

Original scientific paper

MOLECULAR CHARACTERIZATION OF MARE FAMILIES IN THE SERBIAN POPULATION OF THE LIPIZZAN HORSE

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SUMMARY

The Lipizzan horse breed was established in the 16th century in Lipica and later spread throughout Central and Eastern Europe. In addition to six classical stallion lines, 17 classical mare family lines were established in the old Lipica stud. Additional 44 mare family lines, including local lines, were established in other Lipizzan studs. The founders of the classical Lipizzan mare family lines date back to the period between the 18th and 20th century and came from various breeds, including the Karst, Spanish, Italian, Kladruber and Arabian horses. The history of the Lipizzan mare family lines is well described and supported by the availability of 44 nucleotide sequences of the mtDNA control region. In this study, the mtDNA control region of 23 Serbian Lipizzans, representing the established Lipizzan mare family lines, as well as the local mare family line Zora, have been sequenced. The objective of this study was to test whether the Zora mare family line could be distinguished from other Lipizzan mare family lines according to the mtDNA sequence. Sequences were aligned to the entire set of mtDNA control region sequences representing 44 Lipizzan mtDNA haplotypes. Based on the alignment, a median-joining network was constructed. The individuals in this study formed a cluster with the existing Lipizzan mare family lines. However, a total of ten individuals belonging to the local Zora mare family line formed a compact cluster close to the mtDNA haplotypes, representing the classical mare family lines Wera, Betalka, and Monteaura. Additional pedigree data are needed to clarify the origin of the mtDNA haplotype variant identified in this study.

Key words:

Lipizzan horse,
mtDNA haplotype,
maternal lineage,
Zora

INTRODUCTION

The Lipizzan horse breed was established in Lipica in 1580. The goal of the Lipica stud was to breed riding and light carriage horses for the Habsburg court. In the 17th century, Lipizzan horses began to spread throughout Central and Eastern Europe (Nürnberg, 1993). In addition to six classical stallion lines (Conversano, Neapolitano, Favory, Maestoso, Pluto and Siglavy) and two non-classical stallion lines (Tulipan, Incitato), numerous mare family lines were developed following strict breeding rules. Of these, only mare family lines that originated from the Lipica stud prior to World War II are recognised as classical mare family lines (Dovč et al., 2006). Eleven mare family lines were established in the 18th century with mares from studs in Karst (Sardinia, Spadiglia, Argentina), Kladruber (Afrika, Almerina, Presciana, Englanderia, Europa), Kopcan (Stronella, Famosa), and Friederiksberg (Deflorata). In the 19th century, the Arabian horses were used for breeding. There are four classical Lipizzan mare family lines of

Arabian origin (Gidrane, Djebrin, Mercurio and Theodorosta). Two other mare lines were established later (Capriola and Rava) (Druml, 2000). Other mare family lines originated from studs established on the territory of the former Austro-Hungarian Empire (Nürnberg, 1993). Currently, the Lipizzan International Federation (LIF) recognizes 67 Lipizzan mare family lines, 17 classical, 16 Croatian, 17 Hungarian, and 17 Romanian.

