Ann. Anim. Sci., Vol. 19, No. 1 (2019) 49–60 *DOI: 10.2478/aoas-2018-0047*

GENETIC VARIABILITY OF COLD-BLOODED HORSES PARTICIPATING IN GENETIC RESOURCES CONSERVATION PROGRAMS, USING PEDIGREE ANALYSIS*

Grażyna Polak

Department of Horse Breeding, National Research Institute of Animal Production, 00-930 Warsaw, Poland
Corresponding author: grazyna.polak@izoo.krakow.pl

Abstract

Two types of Polish local cold-blooded horses – Sztumski and Sokólski, covered by genetic resources conservation programs, were analyzed for founder's contribution, genetic structure and inbreeding. In both populations, created in the early twentieth century, were used the same stallions, mainly Ardennes, Belgians and Bretons. The aim of the study was to analyze the genetic variability of Sztumski and Sokólski cold-blooded horses, using pedigree information. The reference population contained 2359 horses: 1129 Sztumski and 1230 Sokólski included in conservation programs in 2014. The data set consisted of a total of 12,821 ancestor pedigrees. The results showed that in the analyzed population the number of founders was 1139 for Sztumski and 1118 for Sokólski horses; effective population size and effective number of founders were 688.8; 156.9 and 704.5; 111.4, respectively. The mean coefficient of inbreeding was 1.54 for Sztumski and 1.56 for Sokólski horses. In the gene pool the most important contribution was of Ardennes horses (43.67% in Sztumski and 46.82% in Sokólski horses), followed by Belgian horses (18.32% and 9.3%, respectively). The most important ancestors in both populations were Ardennes sires Rolltan 699 and Gustaw 2807, and German cold-blooded sires Elbgang 1504 and Bär.

Key words: Sztumski and Sokólski cold-blooded horses, local population, inbreeding, founders contribution

In the beginning of the 20th century in Poland, due to an increased demand for massive draught horses, many imported breeding stallions were used. Studies performed in the 1980s (Chrzanowski et al., 1989) showed the existence of around 600 lines originating from Ardennes, Breton and Belgian sires. This fact contributed to creation of the Polish local type of cold-blooded horses: Sztumski and Sokólski (Nozdryn-Płotnicki, 1966; Szuk and Orzeł, 1969; Chachuła, 1962) (Figure 1). The

*Funds of the Ministry of Agriculture and Rural Development, project no. 03-17-14-09.

first studbook for cold-blooded horses, opened in 1964, used the division into local and foreign populations (PZHK, 1964).

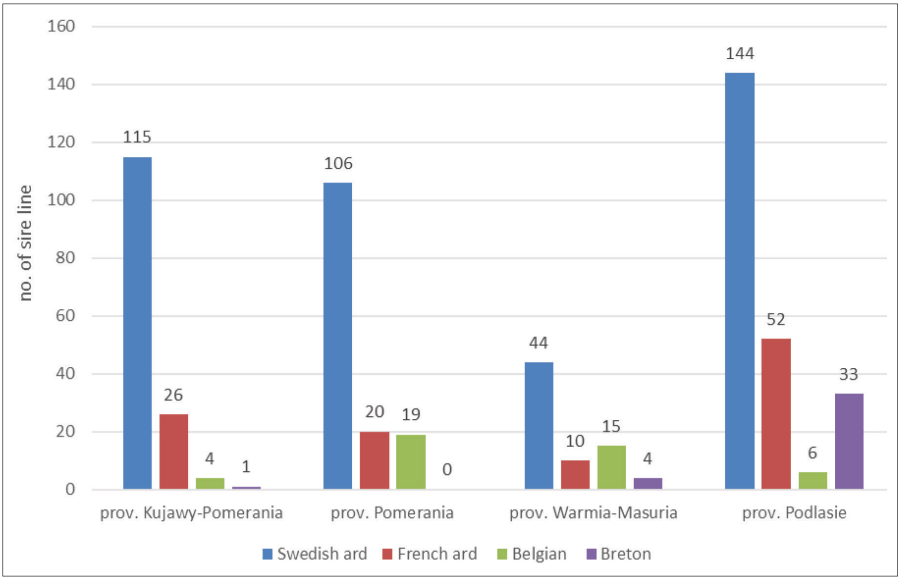

Figure 1. Number of sire lines in Sztumski and Sokólski cold-blooded horses in the middle of the 20th century in north-east regions of Poland (Chrzanowski et al., 1989)

