Theses of Doctoral (PhD) Dissertation

GENETIC ASSESSMENT OF THE HUCUL HORSE BREED

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1 Introduction and goals of the doctoral dissertation

The subject of the research is the Hucul breed, a unique horse breed of European animal breeding. It is unique not only in its appearance but considering also its region of origin. The breed has its origin in the Northeastern region of Wooded Carpathians, the Hucul, with an east-west length of 200 kilometers and a north-south length of 100 kilometers. Before the First World War, the Hucul region (HUZULEI) situated at the border of three countries, namely, Hungary, Bukovina, and Galicia. The impassable source area of the rivers Tisza, Prut, Cseremosz, and Brodina provided genetic isolation to the breed; the genetic structure of the breed was modified only in a negligible way through immigration.

Due to the location of the region Hucul, Hungary has had a bond to the Hucul breed since its existence. Some individuals were also found in the country, at some larger estates. After the peace treaties of the First World War, Hungary bought four Hucul stallions and thirteen Hucul mares from the original Hucul population. These horses, together with some other individuals bought from the larger estates of the country, were accommodated at the military stud farm at Bántapuszta, founded in 1922. Hucul horses bought from Poland were also accommodated at Bántapuszta. Moreover, in 1939, the Hucul breed population of the Stallion Deposit at Turjaremete became the possession of Hungary. Nearly the entire Hungarian Hucul horse population was rescued to Mansbach, Germany from the front of the Second World War. Some individuals were left behind at the estates of the Church. They survived the Second World War. After 1945, Csaba Anghy, former director of the Zoological and Botanical Garden of Budapest, began to conserve the Hucul horse breed with some Hucul horses that were left behind at the estates of the Chruch. Some stallions were also purchased from former Czechoslovakia. In 1992, the reorganization of the civil organization of breeding activities took place and the Pony and Small Horses Breeders' Association was given the right to maintain the Hucul horse breed. Since then, the Association has taken over the breeding of the Hucul horse breed. The priority of the Association was to increase the number of individuals in the population; five mares and two stallions, representing the Ousor and the Goral lines, were bought from Lucina, Romania for breeding purposes. After the war, the families established by Aspiráns and Árvácska expanded to mare families. The genealogical lines were mostly represented by Goral and Ousor. In the middle of the 1990s, a stallion representing the stallion line Polan played a significant role in the breeding of Hungarian Hucul horses. For a long time period, the mentioned three stallion (Goral, Ousor and Polan) and the mentioned two mares (Aspiráns and Árvácska) dominated the Hungarian Hucul horse breed breeding. When the Hungarian Hucul horse population exceeded 200 individuals, the priority of the breeding association became to increase the number of mare families and to provide stallions from each of the seven stallion lines in order to increase genetic diversity.

Today, the number of mares exceeds 300, and there are 37 stallions that represent the stallion lines Hroby, Goral, Prislop, Pietrosu, Ousor, Polan and Gurgul.

After the First World War, the Hucul region was joined to the territory of Romania, Poland, and Czechoslovakia. The Hucul horses of the former stud-farm were shared among Poland, Romania, and Ukraine. Ukraine refused to accept the Hucul horses, and so the Hucul horse population was accommodated in Eichgraben, from where Hucul horses were purchased by breeders from Czechoslovakia and Hungary.

Romania restarted the breeding of the Hucul horse breed and aimed to breed with as much as possible genealogical lines. Besides Hroby and Goral, the genealogical lines Ousor, Pietrosu and Prislop were represented by their stallions. The number of their mares also grew and special attention was drawn to the mating of individuals.

In Poland, the breeding of the Hucul horse breed was restarted after 1924, after the organization of breeding took place outside the stud-farms. In 1937, there were already 450 registered mares. And, a new stallion from the genealogical line Polan was used in the breeding. Thus, the Polish Hucul horse population could be characterized by all the seven genealogical lines. In the 1980s, the number of mares decreased, but, after the 1990s, it started to increase again. Due to successful breeding programs, the Polish Hucul horse breed population is large now. Among the HIF (Hucul International Federation) countries, Poland registers the highest number of Hucul horses.

Czechoslovakia accommodated its Hucul horses, which were bought from the original Lucina stud-farm, in Topolcianky, and one year later, in 1923, in Turjaremete. The Stud-Farm in Topolcianky was dissolved in 1936, however, in 1939, it was founded again. After the dissolution of Czechoslovakia, the breeding of Hucul horses was continued in both the Czech Republic and in Slovakia. In the stud-farm of Topolcianky, only pure-bred Hucul horses were bred. The cross-bred Hucul horses were rejected in the breeding. Also, the Czech Republic aimed to breed with all seven genealogical lines.

In Austria, the breeding of the Hucul horses started in the 1990s. Austria is one of the founding members of the Huzul International Federation.

Pursuant to the decision of the Hucul International Federation, the breeding programs of the countries refuse to accept immigration other than pure-bred Hucul horses. The member countries benefit from the exchange of breeding animals among the countries.

Aim of the research:

- One of the aims of the research was to explore, on the basis of population genetic indicators, how genetic diversity has changed in the Hungarian Hucul horse population.
- Special attention was drawn to explore changes in genetic diversity in the Hungarian Hucul horse population triggered by breeding with breeding animals coming from abroad.
- The other main focus of the research was to assess the degree of genetic differentiation between the populations.

2 Materials and methods

2.1 Materials

Computation was based on an Access database that consists the name, unique identification number (if it exists) and gender of the horse, name of its sire and its dam, its date of birth, its mare family and its genealogical line. The characterization of the Hungarian Hucul horse population was based on the Stud-Book I. and II. (ABAY et al., 2002), Stud-Book I. II. and III. (MIHOK, 2011), and the database of the Pony and Small Horse Breeders' Association. The Slovak Hucul horse population was studied on the basis of the Plemenná Kniha Huculských Koní, Zväzok II 1985-1995 (HUČKO, 1996), Plemenná Kniha Huculských Koní, 1995-2001 (NÁRODNÝ ŽREBČIN s. p. TOPOĽČIANKY, 2001), the Plemenná kniha huculských koní 2002-2007 (HORNÝ et al., 2007), and the most updated data from its breeding association. The assessment of the Czech Hucul horse population was based on the Plemenná kniha huculského koně ČR, I. část, Plemenná klisny, Svazek 1. (JALÍNEK, 2002) and the database of the Czech Hucul Horses. Data about the Romanian Hucul horse population was obtained from the Calul hutul din R. P. R. (RADULESCU, 1957) and the Registru Genialogie Iepe Mame (I-VII. vol.), and data on the broodmares (only broodmares: the dam of the individual, and then her dam, and hers, etc. back to the founder mare) extracted from the studbooks of the stud-farm of Lucsina (I-VII. Volume) (MIHÓK, 2005). This latter source contains data about the mares in Lucina. The database of the Polish Hucul horse population was compiled from the stud-books Linie Genealogiczne polskich koni huculskich (TOMCZYK-WRONA, 2004a), Księga stadna koni rasy huculskiej, (Khc) Tom VIII. (TOMCZYK-WRONA, 2004B), from the online stud-book and an Excel database obtained from the breeding association. The database on the German Hucul horse population was created from an Excel table obtained from the German Breeding Association (Bayerischer Zuchtverband für Kleinpferde und Spezialpferderassen). The database of the Austrian Hucul horse population was based on the book Optimate Huzulendatenbank des Club Hucul (JANSEN and JANSEN, 2009), an Excel table, published in 2013 and documents about the "Huculentag in Stadl-Paura". The genetic diversity of the Hungarian population was explored by using data on the

The genetic diversity of the Hungarian population was explored by using data on the breeding animals found in the stud-book registries in 2002, 2011, 2015 and 2016.

The reference population of 2002 consisted of 85 individuals: 72 mares and 13 stallions that were born between 1979 and 2000.

The reference population of 2011 included 274 individuals, born between 1984 and 2008; there were 251 mares and 23 stallions among them.

In 2015, databases were created for a reference population and an entire population like in the case of the databases of 2002 and 2011. And, there was a database for a reference population that included the breeding animals imported from Poland, and one that excluded them. In the same way, there was a database for the entire population in 2015 that included the breeding animals imported from Poland, and one that excluded them.

