Progress in evaluation of diversity in Pinzgau cattle based on molecular markers

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SUMMARY

The aim of this study was to evaluate the genetic diversity in Pinzgau cattle based on the analysis of genomic data. Biological samples were obtained from a total of 19 proven sires with reliability of the breeding value over 65%. Except one, each of the selected bulls were born in Slovakia. Genomic DNA was genotyped using Illumina BovineSNP50 BeadChip. The analysis of genetic diversity across the selected individuals was carried out based on determination of the heterozygosity level and inbreeding-like effects across group of bulls characterized by F_{IS} fixation index. Moreover the effective population size based on linkage disequilibrium extent was assessed. After applying the quality control of genotyping data, 41,541 of autosomal loci passed the above filtering criteria and were usable for following statistical analysis. The average heterozygosity was observed at level 0.38 ± 0.01. The average negative value of inbreeding-like F_{IS} index signalized higher proportion of heterozygous genotypes across and within all analysed individuals. Both of the selected parameters indicated no reduction of heterozygosity across the evaluated breeding bulls. The analysis of genomic data based on the evaluation of linkage disequilibrium extent may provide an alternative view to the estimation of effective population size in comparison to pedigree data. In the present study, 34,672 pairwise LD values producing by syntenic adjacent marker pairs were useable for estimation of N_e. The observed recent effective population size in the analysed group of bulls was outside the critical limit that describes a breed as being endangered. But the value close to the minimum effective population size signalised the need of tools to monitor the selection process in relation to the control of inbreeding. Results of the present study confirmed the importance of the molecular markers utility in the prediction of

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genetic variability loss and also in managing of breeding programs in small livestock population.

Keywords: Pinzgau cattle, BovineSNP50 Bead chip, genetic diversity, SNP genotyping

INTRODUCTION

Biological diversity in agriculture is an irreplaceable factor in the sustainable development of agricultural production and rural areas. The loss of genetic diversity as results of inbreeding across livestock species including not only cattle breeds is currently a high problem for many breeders. The genetic diversity reflects degree of genetic variation that can be implemented across and within breeds in relation to the maintenance of the highest intraspecific variability. The preservation of genetic variation within livestock species is essential for creation of breeding strategies on national and also global level to obtain a variety of husbandry systems and animals that can adapt to different environmental changes. Moreover the studies of genetic diversity can help to explain the phenotypic variation resulting from the phylogenetic changes that occurred due to the development of each livestock species.

Pinzgau cattle belong to the alpine breeds, and from beginning of exports from Austria around 200 years ago they have been allocated to mountains areas of Slovakia. Pinzgau cattle kept under European conditions traditionally belong to the dual purpose breeds. Preservation of the dual purpose production type of the European Pinzgau population is generally regarded as a basic breeding aim. This breed is characterized by many excellent features, and that is why it has spread from Austria across the whole world. In spite of the fact that the Pinzgau cattle are now endangered population in Europe, many farmers are still interested in its keeping. The breeding aim is set primarily for the purebred population of the Pinzgau cattle. Its development can serve as a base for strategy of the further improvement and distribution under wider European conditions (Kadlečík et al., 2004a). The development of the Pinzgau population size in Slovakia is from long term perspective unfavourable. Due to the decline of active population the Pinzgau cattle can be considered as endangered and is necessary to keep its genetic variability. For monitoring and preservation of its genetic diversity several approaches was taken into account (Kadlečík et al., 2004b; Kasarda et al., 2008).

The aim of present study was to evaluate the genetic diversity in group of Pinzgau proven sires used in current selection schemes and breeding programmes in Slovakia based on analysis of genomic data.

