

Genetic diversity within and gene flow between three draught horse breeds using genealogical information

H. VOSTRÁ-VYDROVÁ², L. VOSTRÝ^{1,2}, B. HOFMANOVÁ¹, E. KRUPA², Z. VESELÁ², J. SCHMIDOVÁ^{1,2}

¹Czech University of Life Sciences Prague, Prague, Czech Republic

²Institute of Animal Science, Prague-Uhřetěves, Czech Republic

ABSTRACT: Three draught horse breeds are maintained in the Czech Republic: the Silesian Noriker (SN), the Noriker (N), and the Czech-Moravian Belgian (CMB). Because the SN and CMB populations are currently closed to outside breeding and are endangered (Genetic Resources), the loss of the genetic variation these horses represent is concerning. Genetic diversity within and between these three breeds and their population structures was analyzed based on pedigree information. Our goal was to identify and quantify factors that affected their genetic variability. The effective population size was analyzed in each breed. The numbers of generations were 22, 32, and 32 for the SN, N, and CMB breeds, respectively, with average equivalent known generations of 9.81, 8.45, and 8.91, respectively. The effective numbers of founders and ancestors contributing to the current genetic pool were, respectively, 69.42 and 22.32 for the SN breed, 98.48 and 42.20 for the N breed, and 43.33 and 23.32 for the CMB breed. The average inbreeding coefficients were 4.6%, 2%, and 4% for the SN, N, and CMB breeds, respectively, and the average inbreeding rate was 0.5% for the SN and CMB breeds and 0.2% for the N breed. The corresponding estimates of effective population size were 95, 195, and 101 for the SN, N, and CMB breeds, respectively. These statistics suggest that the genetic variability has decreased, and without changes in breeding strategy the genetic variability might continue to decline. Using genealogical F -statistics, small genetic differences were identified between the analyzed populations ($F_{ST} = 0.02$).

Keywords: genetic drift; inbreeding; Silesian Noriker; Noriker; Czech-Moravian Belgian horse

INTRODUCTION

The preservation of endangered species is one of the most important goals in current biological science. Conservation programmes are needed to preserve breeds with significant genetic diversity. The genetic structure of a population is shaped by different mechanisms, including gene flow, selection pressure, mutation, and genetic drift, all of which might vary in magnitude over time and be influenced by factors intrinsic or extrinsic to the population. These factors are the most relevant for structuring within-breed diversity.

The Silesian Noriker (SN), Czech-Moravian Belgian (CMB) draught horse breeds, along with the

Old Kladruber and Hutsul horse, belong to a group of endangered horse breeds recognized as Genetic Resources in the Czech Republic (CR). Over the last 120 years, the CMB breed was developed on the CR territory primarily using imported Belgian stallions and, to a lesser extent, Walloons. CMB horses have a medium square frame and mature earlier than other typical draught breeds. The SN breed was developed over the last 100 years from imported Noriker stallions and Bavarian draught stallions. SN horses have a longer than average frame and are a late maturing breed. Another draught breed maintained on a large scale on the CR territory is the Noriker (N), which contributed to the generation of the SN breed and has been

Supported by the Ministry of Agriculture of the Czech Republic (Project No. QJ1510141).

doi: 10.17221/91/2015-CJAS

continuously maintained on the CR territory for 150 years. To a large extent, the SN and CMB breeds have been geographically separated.

World War II negatively impacted breeding programmes because there was a steep decrease in the number of horses. The post-war expansion of farm mechanization caused a further decrease in the numbers. The SN, N, and CMB breeds were registered as separate breeds until the 1960s, but because of their decreasing numbers, all animals were merged into a single studbook – “synthetic breed” called the Czech Draught Horse (CDH). At this time, stallions and mares were used across the original breeds and all offspring including the crossbreeds were considered as “purebred individuals” of this new synthetic population. These genetically crossbred animals were also included in the breeding. However, in 1989, draught horses in the Czech Republic were reassigned into the original three populations (SN, N, and CMB) based on morphological analysis. During this period, although the analyzed breeds were separated, these animals were maintained as open populations and “crossbreeding” was conducted between them. After 1996 and 1999, the populations of SN and CMB horses were certified as rare and endangered breeds (Genetic Resources), and their studbooks were closed. Although SN stallions were still used for breeding with N mares, their offspring, with more than 50% of the SN breed’s genes, were included in the studbook, and these animals were regularly included in the SN breed (until 2013).

Based on the above-mentioned historical development, these three analyzed draft breeds can genetically be considered as a single breed with three subpopulations.

A primary molecular genetic analysis utilizing 13 microsatellite markers (Hofmanova et al. 2014) suggested high genetic similarity between the above-mentioned draught breeds. However, Toro et al. (2009) suggested that these markers were not necessarily good predictors of genomic coancestry because there are problems in estimating genomic heterozygosity based on only a few molecular markers. Until recently, no analysis of the gene flow between these three breeds has been conducted.

The objective of the present study was to describe the level of genetic variability between and within Czech draught horse breeds and to estimate the degree of gene flow and genetic differences among these animals based on genealogical information.

Secondly, we analyzed the genetic relationships between the breeds and between individual horses within these breeds.

MATERIAL AND METHODS

Data. A total of 27 022 pedigree records of registered individuals in studbooks dating from 1900 to June 31, 2013 were provided from the Association of Horse Breeders’ Unions of the Czech Republic. Pedigree analyses were performed using one reference population containing animals potentially contributing to the next generations. The reference population was defined as the entire active population of individuals (stallions and mares) born in the years 1996–2010 ($n = 2202$).

