

Genetic structure and variation of large-type Žemaitukai horse population

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Conservation of genetic variability within a breed is the core problem of the conservation of rare breeds of domestic livestock species. The objective of our study was to examine the genetic differences and genetic variation of the large-type Žemaitukai horse population. The main study was conducted in 1996–2005 at the Institute of Animal Science of Lithuanian Veterinary Academy. The pedigree, gene frequencies of blood group and serum protein markers, homozygosity, and genetic similarities were analysed from 138 horses. The genetic analysis of alleles indicated that the Al^{FB} genotype was dominant in large-type Žemaitukai, the genotypes $Ty^{FF, FO}$ being also frequent. The alleles D^{dghm} , D^{zgm} , D^{dk} , D^{dl} in blood group system were also frequent. The frequency of alleles D^{dk} and Es^J of Kalmanas line was its distinguishing feature. The average homozygosity was 33.41% in the large-type Žemaitukai horse population. The number of effective population size for large-type Žemaitukai is 58; the survival of the population is uncertain.

Key words: horse, population, lines, blood group, serum protein, gene frequency

INTRODUCTION

The conservation of genetic variability within a breed is a core problem in the conservation of rare breeds of domestic livestock species. Rare breeds are particularly susceptible to loss of genetic variation due to the small population size. Large-type Žemaitukai are also endangered from the phenotypic and genetic points of view.

The development of the large-type Žemaitukai horses was started at the end of the 19th century when the demand for a larger horse arose and the old type (small in size) Žemaitukai horse could no longer satisfy the needs of people. At that time, in the north-eastern part of Lithuania, Žemaitukai horses were crossbred with saddle (mostly of the Orlov breed) stud horses or with crossbred stallions of these breeds, and in the south-eastern part of Lithuania Žemaitukai horses were crossbred with North Swedish stallions or crossbred stallions of the mentioned breeds. Such crossbreeding and the later breeding *inter se* led to the development of a larger in size and more solid horse which from 1941 alongside with the old type Žemaitukai was called the local horse. Later, in about 1946, this type of horse was separated from the Žemaitukai and called the harness horse of East Lithuania. In 1949, these horses together with Žemaitukai were given the common name, the Žemaičių (Zh mudka) horse which included both types – the old and the larger in size ones. Since 1985, the large type is no longer called Žemaičių (Zh mudka) but is named large-type Žemaitukai. According to the horse size, large-type Žemaitukai (with height 150 cm) are

taking an intermediate place between Žemaitukai (with height 137 cm) and Lithuanian Heavy Draught (with height 160 cm) horses. A study on the biological and farming qualities has indicated that the Žemaitukai are all-purpose pony-type horses and large-type Žemaitukai are horses of the harness type. The different body measurements, type and use of these horses allow a presumption that the Žemaitukai and large-type Žemaitukai should be treated as horses of two different Lithuanian breeds [1]. Large-type Žemaitukai have a very dangerous risk status; the numbers of stallions, mares and breeding herds until 2000 was decreasing, but in 2001 the conservation was started [2].

In 1923–1924, L. Hirszfeld and F. Przemyschi [3] found that horses also differ in the antigenic composition of blood. The investigation of horse blood typing was started in Lithuania in 1983 [4], but in 1991 the studies were discontinued and later resumed in 1996 when new research methods were adopted. In 1985–1988, immunogenetic studies of horses bred in Lithuania were carried out to determine the blood groups of large type Žemaitukai, Lithuanian Heavy-Draught and other horses bred for equestrian sports [5]. In 2004, microsatellite markers were used for the characterization of local horse breeds at the University of Kentucky.

Blood group antigens and protein polymorphism traits are perfect markers for determining the population development process, population units' difference and similarity. Therefore the purpose of our study was to determine the genetic diversity within the breed, as well

as genetic differences and genetic variation between large-type Žemaitukai population structural units by the method of blood group and protein polymorphism investigation.

