ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 67 130 Number 6, 2019

# LEVEL OF INBREEDING IN NORIK OF MURAN HORSE: PEDIGREE VS. GENOMIC DATA

Radovan Kasarda[1], Nina Moravčíková[1], Ondrej Kadlečík[1], Anna Trakovická[1],
Marko Halo[2], Juraj Candrák[1]

[1] Department of Animal Genetics and Breeding Biology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

[2] Department of Animal Husbandry, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

To link to this article: https://doi.org/10.11118/actaun201967061457
Received: 13. 8. 2019, Accepted: 17. 10. 2019

To cite this article: KASARDA RADOVAN, MORAVČÍKOVÁ NINA, KADLEČÍK ONDREJ, TRAKOVICKÁ ANNA, HALO MARKO, CANDRÁK JURAJ. 2019. Level of Inbreeding in Norik of Muran Horse: Pedigree vs. Genomic Data. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 67(6): 1457–1463.

## Abstract

The objective of this study was to analyse the level of pedigree and genomic inbreeding in a herd of the Norik of Muran horses. The pedigree file included 1374 animals (603 stallions and 771 mares), while the reference population consisted of animals that were genotyped by using 70k SNP platform ($n = 25$). The trend of pedigree inbreeding was expressed as the probability that an animal has two identical alleles by descent according to classical formulas. The trend of genomic inbreeding was derived from the distribution of runs of homozygosity (ROHs) with various length in the genome based on the assumption that these regions reflect the autozygosity originated from past generations of ancestors. A maximum of 19 generations was found in pedigree file. As expected, the highest level of pedigree completeness was found in first five generations. Subsequent quality control of genomic data resulted in totally 54432 SNP markers covering 2.242 Mb of the autosomal genome. The pedigree analysis showed that in current generation can be expected the pedigree inbreeding at level 0.23% ($\Delta F_{PEDi} = 0.19 \pm 1.17\%$). Comparable results was obtained also by the genomic analysis, when the inbreeding in current generation reached level 0.11%. Thus, in term of genetic diversity both analyses reflected sufficient level of variability across analysed population of Norik of Muran horses.

Keywords: horse, genetic diversity, inbreeding, local population, Norik of Muran

## INTRODUCTION

The Norik of Muran is unique draught horse breed mainly intended for work in forestry and agriculture, named for part of the Gemer region in central southern Slovakia on the Hungarian border. This local coldblood horse breed was developed from the mid-20th century by crossing Hutsul and local heavy horses with Norik and Belgian Draught at the Velka Luka stud (Porter *et al.*, 2016). In recent decades, the Austrian Noriker has been also included in the breeding process of the Norik of Muran (Halo *et al.*, 2018). Due to its good character, constitution and excellent manoeuvrability it is now used not only as draught power but also for hippotherapy and sport riding (Pošivák *et al.*, 2015). In Slovakia, the Norik of Muran breed is considered as one of the national biodiversity treasures that significantly contributes to the rural landscape and heritage conservation (Halo *et al.*, 2018). However, its population size is low (current nucleus includes only 174 animals) and the pedigree book of this breed is closed (Porter *et al.*, 2016; Halo *et al.*, 2018). Both of this factors may have a negative impact on the future development of this unique breed mainly with respect to its genetic variability.

An important objective of breeding strategy in such small populations is to keep the highest possible level of biodiversity represented by genetic variability and the minimum level of inbreeding. Previous studies showed that genetic improvement without control of inbreeding can led to the significant loss of genetic variability and inbreeding depression negatively affecting fitness, productive or morphological traits (e.g. Pjontek *et al.*, 2012; Vostrá-Vydrová *et al.*, 2016; Vostrý *et al.*, 2017). By slowing down inbreeding progress and avoiding the mating of related individuals, genetic variability can be maintained or at least its decreasing can be slowed down whereby the preservation of the breed may be attained during a longer period (Somogyvári *et al.*, 2018). Traditionally, the level of inbreeding within livestock populations including horses is estimated based on the pedigree data. But, inaccurate pedigrees may erroneously assign individuals to false lineages or breed memberships resulting in wrong estimates of inbreeding and coancestry. Moreover, discrepancies in pedigree records can lead breeders seeking to limit inbreeding into making misguided breeding decisions (Al Abri *et al.*, 2017). One of the way how to solve this is utilization of the genome-wide SNP panels that provide very useful tool to increase the resolution of lineage assignments and allow the calculation of inbreeding and relatedness on genome-wide level.