MATERIAL AND METHODS

Biological samples

For present study 19 Pinzgau breeding bulls were selected based on following criteria: bulls had to be proven sires present on AI stations with reliability of breeding value over 65% and used in current breeding programs on farms in relation to the maintenance of Pinzgau breed genetic diversity. Except one, each of selected bulls were born in Slovakia from 1998 to 2006. Only one bull originating from lineage Nusil was bought as young sire in Austria. In total, selected bulls represent 13 lineages of Pinzgau cattle. The most frequent lineages were Nobel and Nusil represented by 4 and 3 bulls, respectively.

SNP genotyping and quality control of data

Genomic DNA was extracted for each of selected bulls from semen samples and genotyped using Illumina BovineSNP50 BeadChip containing in total 54,609 SNPs (Illumina, Inc. San Diego, USA) in the commercial lab. The quality control of genotyping data was carried out according to Purcell et al. (2007) in order to select the most appropriate set of loci for following analysis of genetic diversity. Firstly, all SNPs with unknown genomic location and loci assigned to the chromosome X and Y were excluded. In the second step, the quality control for remaining SNPs in dataset was done to remove any SNPs with call rate under 0.95, with more than 10% missing genotypes, monomorphic SNPs and SNPs with minor allele frequency (MAF) under 0.05, and deviation from Hardy-Weinberg equilibrium with limit of 0.001.

Analysis of genetic diversity

After applying quality control of data 41,541 autosomal loci were retained in dataset. The analysis of genetic diversity across selected individuals was performed based on determination of the heterozygosity level and inbreeding-like effects within groups of bulls characterized by F_{IS} fixation index (Weir and Cockerham, 1984) according to Liu and Muse (2001). Subsequently, the extent of linkage disequilibrium (LD) was estimated using SNP & Variation Suite (v7.6.8 Win 64, Golden Helix, Bozeman, MT, USA www.goldenhelix.com). The pairwise r^2 values as measure of the LD extent were estimated using only adjacent syntenic loci marker pairs divided to the distance bins by 50 kb intervals up to 2000 kb. The physical position of SNPs included in the genotyping array is accessible in the official Illumina web sites. In order to reflect the effect of physical distance between loci on the LD extent the average r^2 values were computed for each distance bin (Figure 3). In the final step the effective population size (N_e) was estimated based on the LD (r^2) according to Sved (1971). The genetic distances were derived from intermarker

distances between two considered loci under the simple assumption 1Mb ~ 1 cM. The adjacent syntenic loci marker pairs with values of $0.01>r^2>0.99$ were filtered out from the computation. The past effective population size (N_t) at generation t (t=1/2c) was plotted against the t categories (Figure 3).

RESULTS AND DISCUSSION

After the quality control of genotyping data, 75.71% of the autosomal loci passed the above filtering criteria. The usable part of loci within each chromosome that were selected for analysis of genetic diversity in analysed group of Pinzgau bulls is shown on Figure 1.

SNP density was in average one SNP per 60.27 kb with maximum gap 1962.29 kb and minimum gap 65 bp. The highest number of polymorphic SNPs was found on autosome 1 (BTA 1) and the lowest on BTA 28, what is in accordance with total coverage of loci assigned to the each autosome on genotyping array.

Figure 1 also shows the proportion of loci identified as polymorphic to the total number of analysed SNPs. The observed minor allele frequency (MAF) indicated an almost uniform distribution within each frequency classes (Figure 2) with the average 0.27 ± 0.13, which reflect also the SNPs composition of bovine genotyping array.

The Illumina BovineSNP50 BeadChip was prepared using the loci set that should cover common SNPs validated in economically important beef and dairy cattle breed types and present an average MAF of 0.25 across all loci (Boichard et al., 2012; Wiggans et al., 2012; Kasarda et al., 2015a).

