

# Genetic admixture in pig population observed by microsatellite markers

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## Abstract

The aim of presented study was estimation of genetic relationship within and between 3 mentioned pig breeds bred in the Slovak Republic. Genetic variability at 10 microsatellite loci (SW24, SO107, SO068, SW936, sw353, so386, so355, sw72, tnfb, and SO070) was analysed in 412 pigs of Landrace, Yorkshire and Slovak White Improved breeds. Variation amount of each population was measured with average number of alleles per locus, heterozygosities, polymorphism information content (PIC) value were calculated. Phylogenetic trees were constructed by UPGMA.

The average number of alleles per locus was 11.5, ranging from 7 (SW72) to 16 (SO068). The Slovak White Improved, Landrace and Yorkshire pig breeds showed out high degree of genetic diversity with mean expected heterozygosities of 0.720, 0.697 and 0.705 respectively. Genetic distance ranged from 0.060 between Yorkshire and Slovak White Improved to 0.203 between Landrace and Slovak White Improved breeds.

The scatter diagram from principal component displayed genetic differentiation among all three breeds. A Bayesian method was applied for individual assigning testing. On the base of our results the group of Slovak White Improved population was strongly mixed with Yorkshire breed and the group of all Landrace individuals was presented as enclosed population.

**Keywords:** pigs, microsatellites, genetic variability, biodiversity, admixture

## Zusammenfassung

### Genetische Variabilität von Schweinepopulationen beurteilt mittels Mikrosatelliten-Loci

Ziel der Untersuchung war die Schätzung der genetischen Beziehungen der drei in der Slowakischen Republik gezüchteten Schweinerassen Landrasse (L), Yorkshire (Y) und Slowakisches Weißes Edelschwein (S). Diese Beziehungen wurden durch die Variabilität von 10 Mikrosatelliten-Loci (SW24, SO107, So68, SW936, SW 353, SO368, SO355, SW72, TNFB, SO070) beurteilt. Die Variation zwischen jeder Population wurde als durchschnittliche Anzahl von Allelen je Locus gemessen. Heterozygotie und PIC Wert wurden berechnet. Die phylogenetischen Bäume wurden mit Hilfe von UPGMA konstruiert. Die durchschnittliche

Anzahl an Allelen je Locus betrug 11,5 in der Spannweite von 7 (SW72) bis 16 (SO068). Bei allen drei Schweinerassen fand sich eine hohe genetische Vielfalt mit Heterozygotiewerten von  $S=0,720$ ,  $L=0,697$  bzw.  $Y=0,705$ . Für die genetische Distanz ergaben sich Werte für Y zu S von 0,06 bzw. L zu S von 0,203. Ein Skaterdiagramm der Hauptkomponenten stellt die genetischen Unterschiede zwischen allen drei Rassen dar. Die individuelle Zuordnung erfolgte mit Hilfe der Bayesschen Methode. Die Ergebnisse belegen eine große Nähe der Rassen Y und S, während sich L als geschlossene Population erwies.

**Schlüsselwörter:** Schweine, Mikrosatelliten, genetische Variabilität, Biodiversität, Mischung

## Introduction

The current situation of pig breeds in Europe is marked by coexistence of many small populations of local breeds and relatively small number of intensively selected breeds (e. g. Large White, Landrace and Pietrain) (Foulley *et al.* 2006).

Since early of the 20th century, number of pigs in the Slovak Republic has been drastically decreased due to their lower productivity level comparing to other imported commercial breeds. In our work we use allele length variation at microsatellite loci to investigate the genetic variation in the Slovak White Improved, Landrace and Yorkshire breeds.

Before World War I native Slovak white pigs have been crossbred with imported Yorkshire, Berkshire and Cornwall breeds. Later, there were extensive hybridizations with German White Improved (Deutsche Edelschwein) and German White Improved of Landrace type (Deutsche veredeltes Landschwein) as well as Polish, Swedish and Canadian Landrace. In the recent times, improved types of white pigs were unified to Slovak White Improved breed (Sambraus 2006). Assessment of Slovak breeds' population structure is an important task for animal genetic studies.

Yorkshire is an active outdoor breed. Their descendants could be found in all crossbreeding and rotational breeding programs using two or more breeds throughout the world. The sow component of commercial programmes usually contains half or even more of their blood (Briggs 1983).

