GENETIC STRUCTURE IN THREE BREEDS OF PIGS POPULATIONS USING MICROSATELLITE MARKERS IN THE CZECH REPUBLIC

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Abstract

An analysis of genetic structure in three breeds of small pigs populations in the Czech Republic was a part of a project "The molecular genetic as a tool for effective work in a small populations of pigs." The panel of 10 microsatellite markers was used for the genetic structure analysis of 509 individuals. Two commercial breeds Pietrain (Pn = 152), Duroc (D = 256) and one native breed (a genetic resource) – Přeštice Black Pied (Pc = 101) were assessed in GenAlEx, STRUCTURE and Microsatellite Toolkit software. The observed heterozygosity for Pn, D and Pc was 0.60, 0.55, and 0.72 respectively. The total number of alleles found for the 10 microsatellite markers was 87 in Pn, 75 in D a 74 in Pc. The analysis of population structure indicates there is very little admixture among breeds. The more distanced breed was Duroc. The results of study confirm that Pc breed as a genetic resource represent interesting reservoir of allelic diversity.

Key Words: Genetic diversity, microsatellite markers, population structure, pig breeds.

Many breeds of pigs in the world are on the edge of extinction. Therefore, a conservation of these breeds is important, attention is mainly focused on a preservation of local breeds.

A crucial importance for an ability of population to acclimatize for environmental changes and a pressure of selection has its genetic variability – diversity. One of the first steps during assembling of plans for the preservation of endangered population is a valuation of an actual situation of its genetic variability (TORO et al., 2011). An acknowledge of genetic population structure, among populations and inside population, is essential in order to assign priorities and approaches of conservation and sustainability of small populations.

To study a genetic variability, there are used mainly microsatellite markers (short tandem repeats – STR) nowadays. However, there are known many single nucleotide polymorphism markers (SNP), which could in the future substitute microsatellite markers for a valuation of diversity. To study population structure and variability of some species, these markers have been successfully used.

Actual studies of genetical diversity of pigs are restricted only for using microsatellite markers. It compares a genetic diversity of six chinese native breeds with commercial breeds according to an analysis of microsatellites WANG et al., 2011. NIDUP and MORAN 2011 are summarizing results of studies of genetic diversity in their work by using microsatellite markers in pig breeds in the whole world.

In this work, we have focused on an evaluation of a genetic variability of three small pig populations kept in the Czech Republic and used for a meat production. We have considered an extent of integrity and a similarity of native czech breed/genetic resource Přeštice black Pied pig with imported breeds Pietrain and Duroc. The Přeštice black Pied breed is a native local Czech breed, which has been certificated as a genetic resource since 1992 and has been kept as a closed population since 1996.

To study genetic diversity of these three small populations, ten microsatellite markers have been used, which markers (recommended by ISAG - FAO for monitoring of diversity at pigs) have been included in (FAO, 2011).

Material and Methods

Blood samples were collected from 509 breed boars. Commercial pig breeds Pietrain (Pn=152) and Duroc (D=256), and native breed (genetic resource) Přeštice Black Pied (Pc=101) were used for the analysis. Microsatellite markers were used for genotyping as following: S0068, S0107, SW24, S0355, S0386, SW353, SW936, S0070, SW72, TNFB (PUTNOVÁ et al., 2003). The extraction of genomic DNA from blood cell was carried out by Genomic DNA Mini Kit (Blood/Cultured Cell) (Geneaid) according to the instructions.

Polymerase chain reaction amplification was performed on a Veriti 96 Well Thermal Cycler (Applied Biosystems). The ten microsatellite markers were amplified in multiplex reaction. PCR mixtures contained deionized water, 10 mM dNTP mix, 10 x PCR Buffer I (15 mM $MgCl_2$, 100 mM Tris-HCl pH 8,3, 500 mM KCl, 0,01% gelatine), Taq Gold DNA Polymerase (5U), different primer concentrations and 10 ng extracted DNA, in a total volume of 6,25 µl. Reaction conditions consisted of a 10 min denaturation at 95°C, 30 cycles of 30 s

denaturation at 95°C, 30 s annealing at 58°C, 60 s extension at 72°C, and a final extension at 72°C for 60 min. Fluorescently labelled DNA fragments (GS ROX 500 DNA marker, Applied Biosystems; Hi-Di™ deionized formamid, Applied Biosystems) were separated using an ABI PRISM® 310 Genetic Analyzer. Fragment analysis was carried out using GeneScan 3.7 and Genotyper 3.7 software.

