

HOW BLACK IS REALLY BLACK SLAVONIAN PIG?

Polona MARGETA ¹, Vladimir MARGETA ¹, Kristina BUDIMIR ¹

ABSTRACT

Black Slavonian pig is Croatian autochthonous pig breed, established at the end of the 19th century by crossing locally raised Mangalitsa pigs with Berkshire, Poland China and Large Black pig breeds. Previous study of the main coat colour *Extension* locus in Black Slavonian pigs revealed the presence of the *E^{DI}* allele, typical for black pig breeds of Asian origin. Because the Black Slavonian pig is the only breed with *E^{DI}* genotype raised in Croatia, the genotyping of the *MC1R* gene was used to determine the purity of the Black Slavonian pigs. PCR-RFLP analysis using *NspI* restriction endonuclease performed on 179 pigs from 9 farms in Osijek-Baranja County shows that only 70 pigs were pure black while other 109 pigs were crossbreeds. The method used is simple, fast, low-cost and reliable and for that reasons it will be further used in the selection programme of Black Slavonian pig.

Key words: Black Slavonian pigs / *MC1R* gene / PCR-RFLP genotyping

1 INTRODUCTION

Black Slavonian pig is Croatian autochthonous pig breed, established at the end of the 19th century near Osijek in Slavonia. Its establishment was a result of a planned selection under the supervision of Count Pfeifer, who crossed a locally raised Mangalitsa pigs with Berkshire, Poland China and Large black pig breeds. He created a pig which had excellent characteristics for that time, as confirmed by a gold medal won at Vienna World Exposition in 1873.

The breed was black, resistant and convenient for keeping in extensive (pastures, woods) and half-extensive conditions (pens with some free space). It was also characterised by meat quality, suitable for producing typical traditional meat products like “kulen” (dry cured sausage spiced with red paprika), ham, bacon and other sausages (Karolyi *et al.*, 2007).

After World War II this pig breed was almost forgotten, raised mostly in extensive condition by local farmers, which provided the opportunity for crossmating with lo-

cal wild boar population, and also for uncontrolled crossing with modern pig breeds (Large White, Yorkshire, Pietrain, Duroc). Phenotypic distinction between purebred and F1 crossbred pigs is not possible because of dominant black colour of Black Slavonian pig.

Out of several genes affecting coat colour in domestic animals, the *melanocortin receptor 1 (MC1R)* locus is the most consistently polymorphic. *MC1R* is primarily expressed in melanocytes and plays a key role in melanogenesis by determining the switch between production of red/yellow pheomelanin and dark eumelanin (Robbins *et al.*, 1993; Barsh, 1996). Loss-of-function mutations are associated with recessive red coat color, whereas dominant black coloring is linked with mutations causing the activation of *MC1R* signalling.

The molecular basis at the *Extension (MC1R)* locus in pigs is well defined (Kijas *et al.*, 1998; Kijas, 2001) and described in numerous pig breeds (Fang *et al.*, 2009). The wild-type (*E⁺*) allele allows full expression of both pheomelanin and eumelanin. The dominant black colour results from two different mutations, each of them evolved

¹ J.J.Strossmayer University of Osijek, Kralja Petra Svačića 1d, Osijek, Croatia, e-mail: polona.frajman@gmail.com

independently in Asia and Europe. The E^{D1} allele is Asian in origin and is associated with an L102P missense mutation, and E^{D2} is European and associated with a D124N substitution. The recessive red allele (e) controls two missense mutations A164V and A243T and allele E^P causes black spotting on a red or white background.

Previous study of the *Extension* locus in Black Slavonian pigs revealed the presence of the E^{D1} allele (Margeta *et al.*, 2009). Because the Black Slavonian pig breed is the only one with E^{D1} genotype raised in Croatia, the genotyping of the *MC1R* gene was used to determine purity of the Black Slavonian pigs. The method is simple, fast, low-cost and reliable.

2 MATERIALS AND METHODS

2.1 SEARCH FOR POLYMORPHIC SITES

The previously obtained (Margeta *et al.*, 2009) sequence of the 5' part of the *MC1R* exon from Black Slavonian pig (E^{D1} genotype) was aligned using ClustalX programme (Larkin *et al.*, 2007) with sequences of other pig breeds with different *MC1R* genotype: Wild Boar

(E^+), Large White (E^P), Hampshire (E^{D2}) and Duroc (e) (Fig. 1). Polymorphic sites specific only for Black Slavonian pig breed were further searched for the presence of the restriction endonucleases recognition sites. The search was directed into commercially available endonucleases which cut the sequence once or mostly twice to obtain enzymes useful for fast and reliable screening and determination of the E^{D1} genotype.

