

Finding ‘the long-lost’ Croatian Lipizzan mare families

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Lipizzan horse breed was founded in 1580 in Lipica and introduced to Croatia in 1800 by earl Andrija Jankovich-Bésán. Earl Jankovich founded stallion bloodline Tulip and mare families Czirka, Ercel, Traviata, Margit, Manczi and 502 Moszgo Perla. Herd books showed that South African Lipizzan population is based on Czirka and Ercel mare families that are originally Croatian thus provided potential genetic enrichment of Croatian Lipizzan gene pool. In this study a 648 bp mitochondrial DNA fragment from 50 South African Republic Lipizzan horses was analysed and 249 sequences from Čačić doctoral thesis was retrieved. Mitochondrial DNA analysis of South African Lipizzan horses and their comparison with Croatian Lipizzan horses present that South African Lipizzan horses have five unique haplotypes but still maintain connection with Croatian Lipizzan by sharing a haplotype. Future analysis with high throughput genetic marker such as SNP or WGS will surely provide interesting results.

Keywords: Croatian Lipizzan horse, mitochondrial DNA analysis, shared haplotypes, South African Lipizzan horse

1 Introduction

Lipizzan horse breed is composed of eight stallion bloodlines and 63 mare families (Lipizzan International Federation, 2001) of which one stallion bloodline (Tulip) and 16 mare families (Czirka, Ercel, Traviata, Margit, Manczi, 502 Moszgo Perla, Rendes, Hamad-Flora, Eljen-Odaliska, Miss Wood, Fruska, Mima/Nana, Alka, Karolina, Munja, Rebeca-Thais) have Croatian origin (Čačić, 2008). Today only six mare families are represented in Croatian breeding. Lipizzan horses were first introduced to Croatia in 1800 by earl Andrija Jankovich-Bésán (Steinhausz, 1924). He founded stallion bloodline named Tulip and six mare families Czirka, Ercel, Traviata, Margit, Manczi and 502 Moszgo Perla. Mare families Czirka and Ercel are today present only in South African Republic (SAR) Lipizzan population (Čačić, 2011). Following the political situation in Croatia and Europe in 1929 earl Elemer Jankovich-Bésán migrated to Hungary where he stayed until 1944 then moved to Bavaria (Jankovich-Bésán, 2010), in 1947 moved to England and in 1948 finally to South African Republic (Dalglish, 2011). Along with his family he took 14 Lipizzan horses (nine mares with three

foals belonging to Czirka and Ercel mare family and two 2-year old stallions) that he transported to the Mooi River farm in SAR. Those 14 Lipizzan horses were nucleus that provided today South African Lipizzan population. Investigating breeding records of Lipizzan South African Center it is identified that present SAR Lipizzan population is composed of Czirka and Ercel mare families and that these two Croatian mare families were foundation of Lipizzan breeding in SAR since 1948 until today (Lipizzan South African Center, Breeding books). Since mare families Czirka and Ercel were transported from Croatia to SAR they disappeared not only from Croatian Lipizzan breeding lines but from the Europe breeding. These Croatian mare families represent valuable gene pool that will enrich Lipizzan genetic variability. The aim of this study was to characterize maternal genetic structure of SAR Lipizzan population as well as to compare them with Croatian Lipizzan population.

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2 Materials and methods

DNA of a 68 Lipizzan horse hair samples from South African Republic were extracted with Qiagen Blood and tissue kit (Qiagen, Germany) according to the manufacturer instruction. Sequencing was performed following Aberle et al. (2007). Thus, a 1280 bp mitochondrial DNA D-loop fragment was amplified using forward (5'-AAC GTT TCC TCC CAA GGA CT-3') and reverse (5'-GCA TTT TCA GTG CCT TG CTT-3') primers. The polymerase chain reaction was performed in 20 µl reaction mix containing approximately 50 ng of total DNA, 0.2 µM of each forward and reverse primer and Master Mix. The PCR was carried out in a Mastercycler (Eppendorf, Germany) and consisted of an initial denaturation step at 95 °C for 8 min followed by 38 cycles at 95 °C for 1 min, annealing at 62 °C for 1 min, and elongation at 62 °C for 31 min with a final elongation step of 7 min at 72 °C. PCR products were purified using Wizard® SV Gel and PCR Clean-Up System (Promega, USA) following the manufacturer's recommendations. DNA sequencing was performed from the PCR product on an ABI 3130 DNA automated sequencer (Applied Biosystems, USA) using the ABI Prism Big Dye Terminator 3.1 Sequencing Kit (Applied Biosystems, USA). For a more accurate comparison, total of 249 Croatian Lipizzan horse sequences from doctoral thesis of Čačić (2011), were added for this study. All sequences were aligned with referent sequence X79547 (Xu and Arnason, 1994) using Clustal omega (McWilliam

et al., 2013) and analyses were performed based on the 648 bp truncated fragment. DnaSP v5.10 (Librado and Rozas, 2010) was used to determine unique haplotypes. The haplotype network was performed using PopArt 1.7 (Bandelt et al., 1999).