Pedigree completeness measures. The pedigree completeness and the number of ancestral generations influence the estimated inbreeding coefficients and the relationship coefficients between animals. The pedigree completeness level was assessed as the proportion of ancestors known per generation for each offspring (MacCluer et al. 1983). The number of equivalent complete generations in the pedigree (t) was computed according to Maignel et al. (1996) as follows:

$$t = \sum (1/2)^n$$

where:

n = number of generations between an animal and its ancestor

Probability of gene origin. Several measures of genetic variability were calculated from the pedigree data. The total number of founders (f) for each breed was determined as the total number of ancestors with unknown parents. The effective number of founders (f_e) is defined as the number of founders explaining the same level of genetic diversity as that observed in the reference population (Lacy 1989). This parameter is the reciprocal of the probability that two randomly drawn genes in the population under study originated from the same founder (James 1972). The effective number of founders (f_e) was estimated using the formula:

$$f_e = \frac{1}{\sum_{i=1}^f q_i^2}$$

where:

f = total number of founders

q_i = genetic contribution of the i^{th} founder to the reference population

The effective number of ancestors (f_a) is defined as the minimum number of ancestors, not necessarily of founders, explaining the same genetic diversity as that in the reference population (Boichard et al. 1997). The effective number of ancestors was obtained after determining the marginal genetic contribution of each ancestor. The parameter f_a accounts for losses of genetic variability resulting from bottlenecks caused by unequal contributions among reproductive individuals to future generations. The effective number of ancestors (f_a) was estimated using the formula:

$$f_a = \frac{1}{\sum_{i=1}^a p_i^2}$$

where:

p_i = marginal genetic contribution of ancestor i

a = total number of ancestors

The founder genome equivalent (f_{ge}) is defined as the effective number of founders with a non-random loss of founder alleles resulting in genetic variability identical to that defined in the reference population (Lacy 1989). The founder genome equivalent accounts for both unequal contributions of founders and the random loss of alleles caused by genetic drift (Lacy 1989). The founder genome equivalent (f_{ge}) was calculated using the method of Caballero and Toro (2000):

$$f_{ge} = \frac{1}{2f_g}$$

where:

f_g = average coancestry coefficient for the reference population

According to Caballero and Toro (2000), the average coancestry coefficient (f_g) was estimated using the formula:

$$f_g = \frac{\sum_{i=1}^n \sum_{j=1}^n a_{ij}}{2n^2}$$

where:

a_{ij} = elements of the relatedness matrix

n = number of individuals in the pedigree

The effective number of non-founders (N_{enf}) accounts for the effects of genetic drift in non-founder generations and was computed according to Caballero and Toro (2000):

$$\frac{1}{f_{ge}} = \frac{1}{f_e} + \frac{1}{N_{enf}}$$

where:

f_{ge} = founder genome equivalent

f_e = effective number of founders

Inbreeding coefficient and relatedness coefficient. The inbreeding coefficient of each individual (F_i) was estimated using a tabular method (Falconer and Mackay 1996) based on the method of VanRaden (1992):

$$F_i = a_{ii} - 1$$

where:

F_i = inbreeding coefficient of individual i

a_{ii} = additive genetic relationship of individual i to itself

The average relatedness coefficient of each individual (AR) was computed as the average coefficient integrating the row from the individual in the numerator relationship matrix **A**. This coefficient indicates the probability that a randomly selected allele in the population occurs in a selected individual or among a group of individuals (Goyache et al. 2003).

The realized effective population size (N_e) represents the number of unrelated individuals who would cause the same increase of inbreeding as that of the reference population. The realized effective population sizes (N_e) were estimated based on an individual increase in inbreeding ΔF_i (Gutierrez et al. 2008):

$$\Delta F_i = 1 - \sqrt[t_i]{1 - F_i}$$

where:

F_i = inbreeding coefficient of individual i

t_i = equivalent complete generations

The realized effective population size (N_e) was estimated according to Cervantes et al. (2008) using the formula:

$$\bar{N}_e = \frac{1}{2\Delta F}$$

where:

ΔF = average individual increase in the inbreeding of the reference population

The loss of genetic diversity (gene identity) in the reference population due to genetic drift or unequal founder contribution was derived from f_e and f_{ge} . The total loss of genetic diversity caused by genetic drift or bottleneck (GD) was computed using the function (Lacy 1995):

$$1 - GD, GD = 1 - \frac{1}{2f_{ge}}$$

where:

f_{ge} = founder genome equivalent

doi: 10.17221/91/2015-CJAS

The loss of genetic diversity (gene identity) resulting from an unequal founder contribution (GD^*) was estimated using the function (Caballero and Toro 2000):

$$1 - GD^*, GD^* = 1 - \frac{1}{2f_e}$$

where:

f_e = effective number of founders

The difference between GD and GD^* is the loss of genetic diversity caused by the genetic drift across generations of non-founders and corresponds to the inverse value of $2N_{enf}$:

$$GD - GD^* = \frac{1}{2N_{enf}}$$

where:

N_{enf} = effective number of non-founders

The genetic diversity between breeds was evaluated based on genealogical information using F -statistics (Wright 1969) for the entire metapopulation and for each breed. F -statistics (Wright 1969) were estimated according to Caballero and Toro (2002):

$$F_{IS} = \frac{\tilde{F} - \tilde{f}}{1 - \tilde{f}}, \quad F_{ST} = \frac{\tilde{f} - \bar{f}}{1 - \bar{f}} = \frac{\bar{D}}{1 - \bar{f}}, \quad F_{IT} = \frac{\tilde{F} - \tilde{f}}{1 - \tilde{f}}$$

where:

F_{IS}, F_{ST}, F_{IT} = Wright's fixation coefficients

\tilde{f}, \tilde{F} = average coancestry and inbreeding coefficients for the entire metapopulation, respectively

\bar{f} = average coancestry coefficient of subpopulations

\bar{D} = average genetic distance (Nei 1987)

The F -statistics were standardized according to the sample size (Bartolome et al. 2010).

