

Genetic diversity of the Banija spotted pig breed using microsatellite markers

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Abstract

Banija spotted pig breed was created at the end of 19th century by crossing domestic white sows with drooping ears and/or Turopolje pig sows with black Berkshire. After a period of stagnation in breeding, the population is newly recognized only few years ago and is currently in the process of breed valorisation. The aim of this study was to assess genetic diversity and structure of Banija spotted pig (BS) in comparison with geographically close populations, and to assess the differentiation level of Banija spotted pig. Based on the genotypes obtained for 24 ISAG-FAO recommended pig microsatellite markers, basic genetic diversity indicators were determined for 30 samples of BS, 20 samples of Turopolje and Crna Slavenska pig each, and 17 samples of Landrace population. Observed heterozygosity ranged from 0.36 to 0.6 and was high in BS population (0.58). Average allelic richness (range 2 – 5.2) and the rarefacted number of private alleles (range 9 to 27) were the highest in BS. Inbreeding coefficient in BS was estimated 0.06 (bootstrapping confidence interval 0.022 to 0.123). Structure analysis showed that there are five clusters in the four analysed populations, where BS are clearly distinguished from other populations and substructured. Wright's fixation index estimates are in accordance with the clustering results. In conclusion, the population of Banija spotted pig shows great genetic diversity and is genetically different from neighbouring (geographically close) pig populations.

Keywords: indigenous pig breeds, microsatellites, population structure

Introduction

Genetic variability of a breed or a population is of major concern in planning genetic conservation as well as for breeding. By selecting for economically important traits

genetic variability decreases (Falconer and Mackay, 1996), and highly commercial breeds of domestic animals are expected to have low genetic variability (Arranz et al., 2001). A great number of genetic variations in a population and between different breeds, are an important factor for adaptation to rapid environment changes, or market demand (Food and Agriculture Organization, FAO, 2011). In farm animal genetic resources conservation it is important to discover and valorise new specific genetic groups, which ensure diverse genetic background for the long-term global flexibility of food production (Ojango et al., 2011). Real treasure present in the low-input production systems, such as are preferred on small family farms in Croatia, is still being valorised.

After a period of stagnation in breeding, the BS population is newly recognized only few years ago and is currently in the process of breed valorisation (Salajpal et al., 2017). The aim of this study was to assess genetic diversity and structure of Banija spotted pig in comparison with geographically close populations, and to assess the differentiation level of Banija spotted pig.

Materials and methods

Genotypes were obtained for 24 ISAG-FAO recommended pig microsatellite markers (Table 1). To assess the differentiation level of BS following populations were chosen for comparison regarding the breeds used for the construction of BS: domestic white sows with drooping ears and/or Turopolje pig sows were crossed with black Berkshire. Therefore, random unrelated blood samples of 30 BS, 20 of Turopolje (TUR) and Crna Slavenska pig (CS) each, and 17 samples of Landrace population from continental Croatia (LA) were used to extract the DNA and were genotyped.

Markers and populations were analysed in R 3.2 (R Development Core Team, 2008), with the adegenet 2.1.1 (Jombart, 2008), pegas 0.1.0 (Paradis, 2010), hierfstat 0.04-22 (Goudet, 2005) and PopGenKit (Paquette, 2012) packages. Supervised clustering method (Hubisz et al., 2009) was performed ($k = 1$ to 8, 10 runs per k , 1,000,000 iterations, 10,000 burn-in) using Structure (Pritchard et al., 2000), and StructureHarvester (Earl and vonHoldt, 2012) was used to determine the best K .