The aim of this study was to compare the level of inbreeding resulted from pedigree and genomic data in a herd of 25 Norik of Muran horses.

## MATERIALS AND METHODS

The pedigree file (PF) included overall 1374 Norik of Muran horses (603 stallions and 771 mares), while the reference population (RP) consisted of 25 animals that were genotyped by GGP Equine70k chip. Animals for genotyping were selected based on the pedigree analysis in order to cover the gene pool of current population of Norik of Muran breed in Slovakia.

The completeness of pedigree data was expressed by calculation of four indicators; maximum number of generations, number of full generations, equivalent complete generations and MacCluer's index (MacCluer *et al.*, 1983) by using Endog v4.8 software (Gutiérrez and Goyache, 2005). The quality of genomic data was then analysed by R package *plinkQC* (Anderson *et al.*, 2010) and PLINK v1.9 (Chang *et al.*, 2015) to select only autosomal SNP markers with known chromosomal position with call rate higher than 10% and minor allele frequency (MAF) higher than 1% across animals. The HWE (Hardy-Weinberg equilibrium) limit was set to 0.00001 to adhere to mendelian inheritance patterns.

The pedigree inbreeding ($F_{PED}$) defined as the probability that an individual has two identical alleles by descent were calculated by means of the classical formulas for increase in inbreeding ($\Delta F_{PED}$) and individual increase in inbreeding ($\Delta F_{PEDi}$) using Endog v4.8 software (Gutiérrez and Goyache, 2005). The increase of inbreeding ($\Delta F_{PED}$) for each generation was computed according to Gutiérrez *et al.* (2009) as follows:

$$\Delta F_{PED} = \frac{(F_t - F_{t-1})}{(1 - F_{t-1})}, \qquad (1)$$

where $F_t$ and $F_{t-1}$ are the average inbreeding at the $i^{th}$ generation. The individual increase in inbreeding was computed as:

$$\Delta F_{PEDi} = 1 - \sqrt[t-1]{1 - F_i}, \qquad (2)$$

where $F_i$ is the individual coefficient of inbreeding and $t$ is the equivalent complete generations (Maignel *et al.*, 1996). In addition, the average relationship coefficient (*AR*), defined as the probability that an allele randomly chosen from the whole population in pedigree belongs to given animals, were calculated.

The individual genomic inbreeding ($F_{ROH}$) was calculated by using R package detectRUNS (Biscarini *et al.*, 2018) according to formula proposed by McQuillan *et al.* (2008):

