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Genetic diversity in German draught horse breeds compared with a group of primitive, riding and wild horses by means of microsatellite DNA markers

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Summary

We compared the genetic diversity and distance among six German draught horse breeds to wild (Przewalski's Horse), primitive (Icelandic Horse, Sorraia Horse, Exmoor Pony) or riding horse breeds (Hanoverian Warmblood, Arabian) by means of genotypic information from 30 microsatellite loci. The draught horse breeds included the South German Coldblood, Rhenish German Draught Horse, Mecklenburg Coldblood, Saxon Thuringa Coldblood, Black Forest Horse and Schleswig Draught Horse. Despite large differences in population sizes, the average observed heterozygosity (H_o) differed little among the heavy horse breeds (0.64–0.71), but was considerably lower than in the Hanoverian Warmblood or Icelandic Horse population. The mean number of alleles (N_A) decreased more markedly with declining population sizes of German draught horse breeds (5.2–6.3) but did not reach the values of Hanoverian Warmblood ($N_A = 6.7$). The coefficient of differentiation among the heavy horse breeds showed 11.6% of the diversity between the heavy horse breeds, as opposed to 21.2% between the other horse populations. The differentiation test revealed highly significant genetic differences among all draught horse breeds except the Mecklenburg and Saxon Thuringa Coldbloods. The Schleswig Draught Horse was the most distinct draught horse breed. In conclusion, the study demonstrated a clear distinction among the German draught horse breeds and even among breeds with a very short history of divergence like Rhenish German Draught Horse and its East German subpopulations Mecklenburg and Saxon Thuringa Coldblood.

Keywords diversity, endangered breeds, genetic variation, horse, microsatellite.

Introduction

The German draught horse breeds were developed by interbreeding local working horse populations with other draught horses from neighbouring countries. In fact, controlled breeding of draught horse breeds as we know them in Germany today did not start before the late 19th century (Scharnhölz 2002). Besides the largest German draught horse population, the Rhenish German Coldblood, smaller populations were developed such as the South German, Black Forest and Schleswig Coldblood. Intensification of agriculture and the onset of industrialization at the end of the 19th century led to an urgent demand for these breeds resulting in rapidly increasing population sizes, which peaked between 1920 and 1950. Nevertheless, just a few decades later heavy draught horses had become increasingly unimportant because of the intense mechanization of agriculture and transport systems, and the populations decreased to alarmingly low numbers in the 1970s. Fortunately, governmental support and the dedication of horse breeders helped to save these culturally important breeds from extinction. Currently, seven draught horse breeds are distinguished in Germany, but the numbers of animals recorded are still low for these breeds (Table 1), and all except the South German Coldblood are included in the FAO list of domestic animals to be conserved (FAO, http://dad.fao.org/en/home.htm).

The smallest draught horse population, the Schleswig Draught Horse, is bred mainly in Schleswig-Holstein and Lower Saxony, Germany. Its main origin lies in the Jutland Horse from Denmark. The Mecklenburg, Saxon Thuringa

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Table 1 Population size (N), effective population size (N_e), and inbreeding coefficients (F) for the heavy draught horse populations under study.

Population	N[1]	N_e[1]	F (%)[2]
South German Coldblood	2110	413	2.79
Rhenish German Draught Horse	850	300	1.53
Saxon Thuringa Coldblood[3]	358	113	2.13
Mecklenburg Coldblood[3]	131	34	2.61
Black Forest Horse	799	184	5.75
Schleswig Draught Horse	231	89	4.68

[1]European Association for Animal Production – Animal Genetic Data Bank (EAAP-AGDB), http://www.tiho-hannover.de/einricht/zucht/eaap/index.htm.

[2]Averaged from the data on the horses from each population analysed here in consideration of 11 generations of ancestors.

[3]Subpopulations of the Rhenish German Draught Horse; a further subpopulation of the Rhenish German Draught Horse, the Altmaerkish Coldblood could not be included in the present study because of the low numbers of samples available.

and Altmaerkisch Coldblood are East German subpopulations of the Rhenish German Draught Horse. This breed was founded by breeding primarily with Belgian Draught Horses. Today the largest heavy horse population is the South German Coldblood, a member of the so-called Noric horse group, which also includes the Black Forest Horse, which is bred in Baden-Wuerttemberg.

