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POSTER

Genetic analysis of Croatian autochthonous pig breeds based on microsatellite markers

Margeta, P.; Gvozdanović, K.; Djurkin Kušec, I.; Radišić, Ž.; Kušec, G. and Margeta, V.

Departament for Special Zootechnic, Agricultural Faculty in Osijek, University of Josip Juraj Strossmayer Osijek, Croatia.

SUMMARY

Black Slavonian pig (BS) and Turopolje pig (TP) are two Croatian autochthonous pig breeds. At the beginning of this century attempts were made to preserve and re-establish them, which included also employment of the genetic methods for studying genetic characteristics of both breeds. The number of Black Slavonian breed sows was increased from 237 in 2001 to 1305 last year, while the number of Turopolje breed sows rose from 45 to 132 in the same period. A set of 18 microsatellite markers was used to genotype 20 BS pigs, 20 TP pigs and 20 wild boars. The basic statistical analysis included number of samples, number of alleles for each locus, expected and observed heterozygosity, FIS and their statistical significance FISsign. In observed populations, a relatively high variety of loci was present. Average heterozygosity was between 0.35 and 0.64. The average fixation index (FST) was 0.24. Multilocus FST values indicated that around 24% of the total genetic variation was explained by population differences, with the remaining 78% corresponding to differences among individuals within population. Genetic distances between populations were made with PCA method and it was noticed that studied populations are clearly defined. The analysis of population structure indicates no admixture among analysed breeds. However, some substructuring in the Black Slavonian pig population was observed. Results confirmed usefulness of microsatellites for studying genetic diversity among Croatian autochthonous pig breeds, but for obtaining more accurate results, a study involving larger number of animals and other related breeds would be required.

ADDITIONAL KEYWORDS

Black Slavonian pig.
Turopolje pig.
Croatian wild boar.
genetic markers.

Análise genética de raças suínas autóctones da Croácia através do uso de microssatélites como marcadores

RESUMO

Os suínos Black Slavonian (BS) e Turopolje (TP) são duas raças autóctones da Croácia. No início do presente século foram feitas tentativas para as preservar e reestabelecer, que incluíram o uso de metodologias genéticas para o estudo das características genéticas de ambas as raças. O número de porcas reprodutoras BS aumentou de 237 em 2001 para 1305 no último ano, enquanto que o número de porcas reprodutoras TP aumentou de 45 para 132 no mesmo período. Foram genotipados 20 porcos BS, 20 porcos TP e 20 javalis usando um conjunto de 18 microssatélites. Foi feita uma análise estatística básica em relação ao número de amostras, número de alelos em cada locus, heterozigosidade esperada e observada, FIS e a sua significância estatística FISsign. Nas populações observadas foi detetada uma variedade de loci relativamente elevada. A heterozigosidade média variou entre 0.35 e 0.64. O índice médio de fixação (FST) foi de 0.24. Os valores multilocus do FST indicaram que cerca de 24% da variação genética total era explicada pelas diferenças entre populações e os restantes 78% correspondiam a diferenças entre animais dentro da mesma população. As distâncias genéticas entre populações foram determinadas com o método PCA e verificou-se que as populações estudadas estavam claramente definidas. A análise da estrutura da população indica a ausência de mistura entre as raças analisadas. No entanto foi observada alguma subestruturação na população BS. Os resultados confirmam a utilidade dos microssatélites para estudar a diversidade genética entre as raças suínas autóctones da Croácia mas, para obter resultados mais precisos, será necessário um estudo envolvendo um maior número de animais e outras raças relacionadas.

PALAVRAS CHAVE ADICIONAIS

Porco Black Slavonian.
Porco Turopolje.
Javali da Croácia.
Marcadores genéticos.

INFORMATION

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gkusec@pfos.hr

INTRODUCTION

Turopolje and Black Slavonian are two autochthonous pig breeds in Croatia. Turopolje pig was formed in the early middle age by crossing Siska and Krskopolje breeds. Black Slavonian pig breed was formed in the 19th century by crossing local Mangalitsa pigs with the Berkshire, Poland China and Large black pig breeds. New selection goals in the 20th century aiming at production of leaner pigs with more efficient feed conversion led almost to extinction of these two old breeds. At the beginning of this century attempts were made to preserve and re-establish them, which included also the employment of genetic methods for studying genetic characteristics of both breeds. As a result of preservation attempts, the number of Black Slavonian breed sows increased from 237 in 2001 to 1305 in the last year, while the number of Turopolje breed sows rose from 45 to 132 in the same period. Microsatellites (MS) have been intensely used for population studies in commercial as well as rare pig breeds in the last 25 years (SanCristobal 2006, pp. 189-98) and are convenient markers for evaluating the genetic diversity of domestic animals because of their abundance, even distribution in the genome, high level of polymorphism and ease of genotyping. In pigs, numerous studies of genetic variation between or within different pig breeds were conducted by genotyping multiple microsatellite loci (Li 2004, pp. 368-74; SanCristobal 2006, pp.189-98). MS have higher variability when compared to the same number of bi-allelic markers such as SNPs (Schlotterer 2004, p.66). For that reason, they are often used for parentage assignment and for traceability of different pig breeds in meat products (Jae-Don 2014, pp. 926–931). In this study we used 18 microsatelite markers to detect the genetic relationship between two Croatian autochtonous pig breeds and Croatian wild boar population.