The total number of alleles per marker, allele frequencies, and observed and expected heterozygosities (NEI, 1973) were obtained in GenAlEx version 6.5 software (PEAKALL and SMOUSE, 2012). Polymorphic information content (PIC) of each locus and the probability of exclusion in parentage tests were calculated with Microsatellite Toolkit 3.1.1. (PARK, 2001). A Bayesian clustering method was employed to assess population structure using the program STRUCTURE version 2.2 (PRITCHARD et al., 2000). This approach assumes that an individual may have mixed ancestry from different underlying populations, and uses multilocus genotypes and a Monte Carlo Markov Chain simulation to infer population structure and to assign individuals to the assumed populations. In our case, different numbers of assumed populations (K) were evaluated (from K = 2 to K = 4).

Results

The total number of alleles found for the 10 microsatellite markers was 87 in Pn breed population, 75 in D a 74 in Pc respectively. The polymorphism in all loci were observed in all of the breeds. The number of alleles per locus ranged for Pn between 5 (S0386) and 12 (SW24, S0070), for D between 4 (S0355, S0386) and 12 (S0070, TNFB), and for Pc population between 4 (SW353) and 12 (S0070). The mean for the 10 markers of 8.70 alleles per locus in Pn, 7.50 in D, and 7.40 in Pc. There were some typical alleles for a given breed. The higher number of typical alleles for breed was observed in Pn breed; 22 alleles were unique and did not appear in D a Pc population. On the other hand, the lowest unique alleles 9 have been found in Pc population (Tab. 1).

The observed heterozygosity level ranged from 0.17 for loci SW72 to 0.83 for loci TNFB in Pn population, from 0.01 for loci S0355 to 0.70 for loci TNFB in D population, and finally from 0.44 for loci S0355 to 0.91 for loci S0070 in Pc population. Data on the genetic diversity within the three pig populations studied and presented in Tab. 2. The estimated polymorphism information content (PIC) ranged from 0.17 at SW72 to 0.80 at TNFB in Pn population, from 0.02 at S0355 to 0.73 at S0068 in D, from 0.56 at S0355 to 0.80 in S0070 in Pc. The highest total PIC was detected in Pc (0.69), the lowest in D (0.53) (Tab. 2). The probability of exclusion (PE1) in parentage testing when both candidate parents had a know genotype was greater than 0.99 when combined results of the all 10 markers were used. It is indicating that used microsatellite markers are extremely powerful for this item.

To measure the population structure and degree of admixture, the STRUCTURE algorithm has been applied. All runs from K = 2 to K = 4 showed a pattern allowing a meaningful interpretation. Thus, the status K = 3 corresponds to the picture of breed formation (Fig. 1). Assuming K = 3, the proportional contribution of the assumed ancestral populations to each one of the current breeds was computed, and the corresponding results are summarized in Tab. 3 . Each one of the breeds was very closely identified with one of the "ancestral" population. The contributions of the assumed ancestral populations to the 3 breeds under study are graphically showed in Fig. 1, for values of K ranging between 2 and 4. When K = 2, Pn and Pc where not separated from each other. Only D breed was separated when K = 2. When K increased to 3, the three populations were clustered. For K = 4 , the D breed was nearly subdivided.

