

# Genealogic structure of Slovak Pinzgau cattle population

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*Abstract:* The aim of the study was improve knowledge about genealogical 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. The overall fixation index values per locus ranged from -0.039 (CSRM60) to 0.0535 (BM1824) and average  $F_{IS}$  was close to zero ( $-0.0039 \pm 0.0122$ ). The highest average  $F_{ST}$  was observed in subpopulation divided based on paternal lines. The value 0.0669 from all the loci indicated that 93.31% of the genetic variation was caused by differences among individuals and 6.69% due to differentiation among the origin of animals. Division of the population into logical groups was confirmed and clarified on the basis of genetic analyses. 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 [1]. 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 [2]. 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 [3] and for reconstructing the history of livestock [4].

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 [5], development were monitored [6] and analyses of genetic diversity were performed [7].

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 [8]. Microsatellites have been commonly used to assess within-breed genetic diversity and inbreeding levels, introgression from other species, genetic differentiation, admixture among breeds [9] and to define conservation priorities [2].

The most widely used measures of population structure are Wright's F statistics [10], 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  $F_{IT}$  [2]. 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 [11].  $F_{ST}$  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 [12]. 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 [13].

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. Beef and dual-purpose farming types, as well as purebred and crossbred animals were represented. 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. 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 [14] provided in PowerMarker V3.25 software [15]. 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. Genetic distance according to Nei [16]

was calculated based on similarity matrix for all substructures of selected animals and then visualized to the form of phylogenetic tree using MEGA6 [17].

### Results and discussion

Out of the 8 analysed loci only BM1824 showed highly significant ( $P \leq 0.001$ ) HWE deviations across breed. Overall  $F_{IS}$  ranged from -0.039 for CSRM60 to 0.0535 for BM1824 (see Table 1). Fixation index ( $F_{IS}$ ) measures the reduction of heterozygosity in an individual because of non-random mating within population and hence  $F_{IS}$  values significantly higher or lower than 0 reveal inbreeding or outbreeding, respectively.  $F_{IS}$  is usually estimated from the heterozygote deficit (1 minus the ratio of observed and expected heterozygosity) and hence  $F_{IS}$  values significantly higher or lower than 0 reveal inbreeding or outbreeding, respectively. A positive  $F_{IS}$  may also be the result of genetic subdivision (the Wahlund effect), and negative estimates can arise from crossbreeding. Inbreeding depression, or the reduction of fitness of populations by inbreeding, is caused mainly by homozygosity of deleterious mutations [18]. Whereas the average value of  $F_{IS}$  reached a negative number, generally we can notice there is no reduction of heterozygosity, but this value was close to zero ( $F_{IS} = -0.0039$ ) and few microsatellites have a positive values, what means this situations can be easily changed in the next generations. Positive  $F_{IS}$  values could be derived from inbreeding or from the presence of a substructure within the population.

As is shown in Table 1 the highest average  $F_{ST}$  value reached subpopulation based on paternal lines. The value  $0.0669 \pm 0.0049$  from all the loci indicated that 93.31% of the genetic variation was caused by differences among individuals and 6.69% only due to differentiation among the origin of animals. In subpopulation divisions by farm, breed type and year of the birth were observed following values:  $0.0188 \pm 0.0049$ ,  $0.003 \pm 0.0013$  and  $0.0053 \pm 0.0012$ , respectively. 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 [19], although much smaller values can be significant [2].

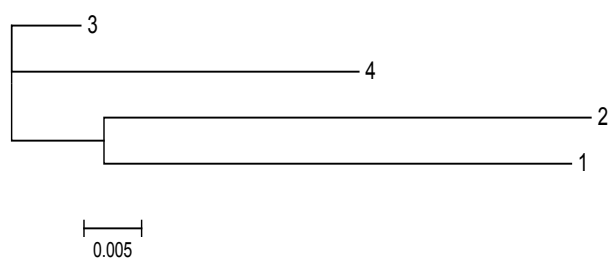
Table 1 Genetic structure of Slovak Pinzgau cattle subpopulations

markers	F <sub>IS</sub>	F <sub>ST</sub>			
		farm	breed type	year of the birth	paternal lines
TGLA227	-0.0318	0.0101	0.0027	0.0039	0.0547
SPS115	0.0139	0.0254	0.0014	0.0129	0.0908
ETH3	-0.0330	0.0232	0.0047	0.0049	0.0582
BM1824 <sup>+++</sup>	0.0535	0.0524	0.0090	0.0046	0.0720
CSRM60	-0.0390	0.0165	-0.0030	0.0067	0.0841
CSSM66	-0.0304	0.0074	0.0069	-0.0005	0.0484
ILST006	-0.0048	0.0178	-0.0016	0.0052	0.0632
TGLA122	0.0399	0.0014	0.0030	0.0059	0.0673
Mean	-0.0039	0.0188	0.0030	0.0053	0.0669
SD	0.0122	0.0049	0.0013	0.0012	0.0049

Legend: <sup>+++</sup>Significance level of Hardy-Weinberg disequilibrium ( $P \leq 0.001$ )

Individuals are characterized by sufficient genetic diversity on separate farms. Slight decrease in heterozygosity is visibly at the farm 4 (PD LČV Čimhová – Vitanová), as indicated by a positive F<sub>IS</sub> value (F<sub>IS</sub> = 0.0207). Farm 3 (Agria a.s. Liptovský Ondrej – Liptovská Porúbka) is dual-purpose type only and 4 beef type, whereas farms 1 (PD Smrečany – Veterná Poruba) and 2 (PD Spišské Bystré – Kvetnica) are changing the type of the farming from beef to dual-purpose, confirming also Fig. 1. Animals from the farm 1 and 2 are genetically closer to each other than animals of the other two farms, because they are both in one cluster, whereas farm 3 and 4 constitute separate cluster.

