SUPPLEMENTARY MATERIALS FOR

Malopolski Horse Stallions: Genetic Diversity Estimated on the Basis of Microsatellite DNA and Class I Markers

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Supplementary Materials Contents

Supplemental Information: History of Malopolski Horse Stallions References Table 3S. Frequency of alleles at erythrocyte antigen loci. Table 4S. Frequency of albumin alleles, protein GC, esterase, glycoprotein XK and transferrin. Table 5S. Observed heterozygosity (H_o) and polymorphic information content (PIC) for class I markers.

Supplemental Information

History of Malopolski Horse Stallions

The Malopolski horse was created in southern and south-eastern Poland over the centuries. Pastureland with a continental climate and very fertile soils were a perfect place for breeding horses. The primitive Polish horse living in these areas influenced the development of the breed and made the horse perfectly adapted to the local environment. Among all the warmblood Polish horse breeds, Malopolski horse possesses most of the features inherited from ancestors (e.g. longevity, fertility, excellent feed utilization, resistance to diseases, adverse climatic conditions and endurance during work). The name of the breed was established in 1962. The Malopolski horse studbook was released the following year. Until that time these horses were of "Malopolski type" - defined as a separate type of an extremely valuable horse group associated to the Malopolska region. Not only the geographical localisation influenced forming the breed, but also the political situation of 17th and 18th century Poland contributed to this process. Horses were used in battles with Tatars. There was a need to breed a light horse capable of overcoming long distances, quickly regenerating and not requiring large amounts of food during many long war expeditions. Also, the territorial neighbourhood of Turkey had an impact on the breed in these times – the breeders used oriental stallions, mainly Persian, Turkmen, Tatar bachmats and purebred Arabian. The new political situation in nineteenth-century Europe ended the era of Turkish influence. Afterwards, the breed was developed on the basis of Austro-Hungarian Halfbreds (Thoroughbreds x Arabian Horses) (SONDIJ 2011; SZYMANSKA 2017; ZABEK et al. 2006). Oriental half-blood lines of Austro-Hungarian origin played an especially important role in Malopolski horse breed creation (TOMCZYK-WRONA 2014).

Four sire lines were mostly involved in Malopolski horse breeding: Furioso, Gidran, Nonius and Przedswit. Three of these (Furioso, Gidran, Nonius) were also involved in the creation of other breeds.

The Furioso line derived from Thoroughbred stallion Furioso (Privateer - Miss Furey). This stallion, as a 5-year old horse, was bought in 1841 for Hungarian national studs. He mated in Babolna and Mezohegyes studs (Hungarian studs) with local mares and gave 95 sons and 81 daughters as a breeding result in total (SONDIJ 2011). Furioso, North Star and English Thoroughbreds sires created the Furioso breed; Furioso breed is present in Hungary, Romania and Slovakia (DAD-IS database 2018; KASARDA *et al.*, 2018).

The next line of Malopolski horse breed is the Gidran sire line. The progenitor of this line was Siglavy Gidran (purebred Arab stallion born in 1810 in the Syrian Arab Republic) imported to Mezohegyes stud (SONDIJ 2011). This stallion was also the founder of the Gidran breed –an Anglo-Arab horse breed from Hungary (POSTA *et al.* 2017). Now this rare native Hungarian horse breed is endangered (SZISZKOSZ *et al.* 2016).

Nonius line originates from the Nonius horse (Hungarian warmblood breed). The famous descendant was called Nonius Senior (Orion (Norfolk Roadster breed) – dam: Norman mare). This Anglo-Norman horse was born in 1810 in France and was located in Mezohegyes stud. He was not very popular for matting – he gave only 18 foals during 8 years. But after all his offspring made him a good reputation. The Nonius horse breed had two types: a small saddle type and a larger, heavier type commonly used for draft work. In Galicia the small type of Nonius horse breed was uncommon. They were rarely used in the Malopolska region until the import of 22 Nonius horse stallions to the national studs in the mid-1930s (KASARDA *et al.* 2018; POLISH HORSE PEDIGREE DATABASE 2018; MORAVCÍKOVA *et al.* 2016; SONDIJ 2011).