The reference population of 2015, including the breeding animals imported from Poland, was based on breeding animals that were found in the stud-book registry in 2015, in total, 318 individuals, among them 307 mares, and 11 stallions. They were born between 1988 and 2015. The entire population of 2015, including the breeding animals imported from Poland, comprised of breeding animals that were born between 1895 and 2015. The database on the entire population of 2015 was created from the founders on and it contained, in total 3,002 individuals: 2,226 mares, and 775 stallions.

The reference population of 2015 that excluded the breeding animals imported from Poland consisted of 165 individuals, 161 mares and 4 stallions. They all were born between 1988 and 2015. The entire population of 2015, excluding the breeding animals imported from Poland, consisted of 2,558 individuals, among them 1,963 mares and 595 stallions. The year of birth was between 1895 and 2015.

Not only the genetic diversity was assessed in these populations, but also the extent, in which the population genetic indicators of the reference population of 2015 and the entire population of 2015 were modified by the breeding animals imported from Poland. Namely, Rapsod (registered as 3139 Polan in the Hungarian Hucul horse breeding in 1996), Parter (registered as 4122 Goral Parter in the Hungarian Hucul horse breeding in 2001), Ploskor (registered as 3883 Goral Ploskor in the Hungarian Hucul horse breeding in 2000), Bryf (registered as 5137 Hroby Bryf in the Hungarian Hucul horse breeding in 2008), Grad (registered as 4677 Hroby Grad in the Hungarian Hucul horse breeding in 2006), Jadeit (registered as 3921 Pietrosu Jadeit in the Hungarian Hucul horse breeding in 2001), and Wilia (registered as Hroby Wilia in the Hungarian Hucul horse breeding in 2008).

There reference population of 2016 included 336 individuals in total, 304 mares, and 32 stallions, born between 1991 and 2015. The entire population of 2016 consisted of 3,026 breeding animals, 2,283 mares and 743 stallions. The year of birth was between 1895 and 2015.

The Eastern-European database consisted of 9,942 individuals in total, 6,490 mares, and 3,452 stallions. The period of analysis lasted from 1895 to 2016 on the following basis (listing is in the order of breeding history; beginning with the country that has the longest breeding history): in Romania between 1895 and 2011, in Poland between 1895 and 2016, in Hungary between 1895 and 2015, in Slovakia between 1895 and 2014, in the Czech Republic between 1895 and 2012, in Austria between 1895 and 2014, and in Germany between 1895 and 2014.

Data of each of the countries were firstly collected in separate databases in Access, then they were aggregated to one common table, according to various queries providing the basis for the study of the entire database. The database of each of the countries was used for the assessment of the relevant Hucul horse population. Thus, the Hungarian database was used for the population genetic assessment of the Hungarian Hucul horse population. Firstly, tables had to be exported from Access to Excel, then data had to be converted into inputs adaptable to the programs used for the calculations. (For instance: 1 = stallion, 2 = mare, 3 = gelding). The common table (later on referred to as Eastern-European database) consists of data on the country of origin of each horse that allowed the computation of genetic distances between the populations. After that, all population genetic indicators were assessed by using the ENDOG (GUTIÉRREZ and GOYACHE, 2005) software. For the graphical representation of the F-statistics and the Nei's standard genetic distance, the RStudio statistical software (RSTUDIO TEAM, 2015) was used.

2.2 Methods

Average relatedness

The average relatedness coefficient shows the likelihood of an allele randomly chosen from the pedigree covering the whole population belonging to an individual.

In order to estimate average relatedness, the algorithm of COLLAU (2002) was used, considering that the ENDOG software uses that, too. It was calculated according to the equation: c' = (1/n) l'A Where c' is row vector where c_i is the average of the coefficients in the row of individual i in the numerator relationship matrix, A, of the dimension n and A is relationship matrix of size $n \times n$.

Inbreeding coefficient

The rate of inbreeding was estimated by the inbreeding coefficient. Its fidelity depends on both the length and the completeness of the pedigree (BOICHARD et al., 1997).

After WRIGHT (1922), the inbreeding coefficient of an individual (X) is:

$$F_{x} = \Sigma \left(\frac{1}{2}\right)^{n+n'+1} (1 + F_{A}),$$

Where "A" is the common ancestor in the chains of origin of the father and mother of the individual X; "n" and "n" are the number of generations between the individual X and the ancestor "A" on the father's side (n) and the mother's side (n'), and F_A is the inbreeding coefficient of the common ancestor. " Σ " means the summary of all common ancestors and ancestry roads in the chains of origin of the individual X's father and mother. Its precision depends on the length and the completeness of the pedigree (BOICHARD et al. 1997).

The inbreeding coefficient in the ENDOG software is computed on the basis of the algorithms of MEUWISSEN and LUO (1992), and VANRADEN (1992) that is - in essence - a calculation of a genetic relationship matrix.

Pedigree completeness

Pedigree completeness expresses in which extent an individual's pedigree is known.

- The *equivalent complete generations* is computed as the sum over all known ancestors of the terms computed as the sum of $(1/2)^n$ where n is the number of generations separating the individual to each known ancestor (MAIGNEL et al. 1996).
- Founders are defined as animals in the pedigree without known sires and dams. The *maximum number of generations traced* the number of generations that can be found between the individual and its most distant ancestor.
- The *number of full generations traced* the furthest generation, in which all ancestors are known.

Effective population sizes

There are multiple ways to estimate the effective population size. An alternative to it is to use the number stallions (N_m) and mares (N_f) that have offspring in the year of the analysis. The algorithm is as follows:

$$N_e = \frac{4N_m N_f}{N_m + N_f} * 0.7 \text{ (FALCONER \'es MACKAY, 1996)}.$$

The other alternative is to use the inbreeding coefficient, as follows:

 $N_e = \frac{1}{2\Delta F_y L}$, in which ΔF_y is the rate of increase, per year, of inbreeding in the population, L is the generation interval.

The estimation of effective population sizes based on individual increase in inbreeding (ΔF_i) was calculated following the approach proposed by GUTIERREZ et al. (2009). The ΔF_i coefficients are simply computed as $\Delta F_i = 1 - \sqrt[t-1]{1 - F_i}$ where F_i is the individual coefficient of inbreeding and t is the 'equivalent complete generations' (MAIGNEL et al. 1996). This estimate of effective population size $(\overline{N_e})$, called 'realized effective size' by CERVANTES et al. (2008), can be computed from ΔF , that can be easily computed by averaging the ΔF is of the n individuals included in a given reference subpopulation, as $\overline{N_e} = \frac{1}{2\overline{\Delta F}}$.

Generation interval

Generation interval shows the average age of parents at the time of their offspring's birth (JAMES, 1977). According to GÁSPÁRDY et al. (2003) generation interval is the time that passes between the birth of a parent and the birth of their breeding offspring, i.e. the average age of the parents – weighted with the number of their further reproducing (effective) offspring – at the birth of their offspring.

Effective number of founders (f_e)

In population genetic calculations, founders are defined as individuals, whose sires and dams are unknown in the pedigree. All individuals can be traced back to the founders that, however, contribute to the gene pool of the population in different proportions. The effective number of founders is defined as the least number of ancestors that would create the same genetic diversity in the reference population, given all ancestors would contribute equally to the gene pool of the reference population. It is given by the software.

ENDOG software uses the following algorithm: $f_e = 1/\sum_{k=1}^f q_k^2$, where q_k is the probability of gene origin of the k ancestor.

Effective number of ancestors (fa)

The effective number of ancestors is defined as the minimum number of ancestors that are responsible for all genetic variability in the reference population. The ancestors are selected on the basis of their contribution to the genetic diversity of the reference population. Since ancestors are not necessarily founders, there can be - due relatedness - overlaps between the genetic contributions of the individuals. In the case of ancestors, their marginal contribution is taken into consideration (their genetic contribution without overlapping).

ENDOG software uses the following algorithm: $f_a = 1/\sum_{k=1}^f q_j^2$, where q_j is the marginal contribution of an ancestor j, which is the genetic contribution made by an ancestor that is not explained by other ancestors chosen before.

The ratio of effective number of ancestors and effective number of founders (f_a/f_e) , the bottleneck effect occurred in the population

The ratio of effective number of ancestors and effective number of founders (f_a/f_e) implies the bottleneck effect suffered. If f_e is higher than f_a , the population suffered a bottleneck effect.

Founder genome equivalent (fg)

The founder genome equivalent (BALLOU and LACY,1995) can be defined as 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.