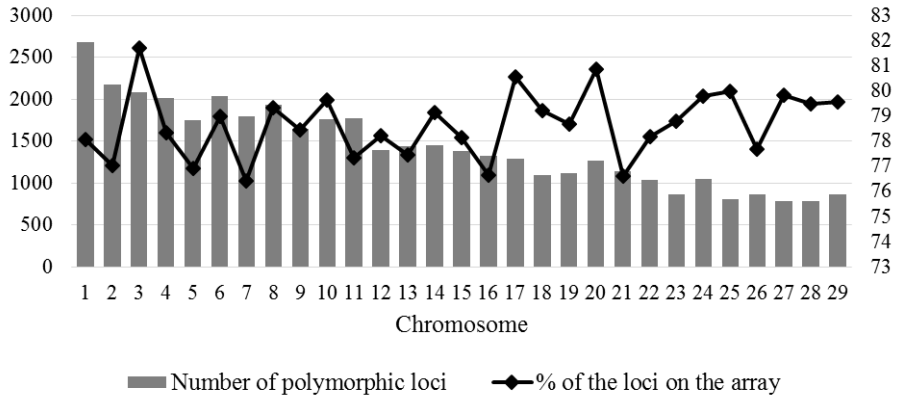

Figure 1. Distribution of the polymorphic loci across autosomes and their proportion to the total number of SNPs on the genotyping array

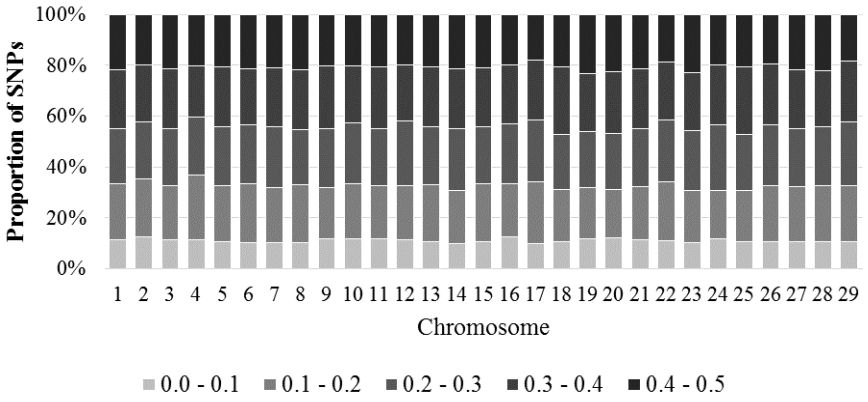

Figure 2. Proportion of polymorphic loci within different MAF classes

The evaluation of genetic diversity across analysed individuals was carried out by determination of the heterozygosity level and F_{IS} index reflecting the inbreeding-like effects within group of selected breeding bulls. Moreover the effective population size based on linkage disequilibrium extent was assessed. The average heterozygosity at level 0.38 ± 0.01 was observed across group of Pinzgau bulls. Similarly, the average negative value of inbreeding-like F_{IS} index (-0.01 ± 0.02) signalized higher proportion of heterozygote genotypes across all analysed individuals.

Sufficient level of heterozygosity in Pinzgau population was reported also by Šidlová et al. (2014a), but the analysis based on identification of 8 microsatellite markers indicated the need of continuous monitoring. By the using of genomic data the inbreeding coefficient for each of the analysed individuals or across whole population can be estimated more precisely based on the assessment of runs of homozygosity (ROH). Runs of homozygosity are contiguous lengths of homozygous genotypes that are present in an individual due to parents transmitting identical haplotypes to their offspring. The extent and frequency of ROHs can inform on the ancestry of an individual and its population (Purfield et al., 2012). In relation to the lengths of ROH segments different inbreeding coefficients are expected mainly in respect to remote common ancestors.

Based on this approach Šidlová et al. (2014b) observed in Slovak Pinzgau cattle decrease of the inbreeding level in comparison to the population originating from Austria that could be due by the development of breeding strategies for Slovak Pinzgau. But the evaluation of selection signatures in population of Slovak and Austrian Pinzgau clearly showed use of uniform selection schemes and only low genetic differentiation in both populations resulting mainly from common ancestors (Kasarda et al., 2015b).