The genetic resources conservation programs for Sztumski and Sokólski cold-blooded horses, introduced in 2008 (IZ PIB, 2015 a, b) were aimed to restore and consolidate specific traits, to preserve the genetic variation and to increase the number of animals in both populations.

Many authors take the position that the main problem in small populations is to prevent the growth of inbreeding rates (FAO, 2013). Langlois (Langlois et al., 2004), who analyzed inbreeding influence, found that an increase by 1% caused reproductive performance to fall by 0.5%, but Janssens (2015) claims that such an increase decreases performance by 1%. Furthermore, the populations run the risk of the bottleneck effect which is associated with increase of inbreeding (Lacy, 1995). Increase of inbreeding was observed in Polish native breeds currently participating in the genetic resources conservation programs, where Polish Konik reached 14.9% (Jezierski, 1993), Hutsul 7.6% (Kwiecińska and Olech, 2008) and in the population of Silesian horses this figure was 1.26%, with fluctuations from 0 to 18.3% (Walkowicz, 2009).

For purebred horses with a closed herd book like Arabian, the coefficient of inbreeding observed between 1987 and 1995 in Poland was 10% (Chmiel et al., 2001). Janssens (2015), considering the whole population of Arabian horses, estimated this coefficient at only 1%. For the Carthusian strain of Spanish Purebred horse, inbreeding coefficient was much higher (13%) due to a small number of individuals (Gutierez et al., 2008). Similarly, Petersen et al. (2013), analyzing 814 horses belonging to 36 breeds, found extremely high inbreeding coefficient in Exmoor pony (56%) and in the Brazilian breed of Mangalarga Paulista horse (24%).

Among the European cold-blooded horses, inbreeding level varied over a wide range: 1.51% for Czech Norico (Vostry et al., 2011), 1.7% for Rhenish (Biedermann et al., 2002), 6.6% for Lithuanian cold-blooded horse (Sveistiene, 2006). A very high coefficient – almost 15% – was found in the 1980s for cold-blooded Jutland horses (Johansen, 1984) and Clydesdale – 26% (Petersen et al., 2013).

The first analysis of the population of Sztumski cold-blooded horses included in the conservation program was carried out in 2013 (Gołębiewska et al., 2013) and showed the average coefficient of inbreeding to be 1.93%.

The objective of this study was to estimate genetic variability and the coefficient of inbreeding for Sztumski and Sokólski cold-blooded horses participating in genetic resources conservation programs in 2014 and to determine the genetic contributions of most important founders, using pedigree information.

Material and methods

The pedigree information on 12,821 ancestors, used for this study was provided by the Polish Horse Breeders Association (cold-blooded horse Stud Book, Volumes I–VI). The 2653 horses involved in the conservation programs in 2014 (1129 Sztumski and 1230 Sokólski) were considered as the reference population.

All the available pedigree information was entered into the Bio_konie horse database. Beginning from these 2359 horses the pedigrees were completed back to those horses, which are considered as founders of two populations. At the end of the procedure the pedigree file contained information about 6534 Sokólski and 6287 Sztumski horses (Table 1). It was assumed that the foreign animals (imported stallions and mares), which were found in the pedigrees, were considered as the founders (f_e) and their ancestors were not included in the database. Also ancestors born in Poland of unknown origin (NN) were considered as founders too.

