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Original Paper

Genomic diversity of beef cattle in Slovakia

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The aim of the study was to determine the state of genetic diversity in Charolais and Limousine populations. The analysis was based on the panel of 49 629 SNPs that were used for genotyping of 85 individuals. For the assessment of the genetic diversity, the genomic inbreeding coefficient resulting from runs of homozygosity distribution in the genome and linkage disequilibrium based effective population size (N_e) were calculated. The results reflected a decrease in recent inbreeding ($F_{ROH > 16\ Mb}$ under 1%) compared to historical ($F_{ROH > 1\ Mb}$ in average 6%). The current effective population size was estimated based on the linear regression using N_e estimates for 50 generations ago. The effective population size across all analysed animals was 33.05 for Charolais breed (decrease of 4.51 animals per generation) and 7.02 for Limousine breed (decrease of 2.81 animals per generation). The estimation of current N_e indicated the endangered status of assessment populations and referred the need for continuous monitoring to increase population size but without reducing genetic diversity as a result of inbreeding.

Keywords: effective populations size, homozygosity, Charolais, inbreeding, Limousine

1 Introduction

Evaluation of genetic diversity on the genome-wide level is currently a widely used method considering recent progress in single nucleotide polymorphism (SNP) chip technology and the development of advanced methodologies in population genetics (Lenstra et al., 2012). The most important parameters in terms of genetic diversity conservation are the effective population size (N_e) as well as the coefficient of inbreeding (Moravčíková et al., 2017). Traditionally, the inbreeding coefficient was estimated based on genealogical information (Kadlečík et al., 2016). However, several authors (e.g. Keller et al., 2011) reported that the use of pedigree data for estimation of the inbreeding intensity in a population might significantly underestimate the true level of inbreeding coefficient. The development of high-performance and modern genotyping technologies led to an increase in interest of genomic inbreeding coefficients evaluation (Forutan et al., 2018). High-performance genotyping allows a new and more accurate assessment of the degree and effect of inbreeding in livestock (Ferenčaković et al., 2013). Using high-density SNP markers, the level of genomic inbreeding may be estimated based on the distribution of runs of homozygosity (*ROH*) in the animal's genome (Kasarda et al., 2017). Wright (1938) defined the effective population size as the size of an idealized population (without the influence of factors such as selection or migration), which would then produce the same increase of inbreeding or the same gene frequencies as in real population. The N_e reported relevant information necessary to monitor genetic diversity in livestock populations. In the absence of pedigree data, Flury et al. (2010) reported the linkage disequilibrium (LD) analysis as an alternative for estimating the effective population size. This study aimed to evaluate the inbreeding coefficient and the effective population size in the Charolais and Limousine breeds that were imported to Slovakia since 1990 (Kadlečík et al., 2016).

2 Material and methods

The Charolais sample consisted of 68 genotyped purebred cows and bulls from two farms, whereas the Limousine sample included 17 purebred cows and bulls from single farm. Both farms are recognized as nucleus herds of particular

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breed in Slovakia for production of future breeding sires and dams. Genomic DNA animal samples (hair roots) were genotyped in a commercial lab using the International Beef and Dairy (IDB) chip for 49 629 SNPs. The quality control of genotypic data was made by software PLINK 1.9 (Chang et al., 2015) when all markers with unknown chromosomal position (Btau 5.0.1) and SNPs located on gonosomes (Moravčíková et al., 2018) were filtered out. In the next step, the individuals and SNPs with a call rate higher than 90% and minimum minor allele frequency (MAF) of 0.01 were only retained. The final dataset included 41 153 SNP markers for Charolais breed and 41 151 SNP for Limousine breed.

The genomic inbreeding was estimated according to McQuillan et al. (2008) using PLINK 1.9 software (Chang et al., 2015). The *ROH* regions were defined as genomic regions with a minimum of 15 consecutive homozygous genotypes and minimum density of 1 SNP per 100 kb (Šidlová et al., 2015). According to Ferenčaković et al. (2013), the maximum distance between homozygous genotypes was set to 1 Mb. The distribution of *ROH* segments in the genome was evaluated separately for five length categories. The heterozygous calls were not allowed, except category >16 Mb. Number of missing genotypes which were allowed in *ROH* was calculated according to Ferenčaković et al. (2013). For categories of *ROH* 1–2 Mb and 2–4 Mb zero missing genotypes were allowed, 1 missing SNPs were allowed for categories 4–8 Mb, the category 8–16 Mb two missing genotypes were allowed and 4 missing SNPs were allowed in the category over 16 Mb.

