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DIFFERENCES AND CHANGES: AN EVALUATION OF THE GENETIC DIVERSITY OF ARABIAN MARES FROM POLISH STATE STUDS BORN BETWEEN 1996 AND 2013*

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Abstract

The goal of our study was to estimate genetic diversity of Arabian horses from Polish state studs which could have changed due to the extensive use of foreign sires in Polish breeding after 1989. The analysis focused on 1,388 Arabian mares born from 1996 to 2013 in the Białka, Janów Podlaski and Michałów studs. Basic parameters of pedigree, mitochondrial DNA and microsatellite analyses were considered in the study. In the pedigree analysis, changes in the gene pool over the years were noted through the considerable increase of contribution of new founders' genes and the considerable decrease of inbreeding level. The microsatellite analysis confirmed progressive changes in the gene pool but, contrary to expectations, only three new microsatellite alleles were detected, and a decreased level of heterozygosity was observed. In turn, mtDNA analysis showed a stable genetic situation in the studs throughout the period analysed. The results of the analyses present a diverse picture of the population, which is clearly divided into the three subpopulations of Białka, Janów Podlaski and Michałów, although the pictures presented differed depending on the analytical methods used. Genetic differences among the studs should be considered a positive feature of the Polish population. In order to preserve the present level of population genetic diversity, changes in breeding policy are needed that aim to stop the loss of genes of the historical ancestors of Polish Arabian horses.

Key words: Arabian horse, genetic diversity, mtDNA, microsatellite, pedigree analysis

The Polish Arabian horse population is a valuable subject of scientific study not only because of its significance to the global Arabian horse industry but also because of its pedigrees dating back to the early nineteenth century, the small magnitude of breeding, with broodmares numbering only in the hundreds, and the closed nature of the population as a result of the state monopoly and the limitation of import of horses from abroad from 1947 to 1989. Consequently, Polish Arabians have been the subject of many studies, including those on the genetic diversity of the population performed based on pedigree data (Głażewska and Jezierski, 2004) or genetic markers such as blood proteins (Kaminski and Tomaszewska-Guszkiewicz, 1978; Żurkowski et al., 1992, 1993, 1998), mtDNA (Bowling et al, 2000; Głażewska et al., 2007; Khanshour and Cothran, 2013), microsatellites (Gralak et al., 1998; Khanshour et al., 2013; Głażewska et al., 2018; Mahmoum et al., 2020), Y chromosome genes (Wallner et al., 2013; Felkel et al., 2019) and SNPs (Cosgrove et al., 2020).

The present study is a continuation of our previous work (Głażewska et al., 2018) on the genetic diversity of state and private Polish Arabian horses used in breeding between 1996 and 2012 which was examined with breeding and microsatellite data. The goal of that study was to evaluate the genetic diversity of the population under the new breeding policy in Poland after 1989 which encouraged the development of private breeding and enabled significant imports of breeding material from other countries. The genetic distinctiveness of Polish Arabian horses in relation to foreign sires and imported mares, progressive changes in the population gene pool, noticeable differences between the state and private horses, and a slight decrease in heterozygosity in horses from the state studs were observed. The decreased heterozygosity was a concern because of the small size of this part of the Polish population, and the situation in particular state studs merits more detailed investigation. This was the starting point for the present analysis of particular studs, i.e. Michałów (established in 1953), Janów Podlaski, further referred to as Janów (established in 1919 and reactivated in 1960) and Białka (established in 1981). The study was conducted using basic parameters of pedigree, mtDNA and microsatellite analyses that were used to observe changes in the gene pools of the studs and to evaluate genetic differences among the studs over the years between 1996 and 2013.

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Material and methods

Research subject

Initially, while establishing the list of horses for analysis, we took into account all Arabian foals born in state studs in 1996–2013, i.e., 1,388 fillies and 1,339 colts. Based on the data from the Polish Arabian Stud Book (PASB) and from the studs, we computed that 49.9% of the fillies but only 3.5% of the colts (n = 47) from this group were used in state breeding in the period up to 2020. Formally, both sexes participate equally in gene transmission to the next generation, but only 47 colts born in the period analyzed were used as sires and remain important for state breeding to this day. Consequently, this meant that at the stage of gene transmission to the next generation, a significant part of parental genes was irretrievably lost, i.e. not only those lost because of unsuccessful matings but also those transferred to 96.5% of the stallions that did not produce offspring. Moreover, because the number of 47 colts is too small to draw statistically significant conclusions (especially since the different scale of the breeding use of sires, leads to a very large disproportion in the number of their offspring), finally, we omitted stallions and focused only on mares as the basic group influencing the gene pool of the next generation.