Table 1. Number of horses in pedigree, number of horses in reference population, number of founders and number of generations of Sztumski and Sokólski cold-blooded horses in 2014

Item	Sztumski horses	Sokólski horses
Total horses	6287	6534
Number of sires	2215	2318
Number of dams	4072	4216
Reference population	1129	1230
mares	917	1017
stallions	212	213
Maximum generation traced	15	17
Number of founders	1139	1118
Pedigree completeness	51.10	45.49

To analyze the genetic variability of the Sztumski and Sokólski cold-blooded horses, covered by the genetic resources conservation programs in 2014, the following parameters were estimated:

Effective population size (N_e) is defined as the size of a hypothetical idealized population that would generate the values of genetic diversity parameters observed for a given population (FAO, 2013) or 'the size of an idealized population which would give rise to the rate of inbreeding, or the rate of change in variance of gene frequencies observed in the population under consideration' (Cervantes et al., 2011). Information such as population size, pedigree information, molecular analysis are important for the correct estimation of the effective population size. Due to the greater availability of data, as well as the economic aspect (the cost of molecular analysis), greater use is made of the Wright method (Wright, 1931), where N_e may be estimated on the basis of the number of females and males:

$$N_e = \frac{4N_m N_f}{N_m + N_f}$$

This model assumes random mating with no selection and no variance in the number of progeny produced by each breeding animal. If selection is present, even selection based on phenotype, this model overestimates N_e. To avoid this mistake, use was made of the formula proposed by Santiago and Caballero (1995), where N_e is decreased by 30%:

$$N_e = \text{original } N_e \times 0{,}7$$

The effective population size can be estimated too using an increase of inbreeding rates (Sölkner et al., 1998):

$$\frac{1}{N_e} = \frac{F_t - F_{t-1}}{1 - F_{e-1}}$$

However, this calculation is conditional on an increase in the average inbreeding rates between successive two generations (reference population and parental generation). Considering the reference population of Sztumski horses and parental population, the inbreeding rate decreased by – 0.197%, This means that estimation based on this method was impossible because of nonsense result (Cervantes et al., 2011).

Effective number of founders was estimated according to Lacy definition (1995), where the number of equally contributing founders is expected to produce the same genetic diversity as in the population under study, where q_i is the genetic contribution of the i^{th} founder to the reference population.

$$f_e = \left[\sum_{i=1}^{N_f} q_i^2 \right]^{-1}$$

Generation interval (GI) is the average age of the parents at birth of their offspring that in turn will produce the next generation of breeding animals (Oldenbroek, 2015). Generation intervals were calculated as the difference between the average age of offspring and parents. GI may differ between male and female parents. for each of the possible paths: sire to sire, sire to dam, dam to sire, dam to dam.

Pedigree completeness (PEC) describes the degree of completeness of pedigree including maternal and paternal lines (MacCluer et al., 1983):

$$PEC_{animal} = 2C_{sire} \times C_{dam} / C_{sire} \times C_{dam}$$

In the analyzed two populations pedigree completeness was assessed for the 5th and last generations.

Gene contribution of foreign population of cold-blooded horses: Ardennes, Belgian, Breton was calculated for reference populations as the proportion of probabilities of gene origins. The calculation was based on the breed to which the founder belonged.

Inbreeding coefficient (F). This is defined as the probability that an individual has two genes identical by descent (Wright, 1931). **F** is the measure of diversity that ranges from 0 (non inbred) to 1 (completely inbred). Average inbreeding depends on the availability of pedigree information, and populations with many generations of genealogy will tend to have the highest average inbreeding (FAO, 2013).

The software R (R Core Team, 2013) was used to calculate generation intervals, effective number of founders, gene contribution and pedigree completeness.

Results

Population size

The population of Sztumski and Sokólski cold-blooded horses under conservation programs was in constant growth since 2008. In the period 2008–2014 populations tripled in size, due to the high attractiveness of the conservation programs, supported by measures in the framework of the Common Agricultural Policy (Figure 2).

Effective population size

Effective population size was 482 for Sztumski and 493 Sokólski horses. Estimation based on the inbreeding growth was impossible due to its variable level, which resulted in the negative value of inbreeding growth.