Results and discussion

High level of genetic diversity was identified in the analysis of the 24 loci (Table 1). In total, 208 alleles were found in 89 genotyped animals. Average number of alleles per locus was 8.7, with markers S0026, S0218 and S0228 having minimal total number of 5 alleles each. The highest average expected heterozygosity (H_e) was recorded for marker S0005 (17 alleles at locus), and the highest observed heterozygosity (H_o) for marker S0097. Most diverse markers were similar as in Šprem et al. (2014). All of the markers were evaluated suitable for further analysis due to their diversity, since none of them had null-allele frequency estimated higher than 0.3 or showed significant deviation from Hardy-Weinberg equilibrium in more than half of the populations (Šalamon et al., 2014). Marker S0218 was fixed in TUR. Markers Sw1067 and S0005 showed the highest number of alleles per population, 9 in BS and 12 in CS. In total, 75 private alleles were sampled: 27 in BS, 23 in CS, 16 in LA

and 9 in TUR. The largest rarefacted mean number of alleles per locus (MNA) when all of the markers are considered jointly was found in BS (5.17) and CS had similar value (5.06), while LA and TUR showed lower diversity (4.38 and 2.92, respectively).

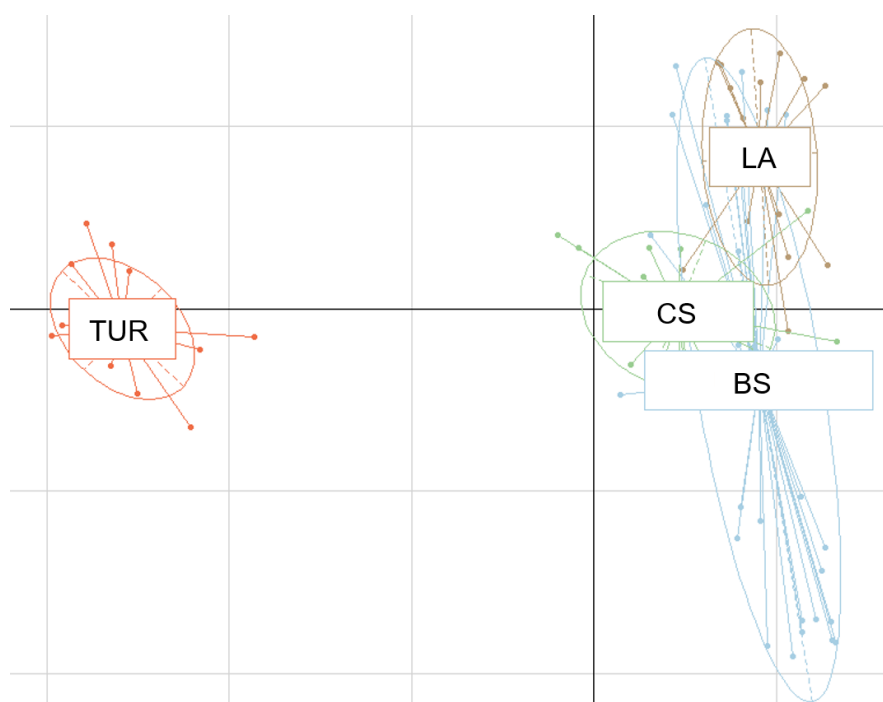
Table 1. Genetic diversity parameters recorded for the 24 microsatellite loci analysed in the 4 pig populations

Markers	A	He	Ho	F(nullB)	Fis
Sw2406	9	0.511	0.326	0.14	0.332
S0228	5	0.379	0.349	0.022	0.035
sw240	10	0.79	0.756	0.02	-0.02
S0097	9	0.813	0.802	0.006	-0.082
Swr1941	8	0.524	0.356	0.123	0.238
S0355	10	0.783	0.724	0.034	-0.042
SO090	8	0.761	0.506	0.169	0.246
S0218	5	0.317	0.221	0.079	0.226
Sw122	8	0.837	0.767	0.039	-0.056
S0026	5	0.601	0.402	0.141	0.223
Sw2410	11	0.84	0.77	0.04	-0.065
S0226	9	0.637	0.54	0.063	0.008
Sw24	8	0.433	0.333	0.075	0.094
Sw72	6	0.758	0.69	0.041	-0.078
Sw830	10	0.812	0.724	0.051	-0.068
Sw632	8	0.616	0.437	0.125	0.144
S0155	8	0.749	0.586	0.102	0.053
S0002	11	0.844	0.483	0.244	0.307
Sw9366	9	0.838	0.674	0.098	0.014
Sw857	8	0.741	0.512	0.152	0.139
Sw1067	10	0.749	0.517	0.153	0.09
S0005	17	0.862	0.667	0.117	-0.038
Sw911	8	0.715	0.471	0.166	0.05
S0101	8	0.584	0.264	0.253	0.264
Overall	208	0.687	0.537		

A - number of alleles per locus; Ho - average observed heterozygosity; He - average expected heterozygosity; F(nullB) - frequency of null alleles for a locus; Fis - coefficient of inbreeding.