The list of mares for the analysis was compiled based on data from the Polish Arabian Stud Book (PASB) volumes XIII-5 – XVII-2, which was divided into six three-year periods (D1–D6). In total, 1,388 mares, including 236 mares from Białka, 507 from Janów and 645 from Michałów, were analysed. The mares were sired by 76 Polish (1,025 daughters) and 37 foreign (363 daughters) sires. Of 113 sires, 44 stallions, with a total of 1,037 daughters, were used in more than one stud, including nine stallions used in three studs. In the period analysed, a growing proportion of foreign sires' daughters was noted from 10.8% in the first period to 41.5% (with a maximum of 62.1% in Janów) in the last period. Special attention is paid to the increase in the proportion of representatives of the Egyptian stallion Saklawi I line, from 10.8% in the first period to 51.2% in the last period.

Molecular analysis covered a smaller number of mares (1,290) because of a lack of molecular data for some horses. Detailed information regarding the number of mares is presented in Table 1.

Pedigree analysis

The pedigree database created for the purposes of previous research projects (Głażewska and Jezierski, 2004; Głażewska and Gralak, 2006) was used in the study. This database, which includes data from the early nineteenth century, was updated based on information from the previously mentioned PASB volumes and from 5-generation pedigrees of the mares' foreign fathers published in an online database at http://www.janow.arabians.pl/en/pedigree-database.php. The data collected was used for a simplified pedigree analysis, which included only two parameters: founder contribution to the gene pool and inbreeding coefficient. A founder was defined here as an ancestor with unknown parents, and his contribution (FC) to the gene pool was the expected proportion of the population's gene pool that was descended from this founder (Lacy, 1989). FC was equal to the value of the coefficient of relationship between the founder and its descendants. The calculations of the total founder contributions were carried out for two groups of founders: "the old", which were the historical founders of the Polish population present in the pedigrees of Polish mares born before 1970, and "the new", which were the founders present in the pedigrees of foreign horses used in Polish breeding in subsequent years. The Egyptian ancestors of horses imported from the Tersk stud (USSR) in the late 1960s and 1970s were the first founders from this group. The Wright (1922) inbreeding coefficients (F), defined as the probability that two alleles at a randomly chosen locus in an individual are identical by descent, were computed based on all the pedigree information in the database. The calculations were performed using GENES version 11.8 (Lacy, 1998), which is presently available as a part of PMx software (Lacy et al., 2012).

mtDNA analysis

The aim of this part of the study was to assess changes in the frequency of mtDNA haplotypes over time, which could result from different breeding preferences for particular dam lines. Mitochondrial DNA diversity was analysed using the indirect method that is based on the affiliation of mares to dam lines of known haplotypes which were established in a previous study of 458 bp mitochondrial D-loop region and then deposited in GenBank under accession numbers EF437553 – EF437566 (Głażewska et al., 2007). The number of mares representing particular

Table 1. Number of Arabian mares analysed according to a stud and a period

	Stud	Period D1	Period D2	Period D3	Period D4	Period D5	Period D6	Total
		1996–1998	1999–2001	2002–2004	2005–2007	2008–2010	2011–2013	
Pedigree and mtDNA analyses	Białka	30	33	34	53	46	40	236
	Janów	67	75	100	99	79	87	507
	Michałów	70	96	119	126	113	121	645
	Total	167	204	253	278	238	248	1388
Microsatellite analysis	Białka	22	30	33	52	46	38	221
	Janów	59	62	92	96	76	86	471
	Michałów	60	78	104	122	113	121	598
	Total	141	170	229	270	235	245	1290

dam lines in each of the studs was calculated, and then, after assigning typical mtDNA haplotypes to each line, haplotype frequencies were calculated. Generally, with this indirect method, discrepancies between the estimated and real levels of mtDNA diversity appear only when there are errors in pedigrees or new mutations, and the scale of these discrepancies depends on the generation in which these events occur (Zielińska and Głażewska, 2015). In the population studied, pedigree errors of close ancestors were excluded because of the parentage control that has been in place since 1987. For distant ancestors, two errors detected by Głażewska et al. (2007) were taken into account in the analysis.

The genetic structure of populations and the genetic relationship among the studs and periods were visualised using principal coordinate analysis (PCoA) based on the Nei distance matrix which was performed in GenAlex 6.5 (Peakall and Smouse, 2006, 2012).

Microsatellite analysis

The aim of the analysis was to assess changes in the gene pools of the studs that could have resulted from the introduction of new alleles into the Polish gene pool by foreign sires. This part of the study was based on the results of microsatellite marker analysis performed for parentage control according to standard methods. Eleven microsatellite markers (AHT4, ASB2, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, VHL20) were included, i.e. the same set as in our previous publication (Głażewska et al., 2018). Currently, a greater number of markers is used in parentage control than was used initially. However, including new markers in our analysis of mares born in the first years of the analysis was impossible because biological material from them was unavailable. Therefore, our analysis was limited only to the above-mentioned markers.