Ratio of founder genome equivalent and effective number of founders (f_g/f_e) , the genetic drift assessed in the population

Genetic drift is a random change in the frequency of an existing gene variant - allele - in a population from generation to generation. The magnitude of genetic drift depends on the effective population size: the smaller the population is, the higher is the magnitude of genetic drift. Changes in the frequency of the allele occur until a specific allele becomes fixed or it disappears completely from the population.

The ratio of founder genome equivalents and the effective number of founders (f_g/f_e) indicated whether the analysed population was affected by genetic drift (BOICHARD et al.1997). An increase in the (f_g/f_e) ratio indicates a decrease in genetic drift, while a decrease in the (f_g/f_e) ratio indicates an increase in genetic drift.

Genetic distance

Nei's standard genetic distance

Genetic divergence between the stallion lines and the mare families, and between the countries' Hucul horse populations were described by the Nei's standard genetic distance. The Nei's standard genetic distance can be written as

$$D^{ij} = D_{ij} - [(D_{ii} + D_{jj})/2] = [(f_{ii} + f_{jj})/2] - f_{ij}$$
 where f_{ij} is the coancestry coefficient between lines i and j .

Wright's F statistics

F-statistics or fixation indices (F) can be calculated on the basis of the characteristics of heterozygosity. Three F-measures were developed by WRIGHT (1965) in order to measure the correlation between genes in a species. The measures F_{IS}, F_{ST}, F_{IT} are computed by the ENDOG software in the following way:

Fig: variance of allele frequencies between individuals within a subpopulation Fis = $\frac{\tilde{F}-\tilde{f}}{1-\tilde{f}}$

 F_{ST} : variance of allele frequencies between subpopulations $F_{ST} = \frac{\tilde{f} - \bar{f}}{1 - \tilde{f}} = \frac{\overline{D}}{1 - \overline{f}}$

 F_{IT} : variance of allele frequencies in the total population $F_{IT} = \frac{\tilde{F} - \bar{f}}{1 - \bar{f}}$

where \tilde{f} is the average coancestry in the total population, \tilde{F} is the average inbreeding coefficient of the total population, and \bar{f} the average coancestry within subpopulations.

The matrix of genetic distances can graphically be represented by multivariate statistical methods, e.g. with cluster analysis. Cluster analysis is the task of grouping a set of data in such a way that data in the same group are more similar in some senses to each other than those in other groups. These groups are called the clusters. Popular notions of clusters include groups with distances or similarities between cluster members. In order to cluster genetic distances, the divisive type of hierarchical clustering was used. This is a "top-down" approach; all data (the entire data set) start in one cluster, and, on the basis of similarity, the largest cluster is split until every data is separate. The result is a diagram, a dendrogram, representing a tree. At one end of the dendrogram, each data can be found as a separate cluster, while at the other end of the dendrogram, one large cluster can be seen. The analysis starts at the root of the tree.

3 Results

3.1 Variation of genetic diversity in the Hungarian Hucul horse population in the analyzed years (2002, 2011, 2015, 2016)

One of the aims of the research work was to explore how genetic diversity changed in the Hungarian Hucul horse population at different dates. Reference populations of 2002, 2011, 2015 and 2016 were compared to each other. These reference populations provide a true picture of the Hungarian Hucul horse population and of the change in its genetic diversity that occurred from one date to the other. The findings of the research work are going to help with creating breeding strategies for the future. Reference populations are defined as individuals in the Hucul horse Stud-Book I. and II. from 2002, those in the Hucul horse Stud-Book I., II. and III. from 2011, and individuals registered as breeding animals in 2015 and 2016.

Average relatedness

The average relatedness of the reference population in 2002 (Stud-Book I, 2002) was estimated at 11.17%, while that of the reference population in 2011 (Stud-Book II, 2011) was computed to 12.58%. The average relatedness of the reference population in 2015 was estimated at 12.7%. This means that the increase in the indicator, within four years, became slower. The average relatedness of the reference population in 2016 was computed to a lower level (12.67%) that is a favorable fact from the point of view of the breeding. Findings revealed a significant increase (p<0.01) throughout the analyzed years.

Inbreeding coefficient

The inbreeding coefficient of the reference population (Stud-Book I, 2002) was estimated at 6.21%. Unfortunately, the inbreeding coefficient of the Hungarian Hucul horse population showed an increasing tendency over the years. In 2011, the inbreeding coefficient of the reference population (Stud-Book II, 2011) was estimated at 6.36%, and five years later, in 2015, the inbreeding coefficient became even higher (6.8%). The inbreeding coefficient increased (p=0,075) by almost one percent (6.21% to 7.18%) within fifteen years (2002-2016). The increasing tendency of the computed values shows clearly that the mating of related individuals was not avoided. It is going to be an increasing challenge for the breeding associations responsible for conserving the breed to slow down

the rate of inbreeding. The responsibility of breeders in conserving the breed is either inevitable.

Pedigree completeness

Three indicators were computed in order to describe pedigree completeness. Findings are summarized as follows: The *equivalent complete generations* was estimated at 7.13 generations in the reference population of 2002 (Stud-Book I, 2002) and 8.27 generations in the reference population of 2011 (Stud-Book II, 2011). The equivalent complete generations was even higher in the reference population of 2015 (8.72 generations). That of the reference population of 2016 was computed to 8.75 generations, similar to the equivalent complete generations of the reference population of 2002. The increase of the equivalent complete generations can be explained by the growing number of generations of the breeding history. On the other hand, the lower equivalent complete generations of the reference population of 2016 can be explained by the shorter pedigrees of the imported breeding animals.

Similar changes were revealed in the *maximum number of generations* as those in the equivalent complete generations. The maximum number of generations of the reference population of 2002 (Stud-Book I, 2002) was estimated at 17.21 generations, while that of the reference population of 2011 (Stud-Book II, 20011) was computed to 18.54 generations. The maximum number of generations of the reference population of 2015 was even higher, 19.46 generations, while that of the reference population of 2016 decreased slightly, to 19.16 generations.

The *number of full generations traced* of the reference population of 2002 (Stud-Book I, 2002) was estimated at 4.36 generations, that of the reference population of 2011 (Stud-Book II, 20011) was computed to 5.21 generations. The even higher increase in the number of full generations traced in the reference population of 2015 (5.45 generations) can be explained by the generational renewal. The value of the indicator improves, namely, by the generational renewal. In the reference population of 2016, the number of full generations traced was also higher than in the reference population of the previous year. It was estimated at 5.5 generations.

Since pedigrees are known since the last third of the 19th century, the pedigree completeness improves in each new generation. Higher pedigree completeness allows more reliable estimations of each population genetic indicator. Over the years, pedigrees become more complete, indicating the importance of the breeding animals' registry.

Generation interval

The generation interval slightly increased (p<0,01) over the years. In the conservation (breeding) of endangered animal species, an effort is made to increase the generation interval in order to decrease the extent of inbreeding depression per unit of time. The computed generation intervals in the reference populations seem to confirm this effort. The generation interval of the reference population of 2002 (Stud-Book I, 2002) did not reach 100, was estimated at 9.98 years. Ten years later, when the number of broodmares was nearly 200 (Stud-Book II, 2011), the generation interval of the reference population was computed to 10.63 years. In the conservation of endangered (protected) animal species (breeds), generation renewal should not necessarily mean an improvement of the population by selection. As a consequence, generation renewal can be delayed. The generation interval of the reference population of 2015 was estimated at 10.98 years, while that of the reference population of 2016 was computed to 10.99 years. The longer the generation interval, the longer a breeding animal is active in the breeding, which facilitates the conservation of genetic diversity within the interval.

