A DEEPER SCREENING OF BAZNA PIGS GENOME REVEALED FIRST EVIDENCE OF THEIR ADMIXED GENETIC BACKGROUND

V.A. Bâlteanu[1*], A.S. Costin[1], M.I. Siteavu[1], A. Zsolnai[2]

[1]*University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,*
Institute of Life Sciences, Cluj-Napoca, Romania
[2]*Hungarian University of Agriculture and Life Sciences Kaposvár Campus, Hungary*

Abstract

The evolutionary history of the European pig breeds is very complex. The current view is that several events might have contributed to their current genetic background. In Romania, Bazna pigs faced a sustained demographic decline, although in the past it was a widely spread breed. Despite its similar appearance with other white belted breeds, it is presumed to originate in 1872 crosses between Mangalitza and Berkshire. They were subsequently improved with British (e.g. Large White, Wessex etc) and German (e.g. Angler Sattleschwein). To obtain preliminary evidence of this possible admixed origin of Bazna pigs, we used two data sets: a mitochondrial set (Bazna versus commercial pigs versus Chinese or Vietnamese breeds) and an autosomal set (Bazna versus Mangalitza, Berkshire and Meishan breeds). The results highlighted a possible contribution of Mangalitza and Berkshire breeds to its current genetic background.

Key words: *Bazna pigs, DNA markers, admixed origin, conservation*

INTRODUCTION

The evolutionary history of the European pig breeds was very complex. Pigs (*Sus scrofa*) were primarily domesticated from wild relatives in southwest Asia (Middle East) during the Neolithic Revolution in the Fertile Crescent, around 10,500-10,000 YBP [1, 2]. Far Eastern Asian local pig breeds were independently domesticated in China [1]. DNA evidence suggests that Neolithic pigs from Fertile Crescent arrived in Europe around 8500 YBP *via* human migration routes, but they were replaced in a few hundred years by locally domesticated pigs in different regions across Europe [3, 4].

The British Islands are recognized as an important formation centre for many local pig breeds (Berkshire, British Saddleback, Large Black, Middle White, Chester White etc). A major turning point in the creation of modern commercial pig breeds (ex. Yorkshire, Large White, Landrace etc) was the admixture of British heritage pigs with Chinese pigs in the 18th-19th centuries, aiming to improve reproductive or production traits [5, 6]. The newly formed British commercial pig breeds subsequently spread across European, American [7] and Asian continents, including China [8], where they were also used to improve some local breeds.

The unequal competition or crossbreeding with more productive commercial pig breeds (ex. Large White, Duroc or synthetic pigs) was one of the major causes of the sustained demographic decline or disappearance of many local pig breeds (FAO, 2015). Moreover, a current evolution of African Swine Fever outbreak [9], in some European countries represents a real treat, since many local pigs are

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[*] Corresponding author: avbalteanu@yahoo.com

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managed in outdoor enclosures, where the risk increases.

A turning point in some traditional pig breeds survival was an increased demand on the European and American markets for cured-ham. In addition, the implementation of scientific-based conservation plans and / or appropriate governmental financial support represented reliable solutions for the survival of some traditional pig breeds: Iberian in Spain, Mangalitza in Hungary and Romania and Cinta Senese in Italy.

In Romania, Bazna pigs faced similar challenges, although in the past it was a widely spread breed. Despite its similar appearance of Bazna pigs with other white belted breeds (ex. British Saddleback, Hampshire, Cinta Senese or Angler Sattleschwein), it is presumed to originate in 1872 crosses between Mangalitza and Berkshire, which were later probably infused in different episodes with Yorkshire, Angler Sattleschwein, Wessex, or Large White. Bazna was established as a distinct breed in 1958 (Figura 1).

Fig. 1 Pictures of Bazna pigs from Romania

To have a broader understanding about the presumed admixed origin of Bazna pigs, which is essential for implementation of a reliable conservation plan, the objective of the current work evaluate the contribution of other pig breeds to its genetic makeup.

MATERIAL AND METHODS

To obtain information about the maternal pig lineages that contributed to Bazna breed genetic makeup we expanded our data set by sequencing the MT-CYB gene in Bazna (BazRo, N=48), Mangalitza Red (RedMaRo, N=48) from the Romanian population and 48 Mangalitza Red (RedMaHu), Blond (BloMaHu) and Swallow-belly (SwbMaHu) pigs with Hungarian origin.

DNA extraction was performed from blood samples collected on K3-EDTA tubes using the ReliaPrep™ Blood gDNA kit (Promega, USA). The amplification of MT-CYB gene was performed using the 2X GoTaq G2 kit (Promega, USA). The PCR reactions were set up at 25 µl final volume, with 10 pmol of specific primers [10] and 50 ng of genomic DNA. The thermal cycling profile was: 94 °C for 5 min; 35 cycles of 94°C for 1 min, 58 °C for 1 min, 72 °C for 1 min. The amplification specificity was assessed on agarose gel electrophoresis, containing 1X SybrSafe (Invitrogen, USA) and in 1X TBE buffer (Lonza, Belgium). The sequencing reactions was performed with the BigDye™ Terminator v3.1 Cycle Sequencing Kit and analysed on a SeqStudio (ThermoFisher Sci., USA).

In the final data set Vietnamese pigs (VieRo, N=10) and several MT-CYB sequences available in GenBank from Berkshire (Berk), Hampshire (Hamp), Yorkshire (York), Landrace (Land), Large White (LaW) and Meishan (Meish) pigs were included. The bioinformatics analysis of MT-CYB was performed with MEGA11 [11] and GenAlEx 6.5 software [12].