The following parameters were calculated for particular studs and periods: allele frequency, the average number of alleles per locus (N_a), observed (H_o) and expected (H_e) heterozygosities, and Wright's F_{IS} statistics according to Weir and Cockerham (1984) as a measure of heterozygosity deficiency or excess. The calculations were performed using FSTAT 2.9.3.2 (Goudet, 2002). To describe either increases or decreases in the frequency of particular microsatellite alleles among the periods analysed, a linear trend was assessed using the least-square linear regression model. For statistical purposes, the usual definition of the regression t-value was used (slope/SE_{slope}) with the null hypothesis that the slope of the regression line is equal to zero, and this trend was considered significant if the P-value associated with the t-value was less than the significance level ($P<0.05$). All statistical calculations were performed in Microsoft Excel for Windows.

Similarly to the mtDNA analysis, principal coordinate analysis (PCoA) based on the Nei distance matrix was performed to visualise genetic relations among studs and periods using GenAlex 6.5 (Peakall and Smouse, 2006, 2012).

Results

Pedigree analysis

Pedigree analysis confirmed the conclusions drawn from the observation of Arabian horse breeding in Poland. The founder contribution (FC) analysis indicated an increase in the contributions of new founders to the gene pool at the expense of old founders which undoubtedly resulted from the extensive use of foreign sires of distinct origin (Figure 1).

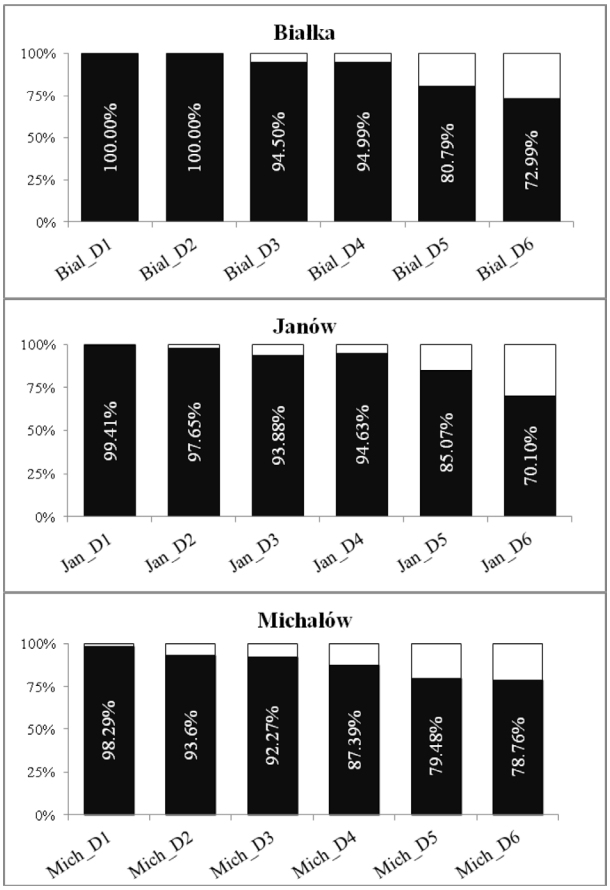


Figure 1. Founder contributions to the gene pools of the Białka (Bial), Janów (Jan) and Michałów (Mich) studs in the periods D1-D6; a group of old founders in black, a group of new founders in white

The FCs of this group of founders increased in particular periods on average from 0.96% in the D1 period to 25.66% in the D6 period, including from 0% to 27.01% in Białka, from 0.59% to 29.90% in Janów and from 1.71% to 21.24% in Michałów. The large increase in the new founder contribution in Janów in the D6 period was notable compared to the previous period. This change resulted not only from the use of more foreign sires in the D6 period, but also from foreign fathers' daughters born in previous periods beginning their breeding careers.

Similar observations arose from inbreeding analysis (Figure 2). The mean inbreeding coefficient of all the mares analysed was 5.46% (from 0% to 16.47%); F = 0% was found in 18 mares and F≥10% in 52 mares, including nine mares with F exceeding 12.5%.

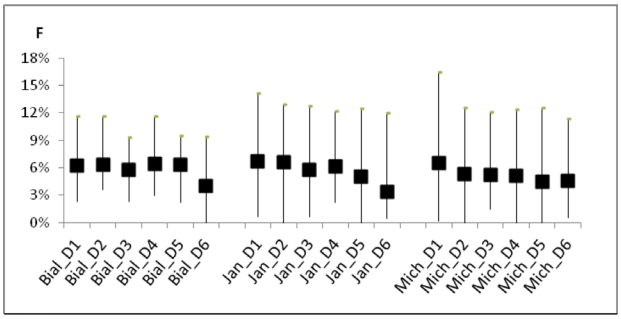


Figure 2. Average, maximum and minimum values of inbreeding coefficient of the mares born in Białka (Bial), Janów (Jan) and Michałów (Mich) in the periods D1-D6

