Production type of Slovak Pinzgau cattle in respect of related breeds

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The objective of this study was to identify the differences in genomic data between Slovak Pinzgau cattle and related breeds in respect to the production type. Genetic structure of 19 Pinzgau bulls has been analysed using 42,854 autosomal SNPs. To provide preliminary investigation the similarity among individuals belonging to the populations that historically contributed to the breed origin was estimated (Holstein, Simmental, Austrian Pinzgau, Ayrshire and MRI). The Bayesian approach as effective detection for number of clusters without reference to prior information of the genetic subdivision was used. Assuming populations distribution based on production type (milk and dual-purpose), two clusters have been chosen as a possible division of six observed populations. Based on this assumption, division to two main populations was observed, Holstein population as a representative of milk production type and Simmental population as a representative of dual-purpose type. Systematic selection due to increasing of milk production is apparent. Genetic insight into the Slovak Pinzgau population is a good indicator for monitoring and preserving of this endangered breed in its original phenotype. Further investigation using high-throughput genotyping data and maintenance of dual-purpose production type is necessary.

Keywords: introgression, local cattle, SNP50 BeadChip

1. Introduction

Pinzgau is an traditional cattle breed of mountainous areas of Slovakia, introduced approximately 2 centuries ago (Kasarda et al., 2008). It was imported from region of the Austrian Alps (Pšenica, 1990). System of cattle recording has started in territory of Slovakia in 1923. The size of population increased and in 1958 it was officially accepted as Slovak Pinzgau breed (Kadlečík et al., 2013). Thanks to its unique traits as longevity, fertility, health, grazing ability it had been bred in mountain regions of northern Slovakia (Pavlík et al., 2013). From the beginning, only purebred animals were used in breeding. Approximately 60 years ago started grading-up crossbreeding with other breeds due to increasing of milk production while unique dual-purpose character was preserved. Jersey breed was used rather experimentally whereas Ayrshire, Meuse Rhine Issel (MRI), Red Holstein have been successfully proved and Red Holstein is used up to now. Nowadays there are used sires born in Slovakia, however, due to their small number also Austrian sires. Austrian Pinzgau has been bred with Red Holstein and Simmental since the second half of the last century. Due to significant decline of the population size in recent years this breed is considered endangered (Kadlečík et al., 2004).

Molecular markers based on DNA have a high polymorphism level, and they have been successfully used for evaluation of genetic diversity and variation in breeding programmes with an impact on the level of genetic conservation schemes (Žídek and Kasarda, 2010). Genomic technologies, such as high-throughput genotyping based on SNP arrays provided background information concerning genome structure in domestic animals (Mastrangelo et al., 2014). Among livestock species, this technology has been applied most successfully in cattle, because factors such as evolutionary history, genetic structure and economics make cattle particularly suitable for the application of genome assisted selection (Nicolazzi et al., 2014). One of the important steps in development of efficient autochthonous breed protection programs is characterization of population genetic variability and to assess the genetic structure (Simčič et al., 2008).

The aim of this study was to characterize the patterns of genetic structure in Slovak Pinzgau bulls population using genotyping array.

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2. Materials and methods

Whole active AI Pinzgau bulls in Slovakia were analysed. DNA of 19 animals was isolated from semen doses. Genomic DNA for each of the semen samples was genotyped at a commercial lab using the Illumina BovineSNP50 v2 BeadChip. The genotyping array contained 54,609 SNPs. To understand the genetic relationship within and among breeds, genotypes from 103 other animals belonging to 5 domesticated bovine breed were used (Decker et al., 2014a; Decker et al., 2014b; Gautier et al. 2010a; Gautier et al., 2010b) without information on sex of individuals (Table 1). Despite the fact that Decker et al. (2014a) and Gautier et al. (2010a) removed closely related individuals from the dataset if pedigree data were available, current numbers of Slovak Pinzgau cattle did not allow us to perform the same exclusion. Moreover, such selection is not needed for this type of analysis, confirming by the studies of Edea et al. (2013) and Mastrangelo et al. (2014).

Due to using more data sets new genetic map was created. In order to minimize risk of genotype errors and excluding poorly performing SNPs individual quality control was performed. All markers with unknown position as well as those that had been assigned to chromosome X were removed. According to Decker et al. (2014) SNPs with minor allele frequency less than 0.0005 were discarded. Excluded were also animals with more than 5% and SNP markers with more than 10% of missing genotypes by PLINK (Purcell et al., 2007). Further analyses were performed including information from 42 854 autosomal SNPs common to all breeds.