Other components of total genetic diversity, including genetic diversity within individuals (GD_{WI}), genetic diversity between individuals (GD_{BI}), genetic diversity within subpopulations (GD_{WS}), and genetic diversity between subpopulations (GD_{BS}) were determined according to Caballero and Toro (2002).

The within-breed coancestry (f_{ii}) and the between-breeds coancestry matrix (f_{ij}) were computed averaging all pairwise coancestry coefficients of the individuals belonging to a given breed i or to two different breeds i and j , respectively (Caballero and Toro 2000).

Based on the above-mentioned methods, Fortran 90 software was developed to compute parameters of inbreeding and genetic diversity. This programme is available upon request.

RESULTS

The number of animals registered in the studbooks between 1960 and 1970 was similar for all breeds, being approximately 20 individuals per year. Between 1970 and 1993, annual registrations increased in the N and CMB breeds. In the SN breed, the number of animals registered peaked in 1997 and decreased thereafter. Between 2000 and 2013, approximately 100 individuals for the SN and CMB breeds and approximately 50 animals for the N breed were registered per year, of which approximately 45% of the animals were stallions and 55% of the animals were mares. The maximum number of registered stallions and mares occurred in 2010 (51) and 2008 (62) for the SN breed, 1994

Table 1. Number of individuals acting as parents in the analyzed breeds

	Whole pedigrees ¹			Reference populations ²		
	SN	N	CMB	SN	N	CMB
SN	0.39	0.08	0.04	0.98	0.45	0.01
N	0.11	0.26	0.04	0.02	0.30	0.01
CMB	0.01	0.04	0.29	0.00	0.02	0.91
CDH	0.08	0.09	0.10	0.00	0.07	0.05
Other	0.41	0.53	0.53	0.00	0.16	0.01

SN = Silesian Noriker, N = Noriker, CMB = Czech-Moravian Belgian, CDH = Czech Drought Horse (synthetic breed including all breeds – SN, N, and CMB – in one studbook)

¹whole pedigrees included all animals in the dataset

²reference populations included animals born in the years 1996–2010

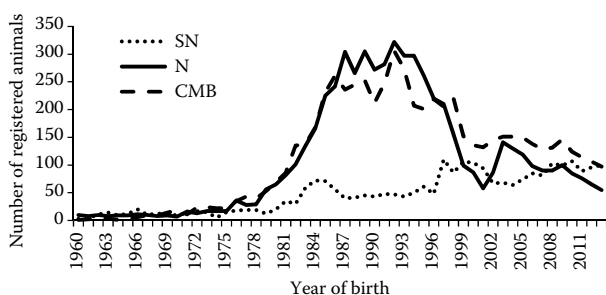


Figure 1. Number of animals registered in the Studbook per year of birth of Silesian Noriker (SN), Noriker (N), and Czech-Moravian Belgian (CMB) draught horses

(117) and 1987 (225) for the N breed, and 1998 (92) and 1993 (213) for the CMB breed (Figure 1).

Table 1 shows the number of individuals acting as parents in the breed studbooks analyzed. A total of 11% of individuals which acted as parents were N, 1% were CMB, 8% were CDH, and 41% of individuals came from other breeds in the SN studbook; a total of 8% of SN, 4% of CMB, 9% of CDH, and 53% of individuals from other breeds acted as parents in the N studbook; a total of 4% of SN, 4% of N, 10% of CDH, 53% of individuals from other breeds acted as parents in the CMB studbook.

The SN population was based on 1235 founders, while the CMB and N breeds had 2379 and 2583 founders, respectively. The effective number of founders (f_e) markedly differed between breeds, with 69.49 in SN, 43.33 in CMB, and 98.48 in N. These values indicate variable genetic contributions from the ancestors. The f_e considerably differed from the effective number of ancestors (f_a) for all breeds, suggesting a past bottleneck event (generational reduction in effective population size). The importance of genetic drift was evaluated based on the ratio of f_{ge} to f_e and the ratio of f_a to f_e . The value of each of these ratios was lower than 1 (Table 2). Particularly, the f_{ge}/f_e ratio suggests that a significant genetic drift occurred at some point in the past for each of these breeds.

The average equivalent of the known generations in the analyzed breeds was 8.91, 8.45, and 9.81 for the CMB, N, and SN breeds, respectively. Pedigree completeness levels were also consistent with the preceding values (Figure 2). The completeness level declined to less than 50% after 17, 15, and 14 generations in the SN, CMB, and N breeds, respectively.

Table 3 shows the cumulative marginal contributions of the most important ancestors. In the CMB breed, two ancestors could explain approxi-

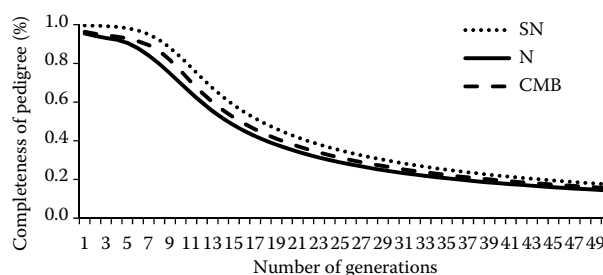


Figure 2. Completeness of pedigree information across generations for Silesian Noriker (SN), Noriker (N), and Czech-Moravian Belgian (CMB) draught horses

mately one quarter of the diversity in the reference population, and ten ancestors accounted for 50% of this diversity. In the N breed, two ancestors contributed by more than 13% to the total genetic variability, while 15 ancestors explained half of the genetic variability in the reference population. In the SN breed, two ancestors contributed by more than 20% to the total genetic variability, and ten ancestors explained more than 56% of the total genetic variability. In the CMB breed, the ten most important ancestors primarily belonged to