The expected effect of the increasing contribution of new founders in the mares' pedigrees was a decrease in the inbreeding level in subsequent periods. Indeed, the results obtained confirmed this assumption. The largest decrease between the first and the last periods was recorded in Janów from 6.71% to 3.33%. In Białka, the F values decreased from 6.20% to 3.96% and in Michałów from 6.47% to 4.54%. Changes in the inbreeding level followed those in the new founders' contributions to the pool. This was particularly visible in the years 2011–2013 (the D6 period) in Białka and Janów, where a large increase in the contributions of new founders and a large decrease in inbreeding level were noted compared to the years 2008–2010 (the D5 period).

***mtDNA* analysis**

The mares represented 15 dam lines in which 14 mtDNA haplotypes (named from A to O) were previously identified (Głażewska et al., 2007). The stable number of dam lines in the entire population, which resulted from the efforts of breeders to maintain all the lines in breeding, and the great differences in the number of representatives of each line (from 9 to 294 mares) were noteworthy. The different representation of dam lines clearly caused differences in the frequency of mtDNA haplotypes, and among the 14 haplotypes present in the entire population, three haplotypes, each represented by more than 190 mares, dominated. This concerned haplotypes D, F and G which were present in 17.22%, 16.28% and 13.83%, respectively, of the total number of mares included in the analysis (in 47.33% in total). In turn, the rarest haplotypes of L and M were found only in 11 and 9 mares, respectively (1.4% of the total number of mares).

The presence of particular dam lines differed among the studs, and, thus, the presence of mtDNA haplotypes also differed. This was a consequence of the decisions taken when establishing the studs to maintain the genetic distinctness of each one. In none of the studs were all the mtDNA haplotypes found, but the number of haplotypes did not depend on the size of the stud. The 11 haplotypes were noted in the largest (Michałów) and smallest (Białka) studs, while 12 were noted in Janów. Similarly to the entire population, basically a constant number of haplotypes was observed in the particular studs throughout the period analysed along with slight changes in haplotype frequency over time (Figure 3).

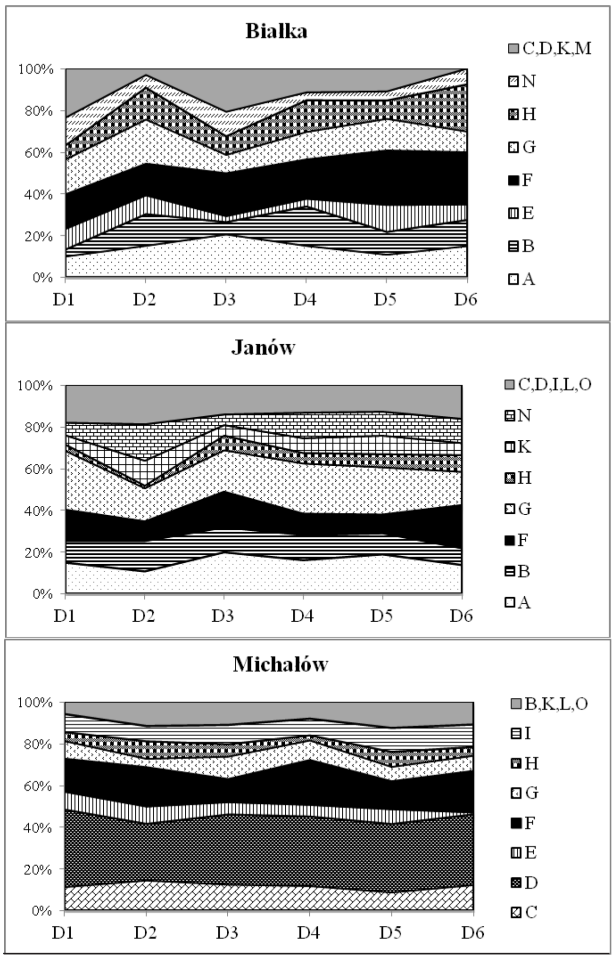


Figure 3. Frequency of mtDNA haplotypes in Białka, Janów and Michałów in the periods D1-D6; haplotypes of a frequency below 5% are shown together