The Landrace breed's development began with the native Landrace type. Native stock in the Slovak Republic was crossed with German, Danish, Dutch, Swedish and Belgian Landrace. In the Slovak Republic as well as in other countries, the 90 % of all marketed pigs are crossbreds, but most attention is given to seedstock.

Pig genetic diversity studies were conducted by genotyping multiple microsatellite loci (Kim *et al.* 2005, Thuy *et al.* 2006). Microsatellites are highly polymorphic co-dominant markers with simple identification, low mutation rates (Ellegren 1995) and uniform distribution throughout genome (Wintero *et al.* 1992). Microsatellite markers have been successfully used to assess the genetic structure and genetic diversity of breeds of domestic animals (Hall & Bradley 1995, Wimmers *et al.* 1999, Aberle & Distl 2004, Switonski 2004, Zsolnai *et al.* 2006, Kadlecik *et al.* 2004, Schwarz *et al.* 2005, Kasarda & Kadlecik 2007, Slaska *et al.* 2008), except the genetic aspects also influence of environmental factors were observed in Slovak pig

breeds (Hajduckova *et al.* 2009, Scirankova *et al.* 2009, Gancarcikova *et al.* 2009, Petrovic *et al.* 2009, Marcincak *et al.* 2003 and 2009).

In the presented study 10 pairs of microsatellite primers were used (Putnova *et al.* 2003). This microsatellite panel is generally used for parentage testing in Slovak Republic as well as for population genetic analyses. Used microsatellite markers were in accordance with the panel of microsatellites recommended by FAO and ISAG and were used for better exclusion ratio in middle European population.

Genetic structure observed by many viewing angles could be powerful tool for breeding programme optimisation. To get a complex view, different statistical methods have been used.

## Material and methods

A total of 412 animals of 3 examined pig breeds raised in the Slovak Republic were distributed as follows: Landrace (n=130); Yorkshire (n=29); and Slovak White Improved (n=253). Individuals used in this study represent the only breeding material allowed to create a Slovak swine population. All individuals included present core genetic material used for insemination and mating program in the Slovak Republic.

Genomic DNA was extracted from whole blood using the Wizard Genomic DNA Purification Kit according to a standard protocol (Promega). DNA microsatellite markers SW24, SO107, SO068, SW936, SW353, SO386, SO355, SW72, TNFB, and SO070 were combined in multiplex-polymerase chain reaction using fluorescently labelled primers (Putnova *et al.* 2003). The reactions were carried out on a thermocycler MJ Research (PTC 200). PCR products were separated on capillary sequencer ABI 310 (Applied Biosystems). Fragment size analysis was performed with GeneScan 3.7 NT software (Applied Biosystems).

Variation amount of each population was measured with average number of alleles per locus and observed ( $H_o$ ), expected ( $H_e$ ) heterozygosity (Nei 1987) and PIC value (Botstein *et al.* 1980) were calculated with the POWERMARKER 3.23 (Liu & Muse 2005). Population structure was evaluated by the hierarchical F statistics— $F_{IS}$ ,  $F_{IT}$ ,  $F_{ST}$  (Weir & Hill 2002) and  $G_{ST}$  using GENETIX software package (Belkhir *et al.* 1996). Distance measures were estimated according to Nei *et al.* (1983). Phylogenetic trees were constructed by using of unweighted pair group method with the arithmetic average (Upgma, Sneath & Sokal 1973) from distance using the POWERMARKER 3.23 (Liu & Muse 2005) and visualized by DENDROSCOPE software 1.0.5 (Huson *et al.* 2007).

To represent geometric relationships among the pig breeds, a principal component analysis (PCA) was applied using gene frequencies of all variable loci by GENETIX software package (Belkhir *et al.* 1996).

Several studies have shown that microsatellites can be used to identify the population origin of an individual (Rannala & Mountain 1997, Cornuet *et al.* 1999). According to Maudet *et al.* (2002), two approaches using observed population allele frequencies to assign individuals to a breed were compared. A »direct« method similar to the one commonly used in livestock studies (Buchanan *et al.* 1994) and a simulation-based method that provides a level of certainty ( $P$ -value) for each animal assigned (Cornuet *et al.* 1999) The assignment tests were carried out by the GENECLASS 2 software (Piry *et al.* 2004). The exclusion method was carried out using the Bayesian approach (Rannala & Mountain 1997) which has showed better

accuracy than the frequency- and distance-based methods (Cornuet *et al.* 1999, Koskinen 2003). Assignment of each individual was tested by the »leave one out« procedure (Efron 1983), what means that each individual was excluded from the data set when performing its assignment.

