Pedigree analysis of the Lipizzan horse populations from Bosnia and Herzegovina and Serbia: Structure, inbreeding and genetic variability

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Abstract: The aim of this study was to evaluate population parameters and to describe the genetic variability in the Lipizzan horse populations from stud farms in Bosnia and Herzegovina (BH) and Serbia (SRB), reported for the first time in the literature. Pedigree data were collected from the national studbooks. Pedigree data for 2 521 horses were used in the analysis, for a reference population covering live animals in the current population. The average generation interval was 10.78 ± 0.36 (BH) and 12.12 ± 0.59 (SRB) years. The equivalent complete generations ranged from 5.49 (BH) to 14.86 (SRB). The average inbreeding values were 3.96 (BH) and 2.12 (SRB), whereas the average relatedness values were 9.24 (BH) and 4.13 (SRB). Values for f_e, f_a, f_g and the f_e/f_a ratio were 21, 12, 6.031 and 1.75 in BH Lipizzan horses, and 79, 20, 9.766 and 3.95 in SRB Lipizzan horses. The number of ancestors explaining 50% of genetic diversity in the BH and SRB reference populations was five and eight, respectively. The effective population sizes obtained from regression on the birth dates were 43.67 (BH) and 211.78 (SRB). The investigated genetic variability parameters were higher in the SRB Lipizzan population than in the BH population. The results of pedigree and genetic structure research indicate the need for a comprehensive approach to monitoring to improve future breeding and genetic diversity in the BH and SRB Lipizzan populations.

Keywords: genetic protection; genetic diversity; pedigree information; population structure

The national stud farm, Vučijak, was founded in Bosnia and Herzegovina (BH) in 1946. Throughout the history of horse breeding in BH, Vučijak Stud has had an important role. The primary reason for establishing the stud farm was to enhance the existing horse population in BH, in the light of creating horses for driving and weight carrying (Rogic et al. 2018). These are some of the reasons why the Lipizzan horses from Vučijak are smaller than other European Lipizzan populations.

The first horses, the founders of the Vučijak stud farm, were imported from the stud farms Lipik and Djakovo, and from private breeders from Croatia. The process of forming the nucleus for Vučijak Stud lasted from 1946 to 1952. Subsequently, stallions and mares from Slovenia and Serbia were imported to Vučijak Stud (Rogic et al. 2018). In Serbia (SRB), the Lipizzan horse has a centuries-old breeding tradition. Currently, the oldest Lipizzaner stud farm is Karadjordjevo, which became a national stud farm in 1921. Karadjordjevo started breeding its own horses in 1929–1930, using stallions and mares from other national stud farms (Lipica, Djakovo, Vucijak and Lipik). Great importance in the breeding of Lipizzan horses in SRB was achieved by Kelebija stud farm, which formed an initial breeding herd from Karadjordjevo stud farm, private Lipizzan breeders and owners mostly from SRB, but also from other state stud farms. The Lipizzan horse is one of the most popular breeds for horse breeding in BH and SRB and it is a valuable genetic resource in these countries.

Bokor et al. (2013) indicated that genetic information, obtained through a pedigree analysis, could be valuable for identification of genetic variability. The pedigree analysis makes it possible to follow the changes that occur through generations, and to assess the population structure and inbreeding level. The tradition of breeding of Lipizzan horses is characterised by small and closed populations, which are under high selection pressure, and by closed studbooks for many years. Considering that all privately-bred Lipizzan horses originated from state-owned nucleus herds, which are bred in closed herds and in the long term, the genetic diversity of this breed can be considered small (Zechner et al. 2001). Genetic drift, bottlenecks and unequal genetic contributions of the founders reduce genetic diversity. Reduction of genetic variability can be assessed by considering the effective number of founders and ancestors (Duru 2017). In the breeding process for any breed, and for small population sizes in particular, genetic variability evaluation serves as a key indicator, especially from the viewpoint of minimizing inbreeding depression (Halo et al. 2018).