Effective number of founders

The effective number of founders (f_e) was 156.9 for Sztumski and 111.4 for Sokólski horses.

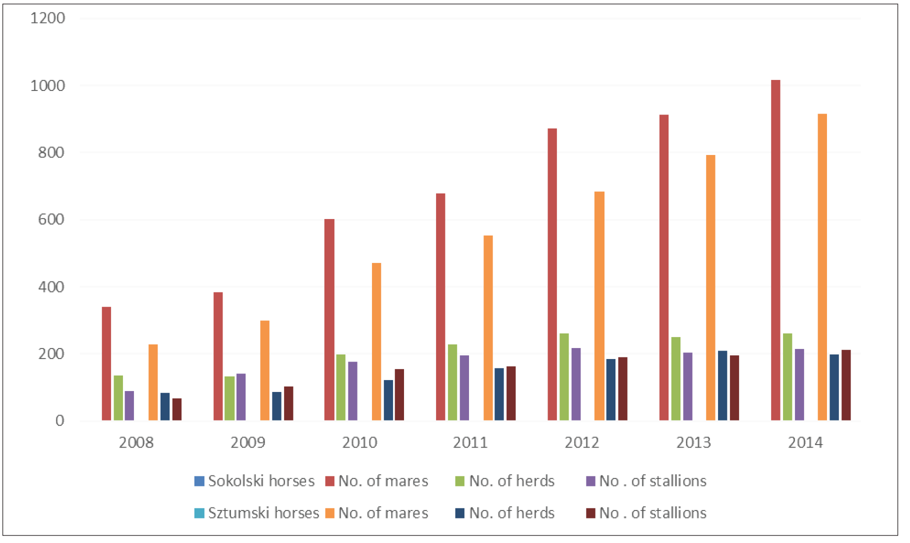

Figure 2. The increase of the Sztumski and Sokólski horses population in 2008–2014

The generation interval

The average generation intervals for both populations was similar: 7.46 years for Sztumski and 7.24 for Sokólski horses (Table 3). The generation intervals were 7.45 years for the pathway Sztumski sire-offspring and 6.8 for Sokólski. The pathway mare-offspring for Sztumski horses was 7.47 and for Sokólski 7.7. The longest pathway was between the Sokólski mares and son (8.75) and the shortest (6.29) between the Sokólski sire and daughter. Among Sztumski horses values of generation intervals were very close on all pathways.

Table 3. Average generation intervals (years) for Sztumski and Sokólski cold-blooded horses in 2014

	Sokólski	Sztumski
Stallions-sons	7.30	7.48
Stallions-daughters	6.29	7.42
Mare-sons	8.75	7.43
Mare-daughters	6.64	7.50
Average	7.24	7.46

Pedigree completeness

In the studied populations the pedigree information was analyzed for all known generations: 16 in Sztumski and 18 for the Sokólski population (Figure 3). From the 4th generation there appeared imported horses, whose pedigrees, according to the assumptions were not entered into the database and these foreign horses were considered as founders. The average pedigree completeness for 5 generations was 98.79 for Sztumski and 98.24 for Sokólski horses. Average pedigree completeness for all known generations was 51.11% in Sztumski horses (16 generations) and 45% for Sokólski horses (18 generations).