Table 1. Alleles identified in Pn, D and Pc breeds

Marker	Pn	D	Pc
S0068	225, **233**, 243, 245, 247, 251, **254**, 255, 257, **259**	225, **229**, 231, 243, 245, 247, 251	225, 231, **237**, **241**, 243, 245, 247, **249**, 251, 255, 257
S0107	166, 188, **192**, 194, 196, 198, 220, **272**, **280**	166, **186**, 188, 194, 198, 220	166, **174**, **176**, **178**, 194, 196, 198, 220
SW24	93, 95, **97**, 99, 101, **103**, 105, 107, 109, 113,117, **166**	93, 95, 99, 101, 105, 107, 109, 113, 117	93, 95, 99, 101, 105, 107, 109, 113, 117
S0355	241, 243, 245, **247**, 255, 263, 269	241, 243, 255, 269	241, 245, **249**, 255, **259**, 263
S0386	164, 166, 172, 174, 182	166, 172, 174, 182	164, 172, 174, 180, 182
SW353	**93**, **99**, **101**, **107**, 140, 144, 148, 150, **156**	**138**, 140, 144, **146**, 148, 150, **152**	140, 144, 148, 150
SW936	93, 101, **103**, 105, 107, 109, 113, **144**, **148**	91, 93, 101, 107, 109, 113	**89**, 91, 93, 101, 105, 107, 109
S0070	**97**, **162**, 260, 262, 266, 270, 272, 274, 276, 280, 290, **292**	262, 264, 270, 272, 276, **282**, **284**, **286**, 290	260, 262, 264, 266, 270, 272, 274, 276, 280, 286, 288
SW72	**91**, **93,** 97, 105, 107, 109, 113	97, 99, 105, 107, 109, 113, **282**, **284**	97, 99, 105, 107, 109, 113
TNFB	156, 159, 162, 174, 177, 180, 183	**97**, **99**, 156, 159, 162, **165**, 168, 174, 177, 180, 183	156, 159, 162, 168, 180, 183

bold=alleles found only in one breed; underlined=alleles with the highest frequency

Table 2. Mean number of alleles per breed (MNA), expected H_e and observed H_o heterozygosity, polymorphism information content (PIC), probability of identity (PI), probability of exclusion when both parents known (PE1), probability of exclusion when only one parent know (PE2), probability of exclusion when exclude both parents (PE3)

Breed	MNA	H_e	H_o	PIC	PI	PE1	PE2	PE3
Pn	8.7	0.6333	0.6046	0.59	$9.4x10^{-9}$	0.9902	0.9608	0.9999
D	7.5	0.5823	0.5518	0.53	$1.6x10^{-7}$	0.9972	0.9158	0.9996
Pc	7.4	0.7322	0.7154	0.69	$1.8x10^{-10}$	0.9994	0.9854	0.9999

Figure 1. Graphical representation of the estimated membership fractions of individuals of the breeds analysed in each of the K inferred clusters, for K = 2 to K = 4.

