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Review Article

Use of High-density SNP analyses to develop a long-term strategy for conventional populations to prevent loss of diversity – review

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The aim was to review obtained results related to molecular-genetic analyses by high-density SNP chips and mtDNA in farm and wild populations within the project APVV-17-0060 Genomic Indicators of extranuclear DNA as a source of Diversity for Animal Breeding. Continuous human activity and subsequent socioeconomic and climatic changes of the environment significantly affect the genetic diversity of livestock on both intra- and inter-population levels. Concerning the conservation of local livestock populations and thus animal genetic resources (AnGR) for future generations is, therefore, necessary to monitor and look for new "more precise" tools to measure the amount of genetic diversity. The expected result will be the identification of SNP markers and spot mutations with a significant effect on the process of development resp. variability of traits. In the case of the dog, identification of regions related to fitness, health, and trainability will be the primary objective.

Keywords: Genetic diversity, economically important breeds, Animal genetic resources, Slovakia

1 Introduction

Continuous human activity and subsequent socioeconomic and climatic changes of the environment significantly affect the genetic diversity of livestock on both intra- and inter-population levels. Concerning the conservation of local livestock populations and thus animal genetic resources (AnGR) for future generations is, therefore, necessary to monitor and look for new "more precise" tools to measure the amount of genetic diversity. The volume of conserved diversity is the primary source of the organism's adaptation ability. The molecular-genetic studies play a significant role from the perspective of effective AnGR management. For this reason, the traditional methods of phenotype evaluation using morphological traits should be completed by modern molecular techniques related to the analysis of particular genetic markers to preserve the gene pool of autochthonous livestock breeds.

The DNA is mainly localised in the cell nucleus; previous research was predominantly focused on nuclear DNA. Currently, the diversity evaluation by DNA sequencing, gene mapping, and genetic markers on the autosomal genome level is very well developed not only in livestock populations but also in wild populations. In Slovakia, diversity studies were mainly based on the microsatellites (Moravčíková et al., 2016; Kasarda et al., 2017; Kukučková et al., 2017). Paternity microsatellites are the most commonly used markers approved by ISAG/FAO executive committee as a standard tool for parentage testing as well as for genetic resources conservation. The commercial introduction of 50K SNP BeadChip in 2007 (Illumina, USA) has increased the interest of cattle breeders around the world to implement the known associations between SNPs and economically significant quantitative trait loci (QTL) into the breeding programs (Lu, 2012; Kasarda et al., 2015). Moreover, the availability of large numbers of SNP markers has resulted in new ways to evaluate genetic diversity in more detail and to improve the prioritisation of animals for genetic diversity conservation (Moravčíková et al., 2017). The preservation of livestock genetic diversity is essential for both intra- and inter-populations point of view (Kasarda et al., 2016; Moravčíková et al., 2016). The use of high-density

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genotyping arrays allows more detailed assessment of diversity on genome-wide level (Engelsma et al., 2012), distribution of autozygosity islands (ROH) (Kukučková et al., 2017; Curik et al., 2014) as well as the loss of heterozygosity (LOH) (Ferenčaković et al., 2016).

Therefore, the aim is to review obtained results related to molecular-genetic analyses by high-density SNP chips and mtDNA in farm and wild populations within the project APVV-17-0060 Genomic Indicators of extranuclear DNA as a source of Diversity for Animal Breeding.

2 Material and methods

Within the first period of the project was following the planned schedule and goals, realised the first phase, i.e., molecular-genetic analyses with the use of SNP arrays and sequencing of mtDNA successfully.

In the first period was collected biological samples and extracted nuclear DNA of multiple animal species, including horses, cattle, and dogs. In the case of cattle, it was samples of beef (Charolais, Aberdeen Angus, Limousine) and dairy (Jersey), selected based on the pedigree information and performance (Moravčíková et al., 2019a). Nuclear DNA of those animals was genotyped using different types of bovine SNP Chips, i.e., 150k (n = 50) and 50K (n = 38) SNP arrays. In horses, samples of three breeds were analysed: Lipizzan, Slovak Thoroughbred, and Norik of Muran (Moravčíková et al., 2019b,c; Moravčíková et al., 2020a; Kasarda et al., 2019a,b). Individuals were selected similarly as in the case of cattle based on pedigree information as well as performance. In total, there were within the first period genotyped 93 biological samples. In the case of dogs were collected samples of 48 animals of Tatra hound. The nuclear DNA of those animals was analysed by the use of 230k SNP array (Moravčíková et al., 2019d).