Molecular techniques have been widely used to analyse phylogenetic relationships among various animal groups and different breeds. Microsatellite loci comprise an attractive potential source of information about population histories and evolutionary processes, as these loci permit simple and accurate typing in combination with high levels of polymorphism and widespread distribution in the genome. The usefulness of microsatellite markers has been documented in many previous equine population genetic studies (e.g. Cañon *et al.* 2000; Aranguren-Méndez *et al.* 2001; Bjørnstad & Røed 2001; Cunningham *et al.* 2001).

We compared German draught horse breeds with several endangered and very old populations as well as with breeds, which have been isolated for a long time, and with common riding horses of different histories and origins. For this comparison, the Sorraia Horse and the Exmoor Pony were chosen as representatives of primitive horse breeds, the Przewalski's Horse as representative of a wild horse and the Icelandic Horse represented for almost 1000 years isolated breed and was included because of origin. However, the captive population of 11 Przewalski's horses was interbred with a domestic horse and a domestic/Przewalski hybrid. The Arabian was included because of its influence on most of all European riding horses and one of the oldest known breeds of riding horses. The Hanoverian Warmblood is the largest riding horse population of Germany and has evolved since 1735 by interbreeding Holsteiner stallions, English Thoroughbreds and other horse breeds such as Arabian, Anglo-Arabian, Trakehner and different warmblood breeds with the regional Hanoverian horses.

The main objective of this study was to show the levels of genetic variability among the German draught horse breeds and to estimate genetic distances between them using a highly polymorphic set of microsatellites representing all autosomes. Furthermore, it should be clarified whether the Rhenish German Draught Horse and its East German subpopulations are distinct enough from each other to justify defining separate breeds.

Materials and methods

Sampling and DNA extraction

A total of 403 animals were analysed from six German heavy draught horse breeds and from a total of six riding, wild and primitive horse populations. Blood or hair root samples were collected from South German Coldblood ($N = 45$), Rhenish German Draught Horses ($N = 45$), Saxon Thuringa Coldblood ($N = 23$), Mecklenburg Coldblood ($N = 22$), Black Forest ($N = 45$) and Schleswig Draught Horses ($N = 45$). To place the results in context, DNA samples were also analysed from Hanoverian Warmblood ($N = 47$), Arabian ($N = 25$), Sorraia Horses ($N = 23$), Icelandic Horses ($N = 45$), Exmoor Ponies ($N = 20$) and Przewalski's Horses ($N = 18$). Because of the very low population size and missing readiness of most breeders to cooperate, there were only a few samples of Altmaerkisch draught horses available and thus we did not include this breed in our analysis. In the German Democratic Republic, the Saxon Thuringa, Mecklenburg and Altmaerkisch Coldblood horses were not bred as different breeds and as early as 1990 the East German breeding organizations of coldblood horses were founded, the forementioned three breeds were distinguished according to the location of the breeding organization of the federal country. For the draught horse breeds, only purebred and unrelated animals were sampled according to pedigree information in order to make their inbreeding coefficients comparable with those of the entire breed. Inbreeding coefficients for the draught horses under study were calculated under consideration of 11 generations of ancestors with the methods described by Aberle *et al.* (2003a,b). The samples were also representative for the present sire lines of each draught horse population.

Genomic DNA was extracted from whole blood using the QIAamp® 96 DNA Blood Kit (Qiagen, Hilden, Germany), and from hair root samples using the DNeasy® Tissue Kit (Qiagen), following the manufacturer's protocol.