The pedigree analysis of horse breeds was the subject of many scientific studies (Zechner et al. 2002; Valera et al. 2005; Pjontek et al. 2012; Mederios et al. 2014; Duru 2017; Halo et al. 2018; Somogyvari et al. 2018; Giontella et al. 2019; Dell et al. 2020; Prochniak et al. 2021; Bramante et al. 2022). Through description of the horse population, attempted by the Copernicus Project, Zechner et al. (2002) worked on the pedigree analysis of Lipizzan horses from eight European stud farms. Pedigrees of Slovakian Lipizzan horses were also the subject of a study by Pjontek et al. (2012). In recent years and according to horse pedigrees, there has been an increase in the number of studies that analysed the gene pool of Lipizzan horses based on microsatellites or SNP markers (Barcaccia et al. 2013; Petersen et al. 2013; Kasarda et el. 2016; Grilz-Seger et al. 2019). Different genetic approaches and methods have been applied to characterise the gene pool, genetic diversity and population structure of the Lipizzan breed.

This is the first study related to the pedigree analysis of Lipizzan horses from BH and SRB. The aim of this work was to analyse the information from the Lipizzan Studbooks in order to gain insight into the structure of the populations and evaluate their genetic variability. The results might be used for implementing conservation strategies for the breed, and should suggest appropriate strategies to monitor matings.

MATERIAL AND METHODS

Study data

The pedigree information on the BH Lipizzan horses was obtained from the Studbook from the Vucijak national stud farm. All pedigree data on the horses enrolled in the Studbook from 1946 to 2019 were used. The pedigree information on the SRB Lipizzan horses from 1900 to 2020 was collected from the database held at the Department of Animal Science, Faculty of Agriculture in Novi Sad, which is a central breeding organization for Lipizzan horses in SRB. Pedigree information from a total of 2 521 (1 264 from BH, and 1 257 from SRB) Lipizzan horses was analysed (Table 1). Each horse was characterized by unique equine life number, dam's number, sire's number, date of birth, sex and generation. Animals with unknown parents were considered as the founders. The analysed reference populations consisted of 286 animals and cover live animals in the current populations, i.e., animals that can potentially contribute to the next generation. Population sizes differentiated by sex,

Table 1. Number of Lipizzan horses included in the analysis

	BH		SRB	
Total population				
Total number	1 264		1 257	
Sex	male	female	male	female
Number	647	616	507	749
Reference population				
Total number	150		136	
Sex	male	female	male	female
Number	59	91	52	84

BH = Bosnia and Herzegovina; SRB = Serbia

reference populations and totals for the two different populations are given in Table 1. All parameters were computed using the Endog v4.8 programme (Gutierrez and Goyache 2005). The appropriate formulas and methods are explained in detail in the software instructions.

Pedigree completeness

The completeness of pedigrees was expressed by the indicators calculated for each animal. The number of generations was calculated in three different ways: (1) number of full generations traced, which represents the number of generations with both parents known; (2) maximum number of generations traced, which represents the number of generations that separate the offspring from its furthest ancestors; (3) number of equivalent complete generations calculated as the sum over all known ancestors based on $(1/2)^n$, where n is the number of generations separating the individual from each of the known ancestors (Boichard et al. 1997). The pedigree completeness level (PCL) was defined by computing for the whole pedigree the proportion of ancestors known per generation for each offspring. The generation interval (GI) was defined as the average age of the parents at the birth of the offspring subsequently used for reproduction (James 1977).

Genetic variability parameters

The individual inbreeding coefficient (F) and the average relatedness coefficient (AR) correspond to the probability that an individual has two identical alleles by descent (Wright 1931) and to the probability that an allele randomly chosen from the whole population in the pedigree belongs to a given animal, respectively. F, the individual inbreeding coefficient, was computed following Meuwissen and Luo (1992).

The AR for each individual was calculated according to the equation:

$$c' = \left(\frac{1}{n}\right) 1' \, \mathbf{A} \tag{1}$$

where:
c' – row vector where c_i is the average of the coefficients in the row of individual i in the numerator relationship matrix $\mathbf{A}$, of the dimension n;
$\mathbf{A}$ – relationship matrix of size $n \times n$.

