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Evaluation of genetic diversity of subdivided genealogical groups in Lithuanian Trakehner horse population using immunogenetic tools

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Abstract: The objective of this study was to evaluate the inter-breed genetic diversity of Lithuanian Trakehner horses. The current population in Lithuania numbers 1 039 Trakehner horses. The study included the immunogenetic data analysis of 316 blood samples. Pedigree was traced back to 7–11 generations. The horses were assigned to genealogical clusters originating from East Prussian Trakehnen Stud, Thoroughbred and Arab sires. The first two clusters were subdivided into nine horse groups according to the most important ancestors. The evaluation of genetic diversity was based on six blood groups and five protein polymorphism systems. The differences between genealogical groups were confirmed by principal component analysis and applied cluster analysis. According to the allelic frequency, the Trakehner population changed very slightly through a 30-year period, and only one rare (0.013) allele Tf^{RR} (frequent in other Trakehner populations) has been newly found. The analysis of genetic polymorphism systems based on a very low rate of alleles Q^{abc} (0.093) and D^{dkl} (0.046), which are typical of other populations, indicated the exclusivity of the Trakehner population in Lithuania. The average expected heterozygosity by blood protein polymorphism and blood groups was 0.409 and 0.441, respectively. The genetic clustering diverged by observed heterozygosity of blood groups and by pedigree data in Pilger through Egoist, Bay Ronald through Dark Ronald and Dampfross through Hyperion subgroups. The determined distinctiveness of subdivided Trakehner horse groups suggests reconsidering the breeding strategies and conservation programme of Trakehner horses. Breeding and selection of subdivided sire lines could be among the appropriate solutions for the maintenance and extension of the genealogical structure.

Keywords: blood group; genealogy; stud; protein polymorphism

The Trakehner breed is one of the oldest horse breeds in the world (Teegen et al. 2009). Trakehner breeding was aimed at creating riding horses, initially for cavalry (Nolte et al. 2019). By the order of King Friedrich Wilhelm I, the studs located throughout Lithuania Minor (East Prussia) had been consolidated since 1732, and thus the well-known Trakehnen Stud was founded (Tautorat 1982). References to historical data indicate that the Trakehnen Stud could initiate its breeding work with the horses from the estate of Sereitlaukis, which was the biggest East Prussian Stud at the time with 143 mares and 11 sires in 1730. At the beginning of the 20th century, the Stud of Sereitlaukis estate belonged to the Konigliche Litauische Landgestutte Georgenbur (Purvinas et al. 2014).

After World War II, the population of Trakehners shrank from over 25 000 to about 1 500 breeding animals in East Prussia (Nolte et al. 2019). Many horses were taken to Germany, the remai-

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ning ones to Kirov Stud, Rostov region of the USSR (Kamzolov 2002). In 1945, the inventory and recording of horses for breeding purposes were started in Lithuania and in total 38 purebred Trakehner horses were counted. A total of 86 Trakehner horses were recorded in studbooks from 1948 to 1974, when 69 Trakehners were purebred. In Lithuania active raising of Trakehner horses was resumed in 1961, breeding was carried out in almost all state studs, and since 1972 Trakehner breeding has been assigned to Nemunas State Stud. Trakehner horses, mostly from Kirov Stud and various Polish studs, were imported for the refreshing of the Trakehner population (Mazonis and Barauskas 1974). The Lithuanian Trakehner Horse Society was established in 1993. In 2010 the supervision of Trakehner horse breeding was laid under the National Conservation Programme.

Purebred Trakehner horses, who were moved from East Prussia to Russia and back to Lithuania, could differ from the current Trakehner populations in other countries and, consequently, represent the old genotype of Trakehners, thus becoming an interesting object for research.

In Lithuania, genetic studies of horse blood groups and protein polymorphism were started in 1985 (Kriksciunas 1990). Blood group and protein polymorphism methods were used to analyse the genetic diversity of Lithuanian local horse populations (Juras et al. 2003) and also different foreign breeds in the Czech Republic, Russia, Poland and other countries (Jiskrova et al. 2002; Khrabrova 2008; Stachurska et al. 2014). The analysis of blood groups and protein polymorphism in the current population allows using the previous data for comparisons and evaluating the genetic changes between the generations of the breed.

The objective of this study was to estimate the inter-breed genetic diversity of the Trakehner horse population in Lithuania on the basis of sire line genealogy and by analysing the differences/similarities among separate groups.

MATERIAL AND METHODS

Genealogical structure of population

In total 3 855 pedigree records in the Studbook of 1 039 live horses born from 1923 to 2019 were analysed to set the genealogical structure and choose the correct proportion of the samples for the inter-breed evaluation of Trakehner population. All the live horses were sorted according to the recorded information on pedigree, place and date of birth and assigned to one of the three genealogical clusters, i.e. those originating from Trakehnen Stud sires, Thoroughbred sires and Arab sires (Table 1). The lines were reconstructed by tracing back the paternal line to the individual's founder stallion. The genealogical clusters and groups of the related horses were created on the basis of Trakehner horse lines described by Kamzolov (2002). High-digit stallion lines were divided into important subgroups (covering up to seven generations).