$$F_{ROH} = \frac{\sum L_{ROH}}{L_{genome}}, \qquad (3)$$

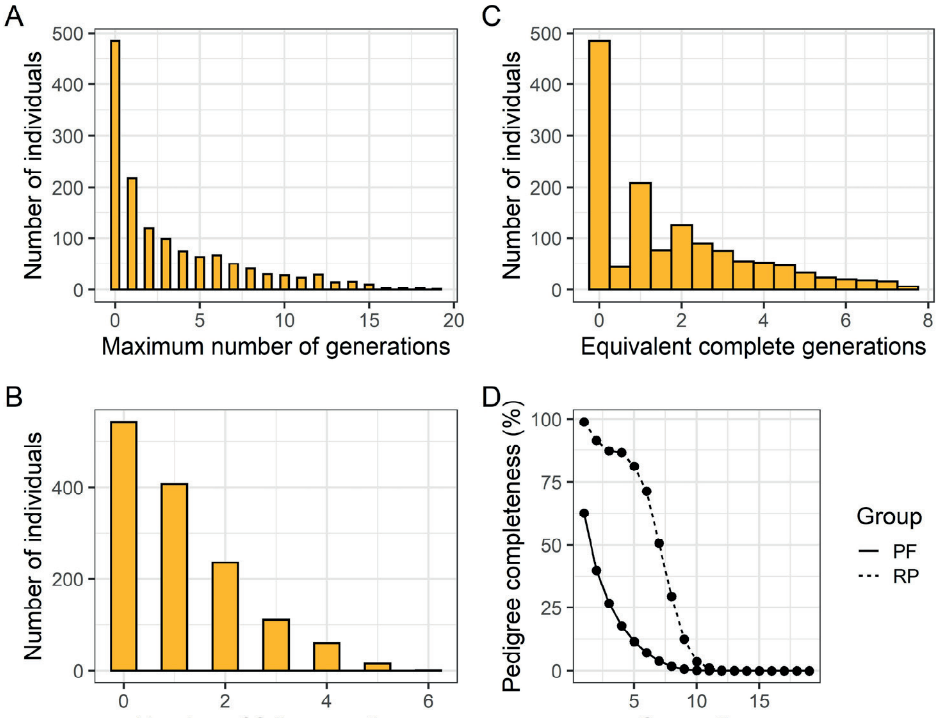
where $\Sigma L_{ROH}$ is the sum of length of all ROH (runs of homozygosity) detected in an individual and $L_{genome}$ is the total length of the genome covered by all autosomal SNP markers left in the database after SNP pruning (2,242,040 kb). The ROH segments were defined as follows: minimum number of SNPs in a run 15, minimum length of run 500 kb, maximum distance between consecutive SNPs in a window 1000 kb, lower density limit 1 SNP per 100 kb and no missing or heterozygous SNPs in a run (Nolte *et al.*, 2019). Based on the assumption that the length of ROHs reflects different generations of ancestors in the past (Ferenčaković *et al.*, 2013), the genomic inbreeding coefficient was expressed separately for five length ROH categories (0–2 Mb, 2–4 Mb, 4–8 Mb, 8–16 Mb, > 16 Mb).