While the history of the Lipizzan mare family lines has been extensively documented based on historical and pedigree data, the study of the nucleotide sequences of the mtDNA control region provides valuable insights into the history of the mare family lines. The maternal inheritance and sequence variation in the D-loop region allow us to trace the Lipizzan mare family lineages back to the 18th century. There are 44 different Lipizzan mtDNA haplotypes based on the 385 bp D-loop region (nt 15450 – nt 15834) (Kavar et al., 1999; Kavar et al., 2002; Kavar et al., 2004, Čačić, 2011). Based on a D-loop sequence analysis, a total of 49 Lipizzan horses, representing 16 mare family lines from the Lipica stud were classified into 13 different mtDNA haplotypes. Sequence analyses revealed a high variability of mtDNA haplotypes within the Lipizzan horse breed, which could be attributed to the heterogeneous origin of the breed (Kavar et al., 1999). For example, the Lipizzan haplotype Capriola has also been found in the Pura Raza Española, Lusitano, Kerry bog pony, Arabian, Norwegian Fjord, Holsteiner, Rhineland Heavy draft, Chillean Criollo, Zemaitukai, Trakehner, Duellmeyer and Barb horse breeds (Kavar & Dovč, 2008). Kavar et al. (2002) sequenced the mtDNA control region of 212 Lipizzans representing 56 mare family lines from eight European traditional Lipizzan studs (Lipica, Piber, Monterotondo, Szilvasvarad, Beclean, Fagaras, Đakovo and Topolcianky). The sequence analysis revealed 37 different mtDNA haplotypes (13 were described in their previous study, and 24 were newly discovered). The Batosta haplotype was present in all the studs, whereas other haplotypes were only observed in one or two studs. Two additional Lipizzan mtDNA haplotypes (Boka and Y) were identified after sequencing 53 Lipizzan horses from private Slovenian breeders (Kavar et al., 2004). In the Croatian Lipizzan population, 23 mtDNA haplotypes were identified after sequencing the D-loop of 249 horses belonging to 24 Lipizzan mare family lines (Čačić, 2011). Most of these belonged to the classical mare family lines such as the Croatian mare family lines (32.5%), Romanian mare family lines (2.8 %), and Hungarian mare family lines (0.8%). However, Čačić et al. (2011) discovered five new Lipizzan mtDNA haplotypes (MH1-5). The Lipizzan mtDNA sequences (n=46) of the 5' region of the D-loop (385 bp long) were deposited to GenBank (Kavar et al., 1999, Kavar et al., 2002, Kavar et al., 2004).

The Karađorđevo stud farm, located in Serbia, was founded in 1885 as a state stud of the Austro-Hungarian monarchy. In the beginning, foals of the Nonius breed were raised in Karađorđevo, which were bought from private breeders from all over the Austro-Hungarian Empire (mainly male foals were brought from the Hungarian stud Mezchegues). The foals were reared for up to a year and then returned to Hungary, where they were used for military purposes. Since 1921, the Karađorđevo stud farm has been the state stud farm of Serbia, breeding Lipizzan horses with animals originating from other state stud farms such as Lipica, Đakovo, Lipik, and privately owned studs. Since its founding, the Karađorđevo stud farm has produced several hundred stallions for breeding, which have been leased to horse cooperatives, associations, equestrian clubs, agricultural farms, and individual breeders throughout Serbia. The selection and breeding have been focused on producing horses with a deep, wide and bony frame, generous and elegant movements, usable in team sports and for riding (Štrbac et al., 2023). Nowadays, there are Lipizzan horses in Karađorđevo representing the mare family lines such as Deflorata and Gidrane, and also Zora and Sikra, which are currently not recognised as Lipizzan mare family lines by LIF. The objective of this study was to determine whether the Zora mare family line can be distinguished from other Lipizzan mare family lines according to the mtDNA sequence.

MATERIAL AND METHODS

Sampling and DNA extraction

Hair roots were collected from 23 Lipizzan horses from Serbia, representing the established Lipizzan mare family lines (n=13) and the local mare family line Zora (n=10). DNA was extracted using the DNA isolation kit (Qiagen DNeasy Blood & Tissue Kit).

Mitochondrial DNA analysis

The primer pair FP (5'- AAC GTT TCC TCC CAA GGA CT-3') and RP (5'- GCA TTT TCA GTG CCT TG CTT-3') were used to amplify a segment of the mitochondrial DNA control region, spanning in the region between nucleotide position 15,402 and 22 (Aberle et al., 2007). The fragments were sequenced by the Sanger sequencing service provider (Macrogen Europe, Amsterdam, the Netherlands).