Table 2. Parameters characterizing the probability of gene origin, effective population size and parameters of inbreeding for Silesian Noriker (SN), Noriker (N), and Czech-Moravian Belgian (CMB) draught horses

	SN	N	CMB
Number of animals	498	729	956
Total number of founders (f)	1235	2379	2583
Effective number of founders (f_e)	69.42	98.48	43.33
Effective number of ancestors (f_a)	22.32	42.20	23.31
Founder genome equivalent (f_{ge})	8.64	18.26	11.01
Effective number of founders/effective number of ancestors ratio (f_e/f_a)	0.32	0.43	0.53
Founder genome equivalent/effective number of founders ratio (f_{ge}/f_e)	0.12	0.19	0.25
Realized effective population size (–)	95.21	195.07	101.37
Average inbreeding coefficient (F_i)	0.046	0.020	0.040
Average relatedness coefficient (AR)	0.12	0.06	0.09
ΔF per generation	0.005	0.003	0.005
Loss of genetic diversity due to:			
unequal founder contribution and random genetic drift	0.06	0.03	0.05
unequal founder contribution	0.01	0.01	0.02
random genetic drift	0.05	0.01	0.03

doi: 10.17221/91/2015-CJAS

Table 3. Marginal genetic contributions of the ten most influential ancestors of Silesian Noriker (SN), Noriker (N), and Czech-Moravian Belgian (CMB) draught horses

ID	SN				N				CMB					
	sex	year of birth	con. (%)	breed ¹	ID	sex	year of birth	con. (%)	breed ¹	ID	sex	year of birth	con. (%)	breed ¹
10322	M	1963	10.9	31	443	F	–	8.4	–	443	F	–	14.1	–
10328	M	1948	9.7	31	10870	M	1952	5.1	33	9500	M	1945	10.3	80
443	F	–	7.2	–	10311	M	1956	4.0	31	11682	F	1969	4.7	80
10870	M	1952	6.3	33	10328	M	1948	3.9	31	13825	M	1974	3.9	80
14731	M	1980	4.8	31	9859	F	1955	3.7	31	9953	F	1949	3.7	80
12294	M	1973	4.4	31	10832	M	1965	3.5	31	13828	M	1977	3.1	80
10324	M	1961	3.7	31	19332	M	1994	3.2	33	10429	M	1961	2.9	80
10311	M	1956	3.1	31	22019	M	1994	3.1	33	12731	F	1976	2.9	86
10389	M	1954	3.0	35	10322	M	1963	2.8	31	12849	F	1977	2.6	86
11440	M	1966	3.0	31	10869	M	1964	2.4	33	15656	M	1984	2.1	86

con. (%) = contribution, ID = identification number, M = male, F = female

¹breeds: 31 = Silesian Noriker, 33 = Austrian Noriker, 35 = Noriker, 80 = Czech Draught Horse, 86 = Czech-Moravian Belgian

original draught breeds. In the SN and N breeds, the majority of individuals were SN horses.

Figure 3 illustrates the average values for the inbreeding coefficient across years of birth. Only small differences were estimated between the birth years of 1960 and 1989. In the SN and N breeds, the average value of F_i ranged from 1 to 3%. In the CMB breed, this value increased between the birth years 1970 and 1982, when average values of F_i ranged from 3 to 5%. After 1990, the average value of F_i increased in the SN and CMB breeds, reaching close to 6% for individuals of both breeds born in 2013. In N, the average value of F_i was only 2–3% since 1990. The increase in F_i from the parent generation to the reference population was lower than 0.01 (Table 2). The average values of the AR (Table 3) in the refer-

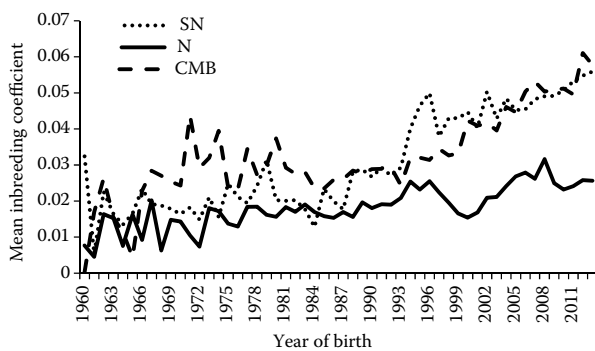


Figure 3. Mean inbreeding coefficient across years of birth of Silesian Noriker (SN), Noriker (N), and Czech-Moravian Belgian (CMB) draught horses

ence population were close to 0.10. The highest value was obtained for the SN breed, and the lowest was obtained for the N breed. The effective population size, derived from an increase in the inbreeding coefficient (Table 2), reached approximately 100 individuals in the reference population of the SN and

Table 4. Parameters describing the metapopulation of Silesian Noriker, Noriker, and Czech-Moravian Belgian draught horses

Self-coancestry	0.517
Inbreeding	0.035
Average coancestry	0.042
Average genetic distance	0.044
Average coancestry over metapopulation	0.025
F_{IS}	-0.008
F_{ST}	0.018
F_{IT}	0.010
GD_T	0.975
GD_{WI}	0.482
GD_{WS}	0.957
GD_{BI}	0.475
GD_{BS}	0.017

F_{IS} , F_{ST} , F_{IT} = Wright's fixation coefficients (Wright 1969), GD_T = total genetic diversity, GD_{WI} = genetic diversity within individuals, GD_{WS} = genetic diversity within subpopulations, GD_{BI} = genetic diversity between individuals, GD_{BS} = genetic diversity between subpopulations