The effective number of founders (f_e) was defined as the number of equally contributing founders that would be expected to produce the same genetic diversity as in the population in the study (Lacy 1989) and it was calculated using the formula:

$$f_e = 1/ \sum_{k=1}^{f} q_k^2 \tag{2}$$

where:
q_k – probability of gene origin of ancestors k;
f – real number of founders.

The effective number of ancestors (f_a) is defined as the minimum number of ancestors (founders or not) necessary to explain the genetic diversity of a population and it indicates the loss of genetic variability caused by the unbalanced use of ancestors for reproduction (Boichard et al. 1997). This parameter was calculated using the expression:

$$f_a = 1/ \sum_{j=1}^{f} p_j^2 \tag{3}$$

where:
p_j – marginal contribution of ancestor j.

The f_e/f_a ratio allows the identification whether the use of breeding animals causes a genetic bottleneck in the population (Biochard et al. 1997). Genetic variability lost to bottleneck effects can be predicted using the $1/2f_a$ equivalence (Lacy 1989; Schurink et al. 2012).

The founder genome equivalent (f_g) is the number of founders that would be expected to produce the same genetic diversity as in the population under study if the founders were equally represented and no loss of alleles occurred (Ballou and Lacy 1995). The f_g was obtained by calculating the inverse of twice the average co-ancestry of the individuals included in our pre-defined reference populations.

The effective population size (N_e) was defined as the number of breeding animals that would lead to the actual increase in inbreeding if they contributed equally to the next generation (Wright 1931). The effective population size was estimated by the individual increase in inbreeding per generation (Gutierrez et al. 2008) and also by the average increase in co-ancestry for all pairs of individuals (Cervantes et al. 2011). Both calculations were done using our reference population animals.

Other estimates of N_e were calculated by computing: (1) the regression coefficient of the individual inbreeding coefficient over dates of birth (Gutierrez et al. 2003); (2) the number of full generations traced; (3) the maximum number generations traced; and (4) the equivalent complete generations (Biochard et al. 1997).

RESULTS

Pedigree completeness

The pedigree completeness for the reference populations per parental generation is illustrated in Figure 1. For the first three generations of ancestors, pedigree knowledge in the BH population
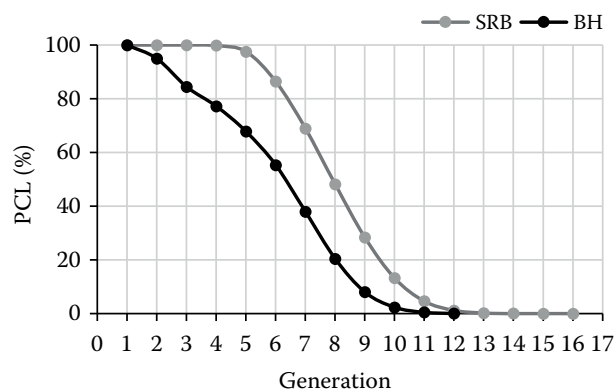

Figure 1. Pedigree completeness level (PCL) for reference populations per generation in Bosnia and Herzegovina (BH) and Serbia (SRB)

was higher than 80%. However, it dropped dramatically after the 6th generation to less than 50%, and after the 9th generation, the percentage of known ancestors was lower than 10%. According to the SRB population, the PCL in the first four generations was 100%. After the 7th generation, it dropped to less than 50%, and after the 10th generation, the percentage of known ancestors was lower than 10%. The number of fully traced generations of Lipizzan horses in the reference populations was 3.59 (BH) and 5.25 (SRB) (Table 2).

We were able to trace the horse origins back to a maximum of 12 (BH) and 16 generations (SRB). The mean maximum number of traced generations varied from 10.40 in BH to 12.84 in SRB. The results of the pedigree depth analysis showed that the average equivalent complete generations for the reference populations were 5.49 (BH) and 14.86 (SRB).