The Bayesian clustering algorithm implemented by the STRUCTURE 2.3.4 software (Falush et al., 2003) was used to infer the population structure. The program enables estimation of a 'hidden structure', that is the number of different clusters (populations) obtained without using any a priori information about individual membership (breed). Furthermore, the program is able to determine the corresponding fraction of an individual's genome derived from an ancestry in one of the clusters (*K*) determined by the program. The program STRUCTURE estimates the natural logarithm of the probability (*Pr*) of the observed genotypic array (*G*), given a preassigned number of clusters (parameter *K*) in the dataset [ln *Pr*(*G*|*K*)]. In a Bayesian set-up the estimate of ln *Pr*(*G*|*K*) is a direct indicator of the posterior probability of having *K* number of clusters, given the observed genotypic array (*G*). Lengths of the burn-in and Monte Carlo Markov Chain were 10^5 steps followed by 10^5 iterations. To obtain a representative value of K in the data set, 10 independent runs of the Gibbs sampler for each *K* between 1 and 5 were performed. In all runs we used default settings, that is, an admixture model with correlated frequencies and the parameter of individual admixture alpha set to be the same for all clusters and with a uniform prior.

Based on level of admixture in observed breeds the classification to groups according production type was investigated.

3. Results and discussion

Genotype data were analysed using a Bayesian clustering algorithm to search for admixture between populations and to infer population structure. In order to illustrate a decision on the most likely parameter *K*, in figure 1, we presented ln *Pr*(*G*|*K*) values for all runs. Over the all 122 monitored individuals, ln *Pr*(*G*|*K*) increased from *K* = 1 to *K* = 3, after which it balanced. It was assumed that the most likely K is that where ln *Pr*(*G*|*K*) is maximised as noted by Druml et al. (2007). Results from analysis of admixture considering a range from 1 through 5 potential clusters pointed out that the greatest average likelihood value with the smallest variance among replicates was obtained for *K* = 3. Similarly the genotyping data have been used by Mastrangelo et al. (2014) for analysis of population structure. They found that whole population observed was divided into the 4 separately clusters with connection of 136 breeds. The optimal number of *K* was caused by different number of investigated cattle populations compared to the present study.

Regardless of the correct outcome of the study was the attention focused on *K* = 2, assuming affiliation individual breeds into one of the production type groups. Historically, investigated populations were used in formation of Slovak Pinzgau breed, particularly in order

Table 1   List of 122 animals used in the study

Breed	Number of individuals	Production type	Citation
1 (Holstein)	54	dairy	Gautier et al. (2010a)
2 (Simmental)	23	dual-purpose	Decker et al. (2014a)
3 (Austrian Pinzgau)	5	dual-purpose	Decker et al. (2014a)
4 (Ayrshire)	18	dairy	Decker et al. (2014a)
5 (MRI)	3	dual-purpose	Decker et al. (2014a)
6 (Slovak Pinzgau)	19	dual-purpose / beef	our data
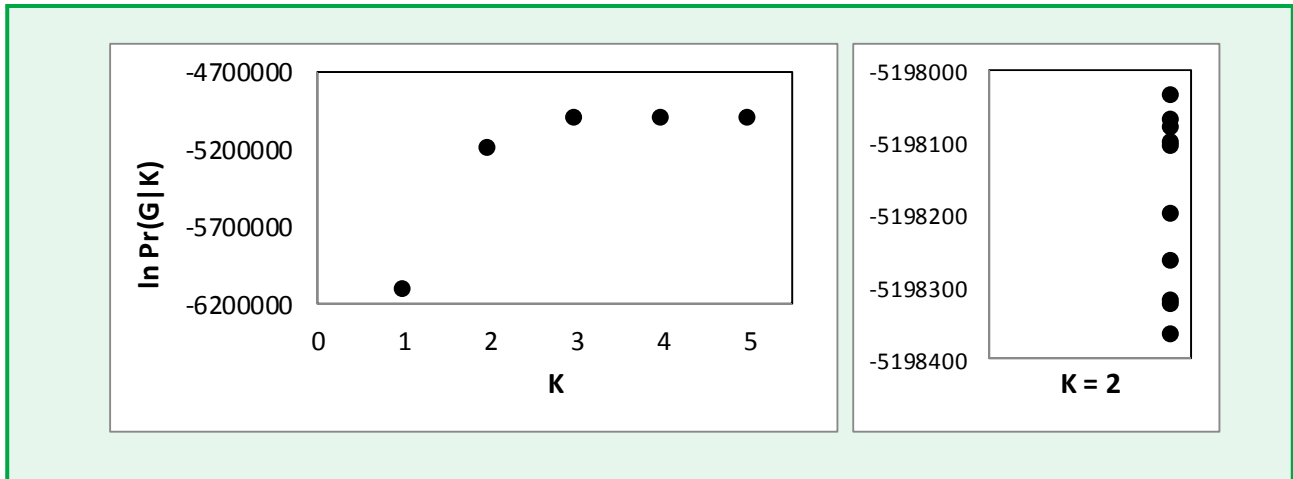
Figure 1   Ln *Pr*(*G*|*K*) values presented as a function of the number of clusters. The largest ln *Pr*(*G*|*K*) values within each *K* (among 10 runs) are presented with circles. In the right sight there are the values of all runs for *K* = 2
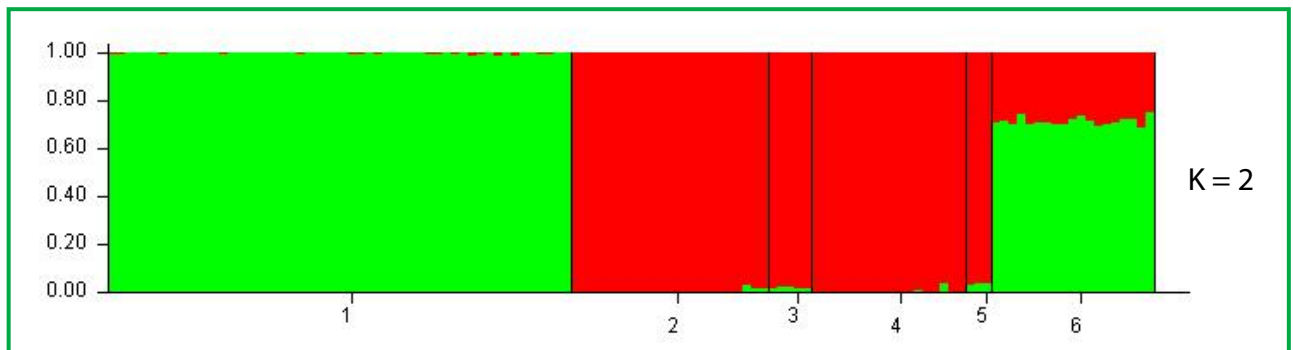
Figure 2   Graphical presentation of the population structure analyses for a sample of 6 cattle populations. Each individual is represented by a single vertical line broken into K colour segments, with lengths proportional to the estimated membership of the inferred cluster