3 Results and discussion

The analysis was performed on the 648 bp long mtDNA control region fragment. During process of DNA extraction, PCR and sequencing we had to discard 18 samples due to the low sample quality. On final data set of 299 Lipizzan horse sequences, 50 sequenced in this study and 249 retrieved from the Čačić (2011) doctoral thesis, 46 different haplotypes and 79 polymorphic sites were found. Sequences retrieved from Čačić doctoral thesis are all Croatian Lipizzan horses with different family origin (classical, Croatian or Hungarian) according to Studbook of the Origins of the Lipizzaner Breed (Spanish Riding school, 2010). SAR Lipizzan horses clustered in six haplotypes, five unique and one shared with horses from Croatian and classical Lipizzan families. Detailed characterization of all 46 observed haplotypes is presented in Figure 1. The D-loop region sequenced in this study was highly polymorphic, showing haplotype diversity (Hd) of 0.938 (sd 0.004) and nucleotide diversity (Nd) of 0,01617 (sd 0,00044). A median-joining network of the Lipizzan haplotypes is showed in Figure 2. As it is concluded from the median-joining network two horse

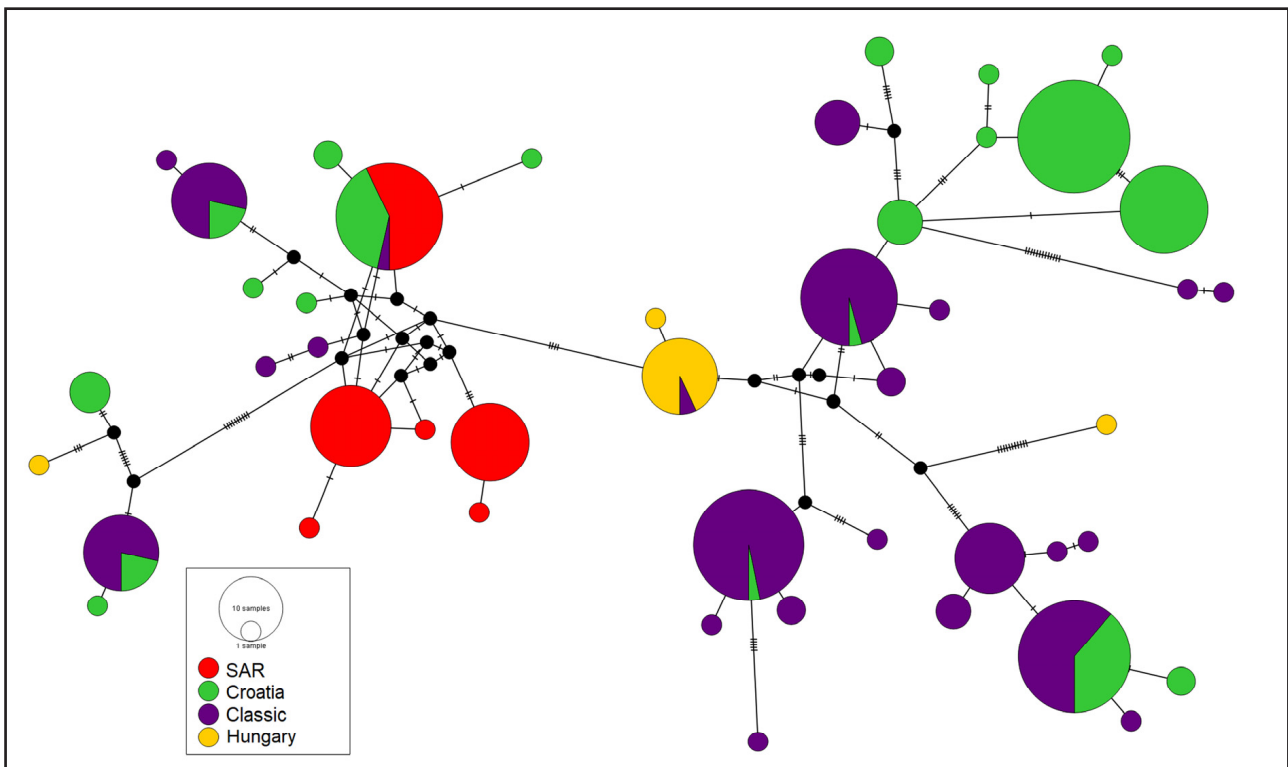


Figure 2 Median-joining network of 46 Lipizzan haplotypes. Little dashes represent the number of mutations

populations are related, even sharing one haplotype. Considering the small size of the Lipizzan population, inclusion of new haplotypes will greatly enrich breed's maternal genetic diversity. This is the first genetic analysis of SAR Lipizzan population and it provides us insight into results that will be obtained with future analysis of whole genome.

4 Conclusions

Old historical records saying that South African Lipizzan horses originate from Croatian breeding lines are confirmed by reviewing the SAR Lipizzan herd books. Croatian mare families Czirka and Ercel are foundation of current SAR Lipizzan breeding. As a first genetic analysis of maternal families, it is visible that SAR population is close to Croatian Lipizzan population. Introduction of the long-lost Croatian mare families will have great contribution to the maternal genetic diversity of Lipizzan population. Surely the future analysis on the whole genome using SNP chip or WGS will give us better understanding of the connection between two populations and will allow us to compare the SAR Lipizzan population with the other European and world Lipizzan populations.

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