The analysis of all the mares in each of the studs revealed that the dominant haplotypes were distinctly different at Janów and Michałów. In Janów these were haplotypes G (21.10%) and A (15.98%), and in Michałów they were haplotypes D (32.87%) and F (16.74%). In Białka, which was created based on mares from the Janów and Michałów studs, three haplotypes dominated: F (20.76%), which was represented numerously in Michałów, and A and G (in 14.41% and 13.98% of the mares, respectively), which were the most common haplotypes in Janów. These observations were reflected in the results of PCoA analysis which confirmed the genetic distinctiveness of the studs (Figure 4). Michałów occupied the most distinct position on the plot, while the distance between Janów and Białka was much shorter, although the differences between these studs were still clearly visible. PCoA was used also to observe changes in the genetic structure of the studs over time, but it failed to deliver unambiguous conclusions. While we can discuss the progressive changes in the genetic composition of the Białka stud in subsequent periods, no unequivocal

trend was observed in Janów despite notable differences among periods. An equally unclear picture was obtained for Michałów, with the mares from periods D1, D4, D5 and D6 located within close proximity in the centre of the left side of the plot and the mares from periods D2 and D3 (years 1999–2001 and 2002–2004, respectively) occupying distant positions on the top and bottom of the plot.

Microsatellite analysis

Sixty-three alleles (from 61 to 63 in different periods) were present in the entire population with 55 to 58 in Białka, 57 to 61 in Janów and 58 to 61 in Michałów (Table 2). Only three new alleles were recorded that had not previously been observed in the state studs. The first was HTG6/M (in 37 mares), which was introduced into the gene pool by an Egyptian-bred stallion and was already detected during the previous analysis (Głażewska et al., 2018). The next two new alleles were HTG4/O and HMS7/O, found only in two daughters of American sires which were born in Janów and Michałów.

With regard to the genetic distinctiveness of the Polish population, the HTG7/M allele, which was designated as typically Polish in a previous study (Głażewska et al., 2018), was particularly interesting. The allele was found in a total of 45 mares, with the highest proportion in Białka (5.51% of all mares) compared to Janów (3.94%) and Michałów (1.86%). A decreasing trend in its frequency was noted in each of the studs, although the changes were not statistically significant in any of them. Changes in the frequency of this allele in Białka fluctuated and varied in subsequent periods between zero and a few percent, but the decline of its frequency in Janów (between 3.51% in D1 to 0.58% in D6) and Michałów (from 5.00% to 0.41%, respectively) suggested the progressive loss of this allele from the gene pools of these studs.

Generally, changes occurred in the frequency of particular alleles in the studs among periods, although only in some cases was this trend statistically significant (Supplementary Table S1). When the mares were considered together, changes in frequency (decreases or increases) were statistically significant for 16 alleles from eight loci (except HMS2, HMS6 and HTG6). However, when each stud was analysed separately, significant changes in frequency concerned different alleles and loci (Supplementary Table S2). In Białka, significant increases were noted in the frequency of two alleles, HTG4/K and VHL20/M, and a significant decrease was noted in HTG6/J. In Janów at least one allele significantly increased or decreased (15 alleles in total) for most markers except HMS2, HTG6 and HTG7. The highest changes with t-values > 5 were observed in AHT4/I, ASB2/Q, HTG4/K, HTG4/L and HTG10/K. In Michałów significant changes in the frequency of alleles were observed in eight markers (11 alleles), except HMS6, HTG6 and HTG7, and no t-value exceeded 5.

Table 2. Genetic diversity parameters estimated for 11 microsatellite loci in the Białka (BIAL), Janów (JAN) and Michałów (MICH) studs in six periods (D1-D6): sample size (N), average number of alleles (N_a), effective number of alleles (N_e), allelic richness (R_s), observed heterozygosity (Ho), expected heterozygosity (He), F_{IS} index (probability of the hypothesis $F_{IS} = 0$; where ^{x} $P<0.05$, ^{xx} 0.01, ^{xxx} 0.001, respectively). Standard errors are given (SE)