## RESULTS AND DISCUSSION

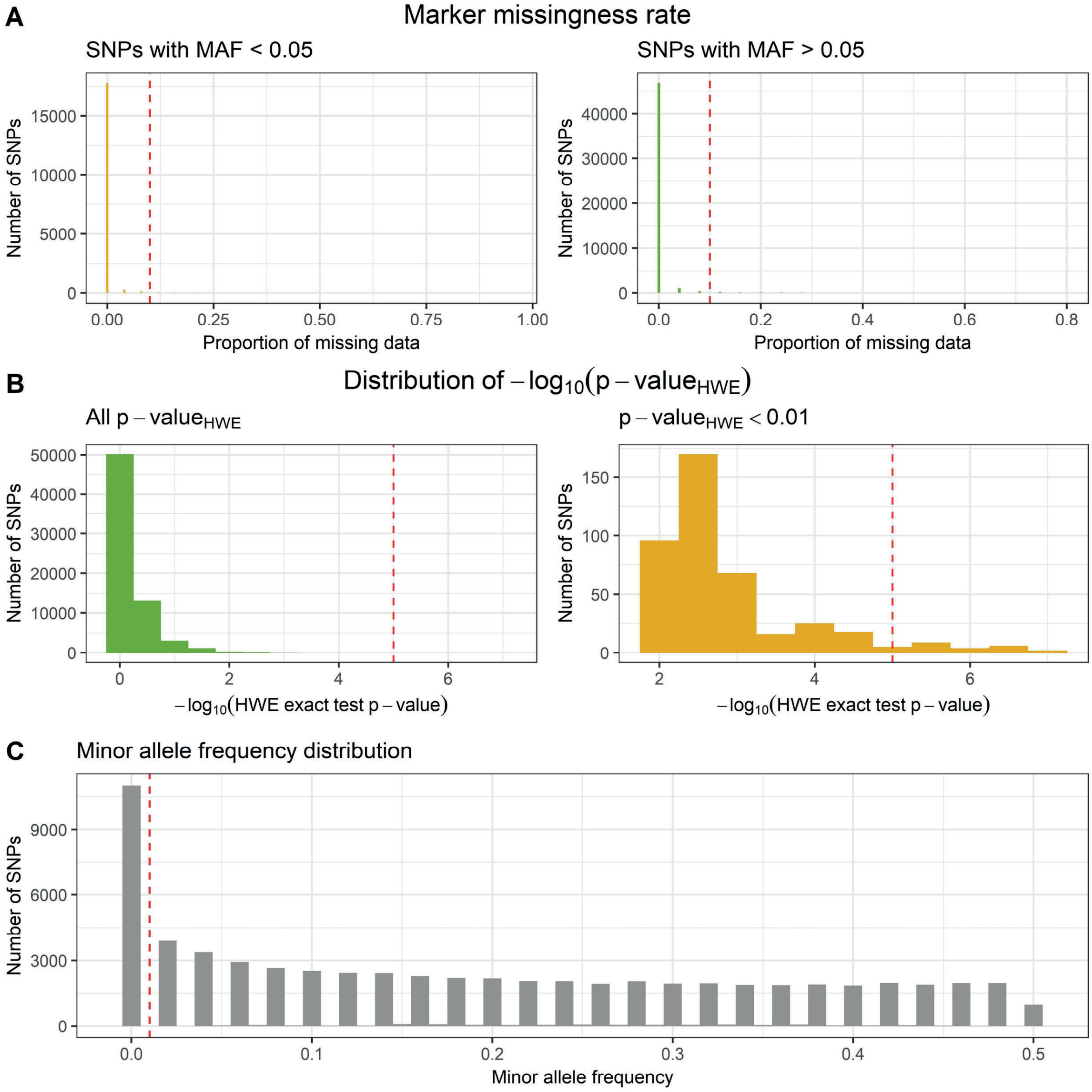
A maximum of 19 generations was found in pedigree file (Fig. 1). The average maximum number of generations traced was 3.12 ± 3.89 and 12.67 ± 1.92 in pedigree file and reference population, respectively. As expected, the average number of full generations traced was higher in reference population (2.75 ± 1.62) compared to pedigree file (1.12 ± 1.21). The equivalent complete generation was 1.72 ± 1.85 in pedigree file, while in reference population 6.14 ± 0.90. The trend of pedigree completeness according to MacCluer's index is shown in the Fig. 1D. The results similarly showed higher completeness of pedigree information in reference population, when the highest level of pedigree completeness was found in first five generations. In first generation the reference population showed totally 98.86% of known ancestors, while in pedigree file only 62.56% of known ancestors was detected. The completeness of genealogical information in pedigree file according to applied indexes is shown in the Fig. 1. The decrease of pedigree completeness across the generations of ancestors in horses was well documented in previous studies (Pavlík *et al.*, 2014; Vostrý *et al.*, 2017; Somogyvári *et al.*, 2018; Halo *et al.*, 2018). Compared to this study, Druml *et al.* (2009) reported for Austrian Noriker higher pedigree completeness. Similar level of completeness was found in Slovak Sport Pony (Pjontek *et al.*, 2012), Czech Sport Pony (Vostrý *et al.*, 2017) or German Paint horse (Siderits *et al.*, 2013).

The Fig. 2 shows the per-marker quality of genomic data. From overall 71947 SNP markers, only 68214 were located on autosomes. Totally, 1762 SNPs were filtered out due to high level of incomplete genotypic information, 11973 markers didn't meet the minor allele frequency threshold and 47 SNPs were excluded due to HWE limit. After the applied SNP pruning left in the final database 54432 SNP markers covering 2.242 Mb of the autosomal genome.