Immunogenetic data

The study of blood typing included 316 samples from the routine parentage testing of Trakehner horses born from 1983 to 2015 in the territory of Lithuania. The horses were selected and grouped individually by the pedigree indicated in the Studbook (minimum seven generations, maximum 11) and assigned to separate groups. Altogether, nine subdivided groups of the tested horses were created (depending on their ancestors), i.e. Landgraf (L) n = 25, Bay Ronald through Dark Ronald (BRDR) n = 31, Bay Ronald through Gainsborough (BRG) n = 14, Dampfross through Hyperion (DH) n = 6, Dampfross through Pythagoras (DP) n = 14, Pilger through Einhard (PE) n = 26, Pilger through Egoist (PEG) n = 5, Pilger through Eol (PEO) n = 50, Pilger through Topkij (PT) n = 60 (Table 1). Eighty-five samples were not included in subdivided horse groups because of a low number of tested horses in the single sire group (1–3 offspring).

Immunogenetic studies were based on blood typing carried out at the Laboratory for Genetic Studies of the Animal Science Institute of Lithuanian University of Health Sciences as a routine horse parentage testing. Agglutination and haemolysis reactions were carried out by the requirements of the International Society for Animal Genetics (ISAG). Blood serum protein polymorphic variants were determined by a standard protein electrophoresis method in polyacrylamide gel. Blood groups were identified using six genetic systems: *EAA*, *EAC*, *EAD*, *EAP*, *EAQ*, *EAU*. The following

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Table 1. The structure of the Lithuanian Trakehner horse population based on sire parentage and sampled horses of each group for immunogenetic analysis

Diversity of particular founders	Population structure				No. of analysed horses
	sires	geldings	mares	total	
Ancestors from East Prussian Trakehnen Stud					
Dampfross through Hyperion	10	8	18	36	6
Dampfross through Pythagoras	12	2	28	42	14
Pilger through Einhard	55	18	105	178	26
Pilger through Egoist	4	11	17	32	5
Pilger through Eol	22	7	40	69	50
Pilger through Topkij	81	23	143	247	60
Non grouped	15	7	33	55	30
Thoroughbred ancestors					
Bay Ronald through Dark Ronald	13	4	29	46	31
Bay Ronald through Gainsborough	36	15	58	109	14
Landgraf	11	2	22	35	25
Non grouped	52	16	89	157	38
Arab ancestors					
Non grouped	7	5	14	26	16
Other ancestors					
Non grouped	2	2	3	7	1
Total	320	120	599	1 039	316

alleles were tested: A^{ad}, A^{abcd}, A^{abd}, A^{b}, A^{c}, A^{bc}, A^{acd}, C^{a}, D^{cgm}, D^{dl}, D^{dkl}, D^{dghm}, D^{bcm}, D^{dfk}, D^{dk}, D^{ad}, D^{bcm}, D^{cfgm}, D^{cfm}, P^{b}, Q^{bc}, Q^{abc}, Q^{b}, Q^{c}, U^{a}. Five blood protein genetic systems were identified: transferrin (*Tf*), postalbumin (*Xk*), vitamin D-binding protein (*Gc*), albumin (*Al*) and serum esterase (*Es*). In the systems above, the following alleles were analysed: *Tf* system – *D*, *F*, *H*, *O*, *R*; *Xk* – *F*, *K*, *S*; *Gc* – *F*, *S*; *Al* – *A*, *B*; *Es* – *F*, *I*, *S* (Juras et al. 2003).

Genetic diversity

Blood groups (coded numerically as codominant data) and protein polymorphism (coded numerically as haploid data) were analysed separately. The number of alleles (NA) and the frequencies for each allele in horse subgroups were counted directly. The Chi-square test was calculated by MINITAB15 v15.1.30 statistical software (Minitab, LLC, State College, PA, USA) and used to determine statistically significant allelic frequency differences between subgroups. FSTAT program (Goudet 2001) was used to estimate allelic richness per locus (R_t). The following analyses and data management were mainly done with GenAlEx v6.5 software (Peakall and Smouse 2012). The arithmetic mean of the allele number across loci (Na) was determined and the effective number of alleles (Ne) was estimated in different horse groups (Brown and Weir 1983) for the meaningful comparisons of allelic diversity across loci with diverse allele frequency distributions. The variability based on blood groups was measured as expected heterozygosity (H_E) or often referred to as genetic diversity, observed heterozygosity (H_O), average expected (Mean H_E) and observed heterozygosity (Mean H_O) within a horse group per locus (Hartl and Clark 1997). H_E and Mean H_E were calculated in blood protein polymorphism. Due to the fact that blood protein data were haploid, it was not possible to calculate the observed heterozygosity. The standard error of the means (SE) was estimated. Wright's fixation index (F_{ST}; Weir and Cockerham 1984) was used to measure a reduction in heterozygosity of the subpopulation due to random genetic drift using the FSTAT program.