2.2 PCR AMPLIFICATION AND RESTRICTION

Samples of 179 Black Slavonian pigs were collected on 9 farms in Osijek-Baranja County. Genomic DNA was isolated from samples with Genomic DNA Miniprep Kit (*Bio Basic Inc.*). A primer pair MERL1 (5'-RGTGCCTGGAGGTGTCCAT-3') and EPIG2 (5'-CGCCCAGATGGCCGCGATGGACCG-3') was used to amplify a 428-bp product from the 5' half of the single exon of *MC1R* gene (Kijas *et al.*, 1998). PCR was performed and 10 μ l of the PCR-products from all 179 animals were digested by restriction endonuclease *NspI* (*Thermo Scientific*) to determine genotype at nucleotide position 282. Only pigs

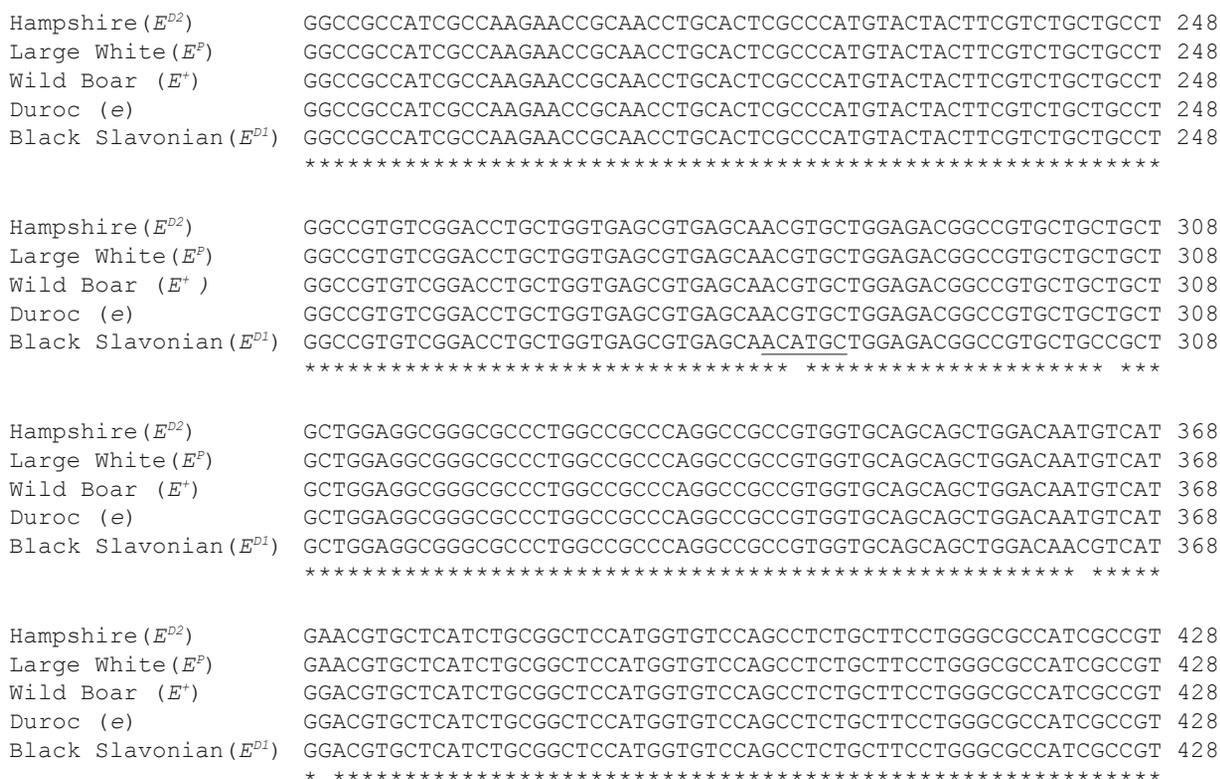


Figure 1: A part of a CLUSTAL 2.1 multiple sequence alignment of the 5' part of the *MC1R* exon1 of pig breeds with different *Extension* locus genotype. Polymorphic sites specific for the Black Slavonian pig breed (genotype E^{D1}) are shaded and *NspI* recognition site is underlined.

with E^{PI} allele holds *NspI* restriction site (RCATGY). The digestion-reactions were checked on 2% agarose gel.

3 RESULTS AND DISCUSSION

3.1 SEARCH FOR POLYMORPHIC SITES

Multiple sequence alignment (ClustalX) of the 5' part of the *MC1R* exon (from nucleotide position 128 to nucleotide position 428 from transcriptional start site) from different pig breeds revealed three polymorphic sites specific for the Black Slavonian pig breed: A-G substitution at nucleotide position 282, T-C substitution at position 305, and T-C substitution at nucleotide position 363, respectively (Fig. 1).