The Przedswit line (Polish name Przedświt) was founded by Przedswit (Knight of the Garter – The Jewel). This English Thoroughbred was born in 1872 in the Malopolska region. After great competition results, he was sold in 1876 to Austrian national studs in Piber. Przedswit was a progenitor of numerous purebred and half-bred sire lines. His half-bred son – Przedswit I (Przedswit - 54 Eclatante (Anglo-Norman mare)) was moved to stud in Radowce. He created his own sire lines: Przedswit II, III, V, VI and VII (POLISH HORSE PEDIGREE DATABASE 2018; SONDIJ 2011).

Sires from stud in Radowce played a great role in the creation of the Malopolski horse breed. This stud was established in the 18th century with typical half-bred horses from Hungary, Moldova, Poland, Turkey and the Caucasus. Most of them were of oriental type. However, the stud was reduced because of war and the political situation throughout the 19th and 20th centuries. Among all horses bred in Radowce stud, stallion Shagya X (born in 1899) had the greatest impact on the Malopolski horse breed.

The Malopolski horse was created using many oriental stallion types and lines (SONDIJ 2011). Today the breed has different proportions of Anglo-Arabian and oriental horses in its pedigree (ZABEK *et al.* 2006).

Moreover, not so long ago the breed had four regional types (variants): lubelski, kielecki, sadecki, dabrowsko-tarnowski. The first two were created using sires from Janow Podlaski, especially

from the Shagya line. At the same time, the lubelski type was influenced by English Thoroughbred, East-Prussian and Wielkopolski horse stallions. Characteristic features of both types were: noble, perfect movement and endurance during work. These light types of horses were used in sports and by cavalry. Sadecki type was formed in Malopolskie voivodeship. Natural conditions had an influence on creating smaller, noble animals with a lean constitution, descendants of Turkish, Hungarian and Transylvanian horses. Sadecki type horses were versatile in usage, durable, with a very balanced temperament. The greatest impact on this type had sires from Klikowa stud – mainly offspring of Furioso and Przedswit lines. Both sire lines are characterised by a pleasing appearance and aesthetic appeal. The sire lines from Klikowa were selected as the best for improvement of the breed (BOROWSKA & SZWACZKOWSKI, 2015; PIESZKA *et al.* 2014; SONDIJ 2011; SMUGALA *et al.* 2006; ZABEK *et al.* 2006)). The dabrowsko-tarnowski variant was of Anglo-Arabian type with high participation of the Gidran line. This type was universal in use and more noble than other types, but possessed the defects of Gidran (like soft back, little intense at withers and often strong lymphatic).

After World War II, the breed of Malopolski horse was kept in six national studs:

• Walewice - stud of dams of Shagya line, mostly offspring of sire Shagya X;

• Janow Podlaski (breeding of half-breed horses since 1919) – stud of half-Arabian sires and dams: mostly form Shagya, Dahomana and Amuratha; after World War II also half-Arabian dams from Babolna stud;

- Trzebienice Gidran line;
- Stubno mostly Przedswit and Furioso sire lines; marginally Nonius sire line;
- Prudnik stud of Malopolski horse with Gidran's dams;

• Ochaby (legal successor of Pruchna stud) – Anglo-Arabian purebred and half-bred dams imported from France in 1946, whose descendants were registered in the Malopolski horse stud book.

Today, breeding is conducted with studs of the Agricultural Property Agency: Walewice, Janow Podlaski, Prudnik and Ochaby. Increasingly valuable breeding material is in the hands of private breeders (SONDIJ 2011). The biggest influence on the Malopolski horse breed had stallions from national studs of stallions in Boguslawice, Bialka and Klikowa (NOGAJ & NOGAJ 2000; SONDIJ 2011).