## Results and discussion

The allele frequencies of 10 microsatellite loci were analysed in 412 pigs of three most common pig breeds raised in the Slovak Republic. A total of 115 alleles combined in 367 genotypes were observed at 10 loci. The average number of alleles per locus was 11.5, ranging from 7 (SW72) to 16 (SO068).

As shown in the Table 1, the Slovak White Improved, Landrace and Yorkshire pigs have exhibited a high degree of genetic diversity with mean expected heterozygosities of 0.720, 0.697 and 0.705, respectively. On the contrary to small allele and genotype number the Yorkshire breed has the top value of observed heterozygosity. Only Yorkshire breed shows no presence of inbreeding.

The overall  $F_{IS}$  values per locus ranged from 0.024 (SO355) to 0.043 (TBFB), showing an overall  $F_{IS}$  of 0.035 (Table 2). The  $F_{ST}$  and  $G_{ST}$  estimates of genetic differentiation were not similar for all microsatellites loci. The  $F_{ST}$  values ranged from 0.092 (SO386) to 0.114 (SO107). The mean  $F_{ST}$  value of 0.105 from all the loci indicated that 89.5 % of the genetic variation was caused by differences among individuals and 10.5 % only due to differentiation among the breeds. The mean  $G_{ST}$  value (0.064) presents another view of analysed population and brings probably smaller differentiation among breeds.

Following swine breeds (represented by first letter of the breed name) were included in our study: S–Slovak White Improved, L–Landrace, Y–Yorkshire.

Table 1  
Genetic variability computed separately for each observed breed

Breed	Genotype	Sample size	Allele	$H_E$	$H_O$	PIC	$F_{IS}$
Slovak White Improved	27	251	10.5	0.720	0.706	0.686	0.020
Landrace	20.9	130	8.7	0.697	0.642	0.661	0.082
Yorkshire	12	29	7.1	0.705	0.745	0.667	−0.038
Mean	19.97	136.7	8.766	0.707	0.698	0.671	0.021

Nei's  $D_A$  genetic distance and mean  $F_{ST}$  estimates between each pair of porcine population were computed (Table 2). The genetic distance ranged from 0.060 (between Yorkshire and Slovak White Improved) to 0.203 (between Landrace and Slovak White Improved). Pair wise  $F_{ST}$  estimates ranged from 0.045 (between Yorkshire and Slovak White Improved) to 0.127 (between Landrace and Slovak White Improved). Computed  $D_A$  and  $F_{ST}$  corroborated our trees and PCA analysis.

BP	SW24	SO107	SO068	SW936	SW353	SO386	SO355	SW72	TNFB	SO070	BP	SW24	SO107	SO068	SW936	SW353	SO386	SO355	SW72	TNFB	SO070	
	SLYS	SLYS	SLYS	SLYS	SLYS	SLYS	SLYS	SLYS	SLYS	SLYS		SLYS	SLYS	SLYS	SLYS	SLYS	SLYS	SLYS	SLYS	SLYS	SLYS	
91				x x							192	x										
93	x x x			x x x							194	x x x										
95	x x x			x x x							195											
97	x										196	x x x										
99	x x x										198	x x x										
101	x x x			x x							200	x x x										
103	x			x							209	x x										
105	x			x x x							225											
107	x x x			x x x							227	x x x										
109	x x x			x x x							233	x x										
111	x			x x							235	x x										
113	x x x			x x							237	x x										
117	x										239	x										
119	x										241	x										
140				x x x							243	x x x										
144				x x x							245	x x x										
148				x x x							247	x x x										
150				x x x							249	x x										
154				x x							251	x x x										
156											253	x										
158				x							255	x x										
159											257	x x										
160				x							259	x x										
162											260											
164				x	x x x						262											x x
165											263											x x x
166	x x x										264											x x
168	x x x										266											x x x
170	x										267											
171											268											
172											269											
174	x										270											
176	x x x										272											x x x
177											274											x x x
178	x x x										276											x x x
180											278											x x
182											280											x x x
183											284											x x x
186	x										286											x
188	x x x										290											x x x
190	x x										292											x x x

Figure 1  
 Number of alleles and molecular size for porcine microsatellite markers observed in three swine breeds.

