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Kaposvár University, Faculty of Agricultural and Environmental Sciences, Kaposvár

Microsatellite analysis of population structure in Slovak Pinzgau cattle

V. Šidlová, R. Kasarda, N. Moravčíková, A. Trakovická, O. Kadlečík
Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources
94976 Nitra, Tr. A. Hlinku 2, Slovak Republic

ABSTRACT

The aim of the study was improve knowledge about population structure of Slovak Pinzgau cattle using genetic markers. Observed population structure was characterized by use of eight microsatellites. Each locus was tested for deviations from the Hardy-Weinberg Equilibrium (HWE). In general, breed was in genetic equilibrium, only locus BM1824 deviated from HWE. Cluster algorithms identify groups of related individuals without reference to prior information of the genetic subdivision. We considered 3 clusters that capture the major structure of the data (representative K value) and most likely reflect genealogical structuring. The chosen set of microsatellites confirmed the suitability for genetic structure assessment and its usefulness in determination of the subpopulations for Pinzgau cattle in Slovakia.
(Keywords: genetic structure, microsatellites, Pinzgau cattle, subpopulations)

INTRODUCTION

Many industrial breeds currently suffer from inbreeding, and genetic resources in cattle, sheep, and goats are highly endangered, particularly in developed countries (*Taberlet et al.*, 2008). Genetic diversity within farm animal species refers to the extent of genetic variation within and among breeds, strains and lines in order to preserve the highest intraspecific variability (*Lenstra et al.*, 2012). Maintaining genetic variation is an important requirement for future animal breeding strategies, to match animals to a variety of husbandry systems and for adaptation to environmental changes. In addition, genetic diversity of livestock species is of considerable scientific interest for understanding phenotypic variation (*FAO*, 2007) and for reconstructing the history of livestock (*Ajmone-Marsan et al.*, 2010; *Groeneveld et al.*, 2010).

Slovak Pinzgau cattle are divided into two separate populations. The first is represented by dual-purpose type (dairy) and the second by beef suckler cows (beef). Pinzgau cattle are an original Alpine breed, which had been imported to Slovakia approximately 200 years ago. Thanks to its unique traits as longevity, fertility, health, grazing ability it had been bred in mountain regions of northern Slovakia, but there is significant decline of the population in recent years. Due to this, the population can be considered endangered and it is necessary to assess genetic variability. Taking in the account the situation alternatively breeding programs were optimised (*Kadlečík et al.*, 2004), development were monitored (*Kasarda et al.*, 2008) and analyses of genetic diversity were performed (*Pavlík et al.*, 2013).

Microsatellite markers have been widely used for population genetic analyses and structure of livestock species, as they are informative and can successfully elucidate the relationships between individuals and populations, including also cattle populations (*Sun et al.*, 2007). Microsatellites have been commonly used to assess within-breed genetic diversity and inbreeding levels, introgression from other species, genetic differentiation, admixture among breeds (*Ginja et al.*, 2009) and to define conservation priorities *(Lenstra et al.*, 2012).

Pritchard et al. (2000) described a Markov chain Monte Carlo (MCMC) scheme clustering individuals into populations and estimating the probability of membership (or, for the admixture ,odel, the proportion of membership) in each population.