Microsatellite amplifications and analysis

The 31 microsatellite markers were chosen from the linkage map generated by Swinburne *et al.* (2000a), from the HORSEMAP database on the INRA Biotechnology

Laboratories Home Page (http://locus.jouy.inra.fr), and from earlier publications on genetic diversity in horses. One microsatellite marker was selected per autosome to avoid linkage between the loci. The selection criteria were defined characteristics such as high heterozygosity level, high number of alleles and ease of amplification. The 31 loci were *AHT34* (Swinburne *et al.* 2000b), *ASB17* (Breen *et al.* 1997), *COR007*, *COR017*, *COR018* (Hopman *et al.* 1999), *COR022*, *COR024* (Murphie *et al.* 1999), *COR045*, *COR056*, *COR058* (Ruth *et al.* 1999), *COR069*, *COR070*, *COR071*, *COR082* (Tallmadge *et al.* 1999), HMS03, HMS07 (Guerin *et al.* 1994), *HTG03*, *HTG06* (Ellegren *et al.* 1992), *LEX07* (Coogle *et al.* 1996a), *LEX33* (Coogle *et al.* 1996b), *LEX34* (Coogle *et al.* 1997), *LEX63* (Coogle & Bailey 1997), *LEX68* (Coogle & Bailey 1999), *LEX73* (Bailey *et al.* 2000), *SGCV16*, *SGCV28* (Godard *et al.* 1997), TKY19 (Kakoi *et al.* 1999), *UCDEQ425* (Eggleston-Stott *et al.* 1997), *UM011* (Meyer *et al.* 1997), *VHL20* (van Haeringen *et al.* 1994), and *VHL209* (van Haeringen *et al.* 1998). The 31 microsatellites were amplified alone or in multiplexes (two to five co-amplified loci) in 11 independent PCR reactions. Each PCR reaction tube with a final volume of 12 μL contained 40 ng genomic DNA, 1.2 μL 10x PCR buffer, 15 mM $MgCl_2$, 0.5% DMSO, 100 μM each dNTP, 0.75 U Taq-Polymerase (Qbiogene, Heidelberg, Germany), 5' IRD700 or IRD800 (IRD: Infra Red Dye) 1–10 pmol labelled forward primer, and unlabelled reverse primer. The amplification was carried out in PTC-100™ or PTC-200™ thermocyclers (MJ Research, Inc., Watertown, MA, USA) under the following conditions: an initial denaturation step at 94 °C for 4 min followed by 35 cycles at 94 °C for 30 s, maximum annealing temperatures for 60 s, and a final extension of 30 s at 72 °C. The dilution of PCR products with formamide loading dye in ratios from 1:6 to 1:30 was determined empirically and carried out prior to size fractionating on 6% denaturing polyacrylamide (Rotiphorese® Gel 40; Carl Roth, Karlsruhe, Germany) sequencing gels. Gelelectrophoresis was performed on an LI-COR 4200S-2 automated sequencer. Allele size was scored against known samples used as standards on every gel. Raw data were genotyped by visual examination and manual input.

Statistical analysis

Allele frequencies, unbiased estimates for expected (H_E) and observed (H_O) heterozygosity, and the number of alleles were computed using MSA (Microsatellite Analyzer, Dieringer & Schlötterer 2003). Hardy–Weinberg equilibrium (HWE) tests were conducted with the GENEPOP package version 3.3 (Raymond & Rousset 1995). Exact *P*-values were calculated along with their standard deviations using Guo & Thompson (1992) Markov-Chain algorithm with 1000 de-memorization steps for 100 batches and 1000 iterations per batch. A Bonferroni-Holm correction (Holm 1979) was applied to the exact *P*-values to maintain a multiple test level. First a correction was performed within each population over all 31 loci, after which the HWE was tested over all population loci combinations (Baumung & Sölkner 2002). If more than one population locus combination deviated from HWE, this microsatellite marker was not used for calculating genetic distances in order to obtain stable phylogenies with a great number of informative loci, without distorting genetic distances because of the significant deviation from the HWE. In addition, the hypothesis was tested that all 12 horse breeds are significantly distinguishable on the basis of genic and genotypic differentiation using GENEPOP. Afterwards differentiation tests were performed between the breeds for each locus to evaluate the significance of genetic differentiation among the populations. Genetic diversity within populations was measured as the mean number of alleles (N_A) per locus, the number of private alleles (PA, alleles found in only one breed), the observed heterozygosity (H_O), and the expected heterozygosity (H_E) under HWE. The subpopulation heterozygosity (i.e. average heterozygosity among subpopulations, H_S), the probability for a locus that two gametes chosen at random will carry different alleles (H_T) and the coefficient of gene differentiation G_{ST} (Nei 1973) were estimated separately for the draught horse populations and the other horse populations included here using the computer programme FSTAT version 2.9.3 (Goudet 1995). The individual observed heterozygosities were regressed on the individual inbreeding coefficients of the draught horse breeds using the Pearson correlation coefficient.