Average H_o values among the populations were high and resembling: 0.58 in BS, 0.6 in CS, 0.59 in LA, except for lower value observed in TUR (0.36). Average H_e values among the populations were similar, and higher than H_o : 0.61, 0.63, 0.6 and 0.38 for BC, CS, LA and TUR, respectively. Similar diversity values were observed for CS and TUR in Druml et al. (2012). Inbreeding coefficients (F_{is}) ranged from -0.082 (S0097) to 0.332 (Sw2406) and were positive in 16 markers. Bootstrapping over loci of population's F_{is} resulted in non-significant F_{is} in CS and LA, while low inbreeding was found in BS (0.075, confidence interval: 0.022-0.123) and TUR (0.074, confidence interval: 0.015-0.119). Overall, low diversity found in TUR is in accordance with the history of the breed (Druml et al., 2012; Šprem et al., 2014).

The genetic differentiation estimates of pairwise Wright's fixation index (F_{st}) were low (0.056 for BS-LA pair) to considerable (0.231 for LA-TUR pair). The largest genetic differentiation was found for the TUR breed (0.191 with CS, 0.197 with BS and 0.231 with LA), which is evident also in the results of the principle component analysis (Figure 1) and the fact that this breed is the first distinguished one in structure analysis at $k = 2$ (not shown). High genetic differentiation of the TUR breed is caused by genetic drift, since this population suffers from low diversity and notable inbreeding. Figure 1 shows that all of the indigenous breeds are distinguished from the LA population with the second principle component axis (0.077 for CS-LA pair). Low BS-LA pairwise F_{st} is possibly caused by geographically narrow sampling of LA. Low differentiation of BS and CS ($F_{st} = 0.06$) is evident in the principle component analysis results as well, in close cluster centers of the BS and CS genotypes.



TUR – 20 genotypes of the Turopolje pig; LA – 17 genotypes of the Landrace pig; CS – 20 genotypes of the Black Slavonian pig, BS – 32 genotypes of the Banija spotted pigs.

Figure 1. Result of the principal component analysis of the pig populations genotypes

As expected according to the results presented above, BS shows high variability and the widest genetic group in Figure 1, capturing noticeable diversity not present in other analysed breeds.

All of the populations own their own clusters at $k = 4$. Structure analysis showed that there are five clusters in the four analysed populations according to Delta K (77.04), and the best value of $\ln \Pr(X|K)$ for $K = 5$ was -4,788.5. Graphical representation of the clustering outcome suggested for $K = 5$ (Figure 2) shows all four populations are each associated with their own cluster, and BS is sub-structured (Šalamon et al., 2018), owning two clusters (shown in light and dark blue). This substructure was found also in phenotype (Salajpal et al., 2017). Admixture of the LA cluster is present in several BS samples (up to $Q = 0.417$). LA, TUR and CS samples correspond to their own clusters ($Q > 0.985$; $Q > 0.756$, $Q > 0.849$, respectively).



Ratio of the colours in the bar is the ratio of genotypes of different clusters found in the admixed individual. Yellow – cluster corresponding to TUR samples; light and dark blue – BS; orange – CS, grey – LA cluster.

Figure 2. Proportion of membership for the four pig populations across the five clusters verified in the structure analysis

Conclusions

Banija spotted pig population shows great genetic diversity and is genetically different from neighbouring (geographically close) pig populations. However, further efforts in conservation of BS pig breed should be focused on controlling of the rate of inbreeding and admixture with commercial breeds. Serious situation remains in the Turopolje pig breed with low diversity and notable inbreeding.

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