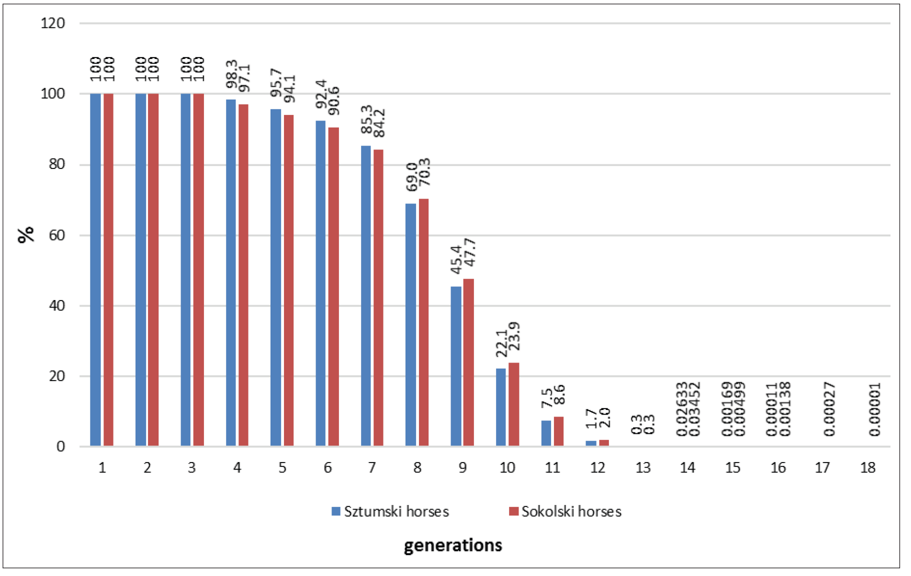

Figure 3. Pedigree completeness of Sztumski and Sokólski horses participating in the genetic resources conservation programs

In 2014 the inbreeding rate of the 1129 Sztumski and 1230 Sokólski cold-blooded horses from the reference population ranged from 0% to 32.7% (Figure 4). The average coefficient of inbreeding for Sztumski horses was 1.54% and for Sokólski 1.56%.

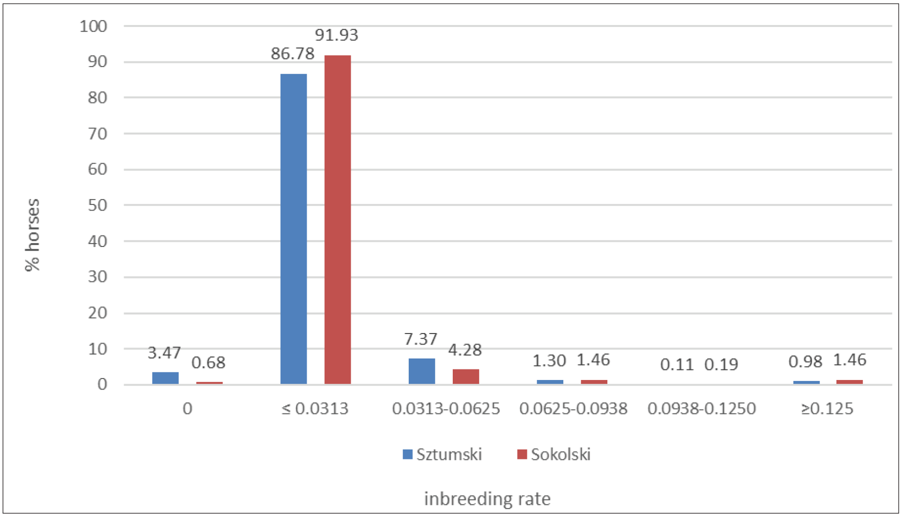

Figure 4. Inbreeding coefficient in the population of Sztumski and Sokólski horses participating in the genetic resources conservation programs in 2014

In the analyzed Sztumski group only 32 horses were not inbred (3.47%). For Sokólski horses this figure was only 7 (0.68%). The main group of Sztumski (803; 71%) and Sokólski (946; 77%) horses accounted for the lowest range of inbreeding, from ≥0 to 3.13%. There were found 24 horses with inbreeding coefficient exceeding 10%: 9 (0.8%) Sztumski and 15 (1.2%) Sokólski. Mostly these horses came from the Pomerania region. The high inbreeding often resulted from the incestuous mating between parents and progeny or from the mating of half-siblings.

Gene contribution and founder line

In both studied populations there was a large share of imported stallions. In general, the stallions imported belonged to the Ardennes, Belgian and Breton breeds (Table 4). There was also a large group of "other cold-blooded" horses, which was formed by Polish horses of mixed origin. Especially high was the contribution of the Ardennes horses, imported from Sweden as well as from France. Their share amounted to 61.9% for Sokólski and 64.2% for Sztumski horses. The second most important founder group were Belgian stallions, 9.3 and 18.3, respectively. There were also many founders of various cold-blooded breeds or local horses of uncertain origin (hybrids), but they did not play an important role as line founders.