MATERIALS AND METHODS

The status of large-type Žemaitukai horse breed is evaluated by means of their monitoring. The monitoring was started in 1996 at the Institute of Animal Science. The level of inbreeding was calculated by the formula of S. Wright [6]. The effective population size was expressed as N_e [7]. The blood of large-type Žemaitukai horses was analysed at the Blood Typing Laboratory of the Institute of Animal Science of Lithuanian Veterinary Academy (IASLVA). The pedigree and blood samples were collected from 138 horses. Blood group and serum protein marker are used for routine horse parentage testing in Lithuania. Blood samples were obtained from each horse on ACD anti-coagulant. A standard immunological procedure involving hemagglutination and complement-mediated hemolysis [8] was used to detect red cell alloantigens at three internationally recognized blood group loci: *A*, *D* and *Q* [9]. The reagents used to detect the antigenic properties were obtained by alloimmunization. Assignment of alleles was based on reagent reaction patterns and followed the internationally accepted terminology.

Standard methods of horizontal polyacrylamide gel electrophoresis [10] were used to identify inherited variants at the following protein loci: albumin (*Al*), esterase (*Es*) and transferrin (*Tf*). The frequency of antigenic factors, allele frequency, genetic similarity (r) and degree of homozygosity (Ca) were computed by conventional methods described by Majjala and Lindstrom [11], Nei [12]. Genetic similarity between the lines was estimated using three blood protein and three blood group systems. The Šachtioras line was compared with the other lines. The χ^2 -test by Zhivotovsky and Machurova [13] was used for estimating the probability of differences between two groups concerning frequency of alleles.

RESULTS AND DISCUSSION

According to the number of effective population (N_e), the large-type Žemaitukai population falls into the category of vulnerable populations when $N_e < 50$. The effective population size for large-type Žemaitukai was 42 in 2001 and 58 in 2005, the survival of the population is uncertain. When the size of the effective population is lower than 100, the genetic diversity of the population starts diminishing. When the size of the effective population is lower than 50, the heterozygosity is reduced by 1% [7]. Genealogical analysis of the large-type Žemaitukai breed indicated that 43.4% of the test horses were obtained by inbreeding. The average coefficient of inbreeding of these horses is 5.1% (0.8–14.1%) by S. Wright and in IV–IV, II–II generations by

Shaporuzh. Line horse breeding was predominant, and therefore the genetic structure of lines was of paramount importance in Lithuania. At the period of the study, three zootechnical stallion lines represented the structure of the large-type Žemaitukai horse breed. These lines were Šachtioras II 235 (61 heads), Agrastas 156 (33 heads) and Kalmanas 185 (30 heads), as well as the Klintas line (10 heads) of the North Swedish breed.

The general characteristics of large-type Žemaitukai horses by the blood group and serum protein data are presented in Tables 1 and 2. Large-type Žemaitukai are characterized by different recurrence of allele and genotype frequencies.

The genetic analysis of large-type Žemaitukai has indicated that the *AB* genotype is dominant in albumin locus and the most frequent genotypes are *FI*, *II* in esterase locus and *FF* and *FO* in transferrin loci (Table 1). The *AB* genotype frequency of large-type Žemaitukai is mostly similar to that of Lithuanian Heavy Draught horses (0.63). A frequent recurrence of *Tf^{DF}* (0.299) genotype is typical of the old type Žemaitukai [14, 15], while in large-type Žemaitukai it amounts to only 0.159 (Table 1). Large-type Žemaitukai horses had 11 genotypes out of the 14 investigated in *Tf* locus, while Žemaitukai horses had only 9 and Lithuanian Heavy Draught was devoid of only *OO* genotype.

In the test group of large-type Žemaitukai, there were found *OO*, *FM* and *HR* with 0.007 genotype frequency and no *DD*, *RR*, *MO* genotypes in the transferrin locus. Large-type Žemaitukai had rather rare *IS* and *FS* genotypes in the esterase locus. The average degree of homozygosity was 35.6% and ranged from 22.31 (transferrin locus) to 49.7% (albumin locus). The *FF* genotype was frequent (82%) in the *GC* locus of large-type Žemaitukai, its genotype frequency being most similar to that of Lithuanian heavy draught horses (80%). Moreover, only the above-mentioned breeds have the *SS* (4%) genotype [16]. Besides, in esterase locus large-type Žemaitukai have rather frequent genotypes *FI*, *II* and a very rare *IS* genotype.