The effective number of founders (f_e) , the effective number of ancestors (f_a) , ratio of the effective number of ancestors and the effective number of founders (f_a/f_e) , the founder genome equivalent (f_g) , and the ratio of the founder genome equivalents and the effective number of founders (f_g/f_e)

The *effective number of founders* in the reference population of 2002 (Stud-Book I, 2002) was 26, while it decreased to 23 as years passed. In comparison with the reference population of 2002 (Stud-Book I, 2002), the number of founders decreased. Fewer founders were responsible for the genetic diversity of the reference populations. The *effective number of ancestors* was computed to 15 in the reference population of 2002 (Stud-Book I, 2002), in that of 2011 (Stud-Book II, 2011) and in that of 2015. It increased to 16 in the reference population of 2016. The increase in the effective number of ancestors in the last year of the analysis reflects the greater role of a new stallion in the breeding, with more offspring in the reference population of 2016. The *ratio of the effective number of ancestors and the effective number of founders* was estimated at 0.5769 in the reference population of 2002 (Stud-Book I, 2002), 0.6521 in the reference population of 2015 and 0.6956 in the reference population of 2016. The ratio revealed that the bottleneck effect occurred in the population. The *founder genome equivalent* was computed to 6.044 in the reference population of 2002

(Stud-Book I, 2002), 5.712 in the reference population of 2011 (Stud-Book II, 2011), and 5.757 and 5.790 in the reference population of 2015 and 2016, respectively. The estimated values of the indicator revealed losses of alleles in the reference populations. The computed genetic drift (*the ratio of the founder genome equivalents and the effective number of founders*) in the reference populations were estimated at the following levels: 0.2324, 0.2483, 0.2503 and 0.2517 in 2002 (Stud-Book I, 2002), 2011 (Stud-Book II, 2011), 2015 and 2016, respectively. It refers to a decrease in genetic diversity.

3.2 The impact of breeding animals imported from Poland on the population genetic characteristics of the Hungarian Hucul horse population on the basis of the reference population of 2015

This chapter aims to evaluate the genetic structure of the Hungarian Hucul horse population, based on pedigree data, using population genetic methods. Particular attention is paid to the effects of the breeding animals imported from Poland to the gene structure of the Hungarian population.

Average relatedness

The average relatedness of the entire Hungarian Hucul horse population was estimated at 10.45%, which, due to the breeding animals imported from Poland, increased (p=0,196) to 10.55%. The degree of average relatedness makes it clear that the mating of related individuals was not avoided. The computed average relatedness of the reference population shows a different tendency. It was estimated at 13.69% without the individuals imported from Poland, and it was computed to 12.7% after including the imported Hucul horses. Thus, the computed value of the indicator decreased (p<0.01).

Inbreeding coefficient

The breeding association responsible for the conservation of the Hucul horse breed chose to increase genetic variation and to slow down the rate of inbreeding in the Hungarian Hucul horse population by immigrating breeding animals from Poland that represented mare families and stallion lines, earlier not present in the Hungarian Hucul horse population. In the research work, the coefficient of inbreeding was computed to both the entire Hucul horse population (3002 individuals) and the reference population (318 individuals). The coefficient of inbreeding of the entire Hucul horse population (from 1895 to 2015) was estimated at 5.65%, while that of the reference population was computed to

6.8%. The findings reveal an unfortunate increase (p<0,01) in the coefficient of inbreeding that drew attention to the increasing tendency of inbreeding depression of the Hungarian Hucul horse population. The coefficient of inbreeding of the Hungarian Hucul horse population, without immigration, was also computed. Findings are not surprising, but they are particularly important. The imported breeding animals modified (p=0.067) the rate of inbreeding of the Hungarian Hucul horse population from 5.83% to 5.65%. The coefficient of inbreeding of the reference population, without immigration, was estimated at 9.06%. Due to the imported Hucul horses from Poland, the coefficient of inbreeding of the reference population reduced (p<0,01) to 6.8%. It is a huge rate of decrease of inbreeding that paves the way for further and safer gen conservation.

Pedigree completeness

The average *equivalent complete generations* of the entire Hungarian Hucul horse population, without immigration, was estimated at 5.62 generations (values were computed between 0 and 10.36), while that, after inclusion of the breeding animals imported from Poland, significantly increased (p<0,01) to 6.06 generations (values were computed between 0 and 10.46). There was no difference (p=0,48) between the average equivalent complete generations of the reference population without and with the breeding animals imported from Poland. The indicator was computed to 8.72 generations (values varied between 4.42 and 10.46).

In both the entire Hungarian Hucul horse population and the reference population, the *number of full generations traced* was 23. The indicator was calculated after the inclusion of the imported individuals in the Hungarian Hucul horse breeding. The indicator value varied between 0 and 23 in the entire Hungarian Hucul horse population, and between 16 and 23 in the reference population. The number of full generations traced of the entire Hungarian Hucul horse population, without immigration, was estimated at 23 generations, too. That of the reference population, without immigration, was computed to 22. After inclusion of the breeding animals imported from Poland in the Hungarian Hucul horse population, ancestors were known in 49%, when they were considered until 20-23 generations. Without the imported breeding animals, ancestors were only known in 38%, when they were considered until 20-23 generations. After inclusion of the breeding animals imported from Poland in the Hungarian Hucul horse population, ancestors were known in 51%, when they were considered until 16, 17, 18 and 19 generations. Without the breeding animals imported from Poland, ancestors were only known in 62%, when they were

considered until 16, 17, 18 and 19 generations. The calculated indicator values can be considered favorable since pedigrees date back to 1895.

Due to the immigration, the *maximum number of generations* significantly increased (p<0.01): that of the entire Hungarian Hucul horse population varied from 13.32 generations to 14.28 generations, while that of the reference population varied from 19.04 generations to 19.46 generations.

Without immigration, the pedigrees of individuals of the reference population, up to generation 4, 5, 6 and 7, was complete in 95%. In the reference population including also the imported animals, pedigrees of individuals were complete in 97%. Up to generation 2 and 3, pedigrees of individuals of the reference population, without immigration, were incomplete only in an extent of 5%. Those of the individuals in the reference population after including the imported animals were incomplete in 3%. Without immigration, the pedigrees of individuals of the entire Hungarian Hucul horse population, up to generation 4, 5, 6 and 7, were complete in 48%. In the entire Hungarian Hucul horse population that included also the imported animals, pedigrees of individuals were complete in 58%. The *number of full generations traced* in the entire Hungarian Hucul horse population, including also the imported animals, increased (p<0,01) from 3.5 generations to 3.77 generations. At the same time, the number of full generations traced in the reference population slightly decreased (p=0,15) from 5.55 generations to 5.45 generations.

Generation interval

Due to the imported breeding animals, the average generation interval decreased slightly (p=0,005) from 11 to 10.98 years. The findings reveal that breeders were happy to benefit from introducing new blood coming from abroad into domestic breeding. The increase in genetic diversity cost the decrease in generation interval.

The longest generation interval was computed to stallions that had broodmare offspring: namely, it was 11.88 years in the population that excluded the imported breeding animals and it was 12.11 years in the population that included the imported breeding animals. The second-longest generation interval was calculated to stallions that had stallion offspring: it was 11.39 years in the population that included the imported breeding animals and it was 11.47 years in the population that excluded the imported breeding animals. The generation interval was computed to a lower value to the broodmares that had stallion offspring: namely, 10.15 years in the population that included the imported breeding animals and 10.35 years in the population that excluded the imported breeding animals. The shortest

generation interval was calculated to broodmares that had broodmare offspring: namely, 9.90 years in the population that included the imported breeding animals and 10.13 years in the population that excluded the imported breeding animals. The generation interval was significantly (p=<0.01) longer in the genealogical line of stallion-offspring than in that of broodmare-offspring by considering the population either with or without the imported breeding animals. In the case of the stallion-offspring line, the generation interval was 11.98 years and 11.80 years, with and without the imported breeding animals, respectively. In the case of the broodmare-offspring line, the generation interval was 9.94 years and 10.17 years, with and without the imported breeding animals, respectively. The difference is explained by the fact that the stallions are subject to stricter selection criteria than the broodmares, for this reason, the generation interval is longer for the stallions than for the broodmares. Apart from the findings detailed above, significant (p<0.01) differences were revealed in both populations (with and without the imported breeding animals). Namely, between the stallions that had stallion offspring and the broodmares that had stallion offspring; between the stallions that had stallion offspring and the broodmares that had broodmare offspring; between the stallions that had broodmare offspring and the broodmares that had stallion offspring; and, between the stallions that had broodmare offspring and the broodmares that had broodmare offspring.