GIs for the reference populations were calculated for all progeny along four different pathways: sire-son, sire-daughter, dam-son and dam-daughter (Table 3). The average age of the parents at birth of the offspring kept for breeding was 10.78 years in BH and 12.12 years in SRB Lipizzan horses. For the BH population of Lipizzan horses, the longest GI was found for the sire-son pathways, 11.73 years. This was followed by sire-daughter (10.99) and dam-daughter (10.51), whereas shorter GIs were calculated for the dam-son pathways (9.98). The longest average GI was found in the SRB Lipizzan horses for the sire-son pathways, 15.32 years. This was followed by sire-daughter (14.35) and dam-son (9.74), whereas a shorter GI was calculated for the dam-daughter pathways (9.69).

Genetic variability parameters

The rates of inbreeding and average relatedness in the Lipizzan horse breed are shown in Table 2. Results showed that the rate of inbreeding and average relatedness increased per generation (Figure 2). The average F value in the reference population was 3.96% and 2.12% in BH and SRB, respectively. The highest calculated individual F value for the reference population was 10.21% in BH and 5.54% in SRB (Table 2). The average relatedness coefficient in the BH reference population was 9.24%, and in SRB, this value was 4.13%. The average F values were lower than the average relatedness in both populations.

Table 2. Measures of pedigree completeness and inbreeding parameters of the Lipizzan horse populations studied

	BH		SRB	
	TP	RP	TP	RP
Equivalent complete generations	3.32	5.49	3.35	14.86
Number of fully traced generations	2.03	3.59	2.31	5.25
Maximum number of traced generations	5.58	10.40	5.54	12.84
Mean inbreeding in all animals (%)	2.14	3.96	1.02	2.12
Mean inbreeding in inbred animals (%)	4.14		3.46	
Maximum computed inbreeding (%)	26.86	10.21	13.40	5.54
Average relatedness (%)	7.99	9.24	2.79	4.13

BH = Bosnia and Herzegovina; RP = reference population; SRB = Serbia; TP = total population

Table 3. Generation intervals calculated for the reference populations of Lipizzan horses

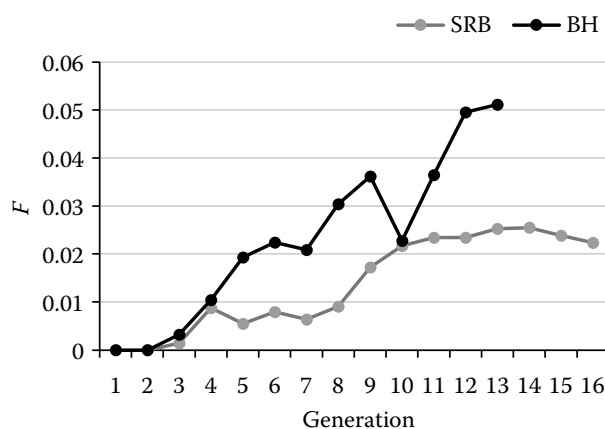|	Number	Interval (years)	SD ± SEM
BH Lipizzan population			
Sire-son	17	11.73	2.7 ± 0.65
Sire-daughter	42	10.99	3.3 ± 0.81
Dam-son	17	9.98	4.8 ± 1.73
Dam-daughter	42	10.51	4.7 ± 1.14
Average	118	10.78	4.0 ± 0.36
SRB Lipizzan population			
Sire-son	9	15.32	5.0 ± 1.67
Sire-daughter	38	14.35	5.3 ± 1.77
Dam-son	9	9.74	6.3 ± 2.11
Dam-daughter	38	9.69	4.9 ± 1.60
Average	94	12.12	5.7 ± 0.59

BH = Bosnia and Herzegovina; SRB = Serbia

Figure 2. Rate of inbreeding per generation (F) in Lipizzan horse populations in Bosnia and Herzegovina (BH) and Serbia (SRB)

