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Genetic diversity in Banija spotted pig: pedigree and microsatellite analyses

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Abstract

Genetic diversity and breed structure of Banija spotted pig (BS) was analyzed trough 721 pedigree records and polymorphism of 23 microsatellites on 30 BS pigs. Two phylogenetic trees were constructed from microsatellite and pedigree information. The inbreeding coefficient obtained by microsatellite markers was 5.6%, while the inbreeding coefficient from pedigree analysis was 3.68%. The rate of inbreeding per generation was 1.74% and effective population size was 28.81. In phylogenetic analyses, relationship coefficients and genetic distances between individuals were calculated using microsatellite and pedigree data. Phylogenetic trees from microsatellite markers and pedigree corresponded well to each other and showed consistency between microsatellite and pedigree information. From the data obtained by microsatellite markers and pedigree, two subpopulations can be observed. The existence of two subpopulations can be explained by two different paths of breed genesis. Thus, future work in the conservation process should include methods such as optimal contribution selection including factorial mating, in order to make genetic progress and control the rate of inbreeding.

Keywords: genetic diversity, microsatellites, pedigree, pigs

Introduction

Main objectives of conservation programs include maintaining the genetic variability by controlling inbreeding rate and the effects of inbreeding on fitness. Due to their high polymorphic nature, microsatellites have become one of the most widely used markers for assessing genetic diversity in populations of farm animals (Groeneveld et al., 2010). The rate of inbreeding obtained from pedigree has been the most frequently used parameter to quantify the rate of genetic drift (Gutiérrez et al., 2003).

In the case when pedigree data is absent or incomplete, is recommended to use data based on genetic marker information (Baumung and Sölkner, 2003). Moreover, the combined use of molecular markers and pedigree is recommended when the available sample size is small and pedigree shallow (Álvarez et al., 2008). This is case with Banija spotted pig (BS), autochthonous Croatian pig breed with origins in the end of 19th century. After a long period of stagnation, conservation efforts enabled the increase in population size in recent years. The aim of this study was to estimate the genetic diversity and breed structure of BS pig breed using microsatellite and pedigree data. This baseline information could be implemented for conservation strategy of BS pig breed in the future.

Materials and methods

Total genomic DNA was isolated from blood samples or ear clips of 30 BS pigs using DNeasy Blood & Tissue Kit (Qiagen GmbH, Germany). 23 microsatellite markers (S0026, S0155, S0005, Sw2410, Sw830, S0355, Sw24, Sw632, Swr1941, Sw9366, S0218, S0228, Sw240, Sw2406, Sw122, Sw857, 0097, Sw72, S0226, Sw911, S0002, Sw1067 and S0101) from the ISAG/FAO recommendation list (ISAG/FAO, 2011) were selected and grouped into three multiplex reactions based on their size and annealing temperature. Multiplex PCR reactions were performed with 2x Type-it Microsatellite PCR Kit (Qiagen GmbH, Germany) following the manufacturer instructions. Microsatellite cycling protocol began with initial activation step 6 min at 95 ºC, followed by 35 cycles of denaturation (30 s at 95 ºC), annealing (90 s at 58, 59 and 59.5 ºC) and extension (60 s at 72 ºC). Cycling program ended with a final extension for 30 min at 60 ºC. Microsatellite multiplex PCR products were analyzed using GeneScan350 ROX internal standard size marker on ABI3730XL capillary gene analyzer (Macrogen Inc., Netherlands). Data processing was performed with the Peak Scanner Software v1.0 (applied Biosystems) software. Allele frequencies, number of alleles, observed and expected heterozygosity, as well as Wright's F_{IS} statistics according to Weir and Cockerham (1984) were calculated using GENETIX 4.05.2 software (Belkhir et al., 2004). Polymorphic information content (PIC) were calculated using the Cervus 3.0.7. software (Kalinowski et al., 2007). An unweighted pair-group method with arithmetic mean (UPGMA) was used to construct the phylogenetic tree based on Nei's (1987) standard genetic distance using Adegenet package in R (R 3.4.0).