Significance (*P*-value) for population differentiation was based on 1 000 randomisations of genotypes among horse groups by FSTAT not assuming the Hardy-Weinberg equilibrium. In this case, contingency tables of alleles by subgroups were gener-

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ated, and the statistics that FSTAT uses to classify them is the log-likelihood statistic G.

Genetic relationship of subgroups

The pairwise genetic differences among subgroups were evaluated based on Nei's (1972) standard distance D using GenAlEx v6.5 software (Peakall and Smouse 2012).

Visualization of group relationships in the allelic frequencies of all the studied genetic blood groups and blood serum protein systems was performed by principal component analysis (PCA). The PCA was performed on the symmetric correlation matrix. The cluster analysis of the observed heterozygosity of horse groups was based on Euclidian distance coupled with Ward's linkage method. MINITAB15 v15.1.30 (Minitab, LLC, State College, PA, USA) was used for the PCA and cluster analyses.

RESULTS

Genealogical structure of population

The population of 1 039 Trakehners in Lithuania comprised 63% of horses with the ancestors from East Prussian Trakehnen Stud (Pilger – 51%, Dampfross – 8%), 33% from Thoroughbred (Bay Ronald – 15%, Landgraf – 3%), 4% from Arab and other ancestors (Table 1). The parentage of Pilger and Dampfross horse groups was traced back to 1926 and 1916, respectively. Bay Ronald sire lines have been bred in Lithuania since 1986. In Lithuania, the breeding of Landgraf group was launched 40 years ago, but the group was not widely developed. The remaining horses were distributed among small groups that were influenced mostly by Arab sires and did not significantly affect the breeding of Trakehner horses in Lithuania.

Genetic diversity

The results of blood groups and protein polymorphism analysis are shown in Table 2. A different NA, ranging from one to 12, was detected for each locus. However, some alleles were rare and were detected in only a few individuals (Tables 3 and 4).

Table 2. The summary statistics of the diversity indices per analysed blood groups and protein polymorphism loci in Lithuanian Trakehner horses

	Locus	NA	R_t	H_E	F_{ST}	*P*-value
Blood groups	*EAA*	7	2.993	0.569	0.006	***
	EAC	1	1.998	0.497	0.002	–
	EAD	10	5.609	0.858	0.020	***
	EAP	1	1.331	0.070	0.044	**
	EAQ	4	2.834	0.472	0.008	–
	EAU	1	1.693	0.179	0.016	*
	overall	24	–	–	0.012	***
Blood protein	*Al*	3	2.271	0.329	0.004	–
	Gc	3	2.316	0.359	0.003	–
	Es	5	2.383	0.333	0.031	**
	Xk	3	1.770	0.173	0.023	–
	Tf	12	5.603	0.851	0.046	***
	overall	26	–	–	0.027	***

F_{ST} = Wright's fixation index; H_E = genetic diversity; NA = number of alleles; R_t = allelic richness

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

The highest values of R_t as well as H_E were observed in *EAD* locus of blood groups and *Tf* locus of protein polymorphism. Low F_{ST} values in blood groups and blood protein polymorphism indicated high connectivity and low migration within the population. Six out of eleven loci showed significant differentiation of the population by permuting genotypes among horse groups.

Table 5 presents calculated diversity indices of the Lithuanian Trakehner population and summary statistics of blood groups and protein polymorphism analysed throughout the subdivided Trakehner horse groups. Na might have been affected by the sample size in each group, because the smallest groups DH and PEG showed the lowest numbers. Na and Ne were similar in blood groups and protein polymorphism systems for all the analysed horses. Horse groups BRG and BRDR of the same Thoroughbred ancestor Bay Ronald showed the lowest (0.370 ± 0.132) and highest (0.487 ± 0.079) values of Mean H_E in blood groups. Concerning Mean H_O, BRG group showed the lowest value as well (0.524 ± 0.187), while the highest value was found in PEG group (0.700 ± 0.191). Mean H_E was lower than Mean H_O for all the tested Trakehner horses. Slight differences between Mean H_E in blood groups and protein polymorphism were observed.

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Table 3. Blood group allelic frequency, expected (H_E) and observed heterozygosity (H_O) of each locus in subdivided Trakehner horse groups and entire population