A summary of genetic variability results is shown in Table 4. The reference populations of BH and SRB Lipizzan horses were derived from 39 and 230 founders, respectively. The number of ancestors contributing to the reference populations ranged from 29 (BH) to 60 (SRB). In SRB, although the number of founders was 230, the effective number of founders was 79. The number of founders in BH was 39, but the effective number of founders was 21. The number of ancestors in SRB was 60, but the effective number of ancestors was 20. In BH, both the number of ancestors (29) and the number of effective ancestors (12) was lower than in SRB. The f_e was higher than the f_a in both countries. The number of ancestors necessary to explain 100% of the observed genetic variability in the reference populations of BH and SRB Lipizzan horses was 12 and 20, respectively. The number of ancestors explaining 50% of genetic diversity in the BH and SRB reference populations was five and eight, respectively. The f_e/f_a ratio was 1.75 (BH) and 3.95 (SRB). The loss of genetic variability due to bottleneck effects was predicted separately for both populations using the ratio $1/(2f_a)$, and it was 4.14% (BH) and 2.50% (SRB). The f_g was lower than f_e and f_a in both reference populations. In order to determine the amount of genetic variability lost to genetic drift, the f_g/f_e ratio was used. The f_e/f_g ratio in the two populations was 3.48% (BH) and 2.05% (SRB).

Values of N_e were calculated in different ways (Table 4). The effective population size was calculated by the inbreeding coefficient by regression against the maximum, completed and average generation number for the total population. These values in the BH population were found to be 119.41,

Table 4. Genetic variability parameters for the reference populations of the Lipizzan horse breed

Parameters/breed	BH	SRB
Number of animals	150	135
Number of founders	39	230
Numbers of ancestors	29	60
Effective number of founders (f_e)	21	79
Effective numbers of ancestors (f_a)	12	20
Founder genome equivalent (f_g)	6.03	9.77
Number of ancestors explaining 50% of genetic variability	5	8
Effective population size: by increase in inbreeding by maximum generation	119.42*	253.00*
Effective population size: by increase in inbreeding by complete generation	31.68*	94.92*
Effective population size: by increase in inbreeding by equivalent generation	50.54*	133.73*
Effective population size obtained from regression on the birth date	43.67	211.78
Effective population size obtained from log regression on the birth date	43.34	198.15
Effective population size computed via an individual increase in inbreeding	72.97	180.12
Effective population size computed via an individual increase in co-ancestry	30.54	69.06

BH = Bosnia and Herzegovina; SRB = Serbia

*Total population

31.68 and 50.54, respectively. In the SRB population, these values were found to be 253.00, 94.92 and 133.73, respectively. The N_e value calculated from regression on the horse birthdays for reference populations ranged from 43.67 (BH) to 211.78 (SRB).

The trend of the effective population size (N_e) across years is shown in Figure 3. The BH population was characterised by a lower N_e than the SRB Lipizzan horse population. The trend of the N_e in the BH population was more consistent, but the value was always < 100, while on the other hand, in the SRB population, N_e was higher, but with large oscillations across the years.
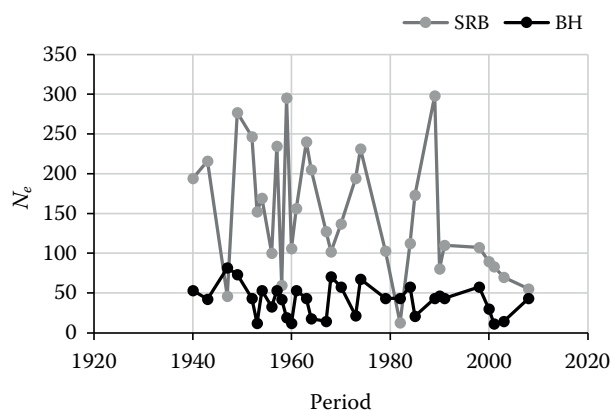

Figure 3. Effective population size (N_e) across decades in Lipizzan horse populations in Bosnia and Herzegovina (BH) and Serbia (SRB)