The most widely used measures of population structure are Wright's F statistics (*Wright*, 1931), which partition the genetic variation in a within-subpopulation component (average subpopulation inbreeding coefficient F_{IS}) and between-subpopulations component (fixation index F_{ST}), with the inbreeding in the total population described by the inbreeding coefficient F_{IT} (*Lenstra et al.*, 2012). In case of heterozygosity decreasing in population F_{IS} value will be positive and opposite, if there is a sufficient number of heterozygotes, this value will be negative (*Hamilton*, 2009). F_{ST} measure provide important insight into the evolutionary processes that influence the structure of genetic variation within and among populations, and they are among the most widely used descriptive statistics in population and evolutionary genetics (*Holsinger and Weir*, 2009). To calculate these indices, one needs first to define groups of individuals and then to use their genotypes to compute variance in allele frequencies. Thus, a fundamental prerequisite of any inference on the genetic structure of populations is the definition of populations themselves. Population determination is usually based upon geographical origin of samples or phenotypes. However, the genetic structure of populations is not always reflected in the geographical proximity of individuals. Populations that are not discretely distributed can nevertheless be genetically structured, due to unidentified barriers to gene flow. In addition, groups of individuals with different geographical locations, behavioural patterns or phenotypes are not necessarily genetically differentiated (*Evanno et al.*, 2005). Bayesian approach uses a Monte Carlo-Markov Chain (MCMC) simulation to infer the most probable number of population clusters and to estimate the proportional contribution of each of the assumed subpopulations to the genotypes of an individual (*Pritchard et al*., 2000).

The aim of this study was to assess genetic structure of Slovak Pinzgau cattle population based on polymorphism at microsatellite loci using statistical programs. This should allow improve our knowledge of population structure and genetic variability with using for preservation of the breed in the original phenotype supported by the current selection schemes and breeding programmes.

MATERIAL AND METHODS

Random selected 302 cows of Pinzgau cattle from four Slovak farms were analysed. Both farming types were represented (beef and dual-purpose), purebred and crossbred animals. DNA was isolated from hair roots and amplified in one multiplex PCR with 8 microsatellites (TGLA122, CSSM66, TGLA227, ILST006, CSRM60, ETH3, BM1824, SPS115). To determine the polymorphism of microsatellite DNA sequences was used fluorescent fragmentation analysis by ABI PRISM 310 Genetic Analyser and the allele sizes were evaluated. Microsatellite analysis using fluorescently-labelled primers and capillary fractionation is the pre-eminent method for the genetic analysis of eukaryotic

organisms. All loci were tested for deviations from the Hardy-Weinberg equilibrium (HWE) using a permutation version of the exact test given by *Guo and Thompson* (1992) provided in PowerMarker V3.25 software (*Liu and Muse*, 2005).

First, observed animals were divided into subpopulations based on farm, where are the animals living, breed type, respectively level of admixture of other breeds, year of the birth and line of father. To describe the properties of a subdivided population F-statistics, genetic identity and distance measures were estimated using above-mentioned software. F_{IS} and F_{ST} values per locus with standard deviation (SD) estimated on 1000 bootstrap replicates were computed. A priori divisions were tested using GENETIX 4.05.2 (*Belkhir et al*., 1996-2004), F_{ST} significance and corresponding analyses showing admixed population was observed.

Second, the Bayesian clustering algorithm implemented by the STRUCTURE 2.1 software (*Pritchard et al*., 2000) was used to infer the population structure. The program enables estimation of a 'hidden structure', that is the number of different clusters (K partitions) obtained without using any a priori information about individual membership (population and/or 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 uses the MCMC method, see also *Falush et al*. (2003), and 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). To obtain a representative value of K for modelling the data, we ran 10 independent runs of the Gibbs sampler for each K between 1 and 8 with a burn-in length of 10^5 followed by 10^5 iterations. 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. After determining the most likely number of subpopulations, the contribution of each K to whole population was estimated.

RESULTS AND DISCUSSION

Out of the 8 analysed loci only BM1824 showed highly significant ($P \leq 0.001$) HWE deviations across breed. The overall average of fixation index was close to zero ($F_{IS} = -0.0039$) which means the reduction of heterozygosity in the whole population was not observed. The F_{ST} has reached following values according to the division method: 0.0188 by farm, 0.003 by breed type, 0.053 by year of the birth and 0.0669 by paternal lines. Detection of possible subpopulation structures provided us with initial view at the genetic structure of Slovak Pinzgau cattle. Positive F_{ST} values indicate a deficiency in heterozygotes in the subpopulations, whereas in the whole population appears to be sufficient heterozygosity, what may imply the Wahlund effect. Generally, F_{ST} values between 0.05 and 0.3 are typical for differentiation of livestock breeds, with a value over 0.15 indicating significant differentiation (*Frankham et al.*, 2002), although much smaller values can be significant (*Lenstra et al.*, 2012). A priori divisions were tested using GENETIX and no statistical significance was observed as well as correspondence analyses showed rather admixed population in all cases.