Analysis of the blood group loci indicated that *D* locus alleles *cgm*, *dk*, *dghm* and *dl* were most frequent (Table 2). The average degree of homozygosity was 31.21% and ranged from 13.81% in *D* locus to 47.66% in *Q* locus. *D^{dghm}* allele frequency (0.418) [14] was recognized as typical of the old type Žemaitukai ($n = 30$), but its frequency is only 0.159 in large-type Žemaitukai. In 1990, B. Krikščiūnas has found that the *D* locus *dghm* (0.310) allele can be used as a breed marker for large-type Žemaitukai [5]. As described in previous studies [17], the *D^{fgkm}* allele was considered to be peculiar to large-type Žemaitukai. Blood group, protein marker and DNA testing will have an important place in the conservation of rare breeds by ensuring that the pedigrees are correct. As Juras and Cothran [18] note, the estimated probability of exclusion of wrongly named parents (PE) was high; for large-type Žemaitukai it was 99.99%. These data imply that the genetic structure of large-type

Table 1. Genotype frequencies of serum protein markers in large type Žemaitukai

Locus	Genotype	All tested horses <i>n</i> = 138	Stallion lines			
			Šachtioras <i>n</i> = 37	Agrastas <i>n</i> = 21	Kalmanas <i>n</i> = 22	Klintas <i>n</i> = 6
Genotype frequency						
Al	AA	0.217	0.162	0.238	0.227	0.167
	AB	0.659	0.784	0.429	0.682	0.833
	BB	0.123	0.054	0.333	0.091	-
Ca%	49.73	64.38	35.15	52.49	72.22	
Es	FF	0.188	0.162	0.143	-	0.167
	FI	0.406	0.541	0.619	0.364	0.500
	II	0.384	0.270*	0.190	0.636*	0.333
	IS	0.007	-	0.048	-	-
	FS	0.014	0.027	-	-	-
Ca%	34.80	39.25	44.20	53.70	38.89	
Tf	DD	-	-	-	-	-
	FF	0.355	0.324*	0.190	0.500	0.333
	DF	0.159	0.162	0.143	0.045	-
	DO	0.065	-	0.286	-	-
	DR	0.014	0.027	-	-	0.167
	FO	0.239	0.270	0.143	0.364	0.333
	FR	0.094	0.162	0.048	-	0.167
	OO	0.007	-	-	0.045	-
	RR	-	-	-	-	-
	FM	0.007	0.027	-	-	-
	HR	0.007	0.027	-	-	-
	FH	0.023	-	0.095	0.045	-
	OR	0.014	-	0.095	-	-
	MO	-	-	-	-	-
	Ca%	22.31	23.24	17.90	38.85	27.78

Ca% – homozygosity, *n* – number of tested horses. **P* < 0.01.

Žemaitukai has changed in the last 15–19 years due to absence of controlled breeding and selection.

Phenotypic differences of lines may reflect their genetic diversity. Higher inbreeding levels may lead to changes in the genetic variation of a breed or line, which also influence the phenotypic differences of individual lines. Therefore, the genealogy of large-type Žemaitukai horses has been analysed and the genetic variation of blood groups and serum proteins in different lines determined.