DISCUSSION

Pedigree completeness

In recent decades, the pedigree analysis has been widely used to analyse population structure, inbreeding and genetic variability, not only in horses, but also in other species. The pedigree analysis provides data that is very useful in evaluating genetic variability, especially for small populations such as Lipizzan horses. The qualities of pedigree information, depth of pedigree and degree of pedigree information quality affect all parameters that describe the probability of gene origin. Zechner et al. (2002) determined the maximum number of traced generations of ancestors in Lipizzan horses from eight European studs (32) and reported the equivalent complete generations of ancestors (15.2). In the population of the Andalusian horse breed, 20 generations of ancestors were reported (Valera et al. 2005), 31 in the Austrian Noriker population (Druml et al. 2009) and 17 in Slovak Lipizzan horses (Pjontek et al. 2012). The average maximum number of traced generations of ancestors for reference populations was 24.56 in the Slovak Lipizzan population (Pjontek et al. 2012), 5.38 in the Noriker of Muran horses (Halo et al. 2018), 8.75 in Hungarian Hucul horses (Somogyvari et al. 2018), and 12.2 in Turkish Arab horses (Duru 2017).

Our study showed similar results for both the BH and SRB Lipizzan populations.

The equivalent complete generation is the best criterion for assessing the quality of pedigree information (Maignel et al. 1996). This parameter in our study for the reference populations ranged from 5.49 (BH) to 14.86 (SRB). The big difference in equivalent complete generations between SRB and BH Lipizzan populations can be explained by the fact that Vucijak stud in BH does not have an electronic database for pedigree data, only a printed studbook, which was used as material for this study. This is the reason why the ancestors from Lipik and Djakovo, founders that were imported to the stud in 1946, were not included in the pedigree. The second reason for greater equivalent complete generations in SRB could also be the fact that the ancestors of the SRB horse population can be traced back to 1900, but in BH, only to 1946. Equivalent complete generations reported in previous research were 1.7 in Brazilian Sport horses (Medeiros et al. 2014), 4.93 in Slovak Sport Ponies (Pjontek et al. 2012), 6.07 in the whole population of Hungarian Hucul horses (Somogyvari et al. 2018), 7.8 in Turkish Arab horses (Duru 2017), 8.26 in Andalusian horses (Valera et al. 2005), 10.25 in Slovak Lipizzan horses (Pjontek et al. 2012) and 15.2 in Lipizzan horses (Zechner et al. 2002). Valera et al. (2005) investigated the population of Andalusian horses and they found that for the first five generations of ancestors, pedigree knowledge was higher than 90%, but dropped to 80% in the 7th generation, and after that it dropped to 33% in the 10th generation. After the 11th parental generation, the percent of known ancestors was lower than 10%. A similar trend was obtained in the present study.

In small populations of horses targeted for breed conservation, which is the case with the Lipizzan horses included in this research, long GIs are advantageous in order to minimize the increase of inbreeding. The increase of GI should lead to better examination of the lower numbers of both stallions and mares (Hamann and Distl 2008). Pjontek et al. (2012) reported average GIs of 9.96 years in Slovak Sport Ponies and 11.61 years in Slovak Lipizzan horses. Halo et al. (2018) found the average GI in the Norik of Muran horses was 10.14 years. In the Andalusian horse population, the average GI was 12.43 years (Valera et al. 2005), and Duru (2017) reported an average GI of 12.4 in the Turkish Arab horses. These findings correspond to the results of our research.