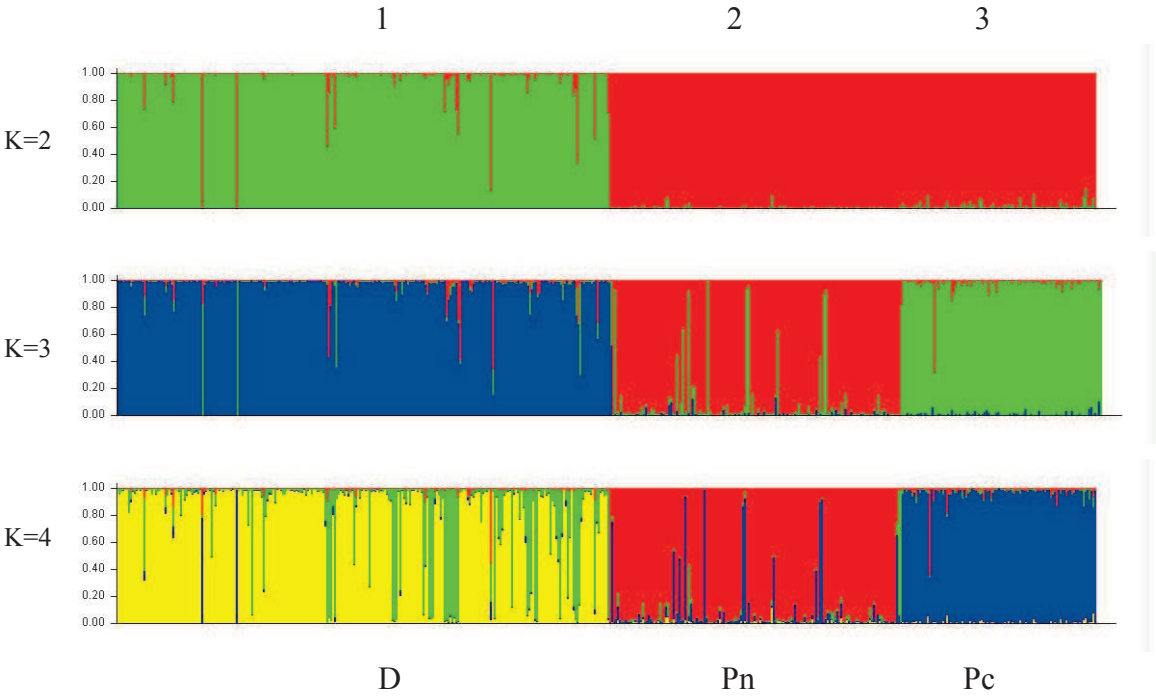

Table 3. Proportional contribution of the inferred clusters (K = 3) to the breed studied

Breed	Cluster		
	1	2	3
D	0.024	0.025	*0.951*
Pn	*0.897*	0.093	0.011
Pc	0.026	*0.962*	0.012

Contribution of the more important cluster per breed is represented in italics

Discussion

In our study, three breeds of pigs have been chosen to distinguish genetic structure. The Pn, D and Pc are part of small pigs population in Czech Republic. The population sizes of Pn, D and Pc according to EFABIS are 1200 – 1400, 800 – 1000, and 1200 – 1400 in 2011 (http://efabis.tzv.fal.de). Since the Pn and D breeds are the world-wide, Pc breed is Czech national native breed and also kept as a genetic resource. Knowledge of the structure of a pigs population in terms of sources of variability amnog and within breeds is essentianl for establishing conservation strategies and priorities (CABALLERO and TORO, 2002).

Genetic markers such as microsatellite markers are suitable for genetic structure studies, because of their

distribution throughout the pigs genome, codominant inheritance, neutrality with respect to selection, large number, and high level of polymorphism (VICENTE et al., 2008).

These markers have proved useful for the analysis of population structure, and have been used for genetic characterization of several species and populations, including European pig breeds (LAVAL et al., 2000; MARTINEZ et al., 2000). In our study, the set of ten microsatellite markers described in PUTNOVÁ et al. 2003 (S0068, S0107, SW24, S0355, S0386, SW353, SW936, S0070, SW72, TNFB) was used for analyzing the genetic structure of three small pig populations of Pn, D, Pc breeds in the Czech Republic.

Numerous studies have reported observed heterozygosity values for different population of Pn and D breeds with values ranging from 0.50 to 0.67 (LAVAL et al., 2000; OLLIVIER et al., 2005; SANCRISTOBAL et al., 2006). In our study, observed heterozygosity for world-wide populations of Pn (0.61) and D (0.55) were lower compared to Pc popolation. The observed heterozygosity for Pc breed was 0.72 and it is indicating that high levels of genetic diversity exist in the genetic resource of Pc breed. The results advert to fact that Czech indigenous pig breed Pc has larger genetic diversity than Pn a D breeds.

The results of the probabilities of exlusions (PE1,PE2, PE3) demonstrate that the microsatelite markes used in this study are extremely powerful for such a usage even in the small populations.

The analysis with STRUCTURE confirms that each of the breeds analysed is closely identified with a single ancestral population, and that there was no admixture between three breeds studied, which are distinct from each other. The results reported here indicate high level of genetic variability and clear breed differentiation, with commercial breeds Pn and D, together with native breed Pc. There was indicated very little admixture between the three breeds studied. The more distanced breed was indicated D population. The same results were reported in VICENTE et al., 2008.

Conclusion

Taken together, our results can be useful in outlining conservation strategies and also conservation programs, even though it was not a resolved aim of this study.

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