Locus	Alleles	Groups									Entire population
		L	BRDR	BRG	DH	DP	PE	PEG	PEO	PT	
EAA	*ad*	0.340	0.339	0.214	0.167	0.286	0.212	0.400	0.320	0.275	0.283
	abcd	0.020	0.000	0.000	0.000	0.036	0.115	0.100	0.020	0.075	0.044
	abd	0.000	0.016	0.036	0.250	0.000	0.000	0.000	0.020	0.000	0.021
	b	0.000	0.016	0.000	0.000	0.036	0.000	0.000	0.000	0.017	0.014
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.003
	bc	0.020	0.016	0.071	0.000	0.000	0.115	0.000	0.030	0.042	0.041
	acd	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.017	0.005
	A^-	0.620	0.613	0.643	0.583	0.643	0.558	0.500	0.610	0.558	0.589
H_E		0.499	0.509	0.533	0.569	0.503	0.618	0.580	0.524	0.604	0.569
H_O		0.760	0.774	0.714	0.833	0.714	0.885	1.000	0.780	0.883	0.823
EAC	*a*	0.440	0.403	0.500	0.417	0.500	0.481	0.500	0.490	0.450	0.464
	C^-	0.560	0.597	0.500	0.583	0.500	0.519	0.500	0.510	0.550	0.536
H_E		0.493	0.481	0.500	0.486	0.500	0.499	0.500	0.500	0.495	0.497
H_O		0.880	0.806	1.000	0.833	1.000	0.962	1.000	0.980	0.900	0.927
EAD	*cgm*	0.220	0.113	0.071	0.083	0.107	0.173	0.100	0.140	0.208	0.150
	dl	0.020	0.097	0.286	0.417	0.000	0.173	0.200	0.080	0.158	0.146
	dkl	0.000	0.065	0.036	0.083	0.071	0.058	0.000	0.060	0.033	0.046
	dghm	0.100	0.258	0.036	0.000	0.143	0.135	0.100	0.090	0.075	0.098
	bcm	0.000	0.000	0.071	0.000	0.071	0.019	0.000	0.030	0.092	0.047
	dfk	0.080	0.081	0.179	0.000	0.143	0.077	0.100	0.070	0.092	0.087
	dk	0.260	0.065	0.036	0.083	0.214	0.135	0.000	0.220	0.108	0.139
	ad	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.005
	cfgm	0.000	0.016	0.036	0.000	0.000	0.000	0.000	0.000	0.008	0.011
	cfm	0.000	0.000	0.036	0.083	0.000	0.019	0.000	0.020	0.025	0.033
	D^-	0.320	0.306	0.214	0.250	0.250	0.212	0.500	0.290	0.183	0.237
H_E		0.765	0.802	0.824	0.736	0.829	0.849	0.680	0.824	0.862	0.858
H_O		1.000	0.968	1.000	1.000	1.000	1.000	1.000	0.980	1.000	0.994
EAP	*b*	0.020	0.145	0.000	0.083	0.036	0.019	0.000	0.010	0.033	0.036
	P^-	0.980	0.855	1.000	0.917	0.964	0.981	1.000	0.990	0.967	0.964
H_E		0.039	0.248	0.000	0.153	0.069	0.038	0.000	0.020	0.064	0.070
H_O		0.040	0.290	0.000	0.167	0.071	0.038	0.000	0.020	0.067	0.073
EAQ	*bc*	0.140	0.242	0.107	0.333	0.214	0.115	0.200	0.150	0.242	0.172
	abc	0.120	0.129	0.071	0.000	0.107	0.115	0.200	0.080	0.058	0.093
	b	0.000	0.016	0.000	0.000	0.000	0.019	0.000	0.030	0.000	0.011
	c	0.000	0.000	0.036	0.000	0.000	0.019	0.100	0.010	0.067	0.024
	Q^-	0.740	0.613	0.786	0.667	0.679	0.731	0.500	0.730	0.633	0.699
H_E		0.418	0.549	0.365	0.444	0.482	0.439	0.660	0.437	0.533	0.472
H_O		0.520	0.774	0.429	0.667	0.643	0.538	1.000	0.540	0.733	0.601
EAU	*a*	0.040	0.210	0.000	0.167	0.107	0.096	0.100	0.100	0.125	0.100
	U^-	0.960	0.790	1.000	0.833	0.893	0.904	0.900	0.900	0.875	0.900
H_E		0.077	0.331	0.000	0.278	0.191	0.174	0.180	0.180	0.219	0.179
H_O		0.080	0.419	0.000	0.333	0.214	0.192	0.200	0.200	0.250	0.199

BRDR = Bay Ronald through Dark Ronald; BRG = Bay Ronald through Gainsborough; DH = Dampfross through Hyperion; DP = Dampfross through Pythagoras; L = Landgraf; PE = Pilger through Einhard; PEG = Pilger through Egoist; PEO = Pilger through Eol; PT = Pilger through Topkij

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Table 4. Allelic frequency and genetic diversity (H_E) of blood protein polymorphism in subdivided Trakehner horse groups and entire population