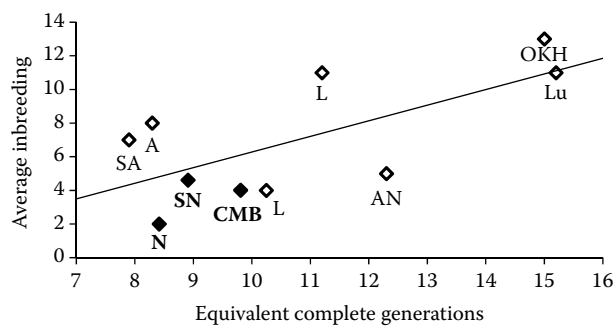


Figure 4. Comparison between mean equivalent complete generations and mean inbreeding coefficients for Silesian Noriker (SN), Noriker (N), and Czech-Moravian Belgian (CMB) as well as for other horse populations (Andalusian (A) – Valera et al. 2005; Austrian Noriker (AN) – Druml et al. 2009; Spanish Arab (SA) – Cervantes et al. 2008; Lipizzaner (L) – Zechner et al. 2002; Pjontek et al. 2012; Lusitano (Lu) – Vicente et al. 2012; Old Kladruber Horse (OKH) – Vostra-Vydrova et al. 2016)

CMB breeds. In the N breed, a two times higher effective population size was estimated than in the SN and CMB breeds. To determine the influence of pedigree, we graphically compared the amount of inbreeding and pedigree completeness obtained for the SN, N, and CMB breeds with the values obtained for other horse breeds (Figure 4). The regression line represents the mean F_i for the horse populations with adjustments made for pedigree quality. The SN, N, and CMB breeds were under the presented regression line.

The total amount of genetic diversity (GD) loss in the analyzed breeds during the last 53 years, due to various reasons, is shown in Figure 5. The average relative GD losses in the reference populations were 6, 3, and 5% for the SN, N, and CMB breeds, respectively. The loss of GD due to genetic drift accumulated over non-founder generations ($GD^* - GD$) was more important than the loss resulting from the unequal contribution of founders in almost all breeds. As shown in Figure 5, a greater loss of genetic diversity was recorded between 1960 and 1980, and the loss of genetic diversity was observed in all breeds in the last year.

The overall difference between breeds expressed as the total value in the metapopulation (F_{ST}) indicates a low differentiation between breeds (Table 4). These values showed that differences between breeds accounted for only 2% of the total genetic variability expressed in the draught horse population of the studied breeds. The remaining

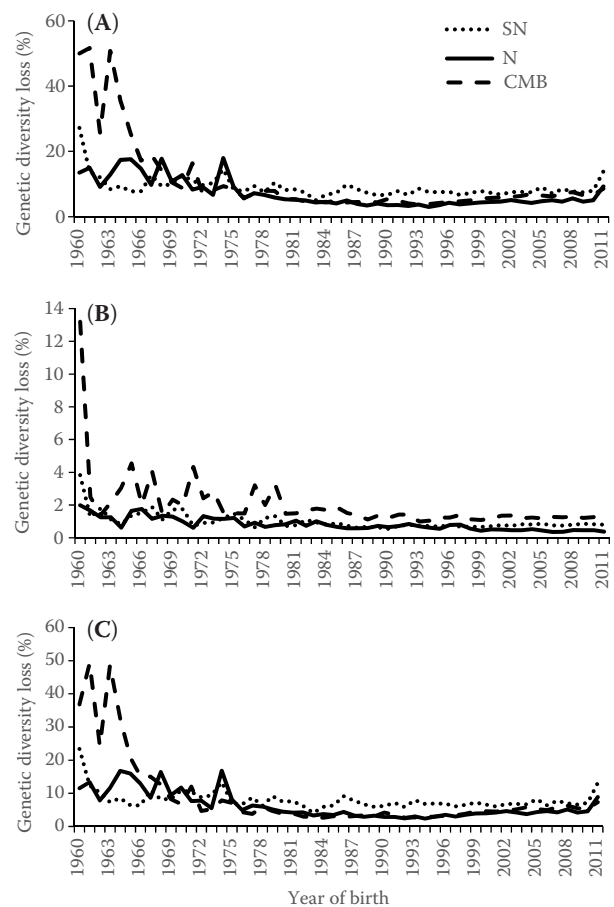


Figure 5. Genetic diversity loss due to unequal founder contribution and random genetic drift (A), unequal founder contribution (B), and random genetic drift (C) in evaluated breeds

SN = Silesian Noriker, N = Noriker, CMB = Czech-Moravian Belgian

98% of the total genetic variability resulted from differences between individuals, regardless of the breed. The population differentiation in Czech draught horse breeds is represented by pairwise F_{ST} coefficients (Table 5). The pairwise F_{ST} coefficients ranged from 0.006 (between SN and N) to 0.019 (between the SN and CMB breeds). Thus, 0.6 to 1.9% of the genetic variability is explained by differences between populations, while the remaining variability is explained by variations within populations. GD_{WS} and GD_{BS} are consistent with these values, in that values of GD_{WS} are significantly higher than those of GD_{BS} (Table 4). The value of GD_{BS} suggests that there is hardly any genetic diversity between groups. The negative values of F_{IS} were estimated for all populations (Tables 4 and 5).

doi: 10.17221/91/2015-CJAS

Table 5. The value of F_{IS} with within-breeds coancestry (in brackets) on diagonal, the value of F_{ST} with between-breeds coancestry (in brackets), and genetic distances between subpopulations (D_{ij} – Nei 1987) below diagonal

	SN	N	CMB
SN	–0.013 (0.058)	0.006 (0.030)	0.020 (0.010)
N	0.012	–0.008 (0.027)	0.013 (0.012)
CMB	0.042	0.025	–0.006 (0.048)

SN = Silesian Noriker, N = Noriker, CMB = Czech-Moravian Belgian

The standard Nei's genetic distance values ranged from 0.01 between the SN and N breeds to 0.04 between the SN and CMB breeds (Table 5). The lowest Nei's genetic distance and the highest genetic identity between the breeds were observed between the SN and N breeds. The coancestry assessed between the analyzed breeds varied between 0.01 for the pair SN–CMB and 0.03 for the pair SN–N. These values indicate low genetic differences overall.