Phylogenetic trees were constructed on the base of Nei's  $D_A$  genetic distance computed for each possible combination of animals separately. Almost all Landrace breed individuals were grouped into the same branches. Group of Slovak White Improved breed was strongly mixed with Yorkshire pigs. Similar results show the relative position of all analysed animals defined by principal component factor scores based on correlation matrix from allele frequency of 10 microsatellites. The x and y axes represented 89.2% and 10.8% of the total variation, respectively. These axes separated the pig breeds into two blocks. Block I contains mostly Landrace breed and block II is created by mixture of Slovak White Improved and Yorkshire breeds. Differences between breeds included in block II are observable only on y axis.

Table 2

Nei's  $D_A$  genetic distance (below the diagonal) and mean  $F_{ST}$  estimates (above the diagonal) between each pair of pig populations

Breed	Slovak White Improved	Landrace	Yorkshire
Slovak White Improved	–	0.127	0.045
Landrace	0.203	–	0.112
Yorkshire	0.060	0.180	–

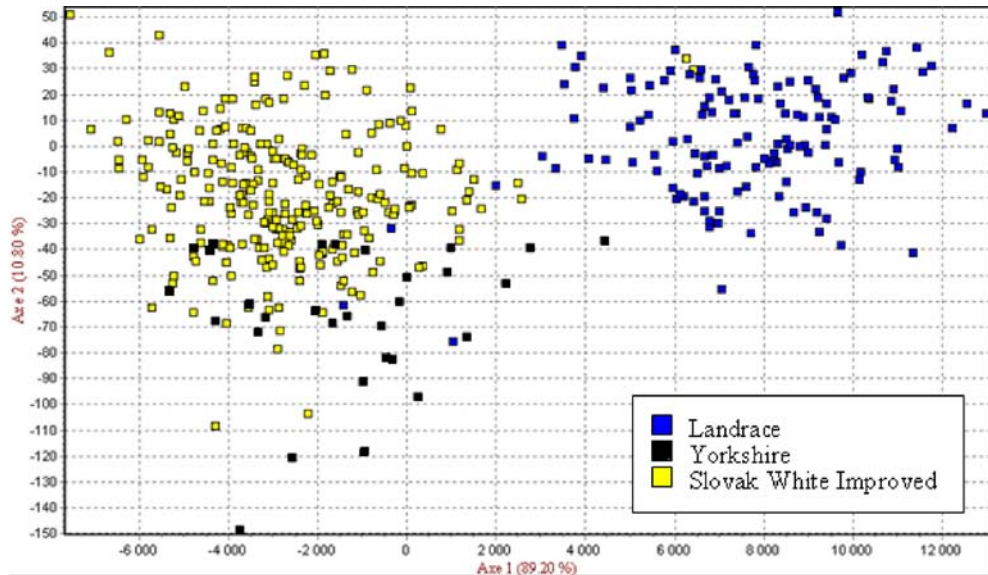


Figure 2

Scatter diagram showing relative position of 412 individuals defined by principal component factor scores based on correlation matrix from allele frequency of the 10 microsatellites.

Table 3

Breed assignment test results, showing the percentage of individuals correctly assigned using the »direct« approach or the »exclusion–simulation« approach using separately 10 microsatellite markers

Item	n	Percentage of individuals correctly assigned		
		Direct	Exclusion–simulation (reject population probability)	
			$P < 0.10$	$P < 0.05$
Landrace	130	95.38	73.85	72.31
Yorkshire	29	72.41	3.45	0.00
Slovak White Improved	253	78.65	30.83	26.09

Table 3 shows the percentage of individuals correctly assigned to their population of origin. All individuals from observed breed were assigned to their breed of origin by using a direct approach, whereas the accuracy of assignment of individuals from Landrace, Yorkshire and Slovak White Improved breeds were 95.38, 72.41 and 78.65%, respectively. By exclusion simulation (10.000 simulated individuals) on confidence level  $P < 0.10$  and  $P < 0.05$  results were

significantly different. No Yorkshire pig was correctly assigned to Yorkshire breed on  $P < 0.05$  level, and only 26.09% of Slovak White Improved pigs were correctly assigned to their breed of origin.