Locus	Alleles	Groups									Entire population
		L	BRDR	BRG	DH	DP	PE	PEG	PEO	PT	
Al	*AB*	0.760	0.742	1.000	1.000	0.857	0.885	0.800	0.760	0.767	0.807
	AA	0.240	0.161	0.000	0.000	0.000	0.077	0.200	0.180	0.117	0.123
	BB	0.000	0.097	0.000	0.000	0.143	0.038	0.000	0.060	0.117	0.070
H_E		0.365	0.414	0.000	0.000	0.245	0.210	0.320	0.386	0.385	0.329
Gc	*FF*	0.760	0.871	0.857	0.333	0.643	0.654	0.800	0.720	0.767	0.778
	FS	0.200	0.129	0.143	0.500	0.214	0.231	0.200	0.200	0.217	0.184
	SS	0.040	0.000	0.000	0.167	0.143	0.115	0.000	0.080	0.017	0.038
H_E		0.381	0.225	0.245	0.611	0.520	0.506	0.320	0.435	0.365	0.359
Es	*II*	0.880	0.677	0.786	1.000	0.786	0.885	0.800	0.900	0.717	0.807
	FF	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.009
	FI	0.120	0.000	0.143	0.000	0.071	0.077	0.200	0.100	0.133	0.108
	IS	0.000	0.290	0.071	0.000	0.071	0.038	0.000	0.000	0.117	0.066
	FS	0.000	0.032	0.000	0.000	0.071	0.000	0.000	0.000	0.017	0.009
	SS	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H_E		0.211	0.456	0.357	0.000	0.367	0.210	0.320	0.180	0.454	0.333
Xk	*KK*	0.880	0.935	0.929	1.000	0.929	0.692	0.800	0.900	0.900	0.905
	KS	0.080	0.065	0.071	0.000	0.071	0.308	0.200	0.080	0.100	0.089
	FK	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.006
H_E		0.218	0.121	0.133	0.000	0.133	0.426	0.320	0.183	0.180	0.173
Tf	*FO*	0.400	0.226	0.214	0.333	0.143	0.154	0.200	0.100	0.333	0.234
	DO	0.320	0.323	0.000	0.333	0.357	0.038	0.200	0.120	0.117	0.155
	DF	0.080	0.032	0.143	0.000	0.143	0.000	0.200	0.320	0.167	0.155
	DD	0.000	0.226	0.000	0.000	0.143	0.346	0.000	0.240	0.133	0.161
	FF	0.000	0.129	0.143	0.167	0.000	0.231	0.200	0.100	0.050	0.098
	DR	0.000	0.000	0.071	0.000	0.143	0.077	0.000	0.080	0.050	0.063
	FR	0.000	0.000	0.071	0.000	0.071	0.154	0.200	0.020	0.100	0.073
	OR	0.040	0.000	0.143	0.000	0.000	0.000	0.000	0.020	0.000	0.013
	OO	0.120	0.065	0.071	0.167	0.000	0.000	0.000	0.000	0.033	0.028
	HO	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
	RR	0.000	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.017	0.013
	FH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
H_E		0.714	0.772	0.857	0.722	0.786	0.772	0.800	0.798	0.813	0.851

BRDR = Bay Ronald through Dark Ronald; BRG = Bay Ronald through Gainsborough; DH = Dampfross through Hyperion; DP = Dampfross through Pythagoras; L = Landgraf; PE = Pilger through Einhard; PEG = Pilger through Egoist; PEO = Pilger through Eol; PT = Pilger through Topkij

Based on the Chi-square test, significant differences ($P < 0.05$, degrees of freedom = 1) between horse subgroups were determined for A^{abd}, D^{dl}, D^{dghm}, D^{dk}, P^{b}, Q^{bc}, U^{a} allele frequencies in blood groups and for Gc^{FF}, Es^{IS}, Xk^{KK}, Xk^{KS}, Tf^{FO}, Tf^{DO}, Tf^{DF}, Tf^{DD}, Tf^{FF}, Tf^{OR} allele frequencies in blood protein polymorphism. Results of the analysis of allele diversity and frequency in blood group genetic systems are presented in Table 3. DH horse group was exceptional due to A^{abd} allele frequency characteristic of the group, while DH related DP group showed the complete absence of this allele. The highest D^{dl} allele frequency was found for DH (0.417) and BRG (0.286) horse groups, while DH related DP group

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Table 5. Descriptive statistics of blood groups and protein polymorphism systems throughout the subdivided Trakehner horse groups and entire population (± SE)

Group	Blood groups				Blood protein		
	Na	Ne	mean H_E	mean H_O	Na	Ne	mean H_E
L	3.167 ± 0.654	2.011 ± 0.480	0.382 ± 0.113	0.547 ± 0.167	3.200 ± 0.735	1.845 ± 0.418	0.378 ± 0.091
BRDR	3.833 ± 0.980	2.344 ± 0.560	0.487 ± 0.079	0.672 ± 0.106	3.200 ± 0.735	2.072 ± 0.593	0.398 ± 0.112
BRG	3.833 ± 1.400	2.233 ± 0.717	0.370 ± 0.132	0.524 ± 0.187	3.200 ± 1.241	2.407 ± 1.152	0.318 ± 0.147
DH	2.833 ± 0.654	2.070 ± 0.382	0.444 ± 0.085	0.639 ± 0.132	2.000 ± 0.632	1.834 ± 0.536	0.267 ± 0.164
DP	3.333 ± 0.803	2.350 ± 0.720	0.429 ± 0.109	0.607 ± 0.159	3.400 ± 0.748	2.162 ± 0.646	0.410 ± 0.114
PE	4.000 ± 1.125	2.545 ± 0.849	0.436 ± 0.120	0.603 ± 0.169	3.400 ± 0.678	2.138 ± 0.581	0.425 ± 0.105
PEG	2.833 ± 0.601	2.111 ± 0.357	0.433 ± 0.114	0.700 ± 0.191	2.600 ± 0.600	2.176 ± 0.706	0.416 ± 0.096
PEO	4.167 ± 1.138	2.297 ± 0.697	0.414 ± 0.115	0.583 ± 0.165	3.800 ± 1.068	2.161 ± 0.708	0.397 ± 0.113
PT	4.667 ± 1.498	2.704 ± 0.932	0.463 ± 0.116	0.639 ± 0.158	4.400 ± 1.249	2.322 ± 0.765	0.440 ± 0.104
Entire population	5.000 ± 1.549	2.587 ± 0.909	0.441 ± 0.115	0.603 ± 0.158	5.200 ± 1.744	2.493 ± 1.056	0.409 ± 0.115