DISCUSSION

A basic assumption for the calculation of a large number of descriptive coefficients derived from the analysis of the pedigree data from a population is that founders are not related. This assumption is not met when the population is small and has been closed for a long time. A more realistic assumption might be that founders originated from diverse remote areas and/or that the number of founders was large (Nagy et al. 2010). Both of these conditions were fulfilled in the analyzed breeds because of regional diversity (western vs eastern part of the Czech Republic) and the long-term import of foreign stallions during the first half of the 20th century. The equivalent of completely known generations of breeds in the present study was comparable with other horse populations: Lipizzaner – 15.2 and 10.25 (Zechner et al. 2002; Pjontek et al. 2012), Austrian Noriker – 12.3 (Druml et al. 2009), Andalusian – 8.3 (Valera et al. 2005), Lusitano – 11.2 (Vincente et al. 2012), and Old Kladruber – 15 (Vostra-Vydrova et al. 2016).

The f_e statistic is an important parameter used to assess if the contribution of the founders is balanced (Lacy 1989). If all founders contributed equally, then the effective number of founders would be equal to the total number of founders.

However, f_e is typically lower than f because f_e reflects unequal contributions of founders to the current population as a result of selection and variations in family size. The f_e and f values less than one indicate a loss of genetic diversity resulting from unequal contributions of the founders. The advantage of some individuals is indicated as f_a/f_e and f_{ge}/f_e ratios describing the extent of bottleneck and genetic drift, respectively (Boichard et al. 1997). The ratios calculated in the present analyses had higher or identical values compared with other reports, including the Austrian Noriker (Druml et al. 2009) – 0.25 and 0.09, Lusitano (Vincente et al. 2012) – 0.47 and 0.21, and Spanish Arabian horses (Cervantes et al. 2009) – 0.46–0.72 and 0.23–0.42. These values are markedly higher than those from other breeds included in the genetic resources of the Czech Republic, including Old Kladruber – 0.27 and 0.05 (Vostra-Vydrova et al. 2016). Rodriganez et al. (1998) reported that the substantial bottleneck and drift effects for swine populations resulted from a closed herd structure and a long evaluation period. The studbooks of the SN and CMB breeds were closed in 1996 and 1999, respectively.

The observed trend of F_i in the analyzed populations corresponded well with their historical development. Until 1960, the breeds were recorded separately, and a low F_i value was maintained through the import of foreign Noriker stallions or mares into the SN and N populations and Belgian horses for breeding into the CMB population. In 1960, based on a political decision, all three breeds were merged under the unified name of Czech Draught Horse, and after that crossing among these animals was common. Such crosses promoted low F_i values, even without the use of foreign stallions and mares. In 1989, the recognition and promotion of individual draught horse breeds was resumed, and cross breeding, particularly in the SN and CMB breeds, was eliminated. Since then, the F_i values in these breeds have increased. The influx of foreign genetic material has maintained the F_i value of the N breed at a low level. As shown in Figure 4, the inbreeding coefficients of all breeds reached a lower F_i value than the average inbreeding estimated from various populations of horses at equivalent completely known generations. In a subdivided population, the mating between some animals is more probable than with others. Thus, the average inbreeding coefficient of the next generation is

higher than the average coancestry of the previous generation. Some studies have demonstrated that the average coancestry is an adequate measure of genetic diversity because this factor accounts for both founder effects and genetic drift (Lacy 1995; Caballero and Toro 2000). Comparing the relationship between average coancestry and genetic diversity demonstrated that selection with the objective of minimizing coancestry in the next generation would maximize genetic diversity (Lacy 1995). Based on a comparison of the average relatedness coefficients within breeds (AR), a decrease of genetic diversity in future generations is expected, particularly in the SN breed.

The average inbreeding values describe changes in the genetic structure of a population across time. When increasing, these values reflect the increased frequency of homozygous loci and the decreased frequency of loci with more than one allele, indicating a loss of genetic variability that might negatively influence fitness characteristics and increase the occurrence of phenotypic defects in subsequent generations. Therefore, the rate of inbreeding (ΔF) (Figure 6) is an important parameter for genetic diversity monitoring. The Food and Agriculture Organization of the United Nations (FAO 1998) stated that the value of ΔF should not exceed 1%. It has been recommended that the N_e (derived from ΔF) of a population should be 50 or higher for the adequate conservation of genetic diversity (Meuwissen and Woolliams 1994; FAO 1998).