Table 4. The ancestors gene contribution in Sztumski and Sokólski population in 2014 (%)

	Sokólski	Sztumski
Ardennes	46.8178	43.6687
Other cold-blooded	19.7712	18.1978
NN	13.4033	11.8248
Belgian	9.2809	18.3243
Sokólski	3.8420	1.0592
Breton	2.7804	0.3179
Döle	1.6020	1.1019
Sztumski	0.7930	3.4598
Nord Swedish	0.7132	0.2870
Mur insulan	0.4416	0.4162
Lidzbarski	0.2001	1.1446
Frederiksborg	0.1138	0.0036
Clydesdale	0.0867	0.0000
Large type	0.0487	0.0278
Konik polski	0.0372	0.0703
Haflinger	0.0334	0.0337
x, don.	0.0281	0.0097
Fiord	0.0067	0.0519
x	0.0000	0.0005
Oldenburg	0.0000	0.0002

The most important founders in the reference population of Sztumski horses were: German cold-blooded sires Elbgang 1504 and Bär; Ardennes sires Rolltan 699 and Geber 5789; Polish cold-blooded Amant 1956 (Table 5). In the Sokólski population the most important were the same Ardennes sires: Rolltan 699 followed by Gustaw 2807, Lingot 4425 and the Breton sire Aiglon 825. Among the line founders the horse that most often appeared in both populations was Rolltan 699, born in Sweden in 1985. His line gave rise to 312 Sokólski and 144 Sztumski mares participating in the genetic resources conservation programs.

Table 5. The 10 most important founders and the number of mares coming from their line and participating in the conservation programs in 2014

Founder	Breed	Year of birth	Sztumski mares	Sokólski mares
ROLLTAN 699	ArdS	1985	144	312
ELBGANG 1504	GC	1981	224	71
GUSTAW 2807	Ard	1939	10	235
BÄR	GC	1977	167	55
GEBER 5789	Ard	1961	139	80
AIGLON 825	Bret	1944	33	153
URAL 5201	ArdS	1956	43	92
AMANT 1956	PC	1944	122	14
LINGOT 4425	Ard	1955	6	130
MOLBER 4821	Ard	1957	98	10

ArdS – Swedish Ardennes; GC – German cold-blooded; Ard – Ardennes; Bret – Breton; PC – Polish cold-blooded.

In both populations there was a large number of Polish founders-mares with no documented origin, which exceeded 40% (Table 6). Compared to the group of stallion-founders there was a very small proportion of imported founders-mares, which did not exceed 2%. This means that almost 98% of the mares were of local, undocumented origin.

Table 6. The Sztumski and Sokólski mares-founders lines breeds (%)

	Sokólski	Sztumski
NN	43.55	43.89
Ardennes	1.05	0.99
Belgian	–	0.11
Lidzbarski	0.13	0.22
Mur insulan	0.13	0.44
Oldenburg	–	0.11
Sokólski	2.63	1.76
Sztumski	2.24	2.09
Large type	0.92	0.44
Thoroughbreds	–	0.11
Other cold-blooded	49.34	49.83

Discussion

In 2014 the coefficient of inbreeding was at one of the lowest levels among Polish local breeds, 1.56% for Sztumski and 1.54% for Sokólski population. In relation to 2008 (Gołębiewska et al., 2013), the level of inbreeding of the Sztumski population decreased by 0.37, from 1.93% to 1.56%. In the Sokólski population there was a higher percentage of horses with low inbreeding coefficient ranging between 0.01 and 2%, because of a large group of mares-founders of unknown origin. As reported by Nozdryn-Płotnicki (1966), after World War II the Sokólski mares often did not have a documented origin, due to the lack of owners' (farmers') interest in breeding documentation. While among Sztumski horses a larger part had inbreeding at the level from 2 to 6%. Therefore it cannot be excluded that the real inbreeding is slightly higher.