Figure 3   Detailed length proportional to the estimated membership of the inferred cluster for each individual from 6 cattle populations

to improve its performance. Pinzgau cattle are divided into two separate populations. The first is represented by dual-purpose type and the second by suckler cows (beef). According the observation all populations have been taken into account, assuming correct classification into one of the populations. A quantification of how likely each breed and each individual belong to specific group is given in figure 2 and figure 3, respectively.

Generally, in table 2, studied breeds created Holstein (classification to cluster 2 = 99.9%) and Simmental (classification to cluster 1 = 99.7%) population. Holstein and Ayrshire are assumed as dairy breeds and others as dual-purpose confirming high ratio of breeds 2, 3 and 5 in cluster 1. Although Ayrshire is originally breed with high milk production, it was separated to the Simmental group, probably caused by its origin in native breeds with small body frame, bad feed utilization and low milk production. Surprisingly, Slovak Pinzgau is more similar to Holstein (milk production) than to Simmental (dual-purpose) despite the fact that primary Pinzgau belongs to dual-purpose or even beef type. Based on genetic insight into Slovak Pinzgau population systematic selection due to increasing of milk production is evident. A similar study was performed by Edea et al. (2013), who successfully distinguished between highland agro-ecology and lowland cattle breeds. Further Grasso et al. (2014) and Perez O'Brien et al. (2015) have demonstrated the assignment of individual groups based on their genetic similarities, thereby providing information about the number of ancestral population underlying the observed genetic diversity.

Table 2 Proportion of membership of each pre-defined population in one of the 2 clusters

Given populations	Inferred clusters (%)	
	1	2
1 (Holstein)	0.1	99.9
2 (Simmental)	99.7	0.3
3 (Austrian Pinzgau)	97.9	2.1
4 (Ayrshire)	99.7	0.3
5 (MRI)	96.3	3.7
6 (Slovak Pinzgau)	28.4	71.6

4. Conclusions

Genetic structure of Pinzgau cattle has been analysed using 42,854 autosomal SNPs. The Bayesian approach as effective detection for number of clusters was used. The average value of ln $Pr(G|K)$ increased up to $K = 3$ and balanced afterwards, assuming 3 clusters as the most suitable division of evaluated populations. However,

the level of admixture is visible just from two separate clusters. Pedigree data present monitored Slovak Pinzgau bulls as 100% purebred, though the historical formation of the breed should be taken into account. Similarity of populations with common history is clear. The results were controversial because Ayrshire as a dairy breed has been assigned to the Simmental group. All animals in that group are known as a Red and White spotted cattle what may denote the historical point explaining the structurness of observed populations. Surprisingly, according to our results Slovak Pinzgau is closer to Holstein (dairy cattle) than to Simmental (dual-purpose cattle) what could be caused by significant "holsteinization" in recent years. Confirming to other studies genetic data are suitable tool for distinction of local breeds of cattle. Detailed character of population structure is a subject of further investigation. It will be focused on finding admixture of populations with exact ratio of each population that contributed to origin of Slovak Pinzgau cattle. This should provide a basis for preservation of the breed in the original phenotype favoured by the current selection schemes and breeding programmes.