We applied STRUCTURE to measure the population structure as the implemented algorithm uncovers 'hidden structure' without using any a priori knowledge about the number of clusters present in dataset. In order to illustrate a decision on the most likely

number of clusters present in the dataset (the most likely parameter K), in *Figure 1*, we presented ln Pr(G|K) values for all STRUCTURE runs. Over the entire cattle population, ln Pr(G|K) increased from K=1 to K=3, after which it began to decline. It was assumed that the most likely K is that where ln Pr(G|K) is maximised. We therefore considered K=3 as being the number of clusters that capture the major structure of the data (representative K value). The difference of ln Pr(G|K) between K=1, K=2 and K=3 are small (less than 200 between K=3 and K=2) so the structure obtained is relatively weak and most likely reflecting genealogical structuring. A quantification of how likely each individual is to belong to each group is given in *Figure 2*.

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 cirkles

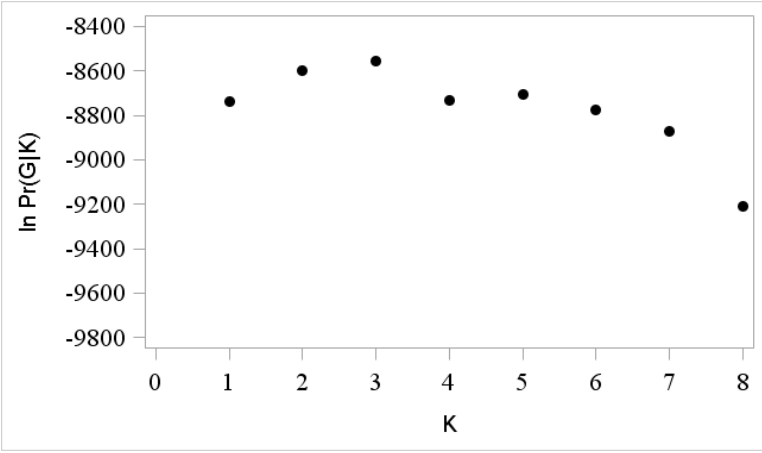
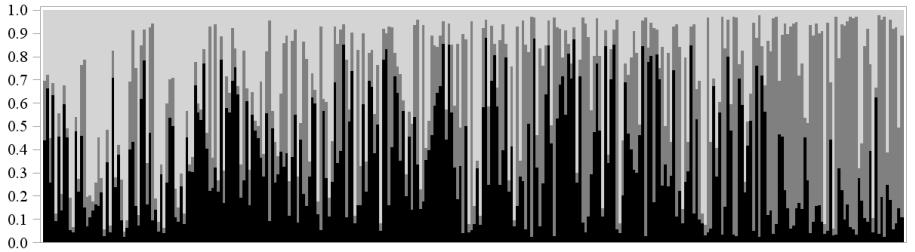


Figure 2

Graphical presentations of the population structure analyses for a sample of 302 Pinzgau cows (without a priori information about subpopulations). Each cow is represented by a single vertical line broken into K colour segments, with lengths proportional to the estimated membership of the inferred cluster



CONCLUSIONS

Genetic structure of Pinzgau cattle population has been analysed using set of 8 microsatellites. The Bayesian approach implemented by the STRUCTURE software was effective in detecting number of clusters. The mean value of ln Pr(G|K) increased up to K=3 and dropped afterwards, indicating the most likely value to be K=3. No of a priori subdivision was significant, however we assumed that population division is based on genealogical information. Concrete character of population structure is a subject of further investigation. The used set of microsatellites can be applied in more detailed studies in the future by analysing more breeds, larger numbers of animals per breed. This should allow improve our knowledge of origin and phylogenetic relationships to other breeds and provide a basis for preservation of the breed in the original phenotype favoured by the current selection schemes and breeding programmes

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REFERENCES

Ajmone-Marsan, P., Garcia, J.F. and Lenstra, J.A. (2010). On the origin of cattle: how aurochs became cattle and colonized the world. Evolutionary Anthropology, 19. 148-57.