Fig. 1 Genetic structure of the population by farm

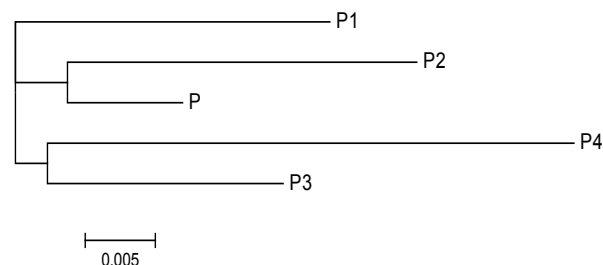


Legend: 1 - PD Smrečany – Veterná Poruba, 2 - PD Spišské Bystré – Kvetnica, 3 - Agria a.s. Liptovský Ondrej – Liptovská Porúbka, 4 - PD LČV Čimhová – Vitanová

Fig. 2 shows the distribution of the animals into five groups by breed type. It is clear that P (purebred animals) is genetically more similar to P2 (12.5% of other breeds) as to P1 (6.25% of other breeds), which may be caused by inaccurate data in the pedigree information. We can observe small reduction in heterozygosity based on F<sub>IS</sub> values in P2 (F<sub>IS</sub> = 0.018) and P3 (25% of other breeds; F<sub>IS</sub> = 0.0034), which may be the result of genetic subdivision or lower number of the animals in these

groups.

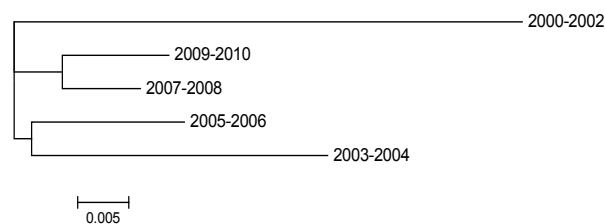
Fig. 2 Genetic structure of the population by breed type



Legend: P - purebred animals, P1 - 6.25%, P2 - 12.5%, P3 - 25%, P4 - 50% of other breeds

Logical division of animals based on year of the birth as shown in Fig. 3 has proved that the oldest animals are genetically the most distant from the younger grades. We can notice that with the passing years, the genetic information preserved in DNA is changing, which may be caused by considerable genetic contributions from migrants while constraining breeding values of the offspring, but also due to type of farming, unification of breed and hence reduction of genetic variability in younger animals. As well positive F<sub>IS</sub> value (0.0207) in the animals born in years 2009-2010 has confirmed decrease of heterozygosity.

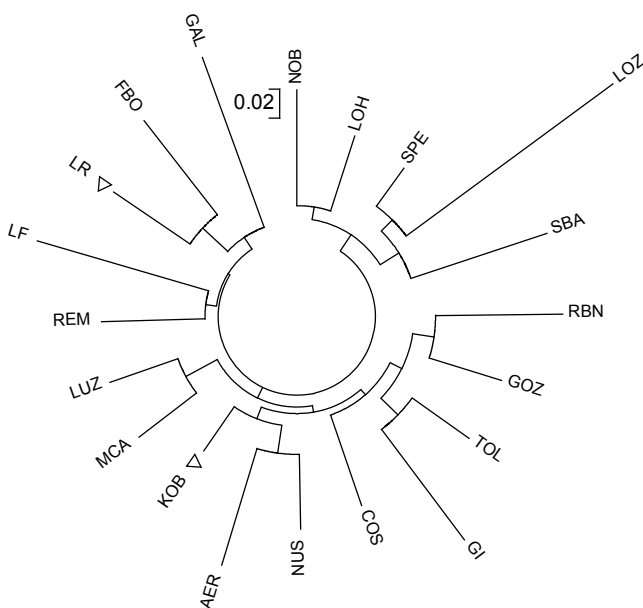
Fig. 3 Genetic structure of the population by year of the birth



A slight reduction of heterozygosity was

occurred in lines LR and KOB based on the positive  $F_{IS}$  value after division of the population into groups based on paternal lines. We can observe distribution of the lines into 3 main clusters. These tree groups are marked in Fig. 4. First cluster is created by lines GAL, FBO, LR, LF and REM. Second one consists from lines NOB, LOH, SPE, LOZ and SBA. The remaining animals belong to the third cluster. All clusters create 2 subclusters, while it is being understood that the each 2 smallest subclusters are genetically more similar to each other than to remaining animals.

Fig. 4 Genetic structure of the population using paternal lines



## Conclusions

Genetic structure of Pinzgau cattle population has been analysed using set of 8 microsatellites.  $F_{IS}$  and  $F_{ST}$  values proved that the population is divided into substructures. In most cases, it was logical division confirmed by analysis of genetic structure. 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 detailed information of 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. 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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