6. References

DECKER, J. E. et al. (2014a) Worldwide patterns of ancestry, divergence, and admixture in domesticated cattle. In *PloS Genetics*, vol. 10, no. 3, e1004254.
DOI: http://dx.doi.org/10.1371/journal.pgen.1004254

DECKER, J. E. et al. (2014b) Data from: Worldwide patterns of ancestry, divergence, and admixture in domesticated cattle. Dryad Digital Repository.
DOI: http://dx.doi.org/10.5061/dryad.th092

DRUML, T. et al. (2007) Individual-based assessment of population structure and admixture in Austrian, Croatian and German draught horses. In *Heredity*, vol. 98, pp. 114–122.

EDEA, Z. et al. (2013) Genetic diversity, population structure and relationships in indigenous cattle populations of Ethiopia and Korean Hanwoo breeds using SNP markers. In *Frontiers in Genetics*, vol. 4, pp. 1–9.

FALUSH, D., STEPHENS, M. and PRITCHARD, J. K. (2003) Inference of population structure using multilocus data: linked loci and correlated allele frequencies. In *Genetics*, vol. 164, no. 4, pp. 1567–1587. DOI: http://dx.doi.org/10.1111/j.1471-8286.2007.01758.x

GAUTIER, M., LALOË, D. and MOAZAMI-GOUDARZI, K. (2010a) Data from: Insights into the genetic history of French cattle

from dense SNP data on 47 worldwide breeds. Dryad Digital Repository. DOI: http://dx.doi.org/10.5061/dryad.2f185

GAUTIER, M., LALOË, D. and MOAZAMI-GOUDARZI, K. (2010b) Insights into the genetic history of French cattle from dense SNP data on 47 worldwide breeds. In *PloS ONE*, vol. 5, no. 9, e13038. DOI: http://dx.doi.org/10.1371/journal.pone.0013038

GRASSO, A. N. et al. (2014) Genomic variation and population structure detected by single nucleotide polymorphism arrays in Corriedale, Merino and Creole sheep. In *Genetics and Molecular Biology*, vol. 37, no. 2, pp. 389–395.

KADLEČÍK, O. et al. (2004) Development of dual-purpose Pinzgau cattle. Nitra: SPU, Slovak Republic, 128 p.

KADLEČÍK, O. et al. (2013) Diversity of cattle breeds in Slovakia. In *Slovak Journal of Animal Science*, vol. 46, no. 4, pp. 145–150.

KASARDA, R., KADLEČÍK, O. and MÉSZÁROS. G. (2008) Trends of endangered population of Pinzgau Cattle in Slovakia. In *Archiva Zootechnica*, vol. 11, no. 3, pp. 82–87.

MASTRANGELO, S. et al. (2014) The genome-wide structure of two economically important indigenous Sicilian cattle breeds. In *Journal of Animal Science*, vol. 92, pp. 4833–4842. DOI: http://dx.doi.org/10.2527/jas.2014-7898

NICOLAZZI, E. et al. (2014) SNPchiMp: a database to disentangle the SNPchipjungle in bovine livestock. In *BMC Genomics*, vol. 15, pp. 123. DOI: http://dx.doi.org/10.1186/1471-2164-15-123

PAVLÍK, I. et al. (2013) Comparison of genetic diversity in dual-purpose and beef Pinzgau populations. In *Acta Fytotechnica et Zootechnica*, vol. 16, no. 3, pp. 69–73.

PEREZ O'BRIEN, A. M. et al. (2015) Low levels of taurine introgression in the current Brazilian Nelore and Gir indicine cattle populations. In *Genetics Selection Evolution*, vol. 47, pp. 1–7.

PŠENICA, J. 1990. *Pinzgau cattle in Slovakia*. Bratislava: Príroda, pp.184 (in Slovak)

PURCELL, S. et al. (2007) PLINK: a tool set for whole genome association and population-based linkage analysis. In *The American Journal of Human Genetics*, vol. 81, no. 3, pp. 559–575. DOI: http://dx.doi.org/10.1086/519795

SIMČIČ, M. et al. (2008) Genetic characterization of autochthonous cattle breeds, Cika and Busha, using microsatellites. In *Acta agriculturae Slovenica*, supplement 2, pp. 71–77.

ŽIDEK, R. and KASARDA, R. (2010). Distribution of genetic distance within groups with different relationship coefficient. In *Acta fytotechnica et zootechnica*, vol. 13, pp. 73–76.