Complete pedigree was built for 721 animal of BS pig based on records provided by the Croatian Agricultural Agency. The reference population contained all pigs born between 2010 and 2017. The CFC software package (Sargolzaei et al., 2006) was used to detect the basic pedigree structure. Quality and integrity of the pedigree information were evaluated by the following parameters: by average number of maximum generations traced back, average number of full generations, and average number of complete equivalent generations. The average relatedness coefficient (Gutiérrez et al., 2003) of each individual is defined as the probability that an allele randomly chosen from the whole population in the pedigree belongs to a given animal. Effective population size (N_e), defined as the number of individuals that would generate the current level of inbreeding, was computed as $N_e = 1/(2\ \Delta F)$ (Falconer and Mackay, 1996) using in ENDOG software (Gutiérrez and Goyache, 2005).

Genetic distances from pedigree records were defined as 1 minus relationship coefficient and were used to construct phylogenetic three.

Results and discussion

Average inbreeding coefficients obtained from pedigree (Table 1) and microsatellite data were moderate. Higher values of inbreeding coefficient (5.6%) were obtained by the microsatellite analysis then from pedigree data (3.68%). The difference may be attributed to the relation between founder animals, which are assumed to be unrelated when coefficient of inbreeding is estimated from pedigree and to the poor completeness of the pedigree. The average maximum number of generations was 2.47, average number of full generations was 1.5, while average number complete equivalent generations was 2. However, more informative measures that describe the loss of the genetic diversity in the population are average relatedness and change in inbreeding per generation. Both indicated fast increase in inbreeding as pedigree becomes more complete. According to the recommendations of the Food and Agriculture Organization of the United Nations (FAO, 2000), ΔF should not exceed 1% and the effective population size (N_e) for a breed should be maintained above 50 animals.

Table 1. Results of pedigree analysis for reference population of Banija spotted pig

Parameters of genetic diversity	Results
Average inbreeding coefficient	3.68%
Average relatedness	9.32%
ΔF	1.74%
N_e	28.81

ΔF - inbreeding rate; Ne – effective population size

According to Barker (1994) and Botstein et al. (1980) in order to maintain genetic diversity microsatellite markers should have minimally four alleles per locus while average heterozygosity in the population should range between 0.3 and 0.8. The average value of H_{obs} for selected microsatellite markers was 0.585 and mean numbers of alleles per locus was 5.913. The mean PIC value was 0.575, which indicates that selected markers have high level of polymorphism information content. Observed heterozygosity and number of alleles per locus indicate existence of genetic diversity in the analyzed sample. However, analysis of the pedigree showed that genetic diversity reduces as pedigree becomes more complete. This might be attributed also to the homogenization of type characteristics and the small number of available breeding individuals (Álvarez et al., 2008). Although marker analysis showed substantial genetic diversity in the population, combined results from genetic marker and pedigree data indicate that future work in the conservation process

should include methods that will enable control the rate of inbreeding. Phylogenetic trees from microsatellite markers and pedigree corresponded well to each other (Figure 1).

