 ± 0.027 | 0.054 ± 0.030 | 0.020 ± 0.011 | 0.269 ± 0.098 |
| ST | 0.896 | 0.928 | 0.397 ± 0.052 | 0.021 ± 0.011 | 0.068 ± 0.032 | 0.295 ± 0.108 |
| MH | 0.922 | 0.947 | 0.980 | 0.369 ± 0.059 | 0.054 ± 0.036 | 0.262 ± 0.103 |
| QH | 0.919 | 0.980 | 0.934 | 0.948 | 0.377 ± 0.060 | 0.246 ± 0.094 |
| PZ | 0.693 | 0.764 | 0.744 | 0.770 | 0.782 | 0.320 ± 0.050 |

* Nei's *I* (genetic identity) is below the diagonal. *D* (genetic distance) is above the diagonal. $J_x$ (average heterozygosity) is on the diagonal. Standard error values are given for *D* and $J_x$

† Loci and frequency values used for domestic horses were from Bowling and Clark[5] and for Przewalski's horses from Tables IV and V

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