The relationship between the effective population size and the genome-wide level of linkage disequilibrium was used to determine N_e status of both breeds analysed. The calculation of LD was based on Pearson's correlation coefficient r^2_{LD} which is expressed as the product of the moments between the frequencies of the two loci. The effective population size was then estimated by the SNeP program (Barbato et al., 2015).

3 Result and discussion

The total length of the autosomal genome covered by SNPs was 2,503,114 kb for the Charolais breed with an average distance of 60.87 kb between adjacent SNPs. For the Limousine breed, the total genome length was 2,503,271 kb and the average distance between adjacent autosomal loci was 62.39 kb. The distribution of ROHs with different length in the genome of analysed breeds was used to express recent and historical inbreeding. As expected based on the previous studies, the ROHs >1 Mb reflecting ~50 generations of ancestors (Peripolli et al., 2018) was in Charolais and Limousine genome most frequent. The inbreeding coefficients calculated from *ROH* with a minimum length higher for 4, 8 and 16 Mb are expected to correspond to reference populations of approximately 12, 6 and 3 generations (Mészáros et al., 2015).

Table 1 Summary of results for each category of *ROH* segments for Charolais breed

ROH category	Average no. of *ROH* ±*SD*	Average length of *ROH* (Mb) ±*SD*	F_{ROH} (%)	Proportion of genome covered by *ROH* (%)
ROH >1 Mb	64.37 ±10.40	159.80 ±35.00	6.40 ±1.40	0.1
ROH >2 Mb	24.15±5.48	105.32 ±32.40	4.21 ±1.30	0.17
ROH >4 Mb	10.18 ±2.61	76.11 ±25.30	3.04 ±1.01	0.3
ROH >8 Mb	2.85 ±1.41	36.04 ±22.30	1.45 ±0.89	0.51
ROH >16 Mb	0.62 ±0.73	15.74 ±21.72	0.63 ±0.87	1.02

Table 2 Summary of results for each category of *ROH* segments for Limousine breed

ROH category	Average no. of *ROH* ±*SD*	Average length of *ROH* (Mb) ±*SD*	F_{ROH} (%)	Proportion of genome covered by *ROH* (%)
ROH >1 Mb	62.82 ±5.93	139.1 ±21.00	5.56 ±0.84	0.12
ROH >2 Mb	19.18 ±4.00	80.43 ±22.54	3.21 ±0.90	0.17
ROH >4 Mb	7.12 ±2.60	50.86 ±19.75	2.03 ±0.79	0.29
ROH >8 Mb	1.71 ±1.16	20.31 ±15.65	0.81 ±0.63	0.48
ROH >16 Mb	0.47 ±0.62	10.21 ±16.01	0.41 ±0.64	0.87