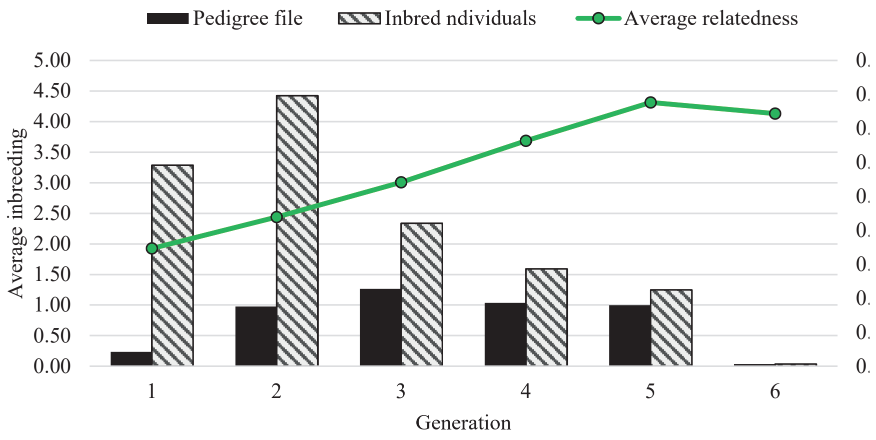
In average, the pedigree inbreeding ($F_{PED}$) was 0.39 ± 1.9% and 0.35 ± 0.59% in pedigree file and reference population, respectively. The individual increase in inbreeding ($\Delta F_{PEDi}$) was higher in pedigree file (0.19 ± 1.17%) than in reference population (0.07 ± 0.11%), while the comparison of mean average relatedness showed that the individuals in reference population were genetically more related (0.01 ± 0.002) than in pedigree file (0.006 ± 0.005). Similar level of individual increase in inbreeding in Norik of Muran population found Halo *et al.* (2018). The Fig. 3 shows the trend of pedigree inbreeding per complete generations. The decreasing tendency of intensity of inbreeding across generations confirmed also the observed average relatedness in population. The results showed that in current generation can be expected the pedigree inbreeding at level 0.23% and average relatedness 0.01, that is positive mainly from the genetic diversity point of view. But, the trend of the average inbreeding of inbred animals across generations can indicate the risk of the increase of inbreeding in the future generations. Due to the fact that the Norik of Muran horse breed can be regarded as small local population with relative low number of founders the obtained level of inbreeding is surprisingly low. For example Pjontek *et al.* (2012) reported for Slovak Sport Pony, Shagya Arabian and Hucul horses bred in Slovakia inbreeding at level 2.67, 3.95 and 6.26%, respectively. Similarly, Somogyvári *et al.* (2018) indicated in small endangered population of Hucul horse average inbreeding coefficient 5.57%.



1: *The completeness of Norik of Muran horse pedigree based on maximum number of generations traced (A), number of full generations traced (B), equivalent complete generations (C) and MacCluer's index (D) (PF – pedigree file, RP – reference population)*



2: *Per-marker quality of genomic data in sample of Norik of Muran horse*



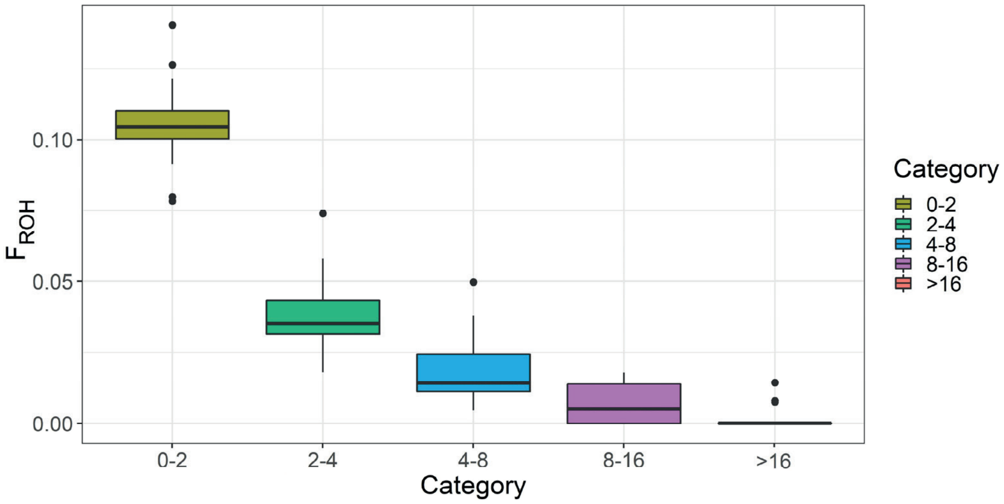
3: *Trend of pedigree inbreeding per complete generations*