A reduced demand for horses to perform practical work, due to the replacement of these animals with machinery, resulted in the merging of draught horses into one breed in 1960. This administrative

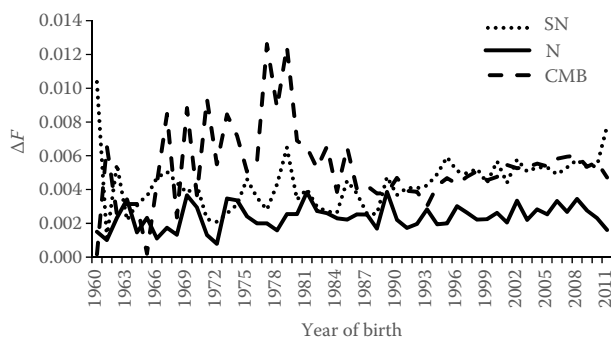


Figure 6. Rate of inbreeding (ΔF) per generation by the year of birth

SN = Silesian Noriker, N = Noriker, CMB = Czech-Moravian Belgian

decision facilitated the use of stallions, regardless of their breed, resulting in a decrease in the number of stallions needed for breeding, leading to both a bottleneck and increased variability in the number of matings per stallion. Genetic distances between breeds were also reduced. The values of pairwise F_{ST} and genetic distances were uncharacteristically low for genetically distinct breeds. The pairwise F_{ST} values suggested weak genetic isolation between these breeds of draught horses. The low pairwise F_{ST} values and genetic distances between breeds resulted from crossing between individuals of particular breeds from 1960 to 1990. However, stallions of the SN breed were used in the N breed populations without restrictions until 2011. Since 2011, the use of SN stallions in the N population has been minimal but has continued. The use of other stallions in the SN and CMB population is not permitted because the SN and CMB populations are now closed. Despite closing the SN and CMB populations, the numbers of sires of individuals belonging to the reference population were 2% of the N stallions in the SN population, 1% of the SN and N stallions in the CMB population, and 45% and 2% of the SN and CMB stallions in the N population (open population). These values indicate considerable gene flow between the SN and N breeds in the current population. The values of Nei's distance are consistent with these conclusions. The gene flow between the analyzed populations was also confirmed by average coancestry value between the SN and N. The average genetic coancestry between the SN and N breeds exceeds the average coancestry within the N breed. This implies a higher average similarity between two individuals in the SN and N breeds than between two individuals in the N breed. This is in accordance with the already mentioned fact that a total of 45% of stallions used as parents were SN in the N studbook (Table 1). Cervantes et al. (2009) showed that the within-breeds coancestry values and the between-breeds coancestry matrix are more informative on the mating policies and histories of the breeds. Note that coancestries are half the additive genetic relationships that are the percentage of genes flow between two individuals or, as here, populations. Taking this into consideration, SN and N share 6% of genes, SN and CMB share 2% of genes, and 2.4% of genes are shared by N and CMB populations.

doi: 10.17221/91/2015-CJAS

The estimated pairwise F_{ST} showed a higher value than that between subpopulations of Catalan donkeys (2.89% – Gutierrez et al. 2005) or in a coloured variety of the Old Kladruber horse (5% – Vostra-Vydrova et al. 2016). The parameter F_{IS} is equivalent to the parameter alpha proposed by Caballero and Toro (2000), showing whether mating between relatives is avoided or not. In the analyzed subpopulations for the selection of the parents for the next generation, breeders followed strict avoidance of mating between close relatives.

In general, genetic diversity was reduced in reference populations of draught horses. The rate of genetic diversity loss resulting from random genetic drift was higher than the rate caused by non-random founder contributions. Based on the rate of inbreeding (ΔF) (Figure 6) and loss of genetic diversity (Figure 5), this genetic drift was primarily generated through bottlenecks that occurred between 1960 and 1980, particularly for the CMB breed. However, in addition to the above-mentioned genetic drift and uneven founder contributions to inbreeding values in the population, the loss of genetic diversity was influenced by the breeding programme and related factors. Fernandez et al. (2005) stated that the loss of genetic diversity within breeds is influenced, to a large extent, by genetic drift, resulting in increased homozygosity and allele fixation in the population. Alvarez et al. (2008) reported that losses of genetic variability occurred soon after setting up the breeding programme, as a significant number of founders did not produce progeny in the next generation.

CONCLUSION

The preservation of biodiversity requires profound knowledge of the basic population parameters and population structure. The results of the present study indicate a serious reduction in genetic diversity among the three Czech draught horse breeds examined, which is most evident from the progressive loss of genetic contributions from the founders and ancestors. Unequal contributions among founders play a major role in the loss of genetic diversity in all breeds, particularly in the SN breed. Random genetic drift also had a substantial impact on the loss of genetic diversity. The small genetic distances between the analyzed breeds were unexpected, likely reflecting the high

frequency of migration between breeds or genetic drift. These results showed that more appropriate breeding strategies should be implemented to increase genetic differences between Czech draught horse breeds.

Acknowledgement. The authors would like to thank F. Goyache, Área de Genética y Reproducción Animal, SERIDA-Deva, Spain, for valuable comments.

REFERENCES

- Alvarez I., Royo L.J., Gutierrez J.P., Fernandez I., Arranz J.J., Goyache F. (2008): Relationship between genealogical and microsatellite information characterizing losses of genetic variability: empirical evidence from the rare Xalda sheep breed. *Livestock Science*, 115, 80–88.
- Bartolome E., Goyache F., Molina A., Cervantes I., Valera M., Gutierrez J.P. (2010): Pedigree estimation of the (sub) population contribution to the total gene diversity: the horse coat colour case. *Animal*, 4, 867–875.
- Boichard D., Maignel L., Verrier E. (1997): The value using probabilities of gene origin to measure genetic variability in a population. *Genetics Selection Evolution*, 29, 5–23.
- Caballero A., Toro M.A. (2000): Interrelations between effective population size and other pedigree tools for the management of conserved populations. *Genetical Research*, 75, 331–343.
- Caballero A., Toro M.A. (2002): Analysis of genetic diversity for the management of conserved subdivide populations. *Conservation Genetics*, 3, 289–299.
- Cervantes I., Goyache F., Molina A., Valera M., Gutierrez J.P. (2008): Application of individual increase in inbreeding to estimate realized effective sizes from real pedigrees. *Journal of Animal Breeding and Genetics*, 125, 301–310.
- Cervantes I., Gutierrez J.P., Molina A., Goyache F., Valera M. (2009): Genealogical analyses in open populations: the case of three Arab-derived Spanish horse breeds. *Journal of Animal Breeding and Genetics*, 126, 335–347.
- Druml T., Baumung R., Solkner J. (2009): Pedigree analysis in the Austrian Noriker draught horse: genetic diversity and the impact of breeding for coat colour on population structure. *Journal of Animal Breeding Genetics*, 126, 348–356.
- Falconer D.S., Mackay T.F.C. (1996): *Introduction into Quantitative Genetics*. Longman House, Harlow, UK.
- FAO (1998): Secondary guidelines for development of farm animal genetic resources management plans. Management of small populations at risk. FAO, Rome, Italy.
- Fernandez J., Villanueva B., Pong-Wong R., Toro M.A. (2005): Efficiency of the use of pedigree and molecular