The extreme cases of very high inbreeding (32%) resulted from close relatedness between horses. These were individual cases that had no effect on the level of inbreeding in the entire population. It should be also noted that in some cases, for consolidating important characteristics, inbreeding may be a desirable phenomenon (Budzyński et al., 1999; 2000). For the Sztumski and Sokólski horses, such important characteristic is consolidation of the body conformation, which is not uniform at present.

It was observed that all Sztumski and Sokólski cold-blooded horses, participating in the genetic resources conservation programs in 2014 had foreign ancestors in the 4th and further generations. This influenced the results of analysis and the relatively high number of founders: 1139 for Sztumski and 1118 for Sokólski population, with the effective number of founders of 156.9 and 111.4, respectively.

Both populations shared a similar generation interval of 7.46 and 7.24 years, but in the Sokólski population large differences were found between particular pathways. The longest distance (8.75) occurred on the pathway mare-son, which indicates a more severe selection of young colts, or the difficulty of getting good offspring. The situation was opposite on the pathway sire-daughter, where the distance was shortest (6.29), which can indicate that a large number of young stallions was brought into breeding, or there was a quick withdrawal of breeding stallions, without precisely determining the level of their breeding values.

Conclusions

The two local types of Polish cold-blooded horses have become genetically similar as a result of using imported stallions, which contributed to improvement of the conformation traits. The use in both populations of the same, foreign sires had negative effect on the distinctiveness of conformation traits and effectiveness of conservation programs. In the last years the careful selection resulted in a positive tendency for the phenotypic differentiation: the Sztumski horse is visibly more massive, while the Sokólski horse is slightly smaller and drier in keeping with the breed standard.

There is no risk of inbreeding increase, which could contribute to deterioration of the productive capacities and breeding value of the studied populations. Taking this into consideration, it is advisable to reduce the number of stallions, whose ratio to the mares is 1:4 in Sokólski and 1:3 in Sztumski population, and to eliminate conformation defects of the offspring (e.g. excessive lymphatic constitution) and lack of characteristic phenotype.

References

Biederman G., Clar U., Finke A., Bickel M. (2002). Analysis of the population of Rhenish German Draught horses. Züchtungskunde, 74: 237–249.

Budzyński M., Gancarz J. (1999). Characterization of reproductive and biometric performance, taking into account inbreeding of mares in Horse Stud Udórz (in Polish). Ann. UMCS Sec. EE, 17: 191–197.

Budzyński M., Kamieniak J., Budzyńska M., Sapuła M., Sołtys L., Krupa W. (2000). Evaluation of the relationship between reproductive indices and in the thoroughbred mare in Horse Stud Rzeczna (in Polish). Ann. UMCS Sec. EE, 18: 133–139.

Cervantes I., Goyache F., Molina A., Valera M., Gutiérrez J.P. (2011). Estimation of effective population size from the rate of coancestry in pedigreed populations. J. Anim. Breed. Genet., 128: 56–63.

Chachuła J. (1962). Sztumski horse (in Polish). PWRiL. Warszawa.

Chmiel K., Gajewska A., Sobczuk D. (2001). Effect of the rate of inbreeding on evaluation results (scoring) of purebred Arabian horses taking part in shows (in Polish). Pr. Mat. Zoot., 59: 135–140.

Chrzanowski S., Chachuła J., Szelagowska-Wąsik U., Oleksiak S., Wilczak J. (1989). Cold-blooded horses in central-eastern and southern Poland (in Polish). PWN, Warszawa, 97 pp.

FAO (2013). *In vivo* conservation of animal genetic resources. FAO Animal Production and Health Guidelines. No. 14. Rome.

Gołębiewska K., Martyniuk E., Polak G. (2013). Genetic analysis of Sztumski horses enrolled in the genetic resources conservation programme in 2008. Ann. Anim. Sci., 13: 687–700.