I: *Summary of $F_{ROH}$ statistic for each length category*

| Category | No. of ROHs | Mean length of ROHs (Mb) | Average $F_{ROH}$ ± SD (%) | $F_{ROH}$ range (%) |
|---|---|---|---|---|
| $F_{ROH0–2Mb}$ | 4418 | 0.85822 | 10.55±1.33 | 7.84–14.03 |
| $F_{ROH2–4Mb}$ | 403 | 2.748423 | 3.79±1.19 | 1.80–7.41 |
| $F_{ROH4–8Mb}$ | 112 | 5.615616 | 1.81±1.11 | 1.06–4.97 |
| $F_{ROH8–16Mb}$ | 30 | 10.65078 | 0.69±0.64 | 0.00–1.79 |
| $F_{ROH>16Mb}$ | 3 | 22.13268 | 0.11±0.03 | 0.00–1.43 |

The individual genomic inbreeding ($F_{ROH}$) was derived from distribution of ROH segments in the Norik of Muran horse genome. The previous studies showed that the ROHs can be used to show the history of population as well as to estimate the level of genomic inbreeding over past generations (Howrigan *et al.*, 2011; Ferenčaković *et al.*, 2013; Curik *et al.*, 2014). Based on this, the genomic inbreeding was calculated for five length categories ($F_{ROH0–2Mb}$, $F_{ROH2–4Mb}$, $F_{ROH4–8Mb}$, $F_{ROH8–16Mb}$, and $F_{ROH>16Mb}$). In cattle, it has been shown that the $F_{ROH>1Mb}$ is related to the proportion of autozygosity originated from ancestors that were born 50 generation ago, while $F_{ROH>16Mb}$ represents the autozygosity derived from chromosomal segments present in ancestor that were born 3–6 generation ago (Ferenčaković *et al.*, 2013).

The number and length of ROH segments detected in the genome of Norik of Muran horses varied across each length category (Tab. I). The shortest segments showed the highest proportion across the all ROHs detected (88.96%), whereas the lowest distribution was found for the ROHs > 16 Mb (0.06%). The mean length of ROHs varied from 0.86 Mb ($ROH_{0–2Mb}$) to 22.13 ($ROH_{>16Mb}$). Obtained proportion of ROHs across the genome of Norik of Muran horse is comparable to other local horse breeds, e.g. Austrian Noriker (Grilz-Seger *et al.*, 2019), Bosnian mountain and Haflinger horses (Druml *et al.*, 2018; Grilz-Seger *et al.*, 2018) or Polish Konik horse (Kamiński *et al.*, 2017). Compared to pedigree analysis, the genomic inbreeding showed the same trend (Fig. 4). The highest level of detected $F_{ROH}$ for the $ROH_{0–2Mb}$ category resulted mainly from the small population size and strong effect of founders that share ROH segments with their progeny. The obtained value of $F_{ROH2–4Mb}$, $F_{ROH4–8Mb}$ and $F_{ROH8–16Mb}$ corresponded mainly to the intensity of selection during the grading-up process of Norik of Muran horse breed in Slovakia. The recent genomic inbreeding ($F_{ROH>16Mb}$) at level 0.11% is slightly lower to pedigree inbreeding in reference population that points out the relatively good quality of genealogical data. In addition, the obtained trend of pedigree as well as genomic inbreeding signalized sufficient level of genetic variability across the population of Norik of Muran horse. Previous studies significantly showed that the distribution of ROHs in the genome can be very good indicator of inbreeding levels in livestock (Kukučková *et al.*, 2017; Mastrangelo *et al.*, 2018; Nandolo *et al.*, 2019). Peripolli *et al.* (2017) reported that the better results of $F_{ROH}$ suggest that $F_{ROH}$ can be used to infer information about the history and inbreeding levels of a population in the absence of genealogical information. The selection of superior animals has produced large phenotypic changes and has reshaped the ROH patterns in various regions of the genome. In horses, the ROHs were used to estimate inbreeding coefficient in several local populations (Kamiński *et al.*, 2017; Druml *et al.*, 2018; Grilz-Seger *et al.*, 2018, 2019).