The chord distance constructed by Cavalli-Sforza & Edwards (1967) (D_C) is one of the best qualified for use with populations of intermediate divergence time as represented by breeds worldwide and in the breeds under study (Eding & Laval 1999). However, standard genetic distance of Nei (1972) (D_S) is the more frequently used distance, and this was calculated to obtain the possibility of comparing our results with those of other studies. The neighbour-joining tree topology was obtained with the PHYLIP software version 3.5 (Felsenstein 1989) using the Cavalli-Sforza distance. Bootstrap values were computed over 1000 replicates, and a consensus tree was drawn.

Results

Levels of variation and HWE

A total of 303 different alleles were detected across the 31 loci analysed. All amplified loci were polymorphic in all breeds except *COR071* and *HTG03*, which were monomorphic (194 and 116 bp, respectively) in the Przewalski's Horse. The number of alleles varied between 4 (*COR022*) and 19 *(ASB17*) with a mean of 9.8 and a standard deviation of 2.7.

HWE was tested for all breed-locus combinations. After a Bonferroni-Holm correction of the exact *P*-values over all

loci and breeds, no locus or population showed a significant heterozygote excess. No significant deviation from HWE ($P < 0.05$) was found within populations considering heterozygote deficiency. Compared not within but across populations, a significant ($P < 0.05$) deviation from HWE was observed for *HMS03* in the Arabian and the Icelandic Horse, and for *COR007* in the Przewalski's Horse. As *HMS03* showed deviations from HWE in more than one population, this locus was excluded from further calculations.

The average gene diversity H_T over all loci in the heavy horse populations was 0.676 while it ranged from 0.317 (*HTG06*) to 0.856 (*VHL20*) for individual loci. In the other horse populations it was 0.785, with a range from 0.608 (*SGCV28*) to 0.894 (*COR058*).

The average expected heterozygosity H_S across all loci in the heavy horse populations was 0.682 and ranged from 0.296 (*HTG06*) to 0.805 (*ASB17*), whereas it was 0.620 with a range from 0.520 (*SGCV28*) to 0.742 (*COR058*) in the other horse populations. The multilocus G_{ST} values in the heavy horse populations indicate that 11.6% of the total genetic variation is explained by breed differences, with the remaining 88.4% corresponding to differences among individuals. The G_{ST} values for single loci ranged in the heavy horse breeds between 0.021 (*SGCV16*) and 0.139 (*VHL209*). In the other horse populations this interbreed genetic variation was with a value of 21.2% much higher, where it ranged between 0.089 (*SGCV28*) and 0.265 (*LEX63*).

The mean number of alleles per draught horse breed varied between 5.20 in the Saxon Thuringa Coldblood and 6.33 in the South German Coldblood (Table 2), which might be explained by the variation in the sample sizes studied (22 and 23 samples of the Saxon Thuringa and Mecklenburg Coldblood as opposed to about 45 individuals of the other draught horse populations). The other horse populations showed mean numbers of alleles between 3.43 in the Sorraia Horse and 6.70 in the Hanoverian Warmblood. Observed heterozygosity ranged from 0.64 (Mecklenburg and Saxon Thuringa Coldblood) to 0.71 (South German Coldblood) in the heavy horse populations, while it varied from 0.47 (Przewalski's Horse) to 0.74 (Hanoverian Warmblood) in the other horse populations. The global population differentiation test showed significant ($P < 0.01$) results for all 30 loci. The pairwise tests, however, revealed that the Mecklenburg and Saxon Thuringa Coldblood are in fact significantly differentiated from the Rhenish German Draught Horse but not from each other. All other population combinations showed significant ($P < 0.01$) genetic differences. The Pearson correlation test performed for the draught horse breeds showed a significantly ($P < 0.05$) negative correlation of inbreeding coefficients and heterozygosity (data not shown).