BRDR = Bay Ronald through Dark Ronald; BRG = Bay Ronald through Gainsborough; DH = Dampfross through Hyperion; DP = Dampfross through Pythagoras; L = Landgraf; mean H_E = average expected heterozygosity; mean H_O = observed heterozygosity; Na = average allele number across loci; Ne = effective number of alleles; PE = Pilger through Einhard; PEG = Pilger through Egoist; PEO = Pilger through Eol; PT = Pilger through Topkij

was completely free of this allele. L group, the ancestors of which were Thoroughbred horses like those of BRG group, had a very low D^{dl} allele frequency. BRDR group was exclusive by its D^{dghm} allele frequency and P^{b} allele frequency.

The analysis of the allele variety and frequency in blood protein genetic systems (Table 4) indicated that BRDR horse group was characterized by the highest Es^{IS} allele frequency (0.290), though in other groups the frequency of this allele was very low or even absent in four horse groups. The Xk^{KS} allele, rare among many analysed groups, was very typical of PE horse group. Moreover, PE horse group carried the Xk^{KK} allele, the rarest compared with all the other analysed groups. Each subgroup showed different combination of dominant alleles under *Tf* system. BRG horse group carried the Tf^{OR} allele, which was completely absent in six horse groups or very rare in L and PEO groups.

Genetic relationship of subgroups

Protein polymorphism indicated higher values of genetic distances according to Nei in comparison with blood groups (Table 6). DH group was the most distant from the other groups, even from those from the same cluster or founder. The genetic relationship was also relatively weak between BRDR and BRG groups (both originated from the same founder) based on blood groups (0.045) and L and PE groups based on protein polymorphism (0.090). Considering protein polymorphism, the smallest genetic distance occurred between PT and other subdivided horse groups, except PE group, though PE and PT groups originated from same ancestor and were indicated as the most numerous groups of the Trakehner horse population in Lithuania.

PCA of the blood groups resulted in seven significant (eigenvalues > 1) principal components (PC) explaining 98% of the total genetic variation. The first and the second component of PCA described 27% and 21% of the total variation, respectively. The visualization of blood groups in a score plot (Figure 1) showed that the horses of PEG, BRG, DH and BRDR groups appeared to be genetically less related than the rest. The highest positive correlations (0.463) were observed between EAA^{c}, EAD^{ad} alleles and component PC4.

PCA of the blood protein polymorphism resulted in eight significant (eigenvalues > 1) principal components explaining 100% of the total genetic variation. The first and the second component of PCA described 24% and 18% of the variance conserved in the dataset, respectively. The score plot of blood serum protein (Figure 2) showed higher divergence of DH, L, DP and PE horses from the other groups.

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Table 6. Pairwise population matrix of Nei's genetic distance based on blood groups above the diagonal and blood protein polymorphism below the diagonal

Groups		By blood groups								
		L	BRDR	BRG	DH	DP	PE	PEG	PEO	PT
By blood proteins	L	0.000	0.026	0.029	0.062	0.009	0.014	0.037	0.004	0.016
	BRDR	0.048	0.000	0.045	0.049	0.019	0.028	0.034	0.022	0.023
	BRG	0.055	0.063	0.000	0.038	0.027	0.014	0.043	0.020	0.019
	DH	0.073	0.128	0.106	0.000	0.058	0.043	0.064	0.046	0.035
	DP	0.046	0.045	0.054	0.064	0.000	0.015	0.038	0.005	0.013
	PE	0.090	0.085	0.065	0.107	0.066	0.000	0.036	0.009	0.009
	PEG	0.035	0.060	0.036	0.109	0.052	0.054	0.000	0.032	0.032
	PEO	0.048	0.057	0.047	0.102	0.034	0.050	0.034	0.000	0.011
	PT	0.028	0.030	0.028	0.095	0.031	0.057	0.026	0.028	0.000

BRDR = Bay Ronald through Dark Ronald; BRG = Bay Ronald through Gainsborough; DH = Dampfross through Hyperion; DP = Dampfross through Pythagoras; L = Landgraf; PE = Pilger through Einhard; PEG = Pilger through Egoist; PEO = Pilger through Eol; PT = Pilger through Topkij