4: *Trend of genomic inbreeding derived from ROHs distribution in Norik of Muran genome*

## CONCLUSION

The intensity of inbreeding is one of the most important parameter in the monitoring of genetic diversity mainly in case of small endangered populations. Both pedigree and genomic inbreeding estimates indicated that the gene pool of Norik of Muran horses isn't significantly affected by mating of relatives. The comparison of results from the pedigree and genomic analyses suggested that the level of inbreeding ranges from 0.23 to 0.11%. Even if the Norik of Muran population is small with relative low number of founders, the level of inbreeding and average relatedness in current generation are low. In Slovakia, the Norik of Muran horse belongs to the traditional breeds that significantly contribute to the rural landscape and heritage conservation. Therefore, the analysis of its biodiversity could contribute to the long-term strategy of animal genetic resources conservation in Slovakia.

Acknowledgements

This study was supported by the Slovak Research and Development Agency (APVV-14-0054 and APVV-17-0060) and VEGA (1/0742/17).

## REFERENCES

AL ABRI, M. A., KÖNIG VON BORSTEL, U., STRECKER, V. *et al.* 2017. Application of Genomic Estimation Methods of Inbreeding and Population Structure in an Arabian Horse Herd. *J. Hered.*, 108(4): 361–368.

ANDERSON, C. A., PETTERSSON, F. H., CLARKE, G. M. *et al.* 2010. Data quality control in genetic case-control association studies. *Nature Protocols volume*, 5(9): 1564–1573.

BISCARINI, F., COZZI, P., GASPA, G. *et al.* 2018. *detectRUNS: Detect runs of homozygosity and runs of heterozygosity in diploid genomes*. CRAN (The Comprehensive R Archive Network).

CHANG, C. C., CHOW C. C., TELLIER L. C. A. M. *et al.* 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience*, 4: 7.

CURIK, I., FERENČAKOVIĆ, M. and SÖLKNER, J. 2014. Inbreeding and runs of homozygosity: A possible solution to an old problem. *Livestock Science*, 166(1): 26–34.

DRUML, T., BAUMUNG, R. and SÖLKNER, J. 2009. Pedigree analysis in the Austrian Noriker draught horse: genetic diversity and the impact of breeding for coat colour on population structure. *J Anim. Breed. Genet*., 126(5): 348–356.

DRUML, T., NEUDITSCHKO, M., GRILZ-SEGER, G. *et al.* 2018. Population Networks Associated with Runs of Homozygosity Reveal New Insights into the Breeding History of the Haflinger Horse. *J Hered.*, 109(4): 384–392.

FERENČAKOVIĆ, M., HAMZIĆ, E., GREDLER, B. *et al.* 2013. Estimates of autozygosity derived from runs of homozygosity: empirical evidence from selected cattle populations. *J Anim. Breed. Genet*., 130: 286–293.

GRILZ-SEGER, G., DRUML, T., NEUDITSCHKO, M. *et al.* 2019. Analysis of ROH patterns in the Noriker horse breed reveals signatures of selection for coat color and body size. *Anim. Genet.*, 50(4): 334–346.

GRILZ-SEGER, G., MESARIČ, M., COTMAN, M. *et al.* 2018. Runs of Homozygosity and Population History of Three Horse Breeds With Small Population Size. *J Equine Vet. Sci.*, 71: 27–34.

GUTIÉRREZ, J. P. and GOYACHE, F. 2005. A note on ENDOG: a computer program for Analysis pedigree information. *J Anim. Breed. Genet*., 122: 172–176.

GUTIÉRREZ, J. P., GOYARCHE F. and CERVANTES, I. 2009. *User´s Guide-ENDOG v 4.6 a Computer Program for Monitoring Genetic Variability of Populations Using Pedigree Information*. ©Juan Pablo Gutiérrez García.

HALO, M., MLYNEKOVÁ, E., HORNÁ, M. *et al.* 2018. Evaluation of Genetic Variability of the Breed Norik of Muran according to Pedigree Information. *Czech J. Anim. Sci.*, 63(5): 195–200.

HOWRIGAN, D. P., SIMONSON, M. A. and KELLER, M. C. 2011. Detecting autozygosity through runs of homozygosity: A comparison of three autozygosity detection algorithms. *BMC Genomics*, 12(1): 460.

KAMIŃSKI, S., HERING, D. M., JAWORSKI, Z. *et al.* 2017. Assessment of genomic inbreeding in Polish Konik horses. *Pol. J Vet. Sci.*, 20(3): 603–605.

