Finding ‘the long-lost’ Croatian Lipizzan mare families

Mato Čačić1, Vesna Orehovački1, Marko Ećimović2, Elemér Jankovich Bésán3, Lilian Moler3, Ivana Drzaic4, Vlatka Cubric-Curik4*, Ino Curik4
1 Croatian Agricultural Agency, Ilica 101, 10000 Zagreb, Croatia
2 Svetog Marka 167, 35211 Trnjani, Croatia
3 Lipizzan South Africa Centre, 1 Dahlia Road, Kyalami 1684, South African Republic
4 University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia

1 Introduction

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
Figure 1  Polymorphic nucleotide positions within 648-bp fragment of the mitochondrial DNA control region found in 249 South African and 50 Croatian Lipizzan horses. Nucleotide positions are numbered according to the reference sequence GenBank X79547 (Xu and Arnason, 1994) presented as H1. H2-H6 are South African haplotypes, H7 is shared haplotype and H8-H47 are Croatian haplotypes.
2 Materials and methods

DNA of 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 TCA 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
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 ErceI 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 longest 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.

Acknowledgements
This study was supported by the Croatian Science Foundation under the Project IP-11-2013_9070 (Utilisation of the whole mitogenome in cattle breeding and conservation genetics; http://mitotauromics.agr.hr/).

References