MATERIAL AND METHODS

The study was performed on 3 pig breeds (Black Slavonian breed - BS, Turopolje breed - TP and Croatian wild boar - CWB). In BS, from 20 samples 16 were sows and 4 were boars, in TP there were 17 sows and 3 boars and in CWB, it was 11 sows and 9 boars. Genomic DNA was isolated from blood samples using GeneJET™ Genomic DNA Purification Kit (*Thermo Scientific™*) following the manufacturer's instructions. Each animal was genotyped for 18 microsatellite markers: SW24, SW857, SO225, SO227, SW240, SO215, SO218, SO005, SW122, SO155, SO226, SO090, SO178, SW911, SO002, SW951, SW632 and IGF1. These markers were chosen based on their quality, size, polymorphism and location on the porcine genome (Archibald 1995, pp.160-2; Rohrer 1996, p. 233-237), representing 15 pig chromosomes including chromosome X (SO218). The PCR products were analysed on an ABI PrismTM 310 Genetic Analyzer (PE Applied Biosystems). Nei's standard genetic distance, observed and expected heterozygosity and F_{IS} values were determined using the GENETIX software (Belkhir 2004). Genetic distances between populations were made with Principal Component Analysis (PCA) method and the analysis of population structure with the STRUCTURE program (Pritchard, Stephens & Donnelly 2000). The program was run 10 times for each K, starting from K = 2 to K = 7. The results presented in this study are based on 10^6 iterations following a burn-in period of 10^5 iterations.

RESULTS

The basic statistical analysis for all 18 loci of observed populations is shown in **Table I**. Marker SO218 is especially signed because of its location on X chromosome and, consequently, modus of inheritance which is specific for sex-linked genes. Between observed populations relatively high variety of alleles

Table I. **Number of alleles (A), heterozygosity (H_{Exp}, H_{Obs}), inbreeding coefficient (F_{IS}) and statistical significance for 18 loci of observed populations of pigs** (Número de alelos (A), heterozigosidade (H_{Exp}, H_{Obs}), coeficiente de endogamia (F_{IS}) e significância estatística para 18 loci nas populações de suínos observadas).

MS locus	**Black Slavonian pig (n = 20)**					Turopolje pig (n = 20)					Croatian wild pig (n = 20)				
	A	H_{Exp}	H_{Obs}	F_{IS}	$F_{IS\ sign}$	A	H_{Exp}	H_{Obs}	F_{IS}	$F_{IS\ sign}$	A	H_{Exp}	H_{Obs}	F_{IS}	$F_{IS\ sign}$
SW24	8	0.61	0.55	0.12		2	0.50	0.85	-0.69		3	0.41	0.3	0.30	
SW857	6	0.72	0.85	-0.15		2	0.35	0.45	-0.28		4	0.61	0.6	0.05	
SO225	6	0.71	0.5	0.32		2	0.10	0.1	-0.03		4	0.47	0.4	0.18	
SO227	2	0.05	0.05	0		2	0.49	0.85	-0.73		6	0.45	0.4	0.13	*
SW240	7	0.78	0.2	0.76	*	4	0.14	0.1	0.32		9	0.86	0.44	0.50	
SO215	1	0	0	NA		2	0.42	0.6	-0.41		3	0.37	0.25	0.34	
SO218	2	0.39	0.25	0.40		2	0.26	0	1	*	4	0.59	0.25	0.59	
SO005	9	0.81	0.6	0.28		2	0.22	0.25	-0.12		11	0.85	0.9	-0.04	
SW122	7	0.82	0.8	0.05		2	0.42	0.6	-0.41		6	0.77	0.85	-0.07	
SO155	8	0.80	0.65	0.21		4	0.59	0.45	0.26		6	0.75	0.5	0.35	
SO226	6	0.82	0.75	0.11		3	0.54	0	1	*	4	0.65	0.35	0.48	
SO090	4	0.64	0.7	-0.08		5	0.55	0.65	-0.16		6	0.79	0.75	0.08	
SO178	8	0.80	0.95	-0.16		5	0.51	0.35	0.34		7	0.76	0.55	0.3	
SW911	6	0.71	0.7	0.04		2	0.10	0	1		5	0.76	0.6	0.23	
SO002	6	0.77	0.75	0.05		3	0.45	0.6	-0.30		8	0.76	0.45	0.43	
SW951	4	0.41	0.4	0.05		3	0.14	0.05	0.66		3	0.22	0.05	0.79	
SW632	7	0.75	0.7	0.09		2	0.26	0.2	0.24		8	0.77	0.9	-0.14	
IGF1	4	0.43	0.45	0.05		2	0.33	0.25	-0.13		6	0.64	0.75	0.25	
Overall	5.61	0.61	0.55	0.12	*	2.72	0.35	0.35	0.09		5.72	0.64	0.52	0.26	*