Breed relationships

The D_C distance ranged from 0.03 to 0.32, and the D_S distance ranged from 0.07 to 1.63 (Table 3). As expected, the most divergent population was that of the Przewalski's Horse, and the next most was that of the Sorraia Horse. Furthermore, the Schleswig Draught Horse was the most divergent among the German heavy horse breeds. The phylogenetic tree indicated the presence of four groups (Fig. 1). The most robust features of the topology were the clusters of Mecklenburg and Saxon Thuringa Coldblood, and the Rhenish German Draught Horse (both with 100% support), and the clusters of the Hanoverian and Arabian and the Sorraia and Exmoor (with 92 and 99% support, respectively).

Discussion

The gene differentiation coefficient (G_{ST}) suggests an overall differentiation of 11.6% between the heavy horse breeds. This is comparable with the 12% differentiation observed between Norwegian horse breeds (Bjørnstad *et al.* 2000) and is somewhat greater than that observed by Cañon *et al.* (2000) between Spanish Celtic horse breeds (8%), and that (3.6%)

Table 2 Sample size (N), observed (H_O) and expected (H_E) heterozygosity (± standard deviations), number of alleles per locus (N_A) and total number of private alleles (PA) averaged over 30 microsatellites in 12 horse populations.

Population		N	H_O	H_E	N_A	PA
South German Coldblood	SG	45	0.707 ± 0.082	0.705 ± 0.109	6.33 ± 1.54	2
Rhenish German Draught Horse	RG	45	0.700 ± 0.099	0.678 ± 0.140	5.97 ± 1.73	–
Saxon Thuringa Coldblood	ST	23	0.685 ± 0.090	0.654 ± 0.140	5.20 ± 1.52	2
Mecklenburg Coldblood	MB	22	0.635 ± 0.074	0.644 ± 0.143	5.43 ± 1.50	–
Black Forest Horse	BF	45	0.696 ± 0.091	0.660 ± 0.130	5.94 ± 1.66	1
Schleswig Draught Horse	SL	45	0.696 ± 0.080	0.685 ± 0.091	5.50 ± 1.45	1
Hanoverian Warmblood	HAN	47	0.741 ± 0.075	0.735 ± 0.103	6.70 ± 1.69	8
Arabian	ARA	25	0.579 ± 0.089	0.574 ± 0.174	4.37 ± 1.27	4
Icelandic Horse	ICE	45	0.716 ± 0.075	0.732 ± 0.103	6.43 ± 1.48	6
Exmoor Pony	EX	20	0.606 ± 0.088	0.560 ± 0.176	4.40 ± 1.48	2
Sorraia Horse	SO	23	0.529 ± 0.076	0.525 ± 0.155	3.43 ± 1.07	2
Przewalski's Horse	PRZ	18	0.468 ± 0.087	0.526 ± 0.192	3.83 ± 1.64	12

Table 3 Genetic distances among 12 horse populations; the Cavalli-Sforza distance (D_C) is given above the diagonal, below Nei's standard genetic distance (D_S).

	BF	SL	ST	MB	SG	RG	ICE	EX	PRZ	SO	HAN	ARA
BF		0.084	0.078	0.076	0.066	0.066	0.128	0.143	0.215	0.179	0.106	0.147
SL	0.312		0.082	0.086	0.076	0.080	0.115	0.142	0.229	0.166	0.100	0.149
ST	0.255	0.220		0.028	0.085	0.042	0.132	0.155	0.242	0.172	0.126	0.172
MB	0.238	0.262	0.067		0.076	0.042	0.121	0.150	0.224	0.175	0.122	0.164
SG	0.221	0.254	0.285	0.245		0.065	0.105	0.124	0.208	0.160	0.090	0.138
RG	0.228	0.249	0.110	0.104	0.230		0.107	0.142	0.233	0.180	0.110	0.155
ICE	0.507	0.393	0.476	0.441	0.370	0.402		0.144	0.220	0.184	0.105	0.152
EX	0.490	0.482	0.545	0.512	0.433	0.538	0.560		0.258	0.159	0.125	0.174
PRZ	0.785	0.894	0.980	0.873	0.754	0.882	0.893	1.048		0.319	0.241	0.261
SO	0.645	0.577	0.602	0.648	0.611	0.644	0.796	0.615	1.628		0.171	0.220
HAN	0.424	0.390	0.503	0.480	0.367	0.459	0.417	0.477	1.058	0.611		0.110
ARA	0.604	0.542	0.674	0.625	0.512	0.622	0.575	0.611	1.007	0.973	0.409	