By close inbreeding were produced 53.3% of all the Agrastas line horses and even 72% of Šachtioras II 235 line horses. The average inbreeding coefficient of the Agrastas line is 4.43 and that of Šachtioras II 235 line 5.8%. Propinquity inbreeding accounts for 50% of the horses of the Kalmanas 185 line. The average inbreeding coefficient of the horses from this line is 6.9%; it is the highest among the lines. Propinquity inbreeding also accounts for even 67% of the Klintas line horses, however, the average inbreeding coefficient is the lowest (1.05%). 80% of the Agrastas line horses possess 1/16 of North Swedish horse blood, and blood immigration in the Kalmanas line horses accounts for only 25%. Genetic variation in a breed or line may be influenced by the increasing level of inbreeding and also by

the phenotypic differences of separate lines. Analysis of the phenotypic traits of individual lines revealed great differences among the lines [13, 15]. Stallions and mares of the Agrastas 156 line are characterized by a beautiful body constitution and energetic disposition. Horses of the Šachtioras II 235 line are distinguished by high working abilities and a strong constitution, however, they have conformation defects (the unproportionally long head). Stallions of this line are characterized as capable of passing their traits to the offspring. Mares and stallions of the Kalmanas 185 line are considered as most typical because of their highly consolidated exterior. Stallions of this line also pass their best exterior and working traits to the offspring very well. The offspring of the Klintas line stallions inherit phenotypic traits, especially the mouse-grey colour, very well.

Allele frequencies of large-type Žemaitukai blood groups and protein polymorphic systems computed for individual stallion lines (Šachtioras II 235, Agrastas 156, Kalmanas 185 and Klintas) are presented in Tables 1 and 2. Analysis of allele frequencies in blood groups (Table 2) indicated that *A* locus *ad* allele was rather frequent (allele frequency 0.405–0.477) in all four lines. Analysis of the 10 alleles in *D* locus showed that horses

Table 2. Allele frequencies of blood group markers in large-type Žemaitukai

Locus	Allele	All tested horses <i>n</i> = 138	Stallion lines				
			Šachtioras <i>n</i> = 37	Agrastas <i>n</i> = 21	Kalmanas <i>n</i> = 22	Klintas <i>n</i> = 6	
Allele frequency							
A	ad	0.431	0.446	0.405	0.477	0.417	
	bc	0.065	0.081	0.119	0.045	0.083	
	b	0.091	0.116	0.071	0.136	0.083	
	c	0.069	0.068	0.024	0.023	0.083	
	A ⁻	0.344	0.283	0.381	0.318	0.333	
Ca%	32.16	30.53	32.9	35.02	30.55		
D	dghm	0.159	0.135	0.190	0.045	-	
	cgm	0.199	0.162	0.190	0.341*	0.167	
	dk	0.185	0.149	0.167	0.295**	0.167	
	ad	0.065	0.108	-	0.045	-	
	dl	0.123	0.176	0.190	-	0.250	
	bcm	0.072	0.068	0.071	0.045	0.167	
	dkl	0.025	0.040	-	0.045	-	
	cdf	0.011	0.013	0.024	0.023	-	
	dfk	0.007	-	0.024	-	-	
	cfgkm	0.047	0.068	0.095	-	0.167	
	D ⁻	0.105	0.081	0.048	0.159	0.25	
	Ca%	13.81	12.69	15.44	23.76	23.62	
	Q	abc	0.036	0.027	-	0.068	0.083
		bc	0.083	-	0.119	0.091	-
		c	0.181	0.257	0.190	0.068	0.167
b		0.022	0.054	-	-	-	
ac		-	-	0.048	0.045	-	
Q ⁻		0.659	0.662	0.643	0.727	0.750	
Ca%	47.66	50.8	46.61	54.87	59.72		

Ca% – homozygosity, n – number of tested horses. *P < 0.05; **P < 0.01.

of the Klintas line had a narrow allele spectrum (5 alleles found), versus even 9 *D* locus alleles determined in the Šachtioras II 235 line. Allele frequency among the lines ranged from 0.013 to 0.477.

Analysis of genotype frequency in the protein polymorphic systems of individual lines (Table 1) indicated that all lines possessed a very frequent *AB* genotype in albumin locus, and the highest genotype frequency was found in the Klintas (0.833) and Šachtioras (0.784) lines, while the lowest in the Agrastas line (0.429). The dominant *II* genotype in esterase locus within genotype frequency (0.636) and genotype *FF* in transferrin locus with the frequency 0.500 were determined for the Kalmanas 185 line. The *FF* genotype in transferrin locus is also characteristic of the Šachtioras line (frequency 0.324).