Gutierez J.P., Cervantes I., Molina A., Valera M., Goyache F. (2008). Individual increase in inbreeding allows estimating effective sizes from pedigrees. Genet. Sel. Evol., 40: 359–378.

IZ-PIB (2015 a). Consolidated text. Genetic resources conservation programme for Sokolski cold-blood (in Polish). http://www.bioroznorodnosc.izoo.krakow.pl/konie/programy-ochrony.

IZ-PIB (2015 b). Consolidated text. Genetic resources conservation programme for Sztumski cold-blood (in Polish). http://www.bioroznorodnosc.izoo.krakow.pl/konie/programy-ochrony.

Janssens S. (2015). The dangers of inbreeding in Arabian horses. Livestock genetics, ECAHO, Prague. http://www.acafrance.org/userfiles/files/2015_GeneticDiversity_StevenJanssens.pdf

Jezierski T. (1993). Effect of inbreeding on reproductive performance of Polish Konik mares (in Polish). Pr. Mat. Zoot., 43: 109–114.

Johansen E.O. (1984). Inbreeding and relationship within the national Danish draughthorse – the Jutland breed. Proc. 35th Annual Meeting of EAAP, The Hague, Netherlands, 6–9.08.1984. Vol. 2. Summaries. (Paper H3b.4): 2 pp.

Kwiecińska K., Olech W. (2008). The inbreeding influence on Hucul mares reproduction results. Annals of Warsaw University of Life Sciences – SGGW, Anim. Sci., 45: 59–63.

Lacy C.R. (1995). Clarification of genetic terms and their use in the management of captive populations. Zoo Biol., 14: 565–578.

Langlois B., Blouin C. (2004). Statistical analysis of some factors affecting the number of horse births in France. Rep. Nutr. Develop., EDP Sciences, 44: 583–595.

MacCluer J., Boyce B., Dyke L., Weitzkamp D., Pfenning A., Parsons C. (1983). Inbreeding and pedigree structure in Standardbred horses. J. Hered., 74: 394–399.

Nozdryn-Płotnicki J. (1966). The Sokolski Horse (in Polish). PWRiL Warszawa, pp. 75.

Oldenbroek K., van der Waaij L. (2015). Textbook Animal Breeding and Genetics for BSc students. Centre for Genetic Resources The Netherlands and Animal Breeding and Genomics Centre, 2015.

Petersen J.L., Mickelson J.R., Cothran E.G., Andersson L.S., Axelsson J., Bailey E. (2013). Genetic diversity in the modern horse illustrated from genome-wide SNP data. PLoS ONE 8(1): e54997.

PZHK (1964). Stud Book of Cold-blooded and Heavy Horses. I Volume (in Polish). PWRiL, Warszawa, 964 pp.

Santiago E., Caballero A. (1995). Effective population size of population under selection. Genetics, 139: 1013–1030.

Sölkner J., Filipcic L., Hampshire N. (1998). Genetic variability of populations and similarity of subpopulations in Austrian cattle breeds determined by analysis of pedigrees. Anim. Sci., 67: 249–256.

Sveistiene R. (2006) Selection on phenotypic traits of potential founders of the new Lithuanian Heavy Draught horse line. Anim. Husb.: Sci. Art., 48: 3–15.

Szuk K., Orzeł T. (1969). The Sokólski Horse Breeding Centre in the context of breeding centres in the Białostockie Voivodeship (in Polish). Koń Polski, 3: 16–18.

Vostry L., Capkova Z., Pribyl J., Hofmanova B., Vydrova H., Mach K. (2011). Population structure of Czech cold-blooded breeds of horses. Arch. Tierzucht, 54: 1–9.

Walkowicz E. (2009). Inbreeding in the modern population of Silesian horses (in Polish). Zesz. Nauk. UP Wroc., Biol. Hod. Zwierz., 58: 159–170.

Wright S. (1931). Evolution in Mendelian populations. Genetics, 16: 97–159.

Received: 21 II 2018
Accepted: 4 X 2018