See Table 2 for abbreviations of breed names.

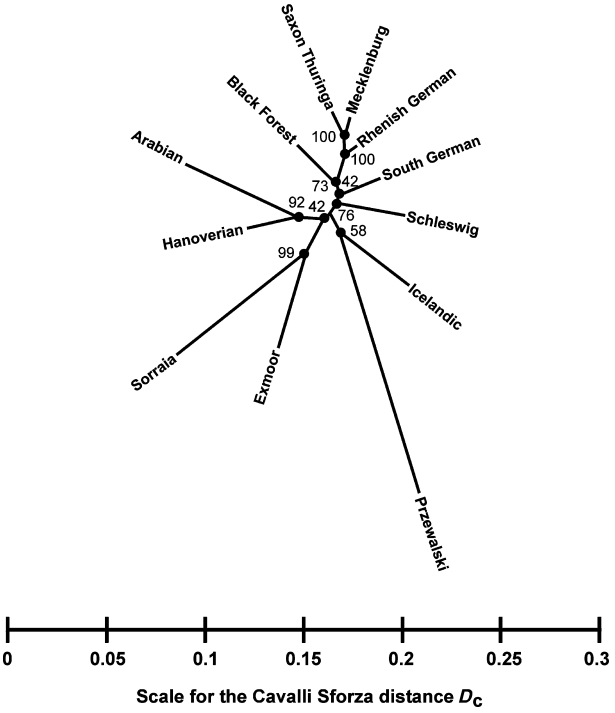

Figure 1 Phylogenetic tree constructed from D_C by the neighbour-joining method showing genetic relationships among 12 horse breeds. Numbers represent the percentage of times that a node occurred in 1000 bootstrap replicates.

found by Aranguren-Méndez *et al.* (2001) among Spanish donkey breeds. The G_{ST} of the other horse populations under study was much higher (21%), which was to be expected, as these populations were more isolated from each other, this is comparable with the results obtained by Saitbekova *et al.* (1999) for goats (17%) and by Cavalli-Sforza *et al.* (1994) for humans (between 10 and 20%).

The significant deviation from HWE observed for HMS03 may not be an inbreeding effect, as it was the only marker deviating from HWE in two populations but might possibly caused by null alleles.

Allele numbers are usually reduced faster than heterozygosity during inbreeding or a bottleneck period (Nei *et al.* 1975). However, we already found a significantly negative correlation between individual heterozygosity and inbreeding coefficients in the German heavy horse breeds. However, the investigation of Curik *et al.* (2003) revealed no significant correlation between these two parameters, although they observed higher inbreeding coefficients in Lipizzan horses (about 10%) than in the heavy horse breeds of the present study.

The observed mean number of alleles is lower in populations where inbreeding coefficients are higher, furthermore, rare alleles disappear during population declines, a phenomenon which was also obvious in all horse populations studied here. The lowest inbreeding coefficients were found in the largest draught horse populations the South German Coldblood (2.79%) and Rhenish German Draught Horse (1.53%), where the mean number of alleles was highest among the heavy horse breeds. The smallest population, the Schleswig Draught Horse, had the least mean number of alleles (5.5) among the four main draught horse breeds, although its inbreeding coefficient averaged 4.68%, which is in fact lower than that in the Black Forest Horses under study (5.75%). But in comparison with an outbred population, such as the Hanoverian Warmblood, losses in mean number of alleles and heterozygosity do not seem to be unduly severe in these populations. Overall, a large amount of genetic variation was observed in the draught horse breeds, and none of them is currently in any danger of losing substantial variation if population sizes do not decline again.