One of the important genetic parameter that can reflect the rate of inbreeding, loss of genetic variation and effectiveness of selection is the effective population size (N_e). The analysis of genomic data based on evaluation of linkage disequilibrium (LD) extent may provide an alternative view to estimation of N_e in comparison to pedigree data. In present study 34,672 pairwise LD (r^2) values producing by syntenic adjacent marker pairs were used for estimation of N_e. Across all adjacent syntenic loci the average r^2 value was 0.20 ± 0.10. Only 0.26% of marker pairs showed complete linkage equilibrium and were excluded from the subsequent analysis. Estimation of the effective population sizes based on LD using the Sved formula (1971) assuming no mutations or selection can leads to difficulties in handling values that are in the limits of the parameter space, because if r^2 is equal to zero, the estimate of N_e is infinite, and if r^2 is one, it is also zero (Uimaro and Tapio, 2010). For this reason only r^2 values between 0.01 and 0.99 was used.

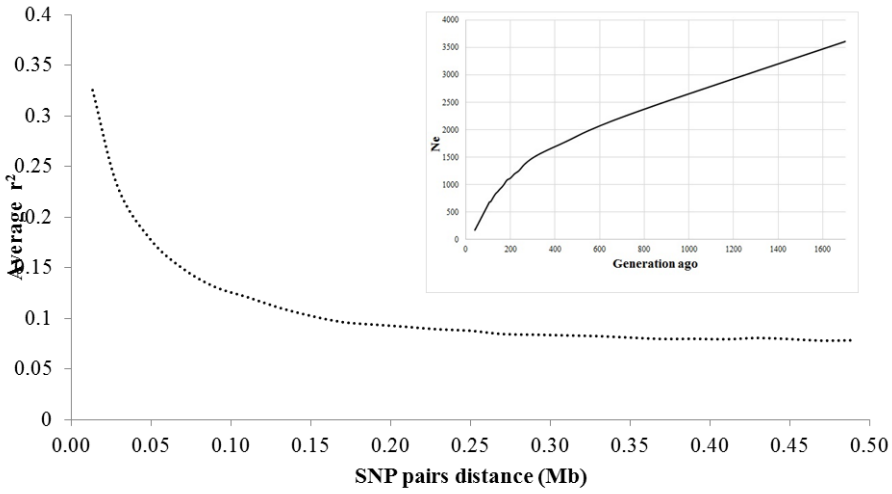

Figure 3. The decay of the r^2 level in relation to the increase of genetic distance and past N_e over time observed in analysed Pinzgau population

Figure 3 shows the observed level of N_e in analysed group of bulls during the past. In relation to the increase of genetic distance the decline of both r^2 and N_e was found (Figure 3). The observed recent effective population size in analysed group of bulls (around 172) was outside the critical limit N_e<100 that describing a breed as endangered (FAO, 1998). The results are in accordance with N_e that was reported for Slovak and Austrian Pinzgau population based on the pedigree data (Kadlečík et al., 2013; Pavlík et al., 2014). Moreover Pavlík et al. (2014) found significant relationship between the increase of inbreeding

and decline of the effective population size in both analysed Pinzgau population. In the analysis of the past N_e (500 generations ago) was found relatively low value (1.992) what can signalise presence of the population bottleneck.

CONCLUSIONS

The results of present study showed that the utility of molecular markers on genomic level can improve the genetic diversity estimation also in small livestock populations that are mainly endangered by the loss of genetic variability. The level of observed heterozygosity in analysed group of Pinzgau breeding bulls seems to be sufficient for their successfully use in future management of Pinzgau population in Slovakia. But the value of recent effective population size close to the minimum required number to preserve the genetic diversity indicated the need of permanent monitoring mainly in relation to the control of inbreeding.