Belkhir K., Borsa P., Chikhi L., Raufaste N. and Bonhomme F. (1996-2004). GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France).

Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14. 2611-2620.

Falush, D., Stephens, M. and Pritchard, J.K. (2003). Inference of population structure using multilocus data: linked loci and correlated allele frequencies. Genetics, 164. 1567-1587.

FAO. (2007). The state of the world's animal genetic resources for food and agriculture. FAO, Rome.

Frankham, R., Ballou, J.D. and Briscoe, D.A. (2002). Introduction to conservation genetics. Cambridge University Press, Cambridge, 617.

Ginja, C., Telo Da Gama, L. and Penedo, M.C. (2010). Analysis of STR markers reveals high genetic structure in Portuguese native cattle. Journal of Heredity, 101. 201-210.

Groeneveld, L.F., Lenstra, J.A., Eding, H., Toro, M.A., Scherf, B., Pilling, D., Negrini, R., Jianlin, H., Finlay, E.K., Groeneveld, E., Weigend, S. and Globaldiv Consortium. (2010). Genetic diversity in livestock breeds. Animal Genetics, 41. 6-31.

Guo, S.W. and Thompson, E.A. (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics, 48. 361-372.

Hamilton, M.B. (2009). Population Genetics. John Wiley & Sous Ltd, West Sussex, UK, 407.

Holsinger, K.E. and Weir, B.S. (2009). Genetics in geographically structured populations: defining, estimating and interpreting F_{ST}. Nature Review Genetics, 10. 639-650.

Charlesworth, D. and Willis, J.H. (2009). The genetics of inbreeding depression. Nature Reviews Genetics, 10. 783-796.

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

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

Liu, K., Muse, S.V. (2005). Integrated analysis environment for genetic marker data. Bioinformatics, 21. 2128-2129.

Lenstra, L.A., Groeneveld, L.F., Eding, H., Kantanen, J., Williams, J.L., Taberlet, P., Nicolazzi, E.L., Soelkner, J., Simianer, H., Ciani, E., Garcia, J.F., Bruford, M.W, Ajmone-Marsan, P and Weigend, S. (2012). Molecular tools and analytical approaches for the characterization of farm animal genetic diversity. Animal Genetics, 43. 483-502.

Nei, M., Tajima, F. and Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. Journal of Molecular Evolution, 19. 153-170.

Pavlík, I., Kadlečík, O., Kasarda, R., Šidlová, V. and Žitný, J. (2013). Comparison of genetic diversity in dual-purpose and beef Pinzgau populations. Acta Fytotechnica et Zootechnica, 16. 69-73.

Pritchard, J.K., Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics, 155. 945-959.

Sun, W.B., Chen, H., Lei, C.Z., Lei, X.Q. and Zhang, Y.H. (2007). Study on population genetic charac¬teristics of Qinchuan cows using microsatellite markers. Journal of Genetics and Genomics, 34. 17-25.

Taberlet, P., Valentini, A., Rezaei, H.R., Naderi, S., Pompanon, F., Negrini, R. and Ajmone-Marsan, P. (2008). Are cattle, sheep, and goats endangered species? Molecular Ecology, 17. 275-284.

Wright, S. (1931). Evolution in mendelian populations. Genetics, 16. 97-159.

Corresponding author:

Veronika Šidlová
Slovak University of Agriculture in Nitra
Faculty of Agrobiology and Food Resources
949 76 Nitra, Tr. A. Hlinku 2., Slovak Republic
Phone: +421-37-641-4289
E-mail: veron.sidlova@gmail.com