Original Research

Runs of Homozygosity and Population History of Three Horse Breeds With Small Population Size

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ABSTRACT

Long consecutive homozygous genotype segments, runs of homozygosity (ROH), are a result of parents transmitting identical haplotypes, which can be used to estimate autozygosity. Based on 612K single-nucleotide polymorphisms, we computed three ROH parameters (genome length covered by ROH, S_{ROH}; number of ROH, N_{ROH}; and autozygosity, F_{ROH}) to investigate different scenarios in contemporary horse breeding: limited census (Bosnian mountain horse), conservation breeding (Posavje horse), and selection within closed studbook (Haflinger). The ROH parameters revealed well-defined differences between breeds. S_{ROH} was highest in the Bosnian mountain horse with 296.32 Mb, followed by the Haflinger sample (S_{ROH} = 270.35 Mb) and the Posavje sample with 192.68 Mb. The highest number of ROH segments (ROHs) was observed within the Haflinger sample followed by the Posavje sample. F_{ROH} ranged at a population level from 8.59% in Posavje, over the Haflinger (mean F_{ROH} = 12.05%) to 13.21% in the Bosnian mountain horse breed. Bottlenecks were detected for Bosnian mountain horse and Haflinger, whereas for the Posavje, a positive effect of the conservation breeding program was documented. Investigating the distribution of ROHs across the genome, we detected four common ROH islands on equine chromosomes ECA 6, ECA 11, and ECA 17, which were present in all breeds. On breed level, the Bosnian mountain horses contained 10, the Posavje, four, and the Haflinger, 11 distinct ROH islands (containing the MC1R locus on ECA 3). With this analysis, we were able to compare genomic levels of inbreeding between breeds differing in management, pedigree completeness, and genes under selection.

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1. Introduction

With the availability of genomewide single-nucleotide polymorphism (SNP) genotyping arrays, genomic inbreeding estimation based on the analysis of runs of homozygosity (ROH) has been established for several livestock species including the horse. A range of studies have proven that the computation of ROH represents a valuable source of information to describe genomic inbreeding (F_{ROH}) [1–6]. Furthermore, it was demonstrated that the identification of ROH segments (ROHs) can be successfully applied to investigate complex population histories and structures and to identify associations with quantitative and qualitative phenotypes [7–12]. Although methodical and technological aspects for the identification of ROH are not fully resolved [13], it is generally hypothesized that long consecutive homozygous segments are a result of identical haplotypes, which were inherited by a common ancestor [3,9,12,14,15].

Compared with pedigree-based inbreeding estimation, the analysis of ROHs provides a range of advantages including the following. F_{ROH} is the quotient of autozygous regions and total genome length; through this definition, it becomes feasible to derive reliable inbreeding coefficients from animals/populations

without pedigree records or incomplete pedigree information. In pedigree analyses, it is assumed that the founder population is unrelated. Runs of homozygosity segments of different length categories reflect to inbreeding events in different time frames, and thus ROH analyses enable a better comparison between fragmented populations and between breeds with different pedigree quality (generation equivalents can vary within horses from 19 in Lipizzans [16] to 3.5 and even less in local breeds, e.g., the Posavje horse [17]).

Based on the high-density SNP genotype information of the 700k Affymetrix Axiom Equine Genotyping Array, we aimed to investigate three different scenarios of contemporary horse breeding in three Slovenian horse populations (Fig.1), which are representatives for a population with extremely limited census, a conservation breeding program, and selection within closed studbook. The Bosnian mountain horse is the oldest autochthonous horse breed of the entire Balkan Peninsula and underwent critical genetic bottlenecks within the period from 1991 to 2018. During these years, the population number decreased due to following reasons: the Yugoslav wars (from 1991 to 1999), the political and economic fragmentation of the original breeding regions, and the privatization and the shutdown of state stud farms in Bosnia and Macedonia. Although horses expressing the same type of the Bosnian mountain horse can be found in Bosnia and Herzegovina, Serbia, Macedonia, and Albania, the number of purebred and registered animals comprises 141 animals and 14 animals in the B-book. The Slovenian Bosnian mountain horse population originates from the former state stud farm Borike in Bosnia and Herzegovina. To increase the effective population size, several Bosnian mountain horses from the country breeding, which matched in type and conformation, were integrated into the Slovenian Bosnian mountain horse population. For those horses, no pedigree documentation exists [18]. The second horse population included in this study is the Slovenian Posavje horse, which belongs to the Posavina breed, originating in the northern Sava flatlands of Croatia. The breeding region also includes the southern part of Slovenia (especially the districts Krsko and Brezice). In 1993, a Slovenian breeding and conservation program was initiated. This was the commencement of pedigree documentation; whereas in the past, only breeding stallions were registered. In contrast to the Bosnian mountain horse, which is threatened by extinction, the Posavje population in Slovenia experiences a period of expansion, as the population increased from 79 breeding mares in 1993 to 627 breeding mares in 2017 [17,19]. Finally we included a sample of the Slovenian Haflinger horse in this study. The Slovenian Haflinger population was established in the 1960s based on imported horses from Austria, and the population constantly increased up to the 1990s. Within the last 2 decades, this breed was exposed to intense selection pressure according to modernized breeding objective including the introgression of foreign stallions. Furthermore, a decrease of the breeding population can be observed in the

Slovenian Haflinger, as the new management decisions do not correspond with the attitudes of traditional Slovenian breeders. The current Slovenian Haflinger population comprises in total 680 registered animals, including 25 breeding stallions and 200 breeding mares.

The aim of this study was to characterize genomic levels of inbreeding based on ROH analysis and to investigate the ROH structure and distribution to analyze population history and to evaluate differences in breeding and selection programs. Furthermore, we identified overlapping ROH regions (ROH islands) within the respective breeds.

2. Materials and Methods

2.1. Sampling

All horses included in this study were selected to represent the genealogical population structure of the breeds based on pedigree information. From the 23 systematically selected Bosnian mountain horses (BMH), 18 were offspring from the Bosnian stud farm Borike, whereas five horses are the offspring of country-bred mares with missing pedigree information. The 28 Posavje horses (POS) and the 18 Haflinger horses (HAF) were sampled according to the genealogical structure (sire lines and mare families) of the two breeds, taken into account the relatedness of animals.

2.2. Single-Nucleotide Polymorphism Genotyping

The SNP genotypes for the 69 horses were determined using the Affymetrix Axiom Equine Genotyping Array [20]. The chromosomal position of the SNPs was determined based on EquCab2.0 reference genome. We did not consider the SNPs positioned on the sex chromosomes (X: 28,017 SNPs and Y: 1 SNP) and the SNPs without known chromosomal position (30,864 SNPs). The SNPs with more than 10% missing genotypes were excluded. This resulted in a total of 611,914 SNPs for each horse.

2.3. Genetic Diversity and Runs of Homozygosity Analysis

To illustrate the population structure, we applied principal component analysis (PCA) on the basis of the genetic relationship matrix (G) with pairwise identities by state between horses as provided by PLINK v.1.7 [21]. The PCA plot was performed using the R platform and statistical analyses, and graphical representations were performed using the software packages SAS v.9.1 [22] and R (www.r-project.org).

Runs of homozygosity segments were determined with an overlapping window approach implemented in PLINK v.1.7 [21] based on the following settings: minimum SNP density was set to one SNP per 50 kb, with a maximum gap length of 100 kb. The final

Fig. 1. Representative horses for the three breeds Bosnian mountain horse (A), Posavje horse (B), and the Slovenian Haflinger (C) (images by Matjaz Mesaric).