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REFERENCES

Boichard, D., Chung, H., Dassonneville, R., David, X., Eggen, A., Fritz, S., Gietzen, K. J., Hayes, B. J., Lawley, C. T., Sonstegard, T. S., Van Tassell, C. P., VanRaden, P. M., Viaud-Martinez, K. A., Wiggans, G. R., 2012 - Design of a Bovine Low-Density SNP Array Optimized for Imputation, PLoS One, 7, e34130

FAO, 1998 - Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans. Food and agriculture organization of the United Nations. Rome, Italy

Kadlečík, O., Hazuchová, E., Pavlík, I., Kasarda, R., 2013 - Diversity of cattle breeds in Slovakia, Slovak Journal of Animal Science, 46, 4, 145-150

Kadlečík, O., Kasarda, R., Hetényi, L., 2004b - Genetic gain, increase in inbreeding rate and generation interval in alternatives of Pinzgau breeding program, Czech Journal of Animal Science, 49, 12, 524-531

Kadlečík, O., Swalve, H.H., Lederer, J. A., Grosu, H., 2004a - Development of dual-purpose Pinzgau cattle, Publishing and Editorial Centre of Slovak University of Agriculture in Nitra, Nitra, 101-105

Kasarda, R., Kadlečík, O., Mészáros, G., 2008 - Trends of endangered population of Pinzgau Cattle in Slovakia, Archiva Zootechnica, 11, 3, 82-87

Kasarda, R., Moravčíková, N., Trakovická, A., Mézsáros, G., Kadlečík, O., 2015b – Genome-wide selection signatures in Pinzgau cattle, Potravinárstvo, 9, 1, 268-274

Kasarda, R., Moravčíková, N., Židek, R., Mészáros, G., Kadlečík, O., Trakovická, A., Pokorádi, J., 2015a - Investigation of the genetic distances of bovids and cervids using BovineSNP50k BeadChip, Archiv Tierzucht, 58, 1, 57-63

Liu, K., Muse, S. V., 2005 - PowerMarker: integrated analysis environment for genetic marker data, Bioinformatics, 21, 2128-2129

Pavlík, I., Sölkner, J., Kadlečík, O., Kasarda, R., Mézsáros, G., Fuerst Ch., Fuerst-Waltl, B., 2014 - Joint genealogical analysis as a tool for diversity evaluation in Pinzgau cattle populations, Archiv Tierzucht, 57, 14, 1-12

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., Maller, J., Skalp, P., De Bakker, P. L., Daly, M. J., Sham, P. C., 2007 - PLINK: a tool set for whole genome association and population-based linkage analysis, Am J Hum Genet, 81, 559-575

Purfield, D. C., Berry, D. P., McParland, S., Bradley, D. G., 2012 - Runs of homozygosity and population history in cattle, BMC Genetics, 13, 70

Šidlová, V., Kasarda, R., Moravčíková, N., 2014a - Genealogic structure of Slovak Pinzgau cattle population, In MendelNet 2014, Mendel University, Brno, 187-191

Šidlová, V., Kasarda, R., Trakovická, A., Curik, I., Ferenčakovic, M., 2014b - Inbeeding coefficient derived from runs of homozygosity estimation in Pinzgau cattle using bovineSNP50 BadChip, Book of abstract International scientific genetic conference „XXVI. Genetic Days“, ČZU Praha, 179-182

Sved, J. V., 1971 - Linkage disequilibrium and homozygosity of chromosome segments in finite population, Theoretical Population Biology, 2, 125-141

Uimari, P., Tapio, M., 2011 - Extent of linkage disequilibrium and effective population size in Finnish Landrace and Finnish Yorkshire pig breeds, Journal of Animal Science, 89, 609-614

Weir, B. S., Cockerham, C. C., 1984 - Estimating F-statistics for the analysis of population structure, Evolution 38, 1358-1370

Wiggans, G. R., Cooper, T. A., Vanraden, P. M., Olson, K. M., Tooker, M. E., 2012 - Use of the Illumina Bovine3K BeadChip in dairy genomic evaluation, Journal of Dairy Science, 95, 1552-1558