3 Results

3.1 Evaluation of genetic variability of livestock and wild populations in Slovakia

a) Evaluation of differentiation level by SNP genotyping

Bioinformatics' analyses of genomic data available for Slovak Pinzgau and Slovak Spotted cattle (50k bovine SNP Chip, microsatellites) were used for the assessment of genetic differentiation (Kasarda et al., 2019c,d; Lehocká et al., 2019; Trakovická et al., 2019). Several previous studies already confirmed that genetic variability of a particular breed, as well as the effective population size, could be reduced due to the bottleneck effect and non-equal use of sires. Therefore mainly the Slovak Pinzgau cattle could serve as the excellent model population for optimisation of methodological approaches for evaluation of genetic diversity loss and impact of inbreeding depression on the genome of small populations. Besides this, genomic data of individuals belonging to the Austrian Pinzgau as well as Slovenian Cika and Piedmontese (n = 179) were also used for better assessment of the level of genetic differentiation (Kukučková et al., 2018).

The Bayesian algorithm implemented in BAPS and Structure software was used to determine the level of genetic differentiation between and within populations. Based on the level of individual membership probabilities within particular populations, gene-flow matrix among identified genetics groups were set up. Obtained results showed that the relatively low level of genetic differentiation (FST = 0.02) reflecting mainly the origin of analysed populations. Expected high gene-flow between populations was confirmed between Slovak Pinzgau and Austrian Pinzgau populations. Moreover, the obtained gene-flow matrix suggested that Piedmontese contributed to the gene-pool of other populations during the early development phase of all breeds; however, its gene-pool was not affected by Pinzgau nor Cika (Kukučková et al., 2018).

Further analysis confirmed that even Slovak Pinzgau and either Austrian Pinzgau gene-pools are unique, as well their cultural and historical value. From that point of view, applied approaches were considered as very beneficial and usable in the quantification of genetic diversity and differentiation in various cases, including wild populations. This idea was used in the study that tested the transferability of bovine 50k SNP array into non-model wild species. The non-model species was represented by three tribes from family Cervidae: reindeer (tribe Rangiferini), moose (tribe Alceini), red deer, wapiti and sika deer (tribe Cervini). Biological samples of red deer (*Cervus elaphus*) included wild animals as well as captive deer from Slovakia. In the present, deer breeding becomes popular, not only in surrounding countries but also in Slovakia. Although the call rate was compared to the cattle significantly lower (61.66%), agreed with those published till now. After quality control, i.e., exclusion of markers and individuals with missingness higher than 10% and markers with minor allele frequency (MAF) lower than %1, were identified in total 1374 polymorphic SNP markers. Obtained results showed that the application of cross-species SNPs genotyping could be regarded as a valuable tool to clarify the proportion of genetic variance conserved within family Cervidae and can also be used to assess the proportion of admixture among closely related species. Applied approaches validated a strong genetic distinction among cervid species at the tribe levels. As expected due to their phylogeny origin, the frequency of alleles varied continuously across three

main genetic clusters composed from genus Cervus, Rangifer, and Alces. Genetic distance matrices revealed the closest genetic affinity between the species from genus Cervus, whereas the highest genetic distance was found between genera Cervus and Alceini. Based on the results could be concluded that the presented methods are from the point of evaluation of the state of genetic diversity informative, and therefore suitable for further application within the project (Moravčíková et al., 2018a; Moravčíková et al., 2019e; Kasarda et al., 2019e).