Group	N	N_a (SE)	N_e (SE)	R_s (SE)	H_o (SE)	H_e (SE)	F_{IS}
BIAŁ_D1	22	5.00 (0.47)	3.38 (0.43)	4.92 (0.46)	0.740 (0.052)	0.670 (0.040)	–0.089^{xx}
BIAŁ_D2	30	5.00 (0.43)	3.33 (0.33)	4.74 (0.40)	0.669 (0.045)	0.671 (0.044)	0.006
BIAŁ_D3	33	5.18 (0.40)	3.26 (0.38)	4.90 (0.37)	0.743 (0.043)	0.662 (0.038)	–0.119^{xx}
BIAŁ_D4	52	5.27 (0.47)	3.16 (0.31)	4.74 (0.40)	0.694 (0.052)	0.649 (0.044)	–0.069^{xx}
BIAŁ_D5	46	5.27 (0.47)	3.03 (0.35)	4.74 (0.40)	0.636 (0.049)	0.621 (0.052)	–0.022
BIAŁ_D6	38	5.00 (0.45)	3.28 (0.33)	4.70 (0.41)	0.693 (0.052)	0.659 (0.048)	–0.048
JAN_D1	59	5.18 (0.48)	3.48 (0.33)	4.81 (0.40)	0.711 (0.032)	0.693 (0.030)	–0.022
JAN_D2	62	5.36 (0.41)	3.52 (0.36)	4.88 (0.41)	0.716 (0.046)	0.685 (0.038)	–0.044^{xx}
JAN_D3	92	5.27 (0.47)	3.50 (0.37)	4.91 (0.42)	0.709 (0.041)	0.679 (0.040)	–0.043^{xx}
JAN_D4	96	5.27 (0.47)	3.41 (0.41)	4.79 (0.43)	0.708 (0.048)	0.661 (0.044)	–0.070^{xx}
JAN_D5	76	5.55 (0.37)	3.44 (0.38)	4.92 (0.39)	0.689 (0.054)	0.664 (0.048)	–0.038^{xx}
JAN_D6	86	5.45 (0.45)	3.63 (0.41)	4.99 (0.44)	0.696 (0.048)	0.682 (0.044)	–0.021
MICH_D1	60	5.36 (0.49)	3.52 (0.39)	4.94 (0.46)	0.708 (0.047)	0.681 (0.040)	–0.036
MICH_D2	78	5.55 (0.43)	3.65 (0.41)	5.02 (0.44)	0.711 (0.053)	0.683 (0.048)	–0.036^{xx}
MICH_D3	104	5.27 (0.52)	3.38 (0.37)	4.66 (0.45)	0.692 (0.052)	0.663 (0.045)	–0.041^{xx}
MICH_D4	122	5.45 (0.47)	3.42 (0.36)	4.75 (0.40)	0.709 (0.053)	0.666 (0.046)	–0.062^{xx}
MICH_D5	113	5.45 (0.41)	3.50 (0.37)	4.75 (0.38)	0.709 (0.051)	0.673 (0.046)	–0.052^{xx}
MICH_D6	121	5.45 (0.41)	3.45 (0.39)	4.83 (0.43)	0.707 (0.057)	0.659 (0.053)	–0.069^{xx}

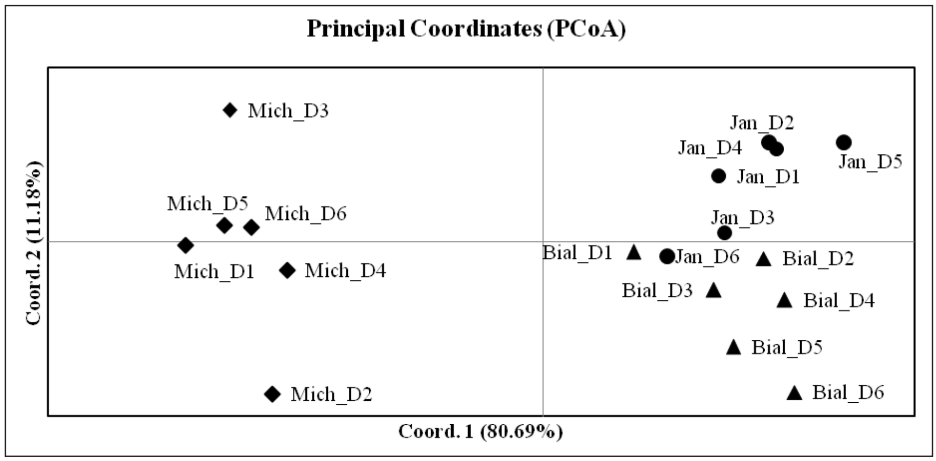


Figure 4. Principal coordinate plot (PCoA) illustrating genetic relations among Białka (Bial), Janów (Jan) and Michałów (Mich) in the periods D1-D6 based on mtDNA data

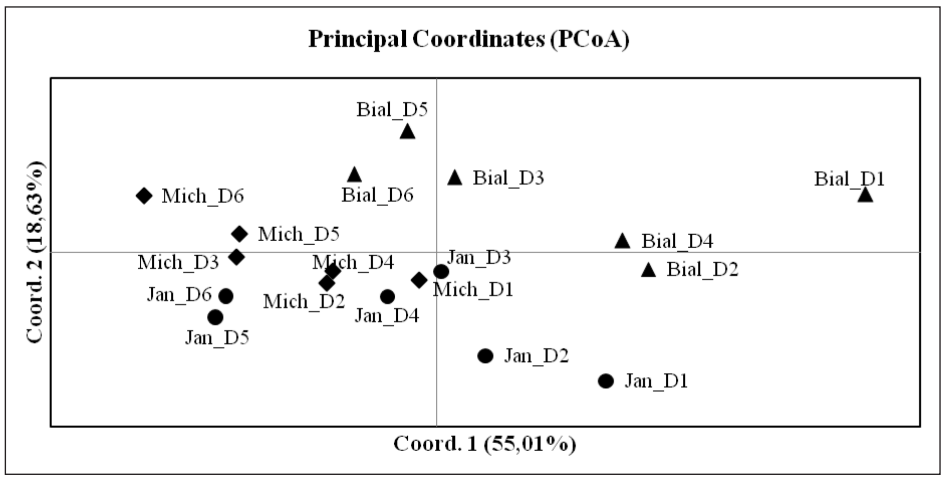


Figure 5. Principal coordinate plot (PCoA) illustrating genetic relations among Białka (Bial), Janów (Jan) and Michałów (Mich) in the periods D1-D6 based on microsatellite data