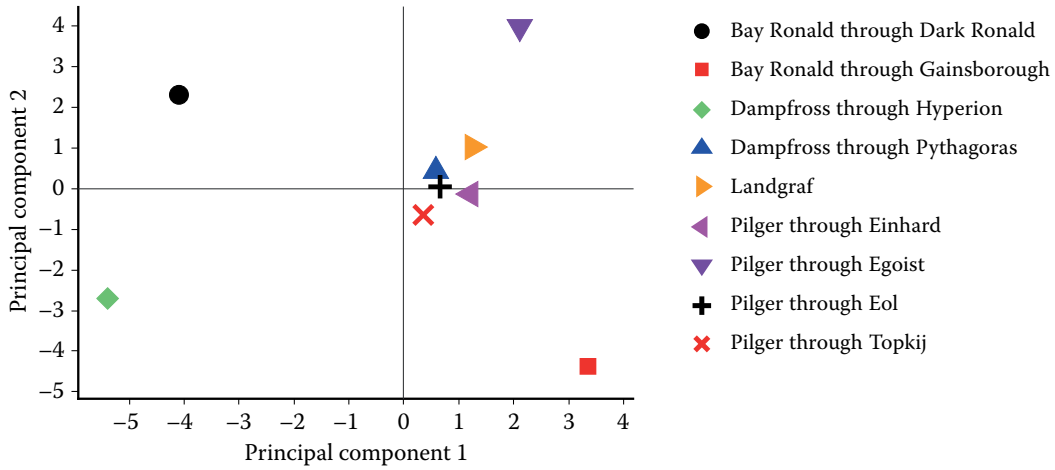

Figure 1. Score plot of the allelic frequency of blood groups describing the relationships among nine Trakehner horse groups

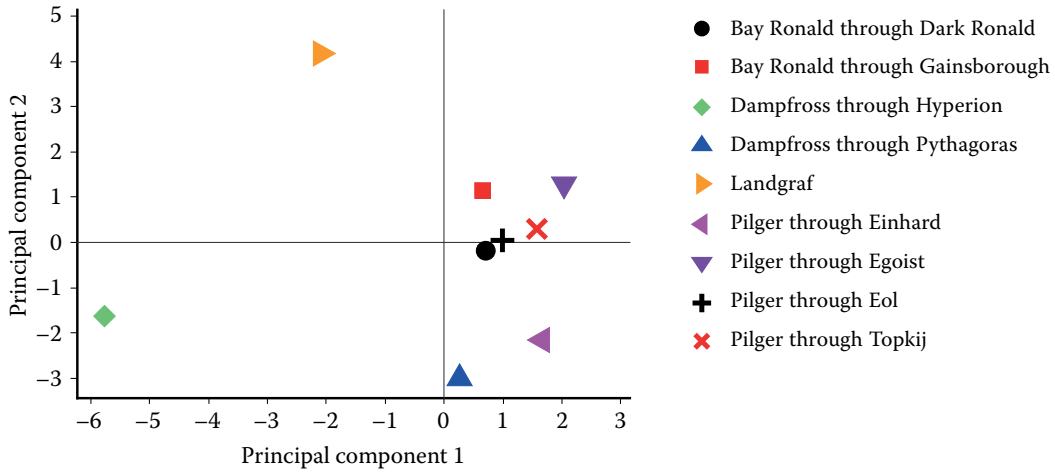

Figure 2. Score plot of allelic frequency of blood protein describing the relationships among nine Trakehner horse groups

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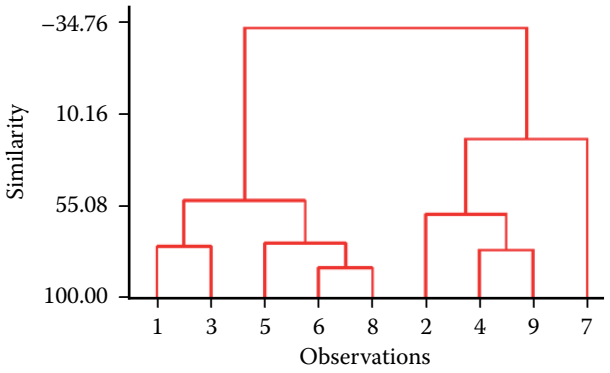

Figure 3. MINITAB dendrogram constructed based on the observed heterozygosity of blood groups (Ward's linkage and Euclidean distance methods)

1 = Landgraf; 2 = Bay Ronald through Dark Ronald; 3 = Bay Ronald through Gainsborough; 4 = Dampfross through Hyperion; 5 = Dampfross through Pythagoras; 6 = Pilger through Einhard; 7 = Pilger through Egoist; 8 = Pilger through Eol; 9 = Pilger through Topkij

The highest negative correlation (–0.473) was observed between Tf^{RR} allele and component PC4.