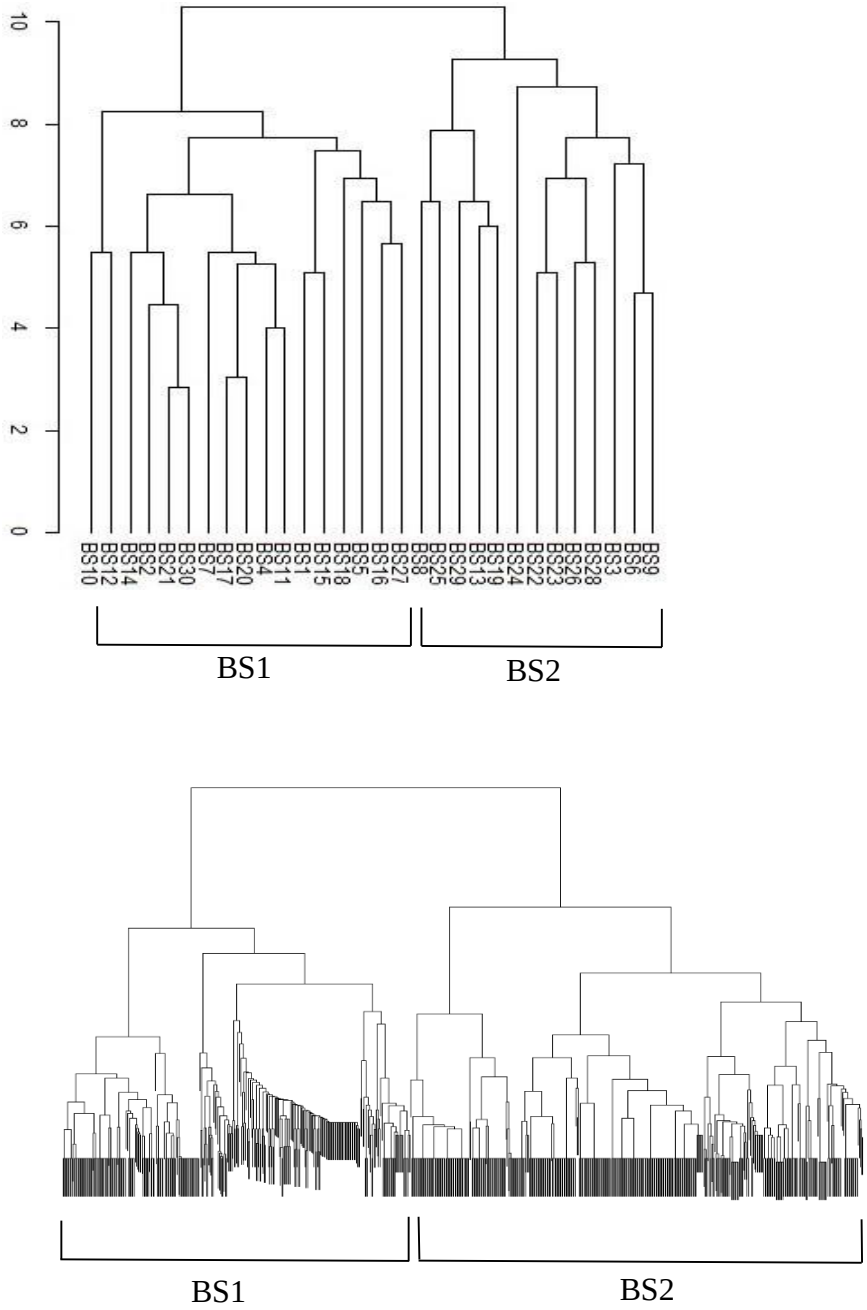

Figure 1. UPGMA tree constructed by 23 microsatellites markers (upper) and pedigree data (lower)

Two distinct lines were observed using both sources of information. The existence of two lines can be explained by two different paths of breed genesis (Salajpal et al., 2017). The first path is crossing of Landrace pigs with Berkshire pigs, while the

second one is crossing of Turopolje pig with Berkshire pigs. Obtained result indicate that use of different types of information could be combined and used to make breeding plan to control and preserve genetic diversity in the population.

Conclusions

Results from the microsatellite and pedigree data showed that further efforts in conservation of BS pig breed should be focused on the creating mating schemes with a goal to control of the rate of inbreeding and further loss of genetic diversity. In phylogenetic analysis, genealogical three obtained from microsatellite marker information clearly reflects the pedigree records suggesting that analysis using microsatellite markers can be a useful tool for determining the genetic structure in combination with pedigree data.

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