The results of microsatellite analysis were reflected in PCoA analysis (Figure 5), which showed an increase in the genetic distance between the mares born in subsequent periods and progressive changes in the genetic structure in each of the studs. The genetic distinctiveness of Białka and growing similarity between Janów and Michałów were observed, however, at the same time, progressive changes in the Michałów population have occurred over the years. In the last period, this led to the mares from the Jan_D6 group being located on the plot closer to the Mich_D2, Mich_D3 and Mich_D4 groups, while the mares from the Mich_D6 group moved outside the genetic map of the population.

An important measure of the genetic diversity of the Polish Arabian horses was the observed heterozygosity (H_o) of 0.701 for the entire population. The analysis showed a decrease in the H_o value between the first (D1) and last (D6) periods in each of the studs, although in the case of Michałów this change was negligible, from 0.708 to 0.707, with a minimum value of 0.692 (Table 2). The largest change in H_o value between the first and the last periods was observed in Białka (from 0.740 to 0.693; min = 0.636), while in Janów this value changed from 0.711 to 0.696 (min = 0.689). A similar decreasing trend was noted in expected heterozygosity (H_e) values. Further, except in the Białka_D2 group (years 1999–2001), H_e in all the other groups was lower than H_o. This resulted in negative F_{IS} values which were statistically significant in 12 cases.

Discussion

The results of the analyses present a diverse picture of the population with its dynamically changing gene pool which is clearly divided into the three subpopulations

of Białka, Janów and Michałów. However, the pictures presented differed depending on the analytical methods used.

Pedigree analysis, the first of the methods applied in the present study, revealed significant changes in founder contributions to the population gene pool and the progressive loss of the "old" genes in subsequent periods. The source of the "new" genes were foreign sires the origins of which were separate from those of the Polish broodmares. The greatest changes were observed in Janów, with an increase in the contribution of the new founder's genes of 29.10%. Pedigree analysis also indicated a significant decrease in the average value of the inbreeding coefficient in each of the studs, with the maximum of 6.71% to 3.33% in Janów.

However, microsatellite analysis did not confirm these observations. While many new alleles were expected to be found in the genotypes of foreign sires' daughters, only three new alleles were recorded, and two of them were found only in single mares. The decrease in the level of inbreeding should have been accompanied by an increase in microsatellite heterozygosity in subsequent periods, but no such trend was observed. While only slight fluctuations in H_o values were noted in Michałów, heterozygosity decreased in Białka and Janów. The discrepancy in the results of these two methods can be viewed from different perspectives. First, the concept of "new genes" refers only to their origin, not to the DNA sequence itself, which could have been the same as in the other founders. Second, pedigree analysis could have overestimated population heterozygosity considering the limited number of generations included in the calculations. Hence, the real number of population founders which brought distinct gene variants into the gene pool could have been significantly lower than the number pedigree analysis indicated. This suggestion is supported by the results of studies by Wallner et al. (2013) on Y chromosome genes in 26 male lines of Arabian horses from many populations, including seven lines present in post-war Polish breeding, in which only two haplotypes were found. The third reason for the discrepancies observed could have been the closed studbook and limited size of the breed, which, with strong selection pressure, could have led to the elimination of rare gene variants and the depletion of the gene pool below the level expected based on pedigree data. It is worth noting that in the period analysed the selection criteria in Polish state studs shifted towards breeding horses almost exclusively for show competition, and this led to the clear dominance in breeding of only one male line, i.e. that of the Egyptian stallion Saklawi I. The decreased heterozygosity currently observed in the state studs could have been caused by the lower heterozygosity of foreign sires; however, this issue was not analysed in the present study. Nevertheless, in the previous study of the whole Polish population (Głażewska et al., 2018), when taking into account weighted data, in which the genotype of a given stallion was multiplied by the number of its weaned offspring, a slightly higher H_o level was recorded in foreign sires as compared to Polish sires (0.697 vs. 0.691).