Effective number of founders (f_e) , effective number of ancestors (f_a) , ratio of the number of founders and the effective number of founders (f_a/f_e) , the founder genome equivalent (f_g) , and the ratio of the founder genome equivalent and the effective number of founders (f_g/f_e)

The *number of founders* in the entire Hucul horse population without the breeding animals imported from Poland was 149. It did not change after including the breeding animals imported from Poland in the Hungarian Hucul population. The *effective number of founders* (f_e) in the population before immigration was 21. That in the population after immigration increased to 22. The number of founders in the reference population without the breeding animals imported from Poland increased from 105 to 106. The effective number of founders (f_e) after immigration increased from 19 to 23. The findings suggest that there is a reduction in genetic diversity since the same genetic diversity was produced by 23 individuals in the reference population (19 individuals without the breeding animals imported from Poland) as with 106 individuals (105 without the breeding animals imported from Poland). In the entire Hucul horse population, the same genetic diversity was produced by 22 individuals

(21 individuals without the breeding animals imported from Poland) as with 149 individuals. Due to immigration, the number of ancestors and the effective number of ancestors (f_a) in the entire Hucul horse population increased from 133 to 137 and from 18 to 20, respectively. Due to the breeding animals imported from Poland, an increase was revealed also in the reference population in terms of both the number of ancestors and the effective number of ancestors. The number of ancestors increased from 62 to 75 and the effective number of ancestors (f_a) grew from 14 to 15. The ratio of the effective number of ancestors and the effective number of founders (f_a/f_e) changed from 0.8571 to 0.9090 and from 0.7384 to 0.6521, respectively. The findings reveal that the bottleneck effect occurred in the population. The founder genome equivalent, due to immigration, increased from 4.778 to 5.757 in the reference population, while that decreased from 9,568 to 9,478 in the entire Hungarian Hucul horse population. Also these findings (5,757 and 9,478) confirm a decrease in genetic diversity. The ratio of the founder genome equivalent and the effective number of founders (fg/fe) decreased - due to the breeding animals imported from Poland from 0,4556 to 0,4308 and from 0,2514 to 0,2503 in the entire Hungarian Hucul horse population and the reference population, respectively. The findings suggest genetic drift. The results confirm that, due to the imported individuals, new founders, earlier not present, were coming to the Hungarian Hucul horse population that contributed to the enrichment of its gene pool.

3.3 Analysis of the European Hucul horse population, assessment of genetic distance between the subpopulations (the Hucul horse population of various countries)

Databases consisting of the Hucul horse population of Austria, the Czech Republic, Germany, Hungary, Poland, Romania, and Slovakia were compiled on the basis of sources detailed in the chapter on Materials and Methods. In total, 9,942 data were registered, referred to as Eastern-European database.

Average relatedness

Average relatedness was estimated at 10.77% (with a minimum value of 0.01% and a maximum value of 17.93%) that is not favorable either.

Inbreeding coefficient

The coefficient of inbreeding estimated on the basis of the Eastern-European population was 6.7% (with a minimum value of 0% and a maximum value of 36.02%). It is higher than the expected value of the indicator (1.70%). This regrettable fact is also confirmed by the inbreeding of a high number of Hucul horse individuals (91.01%).

Pedigree completeness

Three indicators were computed to describe pedigree completeness of individuals in the Eastern-European database. The *equivalent complete generations* of the entire Eastern-European Hucul horse population (of course, it is a theoretical Hucul horse population) was estimated at 7.07 (with a minimum value of 0 and a maximum value of 10.48). The *maximum number of full generations* of the entire Eastern-European Hucul horse population was estimated, on average, 16.34 generations (with a minimum value of 0 and a maximum value of 23 generations). The longest pedigrees can be traced back to 23 generations. The *number of full generations traced* was estimated, on average, at 4.16 generations (with a minimum value of 0 and a maximum value of 7 generations). Throughout time, due to the registry of breeding animals, the completeness of pedigrees become longer and more complete. Unfortunately, many registries and pedigrees disappeared or they were completely damaged, especially during the First World War. For this reason, estimations on population genetic indicators provide a more unfavorable picture about the Hucul horse population than it is in reality.

Effective population size (N_e)

The effective population size of the Eastern-European Hucul horse population was computed to 57.78. That is higher than the effective population size that is considered, by the conservation science, to be critical (50). The critical effective population size for a genetically secure population, in which the increasing tendency of the coefficient of inbreeding can be maintained at an acceptable level, is determined by modeling and experience.

Generation interval

Average generation interval was estimated at 10.35 years. The longest generation interval was observed for the stallions having given offspring that became broodmare (11.42 years). The generation interval was somewhat shorter for the stallions that had offspring having

become stallion (11.27 years). Almost the same values were computed for the generation interval in the case of the broodmares having had offspring that became broodmare (9.28 years) or stallion (9.27 years). In the Eastern-European database, the generation interval was calculated significantly (p<0.05) longer for the stallion-offspring combined lines than for the broodmare-offspring combined lines (11.34 years and 9.27 years, respectively). The difference is explained by the fact that the stallions are subject to stricter selection criteria than the broodmares, for this reason, the generation interval is longer for the stallions than for the broodmares. Significant (p<0.01) differences were revealed in the following genealogical lines: between the stallions that had stallion offspring and the broodmares that had stallion offspring; between the stallions that had broodmare offspring and the broodmare offspring; and, between the stallions that had broodmare offspring and the broodmare offspring and the broodmare offspring and the broodmares that had broodmare offspring and the br

The effective number of founders (f_e) , the effective number of ancestors (f_a) , ratio of the effective number of ancestors and the effective number of founders (f_a/f_e) , the founder genome equivalent (f_g) , and the ratio of the founder genome equivalents and the effective number of founders (f_g/f_e)

The number of founders was 219, the effective number of founders (f_e) was 28. The number of ancestors was 222, the effective number of ancestors was 22. The ratio of the effective number of ancestors and the effective number of founders (f_a/f_e) - 0.7857 - suggests that the bottleneck effect occurred in the population over the years. (The effective number of founders was higher than the effective number of ancestors.) The founder genome equivalent (f_g) was estimated at 9.28. The reduction in genetic diversity is also confirmed by the estimated values: the founder genome equivalent was lower than the effective number of founders and the effective number of ancestors. The founder genome equivalents for each of the subpopulations were estimated as follows: for the population of the Czech Republic: 7.63; Slovakia: 7.12; Romania: 6.65; Poland: 6.25; Hungary: 6.14; Austria: 5.88, and Germany: 4.35. The ratio of the founders' genome equivalent and the effective number of founders (f_g/f_e) was computed to 0.331. That confirms a genetic drift that occurred in the Hucul horse population.

Genetic distance

Nei's standard genetic distance

The Nei's standard genetic distance was estimated at 0,023426 for the entire Hucul horse population (Eastern-European database). The computed value converges to zero. It means that there are many identical allele frequencies referring to the low number of heterozygotes.

The genetic distances computed between the subpopulations were also represented graphically (Figure 1). In the color scale, light colors represent shorter distances, while dark colors represent longer distances between the subpopulations. The shortest genetic distance was observed between the Czech and the Slovakian Hucul horse population. It is explained by historical reasons, since the two countries were, in fact, one for nearly 70 years. Thus, the common basis is characteristic to the two populations. Features, which would be characteristic to one or to the other country, could not be revealed. On the other hand, since 1992, the year of foundation of the HIF, the breeding strategy has been the same in each of the founding members.

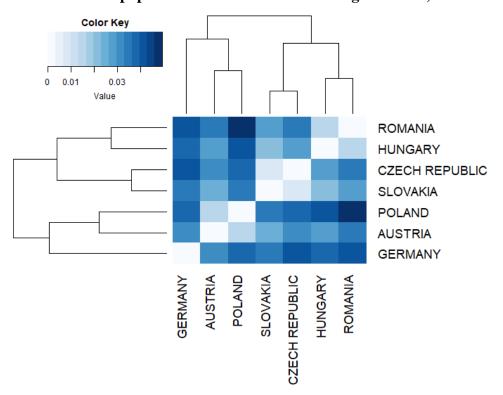
Short genetic distance was revealed between the Austrian and the Polish Hucul horse population, too. Although the Hucul horse population of the Stud-Farm in Lucina was managed by the Imperial and Royal Army and the Hucul horse population of the Stud-Farm in Lucina was rescued from the attacks of the First World War to Kottingbrunn, Austria did not retain any Hucul horses. While Hucul horses have been bred in each of the countries of the Austro-Hungarian Empire for 100 years (except for Austria), the breeding of Hucul horses started only in 1992 in Austria, using breeding animals from Poland. Since the 2000s, Austria acquired, through Hungarian Hucul horse breeders, some individuals also from Lucina, but the tight genetic connection between the Austrian and the Polish Hucul horse population has continued to exist.

