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Towards the identity of different Crna slavonska pig breed gene pools

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Abstract

The microsatellite (MS) genetic diversity studies on CS pig breed showed subclustering of this breed. Structuring results based on MS data of 70 CS pigs were compared to the MC1R coat color gene genotyping results. The MS analysis included also commercial pig breeds to elucidate the relationship of different CS breed gene pools towards commercial pig breeds. The Structure results and the results of Unweighted Pair Group Method with Arithmetic Mean (UPGMA) individual clustering based on genetic distances revealed presence of three gene pools in CS breed. Comparison with the genotyping results of the MC1R coat color gene showed that one gene pool consists of MC1R homozygous black animals, while animals from the other two gene pools (CS2 and CS3) were MC1R heterozygous.

Keywords: Crna slavonska pig breed, MC1R genotyping, microsatellites

Introduction

Crna slavonska (CS) pig breed was established in the second half of the 19th century by crossing Swallow-bellied Mangalitsa sows with Berkshire boars. Only pigs with black hair and skin were kept for further breeding, while pigs of other colors were eliminated from the breeding program (Ritzoffy, 1935). Later in the 20th century, after the 2nd world war, a part of CS pig breed was raised on big farms, where it was crossed with Large Black boars (Balić, 1948). At the same time, the import of modern white pig breeds started and CS pigs were further kept only by local farmers under extensive conditions, where they were often crossed with commercial pig breeds.

In the year 1996, the revitalization of CS pig breed have started, and few years later also genetic characterization of the breed. Genotyping of the MC1R coat color gene showed that pure black CS pigs have black Asian genotype. With the PCR - RFLP genotyping of 179 pigs on MC1R it was discovered that only slightly more than a third of genotyped animals were homozygous for black coat color genotype, while the others were heterozygous (Margeta et al., 2014).

The MS genetic diversity studies on CS pig breed revealed subclustering of this breed. Presence of three gene pools in CS pig population was described by Druml et al. (2012). One pool of CS clustered close to the Turopolje pigs from Austria, while the other two clustered close to the Mangalitsa pigs. Partial overlapping between CS and White Mangalitsa breed was observed in primary component analysis of MS data. Similarly, with the *Structure* results in the genetic diversity study on Croatian autochthonous pig breeds subclustering of the CS pig population at higher K values was confirmed (Margeta et al., 2018).

In this work, structuring results based on MS data of 70 CS pigs were compared to the MC1R coat color gene genotyping results. The MS analysis included also commercial pig breeds to elucidate the relationship of different CS breed gene pools towards commercial pig breeds.

Materials and methods

Total genomic DNA was isolated from ear clips using DNeasy Blood & Tissue Kit (Qiagen GmbH, Germany). MC1R genotyping was performed only on CS breed, while MS analysis included 70 CS, 19 Landrace (L), 14 Large White (LW), 9 Duroc (D), 14 Pietrain (P), 15 PIC and 10 Topigs (TP).

Population structure based on microsatellite multiplex analysis

23 MS 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. Multiplex PCR reactions were performed with 2x Type-it Microsatellite PCR Kit (*Qiagen GmbH, Germany*). 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 to 59.5 ºC) and extension (60 s at 72 ºC). Cycling program ended with a final extension for 30 min at 60 ºC. MS multiplex PCR products were analyzed using GeneScan350 ROX internal standard size marker on ABI3730XL capillary gene analyzer.

The analysis of the population structure was performed with the Structure program (Pritchard et al., 2000). The most likely K-value was identified according to Evanno et al. (2005). Visualization of the Structure results was conducted in Clumpak software (Kopelman et al., 2015). An UPGMA phylogenetic tree based on Nei's (1987) standard genetic distance was constructed using Adegenet package in R (R 3.4.0.; www.r-project.org).

MC1R genotyping

A MC1R genotyping was performed with PCR-RFLP method as already described (Margeta et al., 2014) on 70 Crna slavonska pigs. The same primer pair as for the PCR-RFLP genotyping was used for sequencing of the representatives of different CS gene pools as obtained in the analysis of population structure based on MS data. Sequences were aligned with Clustal 2.1 software (Larkin et al., 2007).

Results and discussion

Population structure based on microsatellite multiplex analysis

To analyze the population structure and degree of admixture, the *Structure* algorithm was applied. The highest log likelihood score was obtained at K-value 8 (for K ranging from 1 to 9). The status K = 8 thus represents the actual composition of the analyzed breeds (Figure 1) showing that the CS breed is split into three different pools which is in consistence with previous findings of Druml et al. (2012) and Margeta et al. (2018).