All three polymorphic sites were further searched for the presence of the restriction endonucleases recognition sites. The search directed into commercially available endonucleases which cut the sequence once or mostly twice revealed only one restriction endonuclease with mentioned characteristics. Restriction endonuclease *NspI* with recognition sequence RCATGY recognizes the polymorphic site at nucleotide position 282 and cuts the sequence in Black Slavonian pigs (ACATGC), while simple substitution abolishes recognition sequence in pig breeds of other *MC1R* genotypes (ACGTGC).

3.2 PCR AMPLIFICATION AND RESTRICTION

PCR amplification of the 428 bp from the 5' site of the *MC1R* exon was performed on all 179 collected samples of Black Slavonian pigs. Results of the restriction digestion of the PCR product with *NspI* restriction endonuclease showed, that out of 179 pigs included in the study, 70 (39.1%) were pure Black Slavonian pigs (genotype E^{PI}), while other 109 (60.9%) pigs were crossbreeds (Fig. 2). The higher number of crossbred pigs was expected, because of unplanned crossings with commercial pig breeds like Large White, Yorkshire, Pietrain and Duroc in the near past. Because of extensive and half-extensive keeping, there were also chances for contact with local wild boar population.

By comparing results from each farm, it could be observed that farms with higher number of pigs had also higher number of crossbred pigs. There was only one farm on which all eight analysed pigs were pure black. Interestingly, that was the farm where we performed *MC1R* genotyping few years ago (Margeta *et al.*, 2009). The owner decided at that time to keep the pigs shown with PCR-RFLP analysis to be pure black and those included in the present study are their descendants.

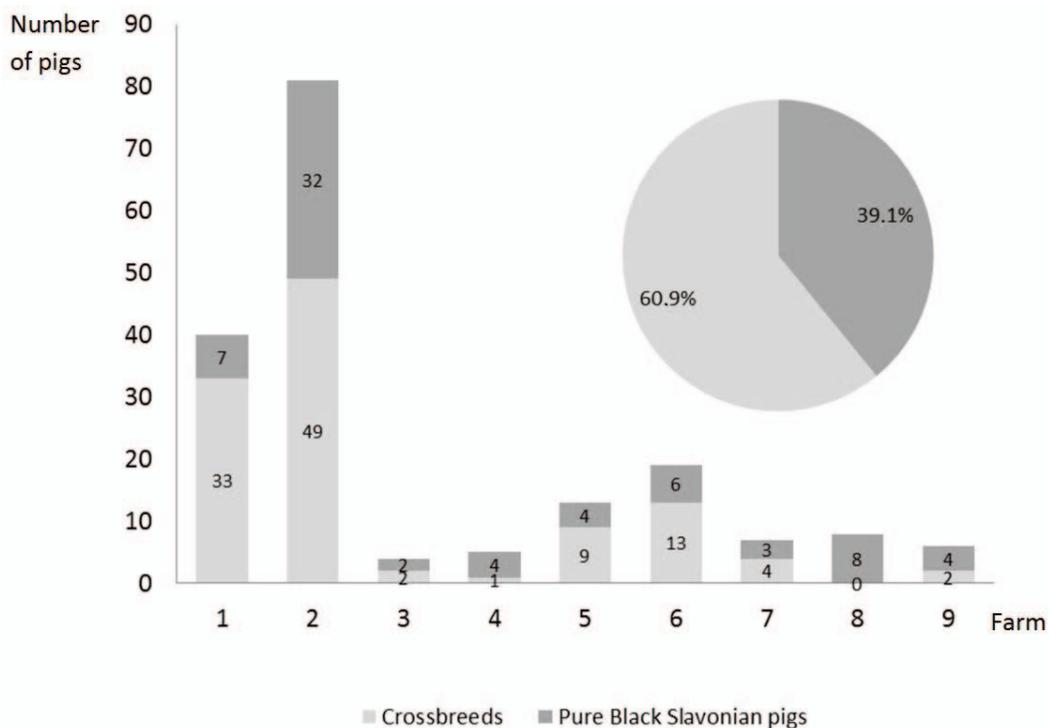


Figure 2: Columns on the chart represent numbers of pure and crossbred pigs on each farm, and the chart-pie represents the percentage of pure Black Slavonian pigs and crossbreeds among all 179 analysed samples

4 CONCLUSION

In the present study, we were able to identify a simple PCR-RFLP method, based on different coat colour *MC1R* gene genotypes, enabling us to detect potential crossings of autochthonous Black Slavonian pig with modern pig breeds or wild boars. Analysis of 179 pigs showed that the majority of Black Slavonian pigs today are crossbred and for that reason the *MC1R* genotyping will be further used in selection of pure Black Slavonian pigs.

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