Genetic variability parameters

In horse breeding, compared to other farm animals, the method of inbreeding is most frequently used, especially in small populations. The inbreeding changes the genetic structure of the population, leading to an increase in homozygosity, which can result in a loss of genetic variability, and finally it can affect the fitness of the population. High levels of the coefficient of inbreeding, the average relatedness coefficient, individual increase of inbreeding and low effective population size values indicate the loss of genetic variability and possible phenotypic expression of genetic defects (Pjontek et al. 2012). The obtained average F values (3.96% and 2.12% in BH and SRB, respectively) for the total populations were somewhat lower than the values reported in some previous research, e.g., 3.9% in Shagya Arabian horses, 4% in Slovak Lipizzan horses (Pjontek et al. 2012), 4.1% in Brazilian Sport horses (Mederios et al. 2014), 4.6% in Turkish Arab horses (Duru 2017), 5% in Austrian Noriker horses (Druml et al. 2009) and 8.5% in Andalusian horses (Valera et al. 2005). However, some F values reported in the literature were lower than those obtained in our study, i.e., 2.6% in Slovak Sport Pony horses, 1.51% in Czech Noriker horses (Vostry et al. 2011), 1.3% in Hanoverian horses (Hamann and Distl 2008) and 1.12% in Norik of Muran horses (Halo et al. 2018). Zechner et al. (2002) analysed the average F value for all, 10 and five generations of Lipizzan horses from eight European studs and reported average F values of 10.81, 5.40 and 2.06%, respectively. A similar study was conducted by Valera et al. (2005) for Andalusian horses, and the reported inbreeding coefficient for the total population was 8.48%, for < 10 generations it was 4.00% and for < 5 generations it was 1.45%. In the Norik of Muran horse breed, the inbreeding coefficient was 1.12%, with average relatedness of 1.72% (Halo et al. 2018). The inbreeding coefficient in the Turkish Arab horse was 4.6% (Duru 2017). The obtained inbreeding coefficient in our BH population was similar to the values reported by Zechner et al. (2002) for 10 and five generations. However, the inbreeding coefficient in the SRB Lipizzan population was smaller than the values reported by Zechner et al. (2002). This could be explained by the SRB population including horses not only from the stud farm, but also from private breeding organizations. The average F values were lower than the average relatedness in both our Lipizzan horse populations.

The loss of genetic variability produced by unbalanced contributions of the founders indicates the effective number of founders (f_e). If the founders were equally used in animal breeding, the f_e would equal the actual number of founders. The effective number of ancestors (f_a) evaluates the loss of genetic diversity caused by a bottleneck in the pedigree. The unequal contribution of founders, bottleneck and genetic drift are factors that reduce genetic variability. Effective numbers of founders and ancestors are used to assess the reduction in genetic variability. The effective numbers of founders and ancestors in the present study were lower than the real numbers of founders and ancestors, indicating decreases of genetic variability. The number of ancestors responsible for 50% of the genetic diversity of the reference population in the BH and SRB Lipizzan horse gene pools was five and eight, respectively, similar to the results from previous studies, for example, eight in Turkish Arab horses (Duru 2017), seven in Hungarian Hucul horses (Somogyvari et al. 2018), six, seven and 11 in Slovak Hucul, Shagya Arabian and Lipizzan horses (Pjontek et al. 2012), and five in Mallorquin horses (Alvarez et al. 2010). Halo et al. (2018) reported 19 ancestors were responsible for 50% of the genetic diversity in Norik of Muran horses.

The results in the present study show that the effective number of founders was higher than the number of ancestors, indicating that bottleneck effects have influenced the genetic variability in both populations. The f_e/f_a ratios, 1.75 in BH and 3.85 in SRB, demonstrated that bottleneck effects have influenced these Lipizzan horse populations, although the contributions of the founders throughout the generations was relatively low, especially in the Lipizzan population from SRB. Selection of stallions for mating based on conformation, so using only the best stallions, could explain the obtained results. In the literature, reported f_e/f_a ratios were 1.08 (Alvarez et al. 2010), 1.8 (Duru 2017), 2.4 (Valera et al. 2005), 2.9 in Slovak Lipizzan horses (Pjontek et al. 2012) and up to 6.2 in Norik of Muran horses (Halo et al. 2018). Genetic variability lost due to bottlenecks in the present study was 4.17% (BH) and 2.50% (SRB), and the losses due to genetic drift were 3.48% (BH) and 2.05% (SRB). The f_e/f_g ratios in the present study were lower than reported for Austrian Noriker draught horses (Druml et al. 2009), similar to those in Spanish Sport horses (Bartolome et al. 2011) and in Brazilian Sport horses (Medeiross et al. 2014), and higher than those reported for Mallorquin horses (Alvarez et al. 2010).