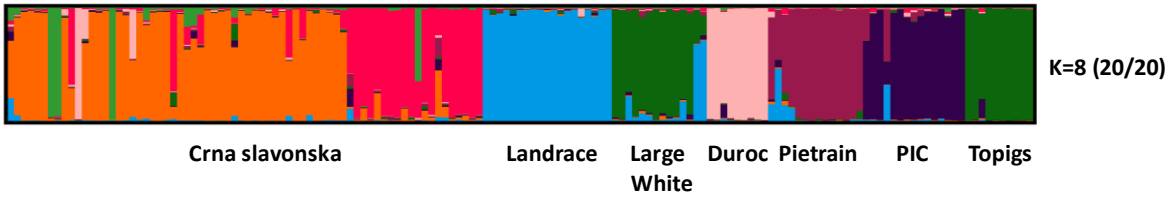

Figure 1. CLUMPAK visualization of the Structure results at K=8 showing substructuring of the Crna slavonska breed

Clustering of CS population began at K = 6. Another three animals clustered partially or completely with Duroc, even at lower K values. Also modern breeds were well defined, except of Large White and Topigs, which clustered together.

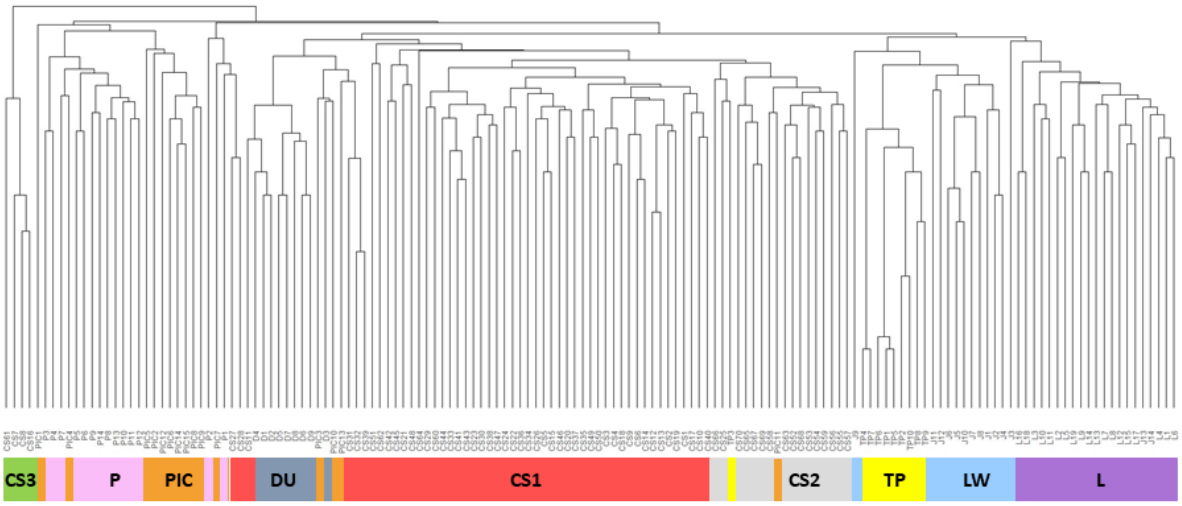

Figure 2. An UPGMA phylogenetic tree based on Nei's standard genetic distance represents subclustering of CS breed (CS1, CS2 and CS3). P-Pietrain, DU-Duroc, CS-Crna slavonska, TP-Topigs, LW-Large White, L-Landrace

The UPGMA tree (Figure 2) represents the individual clustering based on genetic distances. Majority of the CS breed clustered together. The group, recognized by

Structure algorithm as a separate cluster at higher K values, is here represented as a subcluster (CS2). Part of the group CS1 clustered together with Duroc. Four animals, also recognized by Structure as a separate group at higher K values, formed a separate cluster (CS3). Also commercial pig breeds follow similar clustering pattern as obtained by Structure.

MC1R genotyping

MC1R genotyping showed that 27 out of 70 analyzed animals were heterozygous, i.e. possessed only one “black” allele. When compared the MC1R genotyping results with results based on MS analysis, it was observed that MC1R homozygous animals correspond to the major CS gene pool (CS1), while animals from the other two gene pools (CS2 and CS3) were MC1R heterozygous. Only one animal from the CS2 gene pool was MC1R homozygous.