According to the literature, the modern Malopolski horse is mainly an Anglo-Arabian with the features of indigenous horse. Crossbreeding changed their type marginally. Today, Malopolski horse (like the closely related Wielkopolski horse) is a general purpose horse (SEMIK & ZABEK 2013; SMUGALA *et al.* 2006; SONDIJ 2011; STACHURSKA *et al.* 2014).

Malopolski horses have also been used in sport. The first famous Malopolski horse was the stallion Ramzes (Ritterspon xx – Jordi xo), which won jumping contests in Germany during World War II. Since that time many Malopolski horses have been registered in history because of sport results. For example Artemor (Eros xx - Artemiza xo) winner of an Olympics gold medal in Equestrian at the 1980 Summer Olympics in individual jumping (SZYMANSKA 2017).

The breed was also used to create other breeds – for example mares of Malopolski horse (and Wielkopolski horse mares) were used in Polish Halfbred horse breeding (GURGUL *et al.* 2018; PIKULA & BOBIK 2005).

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Tables

Suppl. Table 3S. Frequency of alleles at erythrocyte antigen loci.

EAA adf 0.650 adg 0.110 b 0.040 - 0.200 EAC a 0.956 - 0.044 EAD adlr 0.002 bcmq 0.126 cegimnq 0.088 cgm 0.050 cgmq 0.024 delq 0.150 delq 0.150 delq 0.054 dfklr28 0.038 dghmqr28 0.030 dkl28 0.408 dlnqr 0.006 EAK a 0.020 - 0.980 EAP a 0.278 add 0.118 b b 0.022 d d 0.004 - - 0.578 EAQ b 0.020 c c 0.076 - - 0.516 - EAU a	Locus	Allele	Allele frequencies
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- 0.822		a4	0.144
		-	0.822

Locus	Phenotype	No. of	Phenotype	No. of	Allele
		phenotype*	frequencies	alleles	frequencies
AL	А	23	0.092	ALA 168	0.336
	В	105	0.420	ALB 332	0.664
	AB	122	0.488	-	
GC	F	221	0.884	GCF 471	0.942
	FS	29	0.116	GCS 29	0.058
ES	F	3	0.012	ESF 64	0.128
	FI	57	0.228		
	FS	1	0.004	ESI 424	0.848
	Ι	178	0.712		
	IS	11	0.044	ESS 12	0.024
XK	FK	3	0.012	XKF 3	0.006
	K	243	0.972	XKK 493	0.986
	KS	4	0.016	XKS 4	0.008
TF	D	7	0.028	TFD 100	0.200
	DF1	26	0.104	_	
	DF2	40	0.160		
	DH	5	0.020	TFF1 99	0.198
	DO	14	0.056	_	
	DR	1	0.004	_	
	F1	6	0.024	_	
	F1F2	45	0.180	TFF2 202	0.404
	F1H	3	0.012	-	
	F1O	10	0.040	_	
	F1R	3	0.012	-	
	F2	40	0.160	TFH 34	0.068
	F2H	16	0.064		
	F20	17	0.068	_	
	F2R	4	0.016	TFO 54	0.108
	<u>н 210</u> Н		0.010		0.100
		5	0.020	_	
			0.020	TED 11	0.022
		1	0.004	1 Г К 11	0.022
		3	0.012	_	
	OR	2	0.008		

Suppl. Table 4S. Frequency of albumin alleles, protein GC, esterase, glycoprotein XK and transferrin.

* No. of phenotype is not equal to number of individuals – we did not obtain results for protein alleles for one individual.

Suppl. Table 5S. Observed heterozygosity (H_o) and polymorphic information content (PIC) for class I markers.

Locus	Ho	PIC	
А	0.5238	0.4778	
С	0.0842	0.0808	
D	0.7759	0.7561	
Κ	0.0392	0.0384	
Р	0.5745	0.5117	
Q	0.5772	0.4924	
U	0.3027	0.2737	
AL	0.5770	0.4863	
GC	0.2052	0.1843	
ES	0.4392	0.3848	
A1B	0.0550	0.0544	
TF	0.7897	0.8831	
Mean	0.411	0.3853	