b) Evaluation of differentiation based on mtDNA

Several methodological approaches were tested to evaluate the distribution of mtDNA haplogroups of Tatra Chamois (Rupicapra R. Tatrica) in area of the High Tatras as well as the development of a methodology for reliable differentiation of gene-pools between closely related Alpine Chamois. Haplotypes were identified based on sequencing of the mtDNA control region (D-loop, the final length of 480 bp). In total, sequences of 143 individuals from the High Tatras area were analysed and compared with the haplotypes of seven subspecies belonging to the genus Rupicapra, stored in GenBank. The sequences of *Bos taurus*, Ovis aries and Capra hircus were included in the phylogenetic analysis as outgroups. A consensus sequence was aligned according to the ClustalO methodology. The final length of the aligned region was 428 bp. For the reconstruction of the phylogeny relationship, Neighbour-Joining and Jukes-Cantor models were used. The phylogenetic relationship between haplotypes was then visualised by the Neighbour – Joining tree. The pattern of sequence evolution was analysed using the unrooted phylogenetic network. Despite the relatively short fragment of the D-loop region analysed, reliable differentiation of the particular subspecies was obtained. Thus, our study showed that applied methodology represents an efficient (non-invasive) tool that can differentiate between the Alpine and Tatra chamois to monitor wild populations. Moreover, results indicated in the population of Tatra Chamois' presence of new (till now not explored) haplotype. From the analysis of geographical distribution was observed that population with this haplotype inhabits predominantly Western Tatras (Moravčíková et al., 2020b).

3.2 Detection of selection signatures, identification of mutations and haplotypes based on sequence analysis

Within the present stage of the project, mainly information on nuclear DNA level was used for the detection of selection signatures. Several approaches going out from the evaluation of the distribution of autozygous segments in the genome were tested repeatedly. The aim was to estimate the strength of linkage disequilibrium and identify haplotype structure. The above approaches were tested on the data from the Slovak Spotted population. Information about 37,833 SNP, covering 2,496,829 kb of the genome, were used. The average distance between adjacent markers was 66.05 ±70.27 kb. The minimum distance between markers was 0.001 kb, and the maximum distance was 4,428.95 kb. Analysis of selection signatures was based on the presumption that genomic segments under intense selection pressure developed as the result of the breeding of selected phenotypic traits, defined in the breed standard and breeding goal of Slovak Spotted. Only autozygous segments >4 MB were evaluated based on previous studies because these represent a proportion of autozygosity derived from common ancestors more than 12.5 generations ago. All the applied approaches confirmed, that intense selection act predominantly on specific regions of three chromosomes: BTA5 (59665562-68880383 bp), BTA6 (32489075-42866573 bp and 68546212-72969608 bp) and BTA7 (41321459-46354401 bp). Signals were detected directly in the sequences of genes involved in genetic control of milk production (ABCG2, SPP1, FAM13A), growth and feed conversion (FAM184B, PKD2, PACRGL), reproduction (GDF9) and coat colour (KIT, KDR) (Moravčíková et al., 2018b; Moravčíková et al., 2019f; Kasarda et al., 2019f).

3.3 Estimation of the effect of SNP polymorphism and mtDNA variability on phenotypic variability

Effect of CSN2 polymorphism on milk production, udder health, fertility and type traits was evaluated in Holstein cattle in Slovakia. Linear models were used for the statistical analysis (proc GLM, SAS). Significant positive results were observed in the production and content of milk protein, especially in the case of the A2A2 genotype. Presence of the A2 (A1A2 and A2A2) in genotype positively affected protein content during lactation in case of all animals. Besides, this association study showed that cows with A2A2 genotype achieved significantly lower SCS in comparison to A1A2 or A1A1 genotypes. The significant effect of CSN2 polymorphism was also confirmed in the total score for the udder type and its rear length (Miluchová et al., 2018).

4 Conclusion and recommendation

In the case of nuclear DNA will be genotyping of an individual based on SNP BeadChip application with a high density of markers (bovine SNP50k, bovine SNP150k, bovine IDB, equine SNP70k, canine 250K, etc.). In the case of mtDNA sequencing, special attention will be given to the control (D-loop) region. Besides, the

database of genomic information about individuals of particular populations will be created and database of available phenotypes with the aim of the evaluation of the effect of SNP markers and mtDNA variability on phenotypic variability. The biostatistical analysis will be in the next period oriented on the assessment of the present state of genetic diversity. The degree of their genetic differentiation mainly in connection to genetically related subpopulations (species resp. breeds) and identification of haplotype structure of populations will be evaluated/ monitored. Analysis of SNP effect and mtDNA variability will be realised based on whole-genome association study principles (GWAS), oriented on economically important traits in case of livestock (cattle, horses). The expected result will be the identification of SNP markers and spot mutations with a significant effect on the process of development resp. variability of traits. In the case of the dog, identification of regions related to fitness, health, and trainability will be the primary objective.

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