- marker information in conservation programs. *Genetics*, 170, 1313–1321.
- Goyache F., Gutierrez J.P., Fernandez I., Gomez E., Alvarez I., Diez J., Royo L.J. (2003): Using pedigree information to monitor genetic variability of endangered populations: the Xalda sheep breed of Asturias as an example. *Journal of Animal Breeding and Genetics*, 120, 95–103.
- Gutierrez J.P., Marmi J., Goyache F., Jordana J. (2005): Pedigree information reveals moderate to high levels of inbreeding and a weak population structure in the endangered Catalanian donkey breed. *Journal of Animal Breeding and Genetics*, 122, 378–386.
- Gutierrez J.P., Cervantes I., Molina A., Valera M., Goyache F. (2008): Individual increase in inbreeding allows estimating effective size from pedigrees. *Genetics Selection Evolution*, 40, 359–378.
- Hofmanova B., Pribanova M., Majzlik I., Vostry L., Vostra-Vydrova H. (2014): Population study of three breeds of cold blooded horses in the Czech Republic. In: Proc. 65th Annu. Mtg., European Association for Animal Production (EAAP), Copenhagen, Denmark, 379.
- James J.W. (1972): Computation of genetic contribution from pedigrees. *Theoretical and Applied Genetics*, 42, 272–273.
- Lacy R.C. (1989): Analysis of founder representation in pedigrees: founder equivalents and founder genome equivalents. *Zoo Biology*, 8, 111–123.
- Lacy R.C. (1995): Clarification of genetic terms and their use in the management of captive populations. *Zoo Biology*, 14, 565–578.
- MacCluer J., Boyce B., Dyke L., Weitzkamp D., Pfenning A., Parsons C. (1983): Inbreeding and pedigree structure in Standardbred horses. *Journal of Heredity*, 74, 394–399.
- Maignel L., Boichard D., Verrier E. (1996): Genetic variability of French dairy breeds estimated from pedigree information. *Interbull Bulletin*, 14, 49–54.
- Meuwissen T.H.E., Woolliams J.A. (1994): Effective sizes of livestock populations to prevent a decline in fitness. *Theoretical and Applied Genetics*, 89, 1019–1026.
- Nagy I., Curik I., Radnai I., Cervantes I., Gyovai P., Baumung R., Farkas J., Szendro Z. (2010): Genetic diversity and population structure of the synthetic Pannon White rabbit revealed by pedigree analyses. *Journal of Animal Science*, 88, 1267–1275.
- Nei M. (1987): *Molecular Evolutionary Genetics*. Columbia University Press, New York, USA.
- Pjontek J., Kadlecik O., Kasarda R., Horny M. (2012): Pedigree analysis in four Slovak endangered horse breeds. *Czech Journal of Animal Science*, 57, 54–64.
- Rodriganez J., Toro M.A., Rodriguez M.C., Silio L. (1998): Effects of founder allele survival and inbreeding depression on litter size in a closed line of Large White pigs. *Animal Science*, 67, 573–582.
- Toro M.A., Fernandez J., Caballero A. (2009): Molecular characterization of breeds and its use in conservation. *Livestock Science*, 120, 174–195.
- Valera M., Molina A., Gutierrez J.P., Gomez J., Goyache F. (2005): Pedigree analysis in the Andalusian horse: population structure, genetic variability and influence of the Carthusian strain. *Livestock Production Science*, 95, 57–66.
- VanRaden P.M. (1992): Accounting for inbreeding and crossbreeding in genetic evaluation of large populations. *Journal of Dairy Science*, 75, 3136–3144.
- Vicente A.A., Carolino N., Gama L.T. (2012): Genetic diversity in the Lusitano horse breed assessed by pedigree analysis. *Livestock Science*, 148, 16–25.
- Vostra-Vydrova H., Vostry L., Hofmanova B., Krupa E., Zavadilova L. (2016): Pedigree analysis of the endangered Old Kladruber horse population. *Livestock Science*, 185, 17–23.
- Wright S. (1969): *The Theory of Gene Frequencies: Evolution and the Genetics of Populations*, Vol. 2. Chicago University Press, Chicago, USA.
- Zechner P., Zohman F., Solkner J., Bodi I., Habe F., Marti E., Brem G. (2002): Analysis of diversity and population structure in the Lipizzan horse breed based on pedigree information. *Livestock Production Science*, 77, 137–146.

Received: 2015–11–25

Accepted after corrections: 2016–06–25

Corresponding Author

doc. Ing. Luboš Vostrý, Ph.D., Czech University of Life Sciences Prague, Faculty of Agrobiological Sciences, Kamýcká 129, 165 21 Prague 6-Suchbát, Czech Republic
Phone: +420 224 382 547, e-mail: vostry@af.czu.cz