There was a very short genetic distance between the Hungarian and the Romanian Hucul horse population. The findings confirm what had already been known by experience. For the 1990s, Hucul horses have been continuously imported from Lucina. Through the breeding animals imported from Romania, the Hungarian Hucul horse breeders' association was able to increase the number of mare families and that of the stallion lines in the Hungarian Hucul horse population. Thus, the genetic diversity of the Hungarian Hucul horse population has been increased, which helps with the conservation of the breed.

The degree of genetic difference was the largest between the Polish and the Romanian Hucul horse population. The genetic difference between the Hucul horse population of any of the countries (except for Austria) and Poland was larger than the genetic difference between any other two subpopulations. Although Poland imported Hucul horses from Lucina, the degree of the genetic difference between the two subpopulations did not become lower. The imported individuals did not have any impact on the large Polish Hucul horse population. Experiences confirm this fact. There is a significant difference between the Polish and the Romanian Hucul horse subpopulations also in the appearance (phenotype) of the horses.

In Germany, there is only a small number of breeders breeding Hucul horses. In Germany, Hucul horses are rather used for different kind of activities. The genetic composition of the German subpopulation confirmed that German breeders imported Hucul horses from Poland, Austria, Slovakia, and Hungary. There was a large genetic difference between the German subpopulation and any other countries not mentioned above. The analysis of the genetic composition of the German subpopulation revealed that it is genetically very close to the subpopulation of Poland, Austria, Slovakia, and Hungary. Large genetic difference was revealed between the German subpopulation and that of any other countries not mentioned above.

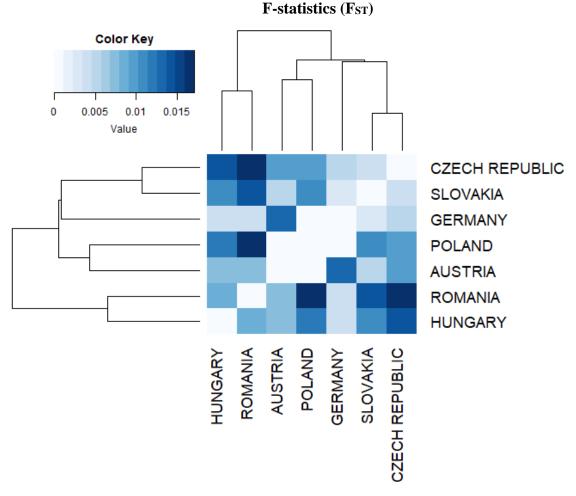
1. Figure: Nei's standard genetic distance between the subpopulations (the populations of each of the breeding countries)



Wright's F-statistics

The genetic difference between the subpopulations and the individuals were also estimated by using Wright's F-statistics. The variance of allele frequencies between the subpopulations (Hucul horse population of the countries) (F_{ST}) was estimated at 0.02476. The computed value converges to zero and it is lower than 0.05 that shows only a slight genetic difference between the subpopulations. The variance of allele frequencies in the total population (F_{IT}) was computed to 0.01393. The positive range of the value refers to minor heterozygosity (supposing also inbreeding) and a low level of genetic isolation. The variance of allele frequencies between individuals within a subpopulation (F_{IS}) was estimated at -0.01110. The negative range of the value refers to the greater proportion of the heterozygotes, which means that the frequency of the heterozygous individuals was larger than it could have been expected on the basis of the Hardy-Weinberg equilibrium.

2. Figure: Variance of allele frequencies between the subpopulations using the



The variance of allele frequencies between the subpopulations using the F-statistics (F_{ST}) was also represented graphically (Figure 2). The largest genetic difference was observed between the subpopulations of Romania and the Czech Republic, and between those of Romania and Poland. There was a large genetic difference between the subpopulations of the Czech Republic and Hungary, Romania and Slovakia, and Germany and Austria. There was a small genetic difference between the subpopulations of Slovakia and the Czech Republic (which is explained by historical reasons), and between Austria and Slovakia. The degree of genetic difference was the smallest in the German Hucul horse population that is explained by the small population size. At the same time, it refers to the small number of generations that was bred in Germany. The computed values (between 0.002 and 0.117 < 0.05) reveal a slight genetic difference within the subpopulation.

Comparison of mare families and stallion lines between the populations of each country

The population to be studied was defined on the basis of the average generation interval (10.35 years). The generation interval was multiplied by 1.5 (15.25 years). This means that the time period of analysis extended from 2000 to 2016. Individuals were analyzed that were born in the specified time period. Differences between the countries are summarized in tables.

Analysis of mare families

There were, in total, 42 mare families that appeared between 2000 and 2016 (see Table 1).

1. TableDistribution of mare families (%) in each of the breeding countries

Mare family	Czech Republic	Austria	Slovakia	Romania	Germany	Hungary	Poland
1 Panca	0.46%	3.51%	0.86%	8.11%		2.90%	0.45%
108 Morza	2.76%						
11 Rotunda	1.38%	0.88%	3.94%	10.81%		3.14%	
11 Zuza	2.30%						
111 Rumina	6.91%						
12 Sarata	0.46%	11.40%		12.16%	55.81%	14.17%	
17 Aglaia	2.76%	1.75%	4.97%	9.46%		1.16%	3.37%
18 Barna	4.61%						
19 Kavka	0.92%		2.74%				
2 Lucina	1.84%	3.51%		4.05%		1.39%	

Mare family	Czech Republic	Austria	Slovakia	Romania	Germany	Hungary	Poland
2 Ritka	0.46%						0.07%
23 Klapta	6.45%						
3 Tatarca						0.35%	
39 Franca	0.46%		0.68%				0.28%
4 Kitca	1.38%	9.65%	0.51%	40.54%	6.98%	14.29%	0.55%
48 Mulica	0.92%		2.23%				
5 Plosca		0.88%		4.05%		1.39%	
70 Sekacka	6.91%		0.34%			0.35%	
71 Róza							0.41%
825 Agla	15.21%	0.88%	17.81%	2.70%	4.65%		0.14%
84 Hurka	1.84%						
84 Polonia	4.15%	20.18%	14.73%		2.33%		18.00%
86 Deremoxa		7.02%	2.57%	8.11%	9.30%	6.16%	
862 Dagmar	17.05%		1.54%		4.65%		
882 Gelnica	3.69%	2.63%	8.73%			8.13%	
90 Machocha		0.88%	13.18%				0.45%
Agatka					4.65%		7.54%
Árvácska			0.34%			26.48%	
Aspiráns		1.75%	0.34%			19.51%	0.03%
Bajkálka		4.39%	0.17%				5.02%
Bukovina	10.60%	0.88%	19.35%		6.98%	0.12%	0.03%
Czeremcha		2.63%					3.30%
Jagoda		0.88%			2.33%		4.54%
Laliszka		11.40%					6.47%
Nakoneczna	0.92%	7.89%	0.51%				4.47%
Reda							2.10%
Szroczka	2.76%	1.75%	4.45%				6.88%
Valuta	2.76%				2.33%		
Wolga		0.88%					10.77%
Wrona		3.51%				0.12%	4.96%
Wydra		0.88%				0.35%	18.38%
Zyrka							1.79%

Romania

In total, nine mare families were represented in the Romanian reference population (by 1 Panca, 11 Rotunda, 12 Sarata, 17 Aglaia, 2 Lucina, 4 Kitca, 5 Plosca, 825 Agla, 86 Deremoxa). With the exception of one mare family (825 Agla), all of them had its origin in Lucina. The mare family 4 Kitca represented 40.54% of the Romanian reference population. The mare families 12 Sarata, 11 Rotunda and 17 Aglaia were significant mare families, too. Their proportion in the reference population was 12.16%, 10.81%, and

9.46%, respectively. The offspring of all four mentioned mare families dominated 72.97% of the reference population.