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Pietrain        TGGTGAGCGTGAGCAACGTGCTGGAGACGGCCGTGCTGCTGCTGCTGGAGGCGGGCGCCC 331
Large White     TGGTGAGCGTGAGCAACGTGCTGGAGACGGCCGTGCTGCTGCTGCTGGAGGCGGGCGCCC 331
Duroc           TGGTGAGCGTGAGCAACGTGCTGGAGACGGCCGTGCTGCTGCTGCTGGAGGCGGGCGCCC 331
Wild boar       TGGTGAGCGTGAGCAACGTGCTGGAGACGGCCGTGCTGCTGCTGCTGGAGGCGGGCGCCC 331
CS1             TGGTGAGCGTGAGCAACATGCTGGAGACGGCCGTGCTGCCGCTGCTGGAGGCGGGCGCCC 331
Large Black     TGGTGAGCGTGAGCAACATGCTGGAGACGGCCGTGCTGCCGCTGCTGGAGGCGGGCGCCC 331
CS2             TGGTGAGCGTGAGCAACRTGCTGGAGACGGCCGTGCTGCYGCTGCTGGAGGCGGGCGCCC 331
CS3             TGGTGAGCGTGAGCAACRTGCTGGAGACGGCCGTGCTGCYGCTGCTGGAGGCGGGCGCCC 331
                ********************* ********************** ********************

Pietrain        TGGCCGCCCAGGCCGCCGTGGTGCAGCAGCTGGACAATGTCATGAACGTGCTCATCTGCG 391
Large White     TGGCCGCCCAGGCCGCCGTGGTGCAGCAGCTGGACAATGTCATGAACGTGCTCATCTGCG 391
Duroc           TGGCCGCCCAGGCCGCCGTGGTGCAGCAGCTGGACAATGTCATGGACGTGCTCATCTGCG 391
Wild boar       TGGCCGCCCAGGCCGCCGTGGTGCAGCAGCTGGACAATGTCATGGACGTGCTCATCTGCG 391
CS1             TGGCCGCCCAGGCCGCCGTGGTGCAGCAGCTGGACAACGTCATGGACGTGCTCATCTGCG 391
Large Black     TGGCCGCCCAGGCCGCCGTGGTGCAGCAGCTGGACAACGTCATGGACGTGCTCATCTGCG 391
CS2             TGGCCGCCCAGGCCGCCGTGGTGCAGCAGCTGGACAAYGTCATGRACGTGCTCATCTGCG 391
CS3             TGGCCGCCCAGGCCGCCGTGGTGCAGCAGCTGGACAAYGTCATGRACGTGCTCATCTGCG 391
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Figure 3. Clustal 2.1 MC1R sequences alignment of three different CS gene pools (CS1, CS2 and CS3) with MC1R sequences of commercial breeds and Wild boar

MC1R sequencing of representatives of all three CS gene pools (CS1, CS2 and CS3) and alignment of observed sequences with MC1R sequences of Pietrain, Large White, Duroc, Large Black and Wild boar revealed that animals from CS1 gene pool possess the same genotype as Large Black (Asian black), while animals from CS2 and CS3 gene pools are heterozygotes between black and white/red/spotted

genotype, suggesting that they are crossbreds between Crna slavonska and commercial breeds (Figure 3). Also previous sequencing of MC1R in black CS pigs revealed above described haplotypes (Margeta et al., 2014), homozygous black was previously denoted as E^{D1} by Kijas et al. (1998). While heterozygous CS are phenotypically still black because of autosomal dominant inheritance of black coat color, the problem arises by crossing two heterozygous animals, leading to part of offspring, which are phenotypically not black. Because Swallow-bellied Mangalitsa has a wild type MC1R genotype (Drögemüller et al., 2006), and all other CS founder breeds (Berkshire, Large Black and Poland China) are black and of Asian origin (possess E^{D1} genotype), our results excluded the possibility that some heterozygotes may have their roots in the CS founder breeds.

PCR-RFLP genotyping on MC1R (Margeta et al., 2014) revealed that only slightly more than one third of CS population is homozygous for black coat color, so excluding all heterozygotes from population would be to rigorous. Nevertheless, implementation of MC1R genotyping for all breeding animals is suggested to prevent crosses between two heterozygotes and occurrence of non-black coat color in the population.

Conclusions

Results of two different methods based on MS data and MC1R genotyping revealed that two CS gene pools obtained by analyzing MS data consisted of MC1R heterozygous animals. Substructuring of CS population occurred at higher K values, showing that heterozygotes are quite integrated into the CS breed and are probably consequence of mating with commercial breeds in the past. Nevertheless, some animals from the CS group clustered together with Duroc, even at lower K values, suggesting that mating with commercial breeds is still present. Because MC1R heterozygous animals are phenotypically the same as homozygous black animals, current breeding program, based only on phenotypic data, cannot successfully deal with this problem. It could be concluded that there is a need to introduce appropriate genetic methods into the breeding and selection program of the CS breed.

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