Studies of protein polymorphism showed that the narrowest genotype structure (9 genotypes) was characteristic of the Klintas line, while the widest structure (14 genotypes) belonged to the Agrastas 156 and Šachtioras lines. Protein polymorphism genotype frequency ranged from 0.027 to 0.833 (Table 1).

The Kalmanas line differed from the others by the high level of significance (P < 0.01) of the frequency of alleles *D^{dk}* and *Es^{II}*. This line is most homogeneous

and considerably exceeds the other lines by the level of homozygosity (*Ca* = 43.12%).

Circular mating inbreeding and selection aimed at line type consolidation might have led to lower heterozygosity, number of alleles and a higher allele concentration in the Kalmanas 185 line. This might have also resulted in a higher homozygosity level (*Ca*) of a line influenced by inbreeding, which was determined as the highest (*F* = 7%) in the Kalmanas 185 line.

The genetic similarity index is the closer to unity, the higher is the relationship of the lines. According to our data, the line similarity index of large-type Žemaitukai varied within 0.44 to 0.70. The highest genetic similarity was determined between the Šachtioras and Klintas lines (*r* = 0.70) and the lowest between the Šachtioras and Agrastas lines (*r* = 0.44). Genetic similarity between the Šachtioras and Kalmanas lines was *r* = 0.47.

The immigration level of North Swedish horse blood is also an indicator of the similarities between the Šachtioras and Klintas lines. The immigration level in the Šachtioras line accounted for even 84% of the horses tested. As expected, least similar were non-related lines.

During our previous studies, a preliminary analysis of large-type Žemaitukai mare families has been carried

out [19]. Analysis of four mare families (Niemka, Gulbė, Danutė and Mėta) has indicated that mare families are genetically unlike. The highest genetic similarity was found between the Niemka and Gulbė families ($r = 0.567$) and the lowest between the Niemka and Danutė families ($r = 0.239$).

Data reported by R. Juras et al. [20], based on the complex of microsatellite DNR markers, indicate a comparatively high genetic diversity of Lithuanian local horse populations. The actual heterozygosity of large-type Žemaitukai horses amounts to 0.758 and of Žemaitukai and Lithuanian Heavy Draught horses to 0.682 and 0.760, respectively. The expected heterozygosity of large-type Žemaitukai is 0.714, of Žemaitukai 0.641 and Lithuanian heavy draught horses 0.718. At twelve microsatellite loci the heterozygosity for large-type Žemaitukai was higher than the mean for domestic horses (0.750 and 0.697, respectively) [20]; in our study the average homozygosity was 33.41%. The currently determined high breed heterozygosity and low homozygosity do not reveal any significant narrowing of the genealogical structure in the population. If no measures are taken to prevent disappearing of the structural units of the breed, a sudden decrease in the number of breeding horses if the future will negatively affect the genetic diversity of the population. As several authors [21, 22] have noted, in a small horse population genetic measures have to be taken, such as, e.g., promoting planned mating with stallions and brood mares that go back to less popular ancestors and founders, in order to avoid reduction of the actual genetic diversity.

CONCLUSIONS

Analyses of blood group and protein polymorphism data indicated large-type Žemaitukai to have a very wide spectrum of alleles. The Kalmanas line is characterized as most phenotypically consolidated and having the highest levels of homozygosity and inbreeding. The highest genetic similarities were determined between the Šachtioras and Klintas lines. In our study of large-type Žemaitukai horse population, the average homozygosity was 33.41%.