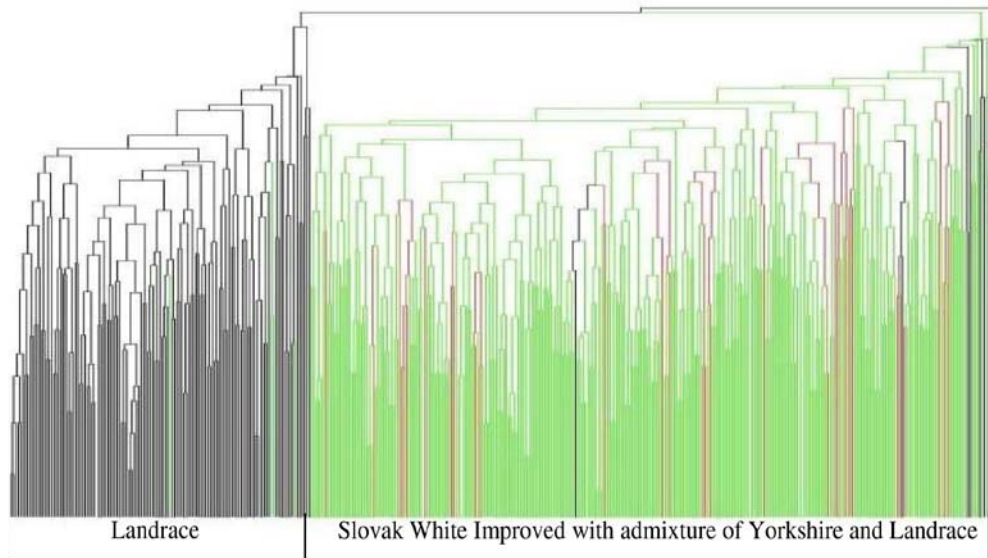


Figure 3  
Dendrogram showing the genetic relationships among 412 pigs based on  $D_A$  genetic distance (Nei *et al.* 1983)

There are many tools suitable for genetic variability observation. As a result of the FAO's recommendation and their availability through genomic mapping, microsatellites have been widely used to characterize genetic diversity (Barker 1999). In present study we have been dealing with the heterozygosity, genetic distance and admixture as an adequate measure of genetic diversity between selected breeds.

Figure 1 shows the fragment length and number of alleles for each microsatellite locus. Alleles unique to at least one breed were found for all microsatellite markers. According to Paszek *et al.* (1998) we cannot confirm specificity of allele 140 (SW353) for Landrace population. Number of alleles and allelic size range were compared to Korean, Chinese and Iberian pig breeds. Number of alleles for marker SO072 (7), SO070 (15), SW936 (10), SW24 (13) were similar to Iberian as well as Korean and Chinese population described by Fabuel *et al.* (2004) and Kim *et al.* (2005). Markedly higher number of alleles for each microsatellite markers described above was observed in Chinese indigenous pig breeds (Yang *et al.* 2003). The observed heterozygosity varied between 0.64 (Landrace) to 0.74 (Slovak White Improved), which represent typical value for European breeds. Observed heterozygosity in Slovak Landrace population is equal to Korean (0.62), Vietnamese (0.60) and German population (Kim *et al.* 2005, Thuy *et al.* 2006). Slovak Yorkshire according to another Yorkshire population presents higher value of observed heterozygosity. Analysed overall microsatellite loci heterozygosity ranged from 0.584 to 0.840. These values are comparable to Pulawska breed (0.54-0.90) described by Babicz *et al.* (2003) and to Indian pigs (0.52-0.90) (Kaul *et*

*al.* 2001). Mean value was equal to Iberian pig (0.69) (Fabuel *et al.* 2004) and just slightly higher than Chinese pig (0.609) (Yang *et al.* 2003). Inbreeding coefficients ( $F_{IS}$ ) were higher in Landrace and Slovak White Improved breeds. Yorkshire population seems to be not affected by inbreeding.

Nei's  $D_A$  genetic distance and mean  $F_{ST}$  estimates between Landrace and Yorkshire breeds were 0.180 and 0.112, respectively. These results are similar to Landrace and Yorkshire population in Korea and China (Kim *et al.* 2005). Small distance between Yorkshire and Slovak White Improved pig (0.060 and 0.045) caused specific result of UPGMA tree. Both breeds were grouped in the same branch and strongly admixed. Hypothesis, that Slovak White Improved breed is still closely related to Yorkshire pig is supported by PCA analysis. Percentage of individual pigs assigned to breed of origin using Bayesian approach shows inability of correct separation of observed breeds. Slovak White Improved population is strongly admixed with Slovak population of Yorkshire pig.

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