The Sorraia and Przewalski's Horse populations, which were recently intensely bottlenecked, show especially severe losses in the mean number of alleles and in the level of heterozygosity. Therefore, we could not confirm the findings of Putt & Whitehouse (1983), Breen *et al.* (1994) and

Bowling & Ryder (1987), who concluded that levels of heterozygosity in the Przewalski's Horse population were similar to those in domestic breeds, despite the breed's narrow genetic base. However, our findings that the number of private alleles was highest in the Przewalski's wild horse correspond to findings by Breen *et al.* (1994). Even compared with the Mecklenburg and Saxon Thuringa Coldblood, mean number of alleles and observed heterozygosities were lower in the Exmoor Pony, Przewalski's Horse, and the Sorraia Horse. This is not surprising as these populations all derive from a very small stock of founder animals. The German Sorraia Horse has even undergone two bottlenecks. These horses derive from 12 Portuguese founder animals, six descendants of which subsequently formed the basis of the German population. Except for one stallion, all Sorraia Horses in the present study descended from those six horses. Oom & Cothran (1994) observed low allelic diversity in both Sorraia subpopulations, but mean observed heterozygosity near the average for other breeds in the present study. In Exmoor Ponies, Cothran (1996) found the genetic variation level to be among the highest observed for any breed of horse. The heterozygosity and mean number of alleles in the Exmoor Ponies observed in this study also reflect a higher genetic variability than that found in the other primitive and wild horse populations, although it is lower than that of the domestic breeds under study. This may be due to the fact that the number of horses analysed here represents only the German population.

Heterozygosity and mean number of alleles in the Arabian Horse are comparable with the findings of Bjørnstad *et al.* (2000) for the Thoroughbred. This is not surprising as both breeds have similar histories. Pure breeding and high degree of inbreeding have long been practised to emphasize or even set special traits in the small number of different strains (Forbis 1980), and this has probably led to the loss of rare alleles and the reduction of heterozygosity. In general, comparisons with other studies have to be taken carefully because different microsatellite markers and partly different types of markers were used.

The group of heavy horses was clearly demarcated from the other horse populations in the neighbour-joining tree. Scharnhölz (2002) suggests two main origins of heavy draught horses. He proposed that draught horses originating from Western European coastal regions were heavier than horses from the alpine area in the region of the former Roman province Noricum. Both the Black Forest Horse and the South German Coldblood are stamped by Noric influence, but the genetic differences between these breeds appear to be greater than expected. A possible explanation for this is the crossbreeding of Black Forest Horses with heavier horses from Belgium and France and with horses of Rhenish German origin that was undertaken at the beginning of the 20th century to obtain heavier draught horses. Later, South German Coldblood Horses were bred with Rhenish German Draught Horses. However, it is doubtful if these crossbreedings with just a few individuals are in fact the only reasons for the close relationship between these breeds. However, the most distinct heavy horse breed was found to be the Schleswig Draught Horse, while the smallest genetic distances were found between the Rhenish German Draught Horse and its subpopulations in East Germany. The Rhenish German Draught Horse breed was created relatively recently (in the 19th century) by breeding local horses primarily with Belgian Draft Horses. Gene flows between breeds from East Germany (Mecklenburg and Saxon Thuringa Coldblood) and the Rhenish German Draught Horse were impossible for four decades as a result of the political division of Germany. The reproductive isolation of these breeds led to significant genic and genotypic differentiations between the Rhenish-German Draught Horse and the East German subpopulations, and thus to the development of a new, genetically distinguishable Eastern German horse population. Furthermore, the Eastern German populations are in fact not significantly genetically distinguishable from each other.

While the East German breeds may be kept as breeds distinct from the Rhenish German Draught Horse, crossbreeding of these breeds would offer a good opportunity to increase genetic variability, to decrease inbreeding coefficients, and to stabilize the population size as these breeds are genealogically of the same origin.

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