Taking into account the results obtained in the present study, previous analyses of protein markers in Polish Arabians born in 1987–1992 were noteworthy. In these studies, the expected negative correlation was not noted between the values of the pedigree-based inbreeding coefficient and protein marker heterozygosity (H_o), and balancing selection pressure that led to a statistically significant excess of heterozygotes was confirmed (Głażewska et al., 2004; Głażewska and Gralak, 2006). In the present microsatellite analysis, excess of heterozygotes and negative F_{IS} values were also noted, and in 12 of 18 instances the values were statistically significant. The negative F_{IS} value, which distinguished the Polish population, was also noted by Khansour et al. (2013) in studies performed using a larger number (15) of microsatellite markers. Results from analyses performed using different types of markers are also interesting. In studies of protein markers H_o values ranged from 0.289 to 0.346 (Żurkowski et al., 1992, 1993; Głażewska and Gralak, 2006). Much higher H_o values, although similar to each other, were obtained in three microsatellite analyses at 0.71, 0.69 and 0.70, respectively, that were conducted with different numbers of markers, i.e. 17 (Machmoum et al., 2020), 15 (Khansour et al., 2013) and 11 (the current analysis). On the other hand, in an analysis of 56,239 SNPs (Cosgrove et al., 2020), H_o was 0.32, which was similar to the values from protein marker studies.

Changes in the gene pool that were visible in the pedigree parameters, were not confirmed by mtDNA analysis. The number and frequency of haplotypes was relatively stable throughout the population as well as in particular studs. This was the result of the studs using mares they had bred themselves (Głażewska et al., 2018), and the breeders' efforts to keep all the dam lines in breeding. Although there were slight changes in the frequency of haplotypes in subsequent periods, we concluded, based on observations of broodmare breeding careers, that this was mainly due to the different number of offspring and unequal sex distribution of the offspring born in a given dam line in subsequent periods. A good illustration of the above is a broodmare from Białka with a rare L haplotype; she started her breeding career by giving birth to four fillies consecutively and ended with three colts which, because of sex, were not considered in our analysis. Previously, the stability of breeding policy, which was observed in the mtDNA analysis, was seen in the results of the morphological analysis by Kownacki et al. (1992) in which no changes in the most important body measures of Polish Arabians were noted starting from 1923.

However, this stability did not mean that there were no differences among the studs. The mtDNA analysis revealed significant differences that were clearly indicated on the PCoA plot in which Michałów occupied a distinctly separate position in relation to Janów and Białka. An analysis of protein markers (Żurkowski et al., 1998)

also showed the Polish Arabian horses to be a population with a stable gene pool, but one that was simultaneously internally different in the number of alleles and their heterozygosity. This translated into values of genetic distance that clearly differentiated the populations of Janów and Michałów and Białka and Michałów. The results of the present microsatellite analysis contrasted with these data, with the distinctiveness of Białka clearly shown on the PCoA plot and the great similarity of Janów and Michałów. Moreover, the distinctiveness of mares from Białka identified with microsatellite analysis corresponded to the results of the morphological analysis published by Sobczuk and Komosa (2012). In this study, PCA analysis showed clear differences between the horses from the Białka stud compared to those from Janów and especially Michałów.

Changes in the gene pool of particular studs over the 18-year breeding period analysed were the last issue considered in our research. In the pedigree analysis, clear trends were noted as old founders' genes were replaced with those of new founders. PCoA analysis of microsatellite data also indicated there were progressive changes in the genetic structure of each stud with horses from the D1 (years 1996–1998) and D6 (years 2011–2013) periods in the most distant positions. However, this was caused not by new alleles in the gene pool but by changes in allele frequencies in subsequent periods. These changes in frequency were partly statistically significant both in the population as a whole and in each stud separately. Interestingly, this applied to different alleles. One possible explanation was the different origin of the founder mares in particular studs, as reflected in mtDNA analysis, the different selection of sires (61% of sires were used only in one stud), and the different breeding policies in each of the studs which could have resulted in different selection pressure on particular genes and genotypes. This, however, resulted in the impossibility of identifying a specific allele or group of alleles as markers of changes in the gene pool of the whole population in the period analysed. Hence, it is justifiable to conduct further studies on the genetic significance of sires used in Polish breeding in recent decades based on detailed pedigree analysis and the analysis of new genetic markers, including those under selection.

We can summarize our study as follows:

In the period analysed, progressive changes in gene pools happened in each of the studs, while the genetic distinctiveness of particular studs was maintained. This distinctiveness should be considered a positive feature of the population. Taking into account reports of the loss of distinctiveness of local Arabian horse populations in the world, the genetic diversity within the Polish population is of particular importance and breeders should strive to preserve it. This is why changes in breeding policy are required that aim to stop the loss of "old" genes originating from Polish ancestors.

We recommend the following:

• maintain the organizational model of state breeding based on three studs with own mares;

• significantly reduce the use of foreign sires from the Saklawi I male line, which has dominated Polish state breeding in recent years;

• increase the number of sires representing historical Polish male lines based on representatives of these lines still living in Poland or abroad.

Conflict of interest statement

All authors declare that there is no conflict of interest associated with this publication.

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