Bioinformatics analysis

After receiving the sequencing data, the quality of the chromatograms was visually inspected and the sequences were proofread using the MEGA 11 software (Tamura et al., 2021). The sequences representing the Lipizzan mtDNA haplotypes were downloaded from GenBank. The accession numbers of short sequences (385 bp) of 44 Lipizzan haplotypes identified in eight European Lipizzan horse studs are as follows: AY057413.1 (Allegra), AF168691.1 (Batosta), AF168689.1 (Betalka), AF168690.1 (Capriola), AF168695.1 (Dubovina), AY057433.1 (Gaetana), AF168696.1 (Gratiosa), AY057416.1 (Monteaura), AF168692.1 (Slavina), AF168694.1 (Strana), AF168698.1 (Thais), AF168693.1 (Trompeta), AF168697.1 (Wera), AY057420.1 (A), AY057421.1 (B), AY057422.1 (C), AY057423.1 (D), AY057424.1 (E), AY057425.1 (F), AY057426.1 (G), AY057419.1 (H), AY057427.1 (I), AY057428.1 (J), AY057432.1 (K), AY057430.1 (L), AY057431.1 (M), AY057434.1 (N), AY057415.1 (O), AY057414.1 (P), AY057417.1 (Q), AY057409.1 (R), AY057408.1 (S), AY057412.1 (T), AY057410.1 (U), AY057411.1 (V), AY057418.1 (X), AY057429.1 (Z), DQ233731.1 (Boka) and DQ233732.1 (Y), KP731354.1 (MH1aL), KP731355.1 (MH1bL), KP731356.1 (MH2L), KP731357.1 (MH3L), KP731358.1 (MH4L) and KP731359.1 (MH5L).

The multiple sequence alignment was generated by incorporating sequences from the current study, the reference sequence (GenBank accession number X79547), and 44 mtDNA 385 bp long haplotype sequences using QIAGEN CLC Genomics Workbench 23.0.1 (<https://digitalinsights.qiagen.com/>). The sequences were categorized into mtDNA haplotypes (mitotypes) using DnaSP v 6.12.03 software (Rozas et al., 2017). Subsequently, a median-joining network was constructed on the basis of the alignment, employing the PopArt 1.7 software (Leigh & Bryant, 2015).

RESULTS AND DISCUSSION

In this study, we focused on the Lipizzan horses bred in Serbia, specifically delving into the Zora mare family line, which we traced back to 1938 (Fig. 1). We sequenced the D-loop of 13 horses from the established Lipizzan mare family lines and 10 horses belonging to the Zora mare family line.

The 385 bp D-loop region (nt 15450 – nt 15834) sequences of horses from the Zora mare family lines show two mutations when compared to Monteaura. At position 15604, Monteaura has a G, whereas Zora has an A (Fig. 2A). Additionally, I, J, K, L, N, Z, Gaetana, and the reference sequence (GenBank X79547) exhibit an A at this position. The second discrepancy from Monteaura emerges at position 15762, where Zora shows an A, while all other Lipizzan haplotypes (along with the reference sequence) exhibit a G. Nevertheless, at position 15762, the chromatograms of all Zora samples indicated a polymorphic site (A/G), indicating heteroplasmy (Fig. 2B). In horses, varying incidences and levels of heteroplasmy have been observed among different populations. The incidence of heteroplasmy might be higher than initially estimated in sequencing studies and within the GenBank dataset (Zhao et al., 2015).