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References

1. Macijauskienė V, Šveistienė R. Veterinarija ir zootechnika 2002; 19(41): 76–81.
2. Šveistys J, Patašiūtė R. Biologija 2000; 3: 72–76.
3. Hirsfeld L, Przesmycki F. Compt Rend Soc Biology 1923; 89: 1360–61.
4. Boveinienė B, Jatkauskienė V, Juras R. Gyvūnų kilmės patikrinimas – dabartis ir perspektyvos. Baisogala, 2001: 22–7.
5. Крикционас Б. Иммуногенетический полиморфизм лошадей Литовской ССР и его использование в племенном деле. Диссертация. Байсогала, 1990: 154.
6. Wright S. Genetics 1931; 16: 97–159.
7. Maijala K. Monitoring animal genetic resources and criteria for prioritization of breeds. The management of global animal genetic resources. Rome, 1999; 104: 73–83.
8. Stormont C, Suzuki Y. Genetics 1964; 50: 915–29.
9. Sanberg K. Guidelines for the interpretation of blood typing test in horses. ISAG recommendation. 1995;7–17.
10. Juneja RK, Gahne B, Sanberg K. Animal Blood Groups and Biochemical Genetic 1978; 9: 29–36.
11. Maijala K, Lindstrom G. Annales Agriculturae Fenniae 1966; 5: 76.
12. Nei M. Genetic distances between populations. Proceed Nat Acad Sci USA 1972; 106: 283–91.
13. Жыватовски ЛА, Мачурова АМ. Методические рекомендации по статистическому анализу иммуногенетических данных для использования в селекции животных. Дубровицы, 1974; 29.
14. Macijauskienė V, Juras R. Animal Science Papers and Reports 2003, 21: 35–46.
15. Juras R, Macijauskienė V. Žemės ūkio mokslai 2002; 1: 58–61.
16. Boveinienė B, Jatkauskienė V, Juras R. Biologija 2000; 3: 19–21.
17. Boveinienė B, Jeninas E, Jatkauskienė V et al. Gyvulininkystė 2000; 37: 29–34.
18. Juras R, Cothran EG. Biologija 2004; 4: 6–9.
19. Šveistienė R, Jatkauskienė V. Animal Breeding in the Baltics. Tartu, 2004; 225–30.
20. Juras R, Cothran EG, Klimas R. J Animal Science 2003; 53(4): 180–5.
21. Poncet PA, Pfister W, Muntwyler J et al. J Animal Breeding and Genetics 2006; 123: 114.
22. Bjornstad G, Gunby E, Roed KH. J Animal Breeding and Genetics 2000; 117: 307.

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STAMBIŪJŲ ŽEMAITUKŲ ARKLIŲ POPULIACIJOS GENETINĖ STRUKTŪRA IR KINTAMUMAS

Santrauka

Genetinio kintamumo saugojimas veislės viduje yra vienas iš pagrindinių žemės ūkio gyvūnų saugojimo koncepcijų. Mūsų darbo tikslas buvo nustatyti genetinius skirtumus ir genetinį kintamumą stambiujų žemaitukų populiacijos viduje. 1996–2005 m. Lietuvos veterinarijos akademijos Gyvulininkystės institute ištirta 138 stambiujų žemaitukų arklių kilmė, kraujo grupių ir serumo baltymų genetinių sistemų alelių dažnis, homozigotiškumas ir linijų genetinis panašumas. Genetinė analizė rodo, kad $A^{I^{AB}}$ genotipas yra dominuojantis, o $Tf^{FF, FO}$ – dažniausiai pasikartojantis. Kraujo grupių genetinėje sistemoje dažniausiai pasikartoja $D^{plghm, cgm, dk, dl}$ aleliai. Tyrimų metu nustatyta, kad eržilo Kalmano linija nuo kitų eržilų linijų skyrėsi pagal D^{dk} ir Es^{ll} alelių dažnumą. Stambiujų žemaitukų populiacija smarkiai sumažėjo. Pagal efektyvų populiacijos dydį ($N_e = 58$) ji patenka į pavojaus kategoriją, nustatytas žemas ($C_a = 33,41\%$) homozigotiškumo laipsnis.

Raktažodžiai: arkliai, populiacija, linijos, kraujo grupės, baltymų polimorfizmas, genų dažnis