Poland

In Poland, 23 mare families were bred out of 42 mare families approved by the HIF. Their mare families were as follows: (1 Panca, 17 Aglaia, 2 Ritka, 39 Franca, 4 Kitca, 71 Róza, 825 Agla, 84 Polonia, 90 Machocha, Agatka, Aspiráns, Bajkálka, Bukovina, Czeremcha, Jagoda, Laliszka, Nakoneczna, Reda, Szroczka, Wolga, Wrona, Wydra, Zyrka) The majority of the most widespread mare families had its origin in Poland. The proportion of the mare families Wydra (18.38%) and 84 Polonia (18%) was particularly high in the reference population. It is interesting to mention that the mare families Wydra, Wolga and Wrona were traced back to Hungarian founders. Six mare families dominated 68.04% of the entire reference population. Other than the above mare families, Wolga (10.77%), Agatka (7.54%), Szroczka (6.88%) and Laliszka (6.47%) were the most significant mare families in Poland.

Hungary

In Hungary, 16 mare families were present out of 42 approved by the HIF. These were 1 Panca, 11 Rotunda, 12 Sarata, 17 Aglaia, 2 Lucina, 3 Tatarca, 4 Kitca, 5 Plosca, 70 Sekacka, 86 Deremoxa, 882 Gelnica, Árvácska, Aspiráns, Bukovina, Wrona, Wydra. The number of mare families in Hungary was less than that of other countries, but, it is still significant considering the breeding history of the Hucul horse breed in the country. The Second World War caused serious damages in the Hungarian Hucul population. Only two mare families were created after the War and they had to be approved by the HIF. The breeding association has made a huge effort to increase genetic diversity in the population and to increase the number of mare families. The Hungarian Hucul horse reference population was dominated by two Hungarian mare families: The mare family Árvácska represented 26.48% of the reference population, the mare family Aspiráns represented 19.51% of the reference population. The Romanian mare families 4 Kitca, 12 Sarata and 86 Deremoxa represented a significant proportion of the Hungarian Hucul horse reference population. Their contribution was estimated at the following percentages: 14.29%, 14.17%, and 6.16%, respectively. The Slovakian mare family 882 Gelnica (8.13%) has also a significant proportion in the Hungarian Hucul horse reference population. The specified six mare families dominated, in total, 88.74% of the Hungarian Hucul horse breeding.

Slovakia

Slovakia has several mare families to breed with. Out of 20 mare families, the Slovakian mare families were the largest. Their mare families were known as: 1 Panca, 11 Rotunda, 17 Aglaia, 19 Kavka, 39 Franca, 4 Kitca, 48 Mulica, 70 Sekacka, 825 Agla, 84 Polonia, 86 Deremoxa, 862 Dagmar, 882 Gelnica, 90 Machocha, Árvácska, Aspiráns, Bajkálka, Bukovina, Nakoneczna, Szroczka. The largest Slovakian mare families were Bukovina, 825 Agla, 90 Machocha, and 882 Gelnica, which represented 19,35%, 17,81%, 13,18%, and 8,73%, respectively, of the entire Slovakian Hucul horse reference population. They were followed by the Polish mare family, 84 Polonia, representing 14.73%. The specified five mare families dominated, in total, 73.8% of the Slovakian Hucul horse reference population.

Czech Republic

The highest number of mare families, in total 25, were found in the Czech Republic. These were known as: 1 Panca, 108 Morza, 11 Rotunda, 11 Zuza, 111 Rumina, 12 Sarata, 17 Aglaia, 18 Barna, 19 Kavka, 2 Lucina, 2 Ritka, 23 Klapta, 39 Franca, 4 Kitca, 48 Mulica, 70 Sekacka, 825 Agla, 84 Hurka, 84 Polonia, 862 Dagmar, 882 Gelnica, Bukovina, Nakoneczna, Szroczka, Valuta. The five largest mare families were 862 Dagmar, 825 Agla, Bukovina, 70 Sekacka and 111 Rumnia representing 17.05%, 15.21%, 10.60%, 6.91%, and 6.91%, respectively, of the Czech Hucul horse reference population. They dominated, in total, 56.68% of the reference population. The specified mare families had their origin in Slovakia, which can be explained by the history of the two countries.

Austria

More than half of the number of all existing mare families were found in Austria. In total, 23 mare families were bred. These were known as: 1 Panca, 11 Rotunda, 12 Sarata, 17 Aglaia, 2 Lucina, 4 Kitca, 5 Plosca, 825 Agla, 84 Polonia, 86 Deremoxa, 882 Gelnica, 90 Machocha, Aspiráns, Bajkálka, Bukovina, Czeremcha, Jagoda, Laliszka, Nakoneczna, Szroczka, Wolga, Wrona and Wydra. The mare family 84 Polonia having Polish origin, which can be found in seven Hucul horse breeding countries, was the largest mare family in Austria. It was followed by the Romanian 12 Sarata, the Polish Laliszka, the Polish Nakoneczna and the Romanian Deremoxa representing 11.4%, 11.4%, 7.89%, and 7.02%, respectively, of the reference population. These five mare families dominated, in total, 57.89% of the reference population.

Germany

In Germany, the breeding of Hucul horses did not have a long history. This can explain, why there were only 10 mare families to breed with. These mare families were: 12 Sarata, 4 Kitca, 825 Agla, 84 Polonia, 86 Deremoxa, 862 Dagmar, Agatka, Bukovina, Jagoda, Valuta. It had been supposed in Hungary that German breeders procured breeding animals, particularly, from Poland. The findings of the analysis refuted this perception. The Romanian mare family, 12 Sarata, was responsible for 55.8% of the gene pool of the reference population. It was followed by the Romanian 86 Deremoxa having represented 9.30% of the reference population. There were only a few individuals in Germany that represented the other eight mare families.

The most endangered mare families

2. Table

The proportion (in %) of the mare families of all of the analyzed subpopulations studied between 2000 and 2016 (%)

Mare families	%	Mare families	%	Mare families	%
2 Ritka	0,06	70 Sekacka	0,42	825 Agla	3,04
3 Tatarca	0,06	2 Lucina	0,48	Wrona	3,10
84 Hurka	0,08	862 Dagmar	1,00	17 Aglaia	3,17
11 Zuza	0,10	Zyrka	1,08	Bajkálka	3,17
108 Morza	0,13	1 Panca	1,13	12 Sarata	3,52
Valuta	0,15	Reda	1,27	Aspiráns	3,60
18 Barna	0,21	11 Rotunda	1,29	4 Kitca	3,94
71 Róza	0,25	86 Deremoxa	1,79	Laliszka	4,19
39 Franca	0,27	90 Machocha	1,90	Agatka	4,61
23 Klapta	0,29	Czeremcha	2,06	Árvácska	4,79
111 Rumina	0,31	882 Gelnica	2,75	Szroczka	4,88
48 Mulica	0,31	Jagoda	2,79	Wolga	6,54
5 Plosca	0,33	Bukovina	2,96	Wydra	11,21
19 Kavka	0,38	Nakoneczna	3,00	84 Polonia	13,38

Table 2 summarizes the proportion (in %) of the mare families of all of the analyzed subpopulations studied between 2000 and 2016. The data in the table reveal the most endangered mare families. The most endangered mare families were considered those, 28

whose proportion was below one percent in the studied interval. Namely, these are the following mare families: 2 Ritka, 3 Tatarca, 84 Hurka, 11 Zúza, 108 Morza, Valuta, 18 Barna, 71 Róza, 39 Franca, 23 Klapta, 111 Rumina, 48 Mulica, 5 Plosca, 19 Kavka, 70 Sekacka, and 2 Lucina. At the other end of the spectrum, the most populous mare families can be found. The mare family 84 Polonia represented the highest proportion (13.38%) in the Eastern-European population, followed by Wydra and Wolga. Nowadays, Poland has the most populous Hucul horse population and this tendency is shown clearly in the proportion of the mare families, as well.

The mare family 4 Kitca was found in each of the member countries of the HIF. The mare families 1 Panca, 17 Aglaia, 825 Agla, and Bukovina were bred in six countries. The mare families 108 Morza, 11 Zuza, 111 Rumina, 18 Barna and 84 Hurka were only found in the Czech Republic. The mare family 3 Tatarca was bred only in Hungary, while the mare family Zyrka was bred only in Poland. There were typically 4-6 mare families that represented half of the reference population in each of the member countries of the HIF. It is also inevitable that the offspring of founders of countries having longer breeding history were more significantly present in the breeding.

Analysis of the stallion lines

In the time period of the analysis, expanding from 2000 to 2016, each of the founders of the stallion lines - (Hroby (1895), Goral (1898), Gurgul (1927), Ousor (1929), Polan (1929), Pietrosu (1930) and Prislop (1932) were represented (see Table 3).