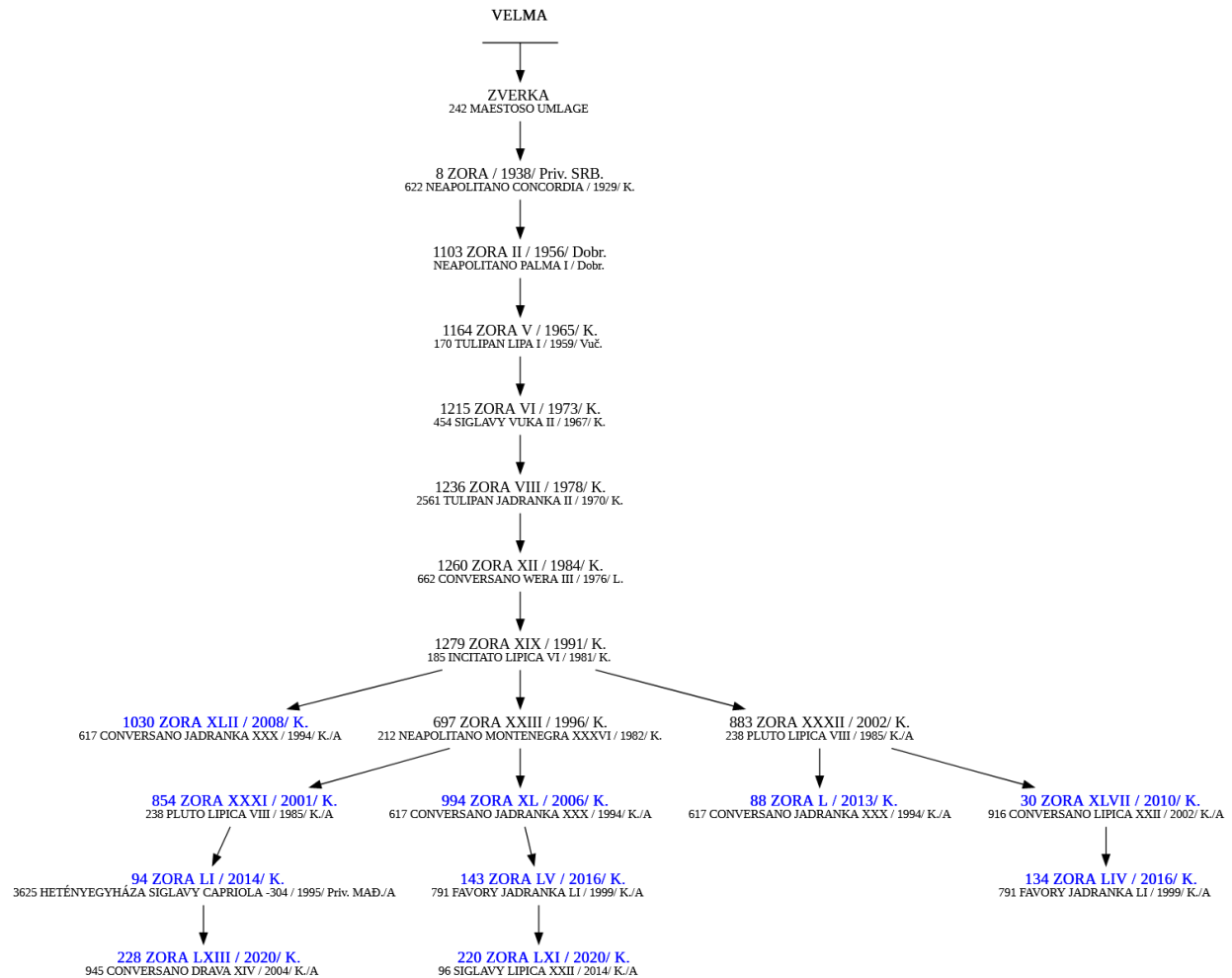


Figure 1. The Zora pedigree presented in a tail-female format

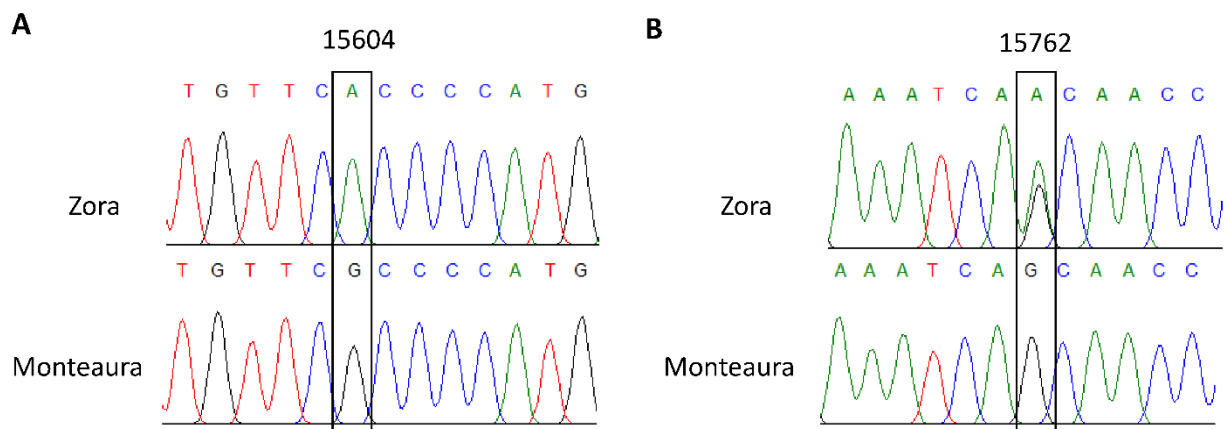


Figure 2. Two mutations of the Zora mare family lines when compared to Monteaura: A) At position 15604, Monteaura shows a G, whereas Zora displays an A. B) At position 15762, Zora showcases both A and G, while all other Lipizzan haplotypes (including the reference) solely exhibit G. The chromatogram indicates a polymorphism, suggesting heteroplasmy at this position

The individuals in this study formed a cluster with the existing Lipizzan mare family lines. However, all ten individuals belonging to the local Zora mare family line formed a compact cluster close to the mtDNA haplotypes, representing the classical mare family lines Monteaura, Betalka, Allegra, and Wera (Fig. 3).

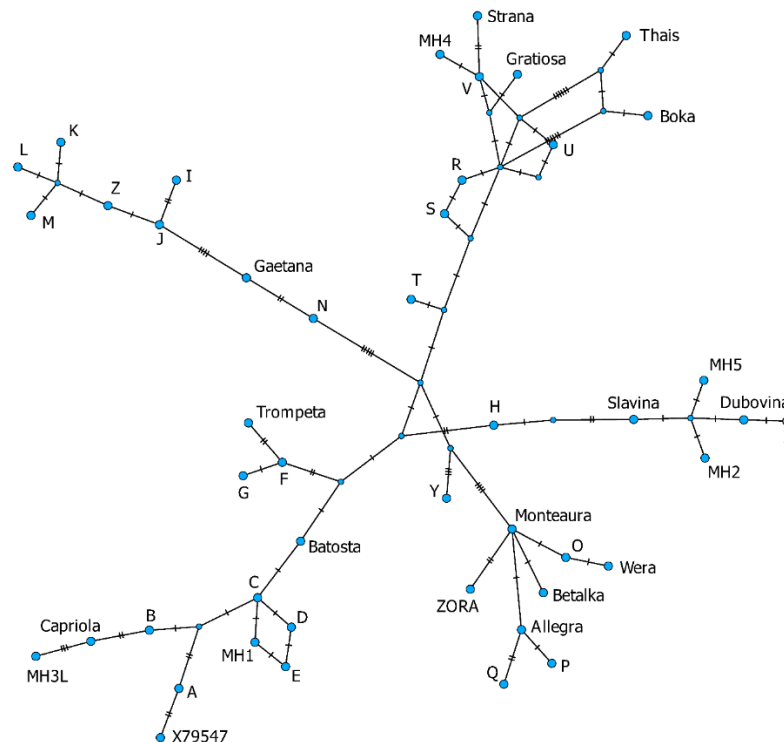


Figure 3. Median-joining haplotype network based on the 44 Lipizzan mtDNA haplotypes, the reference sequence (GenBank X79547) and the haplotype of Zora mare family line. The number of hatch marks indicates the number of mutational differences between the haplotypes

CONCLUSION

The mtDNA haplotype found within the Zora mare family line falls into the cluster of classical mare family lines (Monteaura, Wera, Betalka, and Allegra). Notably, there are two mutations compared to Monteaura, one of which is polymorphic, thus suggesting heteroplasmy. Further genotyping is required to confirm heteroplasmy and to estimate the proportion between the two variants. However, additional pedigree data are needed to clarify the origin of the potentially novel mtDNA haplotype identified in this study.

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Conflict of interest: The authors declare that they have no conflict of interest.

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