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Short communication

Assessment of genomic inbreeding in Polish Konik horses

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Abstract

The aim of this study was to assess the inbreeding coefficient of Polish Konik horses based on runs of homozygosity (ROH). Ninety six horses kept in 6 herds located across Poland were genotyped with the use of EquineSNP60 BeadChip (Illumina). SNP markers with a Minor Allele Frequency lower than 0.01 and SNPs assigned to chromosome X or Y were excluded from the study. A total of 50 708 SNPs were included for statistical analysis (SVS software, Golden Helix). The analysis showed that the population is in genetic equilibrium, with H_e and H_o estimates both equal to 0.3086. Seven categories of Runs of Homozygozity (ROH) length were defined: >0.5, >1, >2, >4, >8, >16, >25 Mb. The genomic inbreeding coefficient derived from ROH (F_{ROH}) calculated for each ROH length ranged from 15.96% based on the shortest ROH (>0,5Mb) to 2.71% for the longest ROH (>25Mb). Among individual horses, the inbreeding coefficient ranged from 5.25% to 22.41% (for ROH >1Mb). Analysis of ROH in Polish Koniks allows for more effective management of their inbreeding in the future.

Key words: horse, Polish Konik, runs of homozygozity, inbreeding, SNP

Introduction

Polish Koniks are a primitive breed of horses closely related to the extinct wild Tarpan (*Equus ferus*; Komosa et al. 2013). Part of the population is kept in stabled conditions and part is living in the wild. The breed is included in the Global Strategy for the Management of Farm Animal Genetic Resources, established by the Food and Agricultural Organization (Jezierski and Jaworski 2008). The stud book for this breed is closed and therefore one of the most important concerns in the Polish Konik population is constant monitoring of inbreeding. Accessibility of a microarray of SNPs covering evenly the whole equine genome opens a new possibility of verifying the inbreeding coefficient of Polish Koniks calculated so far on the pedigree records or microsatellite markers (Mackowski et al. 2015, Szwaczkowski et al. 2015). In this study we attempted to use SNP markers to reveal runs of DNA homozygosity and apply this data to assess genomic inbreeding of Polish Koniks.

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Table 1. General characteristics of the genetic diversity in a sample of Polish Koniks.

Characteristics	Value
Genome Size (Mb)	2240.58
Number of SNPs	50 708
Mean distance between SNPs (kb)	44.19
Average call rate sample (%)	99.98
Average call rate SNP (%)	99.00
MAF	0.22
H_e	0.3086
H_o	0.3086
Het Rate	0.31

MAF – Minor Allele Frequency, H_e – heterozygosity expected, H_o – heterozygosity observed, Het – heterozygosity.

Table 2. Inbreeding coefficient (F_{ROH}) of the Polish Konik based on different ROH size.

ROH size (Mb)	N	F_{ROH}			
		mean	median	min	max
>0.5	96	0.1596	0.1552	0.0987	0.2585
>1	96	0.1215	0.1172	0.0527	0.2241
>2	96	0.1019	0.0946	0.0343	0.2120
>4	96	0.0896	0.0842	0.0278	0.2071
>8	96	0.0688	0.0624	0.0211	0.1710
>16	92	0.0376	0.0348	0.0071	0.1216
>25	72	0.0271	0.0234	0.0110	0.0850

N – number of horses; ROH – runs of homozygosity; Mb – million of base pairs.

Materials and Methods

Ninety six horses (59 mares, 24 stallions and 13 geldings) kept in 6 herds located across Poland were included in the study. All horses were genotyped with the use of EquineSNP60 BeadChip (Illumina) containing 65 157 SNP markers. After routine quality control, SNPs were filtered by MAF (Minor Allele Frequency): SNPs with MAF lower than 0.01 were excluded. Also, SNPs assigned to chromosomes X or Y were removed from statistical analysis. Finally, 92 samples and 50 702 SNP were included for further statistical analysis performed by SVS software (Golden Helix). Runs of Homozygosity were defined as stretches of DNA containing a minimum of 15 homozygous SNPs and a maximum distance of 1 Mb between adjacent SNPs. Autozygosity was estimated according to McQuillan et al. (2008):

$$F_{ROH} = (\Sigma_{j=1}^{n} L_{ROHj})/L_{total}$$

where: L_{ROHj} is the length of ROH j, and L_{total} is the total size of the genome covered by markers, calculated from the sum of intermarker distances (database EquCab2.0, 09/2007) available in SVS software (Golden Helix). For each animal, F_{ROH} was calculated based on ROH of different minimum lengths: 0.5, 1, 2, 4, 8, 16, 25 Mb.

Results and Discussion

The analysis showed that the population was in genetic equilibrium, with H_e and H_o estimates both equal to 0.3086. SNPs were evenly distributed across all autosomes with the average distance of 44.19 kb between SNPs. Average MAF and heterozygosity rate amounted to 0.22 and 0.31, respectively (Table 1). For ROH>1 Mb, ROH coverage per chromosome ranged between 9% (chromosome 6) to 17.77% (chromosome 27) (data not shown). Short and medium ROHs (between >0.5Mb and >8 Mb) occurred in all horses. The longest two categories of ROHs, >16Mb and >25Mb occurred in 92 and 72 horses, respectively. A number of ROHs showed distinct differences among individual horses (data not shown). For example, the number of ROH>8Mb ranged from 1 to 21. The genomic inbreeding coefficient derived from ROH (F_{ROH}) calculated for each ROH length ranged from 15.96% based on the shortest ROH (>0,5Mb) to 2.71% for the longest ROH (>25Mb) (Table 2).

Our analysis is the first attempt to characterize genetic variation of Polish Konik at the genome level. In spite of the fact that Polish Konik was almost an extinct breed, its population has increased sufficiently to achieve genetic equilibrium (H_e and H_o were equal to 0.3086). The genomic inbreeding

coefficient based on ROH was relatively high and ranged from 2.2% to 15.96%. These estimates encompassed an inbreeding coefficient of 9.3% calculated with the use of 17 microsatellites by Szwaczkowski et al. (2015). Mackowski et al. (2015) found that the inbreeding coefficient in Polish Koniks doubled between 1980 and 2010 from 5% to almost 10%. To compare genomic inbreeding assessments with the traditional method, we have to assume which ROH category represents homozygosity of the genome in the best way. For the bovine genome, Ferencaković et al. (2013) and Gurgul et al. (2016) found that an inbreeding coefficient based on ROH >8 Mb showed the best correlations with inbreeding calculated from the pedigree records. If we assume the 8Mb size of ROH as the most representative for autozygosity, then we can roughly assess the actual average genomic inbreeding coefficient in Polish Koniks at 6.88% (Table 2). It seems that the population of Polish Koniks is far from achieving the level of 15% of inbreeding, which is thought to be the borderline initiating depression for many phenotypes (Pluta et al. 2016). Individual diversity expressed in the number of ROHs and inbreeding coefficient can be applied to optimize the best contribution of animals to the next generation, in order to reduce the pace of inbreeding in the stud or population. We believe that genomic inbreeding assessment can be used as additional information assisting traditional pedigree data stored in the stud book, especially in the situation where breeders intent to mate horses of relatively high inbreeding. We conclude that genomic inbreeding of Polish Koniks reflects their actual inbreeding level and might be used as a new and efficient tool assisting in the management of inbreeding.

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