To estimate genetic variability for preservation purposes, the effective population size is one of the most commonly used indicators in population genetics. Demographic information, pedigree or molecular data can be used to estimate N_e. To know the influence of temporal mating policies, estimated N_e using regression on inbreeding values based on the birth date can be useful. The N_e obtained from regression on the birth dates was 43.67 (BH) and 211.78 (SRB). Gutierrez and Goyache (2005) reported that when pedigree information is scarce, N_e can be estimated based on maximum, complete and equivalent generations. In the present study, N_e by increased inbreeding according to the maximum generation was 119.42 and 253.00 in BH and SRB, respectively, which can be considered as the upper limits, N_e by increased inbreeding according to complete generations was 31.68 and 94.92 in BH and SRB, respectively, which can considered as the lower limits, and N_e by increased inbreeding according to equivalent generations was 50.54 and 133.73 in BH and SRB, respectively, which can be considered as the real value. The N_e calculated through the increase of inbreeding is dependent on F, the individual inbreeding coefficient, i.e., the higher the inbreeding coefficient, the lower the effective population size. For example, Vostry et al. (2011) reported the effective population size of 36.3 for the Silesian Noriker, 462.3 for the Noriker and 104.4 for the Czech-Moravian Belgian horses; the average inbreeding increase was 1.22%, 0.35% and 1.01%, respectively. These values correspond to the results of the present study, in which the BH Lipizzan horse population had the higher F value and lower N_e, but the SRB population produced converse results. The effective population size in the BH Lipizzan population ranged from 30.54, computed via an individual increase in co-ancestry, to 119.42, computed by an increase in inbreeding according to the maximum generation. The obtained results indicate the BH Lipizzan population has a higher probability of extinction than the population in SRB. Different approaches and methodologies to calculate effective population sizes have led to differences between the results obtained for our two Lipizzan horse populations. The N_e estimated by an increase in inbreeding according to the equivalent generation can be considered as the real value. For the BH population, this value is slightly higher than 50,

which is considered as the limit for sustainability and predicts the possibility of maintaining the breed in the following generations.

CONCLUSION

In the present study, the pedigree information on the Lipizzan horses from BH and SRB was analysed to estimate population structure, inbreeding level and genetic variability. The investigated genetic variability parameters were higher in the SRB Lipizzan population than in the BH population. However, the pedigree completeness of the BH Lipizzan population needs to be improved. For this, an electronic database must be created and the founder horses of the stud from 1946 need to be connected with their ancestors in order to obtain better pedigree data. Genetic drift and bottleneck effects were supported by the f_g, f_a/f_e and f_g/f_e values reported in our study. A trend of increasing inbreeding indicates that in the future this might lead to reductions in genetic variability, especially in BH Lipizzan horses. Higher values of relatedness coefficient in the BH Lipizzan population will be reflected in the improvement of inbreeding coefficients in the next generation. Moreover, annual calculation of the level of relatedness of the foals and their inbreeding coefficients is highly desirable. In that manner, mating could be planned based on these calculations to control rates of inbreeding of the future foals. The exchange or purchase of breeding stallions among other Lipizzan stud farms is also recommended in order to facilitate the population's long-term sustainability. Finally, the analysis of genetic variability using molecular markers is recommended in the future.

The most important contribution of this research is that it indicates the level of genetic variability in the two Lipizzan horse populations, emphasizing the importance of continuous monitoring and implementation of measures for their preservation. Effective population management should be seen as establishing a balance between conservation and genetic improvement, and therefore, the balanced use and introduction of horses in breeding, and limited mating between close relatives are the most important factors in making mating plans that should ensure the survival of the populations in the long term. For this purpose, through national breeding programs, it is necessary to define measures for the preservation of genetic variability, which most often include criteria for the continuous selection of quality breeding stallions and mares with an optimal share of stallion lines and mare families, the implementation of an annual breeding plan (primarily at studs) and monitoring the level of inbreeding.

Conflict of interest

The authors declare no conflict of interest.

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