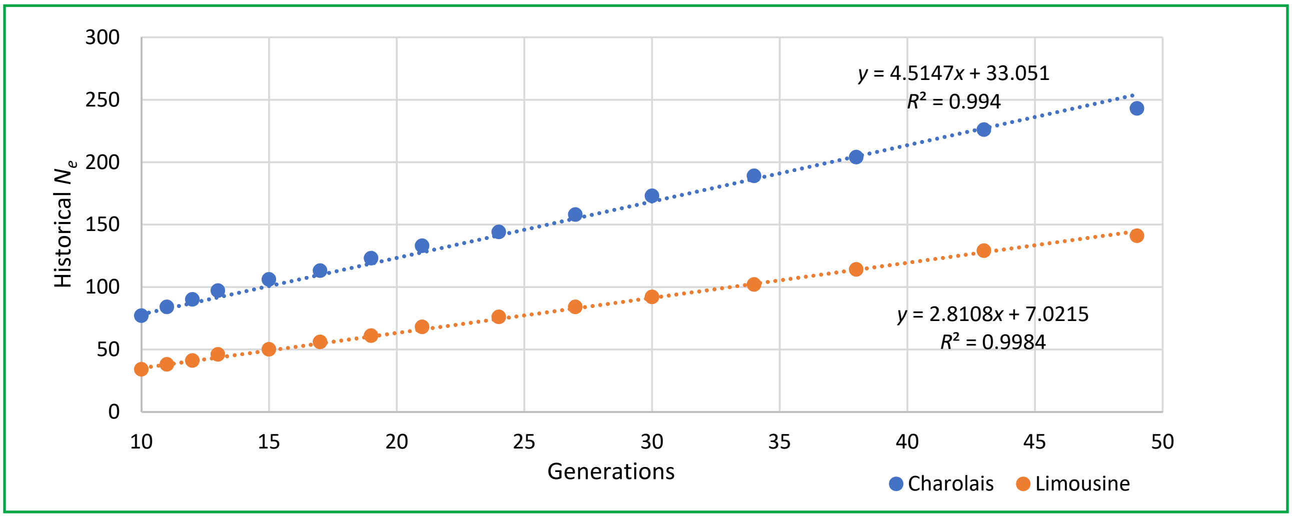

Figure 1 The estimation of N_e trends across generations based on the linear regression

The proportion of genome covered by ROHs was higher in Charolais (0.1–1%) compared to Limousin breed (0.1–0.8%). The summary F_{ROH} statistics for each category analysed are shown in Tables 1 and 2. Generally, the proportion of ROHs >16 Mb in the livestock autosomal genome is relatively high compared to humans mainly due to intensive artificial selection and reduced effective population size (Curik et al., 2014). The results showed that the F_{ROH} values decreased with increasing minimum length of *ROH*. The F_{ROH} across all length categories was slightly higher in Charolais breed, but still under the limit defining the population as endangered by significant diversity loss.

Compared to the study of Szmatoła et al. (2019), the results showed a decrease of genomic inbreeding for analysed beef breeds mainly in case of *ROH* categories >8 Mb and >16 Mb. Metzger et al. (2015) reported that long *ROH* segments reflecting intense selection pressure in population, while short *ROH* segments point to the loss of genetic diversity due to bottleneck (Al-Mamun et al., 2015). Our study confirmed the fact that breeding programs of analysed breeds have been designed in the past to increase the frequency of desired homozygous alleles, but also to keep the inbreeding intensity under 1%.

The LD was estimated for adjacent SNPs, separately for each autosome. The value of decreased with increasing genetic distance in both breeds. The total level of LD was higher in the Limousine breed (6.47 ±0.18) compared to the Charolais (4.70 ±0.15). The differences in estimated LD can be attributed to different selection strategies applied during the breeding processes in the past (Lee et al., 2011). The estimates of historical and current N_e for Charolais and Limousine breeds are shown in Figure 1. The current N_e has been predicted based on a linear regression using N_e estimates for 50 generations ago. The estimates of the historical N_e for the evaluated populations showed a linear decrease, which may be due to a loss of genetic variability in the observed populations.

Analysis of the N_e across all analysed individuals was 33.05 and decrease of 4.51 animals per generation was estimated for Charolais breed. Vice-versa current N_e was 7.02 and was calculated decrease of 2.81 animals per generation for Limousine breed. Compared to the several studies (Bouquet et al., 2011; Lu et al., 2012), the results indicated reduced N_e, due to the reduced size of the assessment populations. The decrease of N_e in evaluation populations can be explained by the influence of bottlenecks, which are associated with domestication, selection, grading up and endangerment of the breed.

4 Conclusion

The analysis of genomic inbreeding based on *ROH* segments suggested the decrease of recent inbreeding in estimated populations. Across all length groups *ROH* segments with a length 1Mb ($F_{ROH >1\ \mathrm{Mb}}$) covered on average 0.1% of the genome, while inbreeding estimates >16 Mb ($F_{ROH >16\ \mathrm{Mb}}$) reached up to 1% for both evaluated breeds. Analysis of the effective population size reflected a decreasing N_e trend over time. It is important to observe that the N_e values can be due to the relatively small sample size for Charolais and Limousine breeds associated with various historical events such as gene flow and selection. The results reflect the need for continual monitoring to increase population size but without reducing genetic diversity in estimation populations Charolais and Limousine. Information of genetic diversity is necessary for genetic improvement, understanding of environmental adaptation and for the conservation of cattle breeds. However, Charolais and Limousin are not native to Slovakia, monitoring of these breeds on the national level is important. From the point of breedingit is positive that levels of inbreeding are acceptable in context to global sustainable trend. Selection of animals in our geographical conditions (after several generations) may contribute to the diversity of the breed as a whole.

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