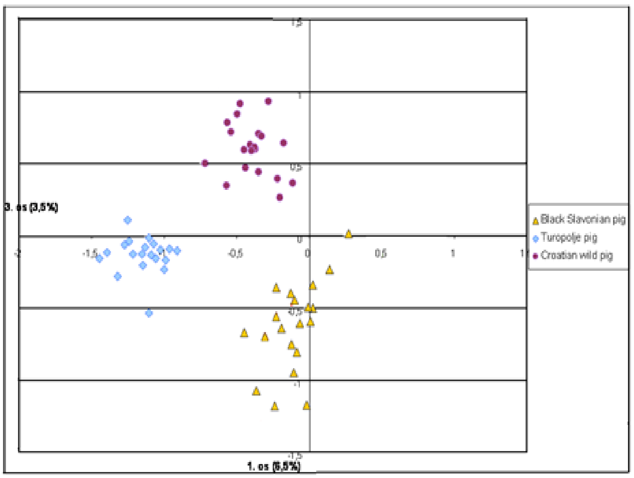

Figure 1. Graphical presentation of genetic distances between observed pig populations made with Principal Component Analysis (PCA) method (Representação gráfica das distâncias genéticas entre as populações de suínos observadas obtida pelo método de Análise de Componentes Principais (PCA)).

is present and the average number of alleles per locus was 4.68, ranging from 1 (locus SO215, Black Slavonian) to 11 (locus SO005, Croatian wild pig). Average heterozygosity (expected and observed) was between 0.35 and 0.64. Mean F_{ST} estimates and signification values between observed populations were calculated. The average fixation index (F_{ST}) was 0.24. Multilocus F_{ST} values indicated that around 24% of the total genetic variation was explained by population differences, with the remaining 78% corresponding to differences among individuals within population. Genetic distances between populations were made with Principal Component Analysis (PCA) method and are shown in **Figure 1**. Although only 10% of the variance has been explained by the PCA analysis, it can be noticed that studied populations are mostly clearly defined. To measure the population structure and the degree of admixture the STRUCTURE algorithm was applied. The highest log likelihood score was obtained for K = 5 for K ranging from 2 to 7. The status K = 5 represents the actual composition of the Croatian breeds **(Figure 2)** showing that the Black Slavonian breed is split into three different pools.

DISCUSSION

Barker (1994, p.501-8) suggested that microsatellite loci used in studies of genetic distance should have no fewer than four alleles in order to reduce the standard errors of distance estimates; on the other hand, Takezaki and Nei (1996, p.392) determined that MS markers have an average heterozygosity of between 0.5 and 0.8 in the population. Considering the foregoing we can conclude, that markers used in our study are suitable for genetic diversity analysis. The heterozygosity observed in Croatian pig populations is similar to the values reported for microsatellites in European pig breeds (Laval 2000, p.193), and to some extent lower than the values reported for Chinese pig breeds (Yang 2003, p.664) Multilocus F_{ST} values indicated that around 24% of the total genetic variation was explained by population differences, which corresponds to results of Laval (2000, p.195). They reported that genetic divergence among European breeds is relatively small (0.092 to 0.279). The results of the STRUCTURE program confirmed previous findings of Druml (2012, p.5) that Black Slavonian breed is split into tree gene pools. Such results are also supported by our previous researches on MC1R coat colour gene, which showed that less than half of Black Slavonian pigs are homozygous for black coat colour (Margeta 2013, p.27), probably due to the uncontrolled crossing with commercial white pigs in the near past. The results from present study demonstrated that microsatellites used in this study are mostly suitable to study genetic diversity among Croatian autochthonous pig breeds. This set of microsatellites may be used for identifying individuals and for genetic diversity studies for selection and conservation of Croatian pig breeds. However, markers SO215, SO227 and SO225 are represented with less than four alleles in at least one of the populations and for that reason it should be considered to exclude them or to replace them by other markers in genetic diversity studies in the future. Further studies including other pig breeds need to be performed to clarify the origin of different genetic pools in Black Slavonian breed.

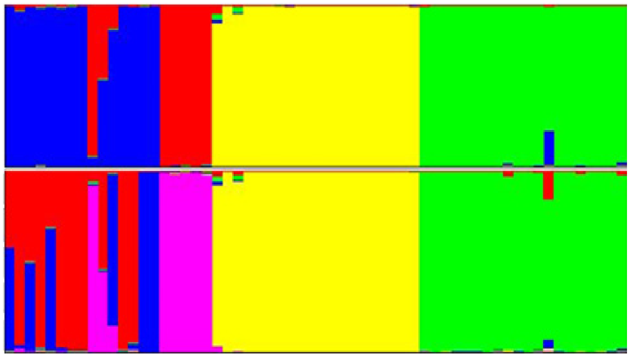

Figure 2: Graphical presentation of the clustering obtained from STRUCTURE software (Representação gráfica dos agrupamentos obtidos através do software STRUCTURE).

ACKOWLEGMENTS

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