The applied cluster analysis for the observed heterozygosity in blood groups displayed a dendrogram with the amalgamation steps (Figure 3), which visually confirmed differences between the subdivided horse groups of the Trakehner population in Lithuania. According to the genealogical analysis, the horses assigned to one sire line (BRG and BRDR, DP and DH, PE, PEO and PT, PEG horse groups) were arranged in two distinctive clusters in the study of immunogenetic data.

DISCUSSION

In 1985–1988, Kriksciunas (1990) studied Lithuanian horse populations, including Trakehner horses, by their blood protein polymorphism. The comparisons of the data in the present study and the findings of Kriksciunas (1990) showed that typical Trakehner alleles such as Tf^{FO} (0.226), Tf^{DF} (0.014) or the alleles with a very low frequency – Tf^{OR} (0.053), Tf^{DR} (0.077), Tf^{FH} (0.006) – had not changed in the previous years. It could be only observed that Tf^{RR} allele was not detected in Trakehner and Thoroughbred populations in the study of Kriksciunas. However, in our study only two horses with Thoroughbred ancestors had the rare Tf^{RR} allele in BRG (0.143) group and one horse with the ancestors from Trakehnen Stud in PT (0.017) horse group. Jiskrova et al. (2002) found that Tf^{RR} allele was frequent (0.231) in the Czech Trakehner population.

The research of Jiskrova et al. (2002), who characterised the Czech Trakehner population based on the analysis of genetic polymorphism systems, indicated that alleles Q^{abc} (0.615) and D^{dkl} (0.423) were very frequent in the Czech population, but that was not typical of the Lithuanian Trakehner population with the frequency of 0.093 and 0.046, respectively. According to Khrabrova (2008), D^{bcm} allele was frequent (0.286) in Thoroughbred horses in Russia. However, in the Lithuanian Trakehner population D^{bcm} allele was rare (0.047) and it was found only in one horse group with Thoroughbred ancestors at a low frequency (0.071) and in four groups originating from Trakehnen Stud with a frequency from 0.019 to 0.092.

We observed differences between the related groups in some alleles (e.g. A^{abd}, D^{dl}) that could be explained by the variety of maternal origin. In the present study, the maternally inherited genetic variation was not analysed, but the literature survey indicated that this genetic variation should be very high (Kvist et al. 2019).

In comparison with other populations, the Lithuanian Trakehner horse population was more heterozygous (0.441) than Trakehners in Poland and Czech Republic. In the Polish Trakehner horse population the expected heterozygosity was 0.363, while the observed one was 0.302 (Iwanczyk et al. 2006). In the Czech Trakehner population the level of heterozygosity was 0.319 (Jiskrova et al. 2002). Higher diversity of the Lithuanian Trakehner horse population in comparison with other countries may have been influenced by accurate selection and long-lasting breeding in the National Stud Nemunas, which has been supported by the Ministry of Agriculture. According to pedigree data, 70% of Trakehner horses in Lithuania were bred by the National Stud and 30% by private breeders. Druml et al. (2007) concluded that populations located in the areas with less intensive breeding had greater diversity within the population and further differentiation among the populations was mainly caused by political and geographical factors. The differences between the pedigree cluster (PEG and DH subgroups) and genetic clustering in the observed heterozygosity were most likely due to different geographical origins of the ancestors and low breeding intensity. PEG is dis-

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tant from the Trakehnen Stud cluster with the same ancestor Pilger possibly because a Trakehner sire from Estonia was used in PEG group. DH group was distant from the groups of the same cluster and even the founder. This might be explained by the fact that DH group is newly restored from the sire Abdullah who was born in North America, while the founder of PEO group was born in Russia. The immigration of different horses affects genetic parameters (Vostry et al. 2011).

In our study some groups such as L and BRG (Thoroughbred cluster) had the lowest heterozygosity in blood groups. Trakehner horses have been close to purebred horses for 250 years. Foreign sires are seldom accepted into the studbook and generally English Thoroughbreds and Arabians are used for refinement (Nolte et al. 2019), which could generate low heterozygosity in some subpopulations.

The current conservation programme administered by the National Stud comprises preservation and selection of all the possible different units of the breed, even those which are not popular among private breeders. Currently, six stallion genealogical lines and 10 mare families from the National Stud are under the conservation programme. Blood protein polymorphism and blood group studies of the Lithuanian Trakehner horse population showed that strict management of breeding and selection allowed preserving the genetic diversity of the breed. Due to a small size of some structural units and specificity of alleles, additional management of the genetic diversity within the population is recommended. The determined distinctiveness between the subgroups of the Trakehner population suggests including segregated sire lines in long-term conservation strategies.

Conclusions

This paper was the first study of the inter-breed genetic diversity of the Trakehner horse population in Lithuania. The obtained results of the genetic relationship (PCA and genetic clustering) indicated that subgroups diverged from clustering by pedigree data. According to Genetic Resources Conservation Programme, which is aimed at maintenance and extension of the genealogical structure, the breeding strategies of Trakehner horses should be reconsidered. Breeding and selection of subdivided sire groups could be among the appropriate solutions.

Conflict of interest

The authors declare no conflict of interest.

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