3. Table
Distribution of stallion lines (%) in each of the breeding countries

Stallion line	Czech Republic	Austria	Slovakia	Romania	Germany	Hungary	Poland
Goral	40.25%	39.47%	22.82%	4.76%	29.55%	21.38%	20.91%
Gurgul	21.16%	12.28%	22.99%		63.64%	1.03%	23.16%
Hroby	9.54%	7.89%	31.20%	23.81%		36.55%	23.50%
Ousor	24.07%	4.39%	14.12%	25.00%	4.55%	15.63%	10.32%
Pietrosu	1.24%	13.16%		29.76%		7.59%	14.92%
Polan		5.26%				8.85%	3.75%
Prislop	3.73%	17.54%	8.87%	16.67%	2.27%	8.97%	3.44%

Romania

In Romania, there were five stallion lines in the breeding in the time period of the analysis. The stallion line Pietrosu represented the highest proportion of the reference population (29.76%). It was followed by Ousor (25.00%), Hroby (23.81%), and Prislop (16.67%).

Poland

All seven stallion lines were bred in Poland in the time period of the analysis. However, the offspring of only three stallion lines represented, in total, 70% of all stallions (Hroby 23.50%, Gurgul 23.16%, and Goral 20.91%). Within the stallion line Gurgul, there were many offspring (including grand-offspring and grand-offspring) of the stallion Gurgul Jasmin born in 1977. The offspring of the stallion line Pietrosu represented 14.92%, while those of the stallion line Ousor represented 10.32% of the stallions in the reference population.

Hungary

All seven stallion lines were observed in the Hungarian Hucul horse reference population in the time period of the analysis. The stallion line with the highest proportion was Hroby (36.55%). It was followed by the stallion lines Goral (21.38%) and Ousor (15.63%). The offspring of the stallion lines Prislop, Poland and Pietrosu represented similar proportions among the stallions. Their observed proportions were 8.97%, 8.85%, and 7.59%, respectively. There were no significant differences revealed among these stallions. There had been only one stallion offspring of the stallion line Gurgul for a long time.

Slovakia

In Slovakia, the offspring of five founders were found in the breeding in the time period of the analysis. The most significant stallion line was Hroby (31.20%) followed by Gurgul (22.99%) and Goral (22.82%). The stallion lines Ousor and Prislop were represented in a smaller proportion: 14.12% and 8.87%, respectively.

Czech Republic

In the Czech Republic all stallion lines, except for Polan, were found in the breeding in the time period of the analysis. The offspring of the founder of the stallion line Goral were the highest in number, representing 40.25%. The offspring of the founder Ousor and those of the founder Gurgul represented similar proportions in the reference population in the time

period of the analysis (24.07% and 21.16%, respectively). The offspring representing the founder Hroby were present in 9.54% of the reference population. The stallion line Pietrosu was represented in the smallest proportion in the reference population (1.24%).

Austria

In Austria, all stallion lines were represented in the reference population in the time period of the analysis. The most significant stallion line was Goral (having represented nearly 40% of the reference population). It was followed by Gurgul, Prislop and Hroby having been responsible for 17.54%, 12.28%, and 7.89%, respectively, of the reference population in the time period of the analysis. The number of the stallions of the stallion line Ousor was the lowest (having represented only 4.36% of the reference population). But, the proportion of the stallion line Polan was also small, it was estimated at only 5.26%.

Germany

In Germany, there were only four stallion lines observed in the reference population in the time period of the analysis. Moreover, only one stallion line, the Gurgul, was responsible for more than half of the reference population (63.64%). The stallion line Goral was also significant in the breeding in the reference population in the time period of the analysis (29.55%). The role of the stallion lines Ousor and Prislop in the breeding was not significant, however, they contributed to the genetic diversity of the breed. The offspring of these founders represented 4.55% and 2.27% of the reference population.

The most endangered stallion lines

Based on aggregate data, it is the stallion line Prislop that was the least populous in the studied period. His proportion was 4.23%. The second least populous stallion line was that of Pietrosu that represented 7.7%. The stallion line Ousor represented 9.33%. The stallion lines of Gurgul, Goral, and Polan all represented a proportion of above 10%. Namely, 14.08%, 16.77%, and 17.03%, respectively. The most populous stallion line was that of Hroby that represented 30.85%, almost one-third of the entire stallion population.

All seven stallion lines were bred in Austria, Hungary, and Poland. In the Czech Republic, six stallion lines were represented in the breeding. There were no offspring of the founder Polan in the reference population in the time period of the analysis. In Slovakia, the stallion lines Pietrosu and Polan, while in Romania, the stallion lines Gurgul and Polan were not

represented in the breeding in the time period of the analysis. In Germany, the offspring of the founders Goral, Gurgul, Ousor, and Prislop were observed in the reference population. In seven Hucul horse breeding countries, the most popular stallion line was Hroby followed by Goral, Polan, and Gurgul. These latter mentioned founders, however, were represented by half the number of offspring than that of the stallion line Hroby. The least popular stallion lines were Ousor, Pietrosu, and Prislop in the reference population in the time period of the analysis.

4 New scientific results

1. The genetic diversity of the Hungarian Hucul horse population did not change favorably since the average relatedness significantly (p<0.01) increased from 11.17% to 12.67%, and the coefficient of inbreeding changed from 6.21% to 7.18% (p=0.075) between 2002 and 2016. The effective number of founders decreased from 26 to 23, while the effective number of ancestors increased from 15 to 16. The findings confirmed that the bottleneck effect occurred in the population and there were no doubts about a genetic drift either.

It is a favorable fact that the generation interval significantly (p<0.01) increased, from 9.98 years to 10.99 years, that ensures the success of the breed conservation in terms of the period (and not in the terms of the generations).

- 2. Seven Hucul horses were imported from Poland, which had a positive effect on genetic diversity. They significantly (p<0.01) decreased the average relatedness and the coefficient of inbreeding of the reference population. The stallion line Polan had a favorable effect on the Nei's standard genetic difference between the stallion lines and the mare families.
- 3. Research findings revealed that the average relatedness and the coefficient of inbreeding of the individuals in the Eastern-European database were higher than it would have been desirable. The ratio of the effective number of ancestors and the effective number of founders confirmed that the bottleneck effect occurred in the population. The decrease in genetic diversity was confirmed by the founder genome equivalent.
- 4. The effective population size (57.78) seemed to give hope since it was observed at a higher level than would have meant to be critical. Between the Hucul horse populations of the countries, the Nei's genetic distance was short. The F-statistics made it clear that individuals originating from the same breeding center stayed in close genetic relationships even after 100 years. There was a small genetic difference observed between the Hucul horse populations of the countries.

5. There were only 4-6 mare families that dominated more than half of the population. Some mare families were bred only in some countries, while others in each of the countries, where Hucul horses were bred. The most endangered mare families in the analyzed period were 2 Ritka, 3 Tatarca, 84 Hurka, 11 Zúza, 108 Morza, Valuta, 18 Barna, 71 Róza, 39 Franca, 23 Klapta, 111 Rumina, 48 Mulica, 5 Plosca, 19 Kavka, 70 Sekacka, and 2 Lucina. All seven stallion lines of the breed were bred only in Poland, Hungary, and Austria. The least populous stallion lines are that of Prislop and Pietrosu.

5 Practically applicable results

- The completed work will help to develop a breeding program for the Hungarian Hucul population, and to consciously modify the existing one. Due to the research, individuals with high inbreeding coefficient were detected. Conscious breeding with these individuals allows to slow down the growth of inbreeding in the population.
- 2. Estimations on Nei's genetic distances enable to determine the direction of migration. Findings revealed about the mare families and the stallion lines make the exchange of breeding animals among the breeding countries conscious. Thanks to the completed work, the breeding association has clear ideas about the measures that are necessary to take for long-term gene conservation.
- 3. In order to increase genetic diversity, it is necessary to establish as many mare families and stallion lines, as possible. The completed work revealed which breeding countries can support other breeding countries by lending mares and/or stallions, and thus, contribute to populating the rare mare families and/or stallion lines.
- 4. The completed work revealed information about the generational renewal. The findings draw the attention of the breeding organizations to the short generational renewals, and, in the same time, to the potential in longer generational renewals. If breeders succeed in delaying the generational renewal, the rate of loss in genetic diversity per unit of time can be reduced.

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7 List of publications



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