KUKUČKOVÁ, V., MORAVČÍKOVÁ, N., FERENČAKOVIĆ, M. *et al.* 2017. Genomic characterization of Pinzgau cattle: genetic conservation and breeding perspectives. *Conserv. Genet.*, 18(4): 893–910.

MACCLUER, J. W., BOYCE, A. J., DYKE, B. *et al.* 1983. Inbreeding and pedigree structure in Standardbred horses. *J Hered.*, 74: 394–399.

MAIGNEL, L., BOICHARD, D. and VERRIER, E. 1996. Genetic variability of French dairy breeds estimated from pedigree information. *Interbull Bulltein*, 14: 49–54.

MASTRANGELO, S., CIANI, E., SARDINA, M. T. *et al.* 2018. Runs of homozygosity reveal genome-wide autozygosity in Italian sheep breeds. *Anim. Genet.*, 49(1): 71–81.

MCQUILLAN, R., LEUTENEGGER, A., ABDEL-RAHMAN, R. *et al.* 2008. Runs of homozygosity in European populations. *Am. J Hum. Genet.*, 83: 359–372.

NANDOLO, W., MÉSZÁROS, G., BANDA, L. J. *et al.* 2019. Timing and Extent of Inbreeding in African Goats. *Front. Genet.*, 10: 537.

NOLTE, W., THALLER, G. and KUEHN, C. 2019. Selection signatures in four German warmblood horse breeds: Tracing breeding history in the modern sport horse. *PLoS One*, 14(4): e0215913.

PAVLÍK, I., SCHUBERTOVÁ, Z., KADLEČÍK, O. *et al.* 2014. Pedigree analysis of Thoroughbred horses in Slovakia. *Acta fytotechn. zootechn.*, 17(4): 122–126.

PERIPOLLI, E., MUNARI, D. P., SILVA, M. V. G. B. *et al.* 2017. Runs of homozygosity: current knowledge and applications in livestock. *Anim Genet.*, 48(3): 255–271.

PJONTEK, J., KADLEČÍK, O., KASARDA, R. *et al.* 2012. Pedigree analysis in four Slovak endangered horse breeds. *Czech J. Anim. Sci.*, 57(2): 54–64.

PORTER, V., ALDERSON, L., HALL, S. J. G. *et al.* 2016. *Mason's World Encyclopedia of Livestock Breeds and Breeding, 2 Volume Pack*. Oxfordshire: CABI.

POŠIVÁK, J., STYKOVÁ, E., NOVOTNÝ, F. *et al.* 2015. Comparison of some biochemical and mineral indices among Norik breed Muráň Plain type and Hucul breed mares. *Acta Veterinaria Brno*, 84(2): 113–117.

SIDERITS, M., BAUMUNG, R. and FUERST-WALTL, B. 2013. Pedigree analysis in the German Paint Horse: Genetic variability and the influence of pedigree quality. *Livest. Sci.*, 151: 152–157.

SOMOGYVÁRI, E., POSTA, J. and MIHÓK, S. 2018. Genetic Analysis of the Hungarian Population of Endangered Hucul Horses. *Czech J. Anim. Sci.*, 63(6): 237–246.

VOSTRÁ-VYDROVÁ, H., VOSTRÝ, L., HOFMANOVÁ, B. *et al.* 2016. Genetic diversity within and gene flow between three draught horse breeds using genealogical information. *Czech J. Anim. Sci.*, 61(10): 462–472.

VOSTRÝ, L., VOSTRÁ-VYDROVÁ, H., HOFMANOVA, B. *et al.* 2017. Genetic diversity of Czech Sport Pony based of genealogical information. *Acta fytotechn. zootechn.*, 20(4): 84–89.

Contact information

Radovan Kasarda: radovan.kasarda@uniag.sk
Nina Moravčíková: nina.moravcikova1@gmail.com
Ondrej Kadlečík: ondrej.kadlecik@uniag.sk
Anna Trakovická: anna.trakovicka@uniag.sk
Marko Halo: marko.halo@uniag.sk
Juraj Candrák: juraj.candrak@uniag.sk