To evidence a possible autosomal signature in Bazna pigs of Mangalitza and Berkshire pigs, which might be consistent with the first crossings mentioned by historical records, we comparatively analysed an Illumina Porcine SNP60 BeadChip data set composed of BazRo (N=5), RedMaRo, (N=25), RedMaHu (N=20), BloMaHu (N=24) and SwbMaHu (N=25), Berkshire (N=25) and Chinese Meishan (N=32). Autosomal SNP data processing was performed using PLINK v. 1.07 [13], Admixture [14] and Structure [15].

RESULTS AND DISCUSSIONS

MT-CYB variation in Bazna pigs was surprisingly high, considering the size of the actual population (less than 400 pigs). Seven haplotypes were identified, which suggests a contribution of several maternal lineages to the current genetic background of Bazna pigs.

The phylogenetic tree, generated using representative MT-CYB haplotypes data, evidenced two major clusters *i.e.* European and Asian. A first observation indicates that the only MT-CYTB haplotype identified in Mangalitza pigs (RedMaRo) from Romania clustered in the European group, while those found in Vietnamese (VieRo) and Meishan (Meish) pigs clustered in the Asian group, in agreement with their genetic origin (Figure 2).

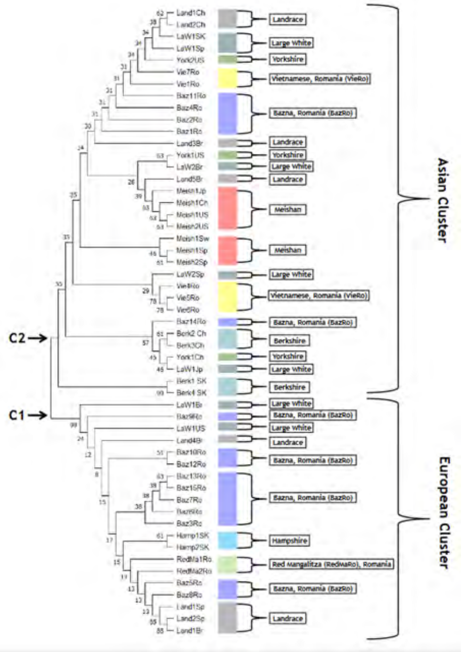

Fig. 2 Phylogenetic tree generated using MT-CYTB sequences evidencing the genetic relationships between Bazna pigs *versus* possible contributing breeds to its formation *i.e.* Mangalitza, traditional or commercial British and American breeds and Asian breeds

The British pig breeds *i.e.* Yorkshire (York), Large White (LaW), Landrace (Land), have clustered either in the European or Asian cluster. This observation is consistent with in the 18th-19th centuries gene flow from Chinese pigs, which are carriers of Asian MT-CYTB haplotypes [5, 6].

In the European cluster, some Bazna pigs MT-CYTB haplotypes grouped alongside some haplotypes identified in Hampshire (Ha), Large White (LaW) or Landrace (Land) pigs, while others in the Asian cluster alongside some identified in Berkshire (Berk), Yorkshire (York), Large White (LaW), Landrace (Land) pigs. The presence of both European and Asian mitochondrial haplotypes in the genetic background of Bazna pigs could probably be explained by the contribution of British breeds to its formation by admixture events, as suggested by historical records.

Some Bazna pigs clustered within the Mangalitza group, which is consistent to the maternal contribution of Mangalitza to its genetic background. The clustering of some Bazna pigs in the Asian cluster, close to some Vietnamese pigs, confirms the presence of Asian haplotypes to its genetic makeup, which were probably introgressed *via* contributing British breeds.

PCo Analysis was performed based on the entire MT-CYTB data set available (Figure 3).

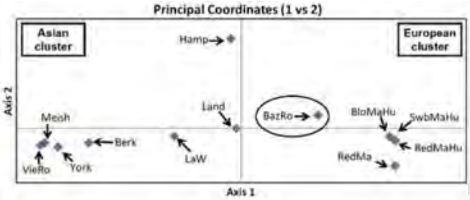

Fig. 3 PCo Analysis plots evidencing the genetic differentiation between Bazna *versus* other pig breeds

The results evidenced a clear differentiation between pig breeds with European genetic background, represented by Mangalitza (RedMaRo, BloMaHu, RedMaHu and SwbMaHu) and those with

Asian genetic background, represented by Vietnamese and Meishan pigs (Figure 3).

The Bazna pigs grouped in an intermediate position between Mangalitza pigs and Landrace or Large White breeds. This is consistent with a contribution of Mangalitza and British breeds gene pool to its genetic background. The clustering of Landrace, Large White, Yorkshire or Berkshire breeds closer or farther away from the European cluster is not unexpected since the majority of the available GenBank sequences used in this study are from China or US. It is likely possible that these populations are carrier of Asian pig haplotypes at higher frequencies, due to bidirectional importation mentioned before [5, 8].

The SNP genotyping data were used to evaluate the genetic relationships between Bazna pigs *versus* some presumed contributing breeds mentioned by historical records *i.e.* Mangalitza and Berkshire. To evaluate the presence of the Asian genetic component, we introduced in the data set some Meishan pigs (carriers of Asian autosomal signature).

The principal component analysis (PCA) visible in the 3D MDS plot (Figure 4) revealed the presence of several clusters formed by breeds respectively populations within the same breed (e.g. RedMaRo and RedMaHu). In Hungary, Mangalitza coloured varieties (Red, Blond and Swallow-belly) are considered distinct breeds [16]. A first observation indicates a clear differentiation of the Meishan breed, with Asian genetic background from the Mangalitza breed, with European genetic background. Within the Mangalitza breed, a clustering of individuals from the colour varieties into four subpopulations was observed, which signifies that they have some degree of differentiation (Figure 4).

Within the Mangalitza breed a certain degree of differentiation between the populations from Romania (RedMaRo) and Hungary (RedMaHu) was observed (Figure 4).

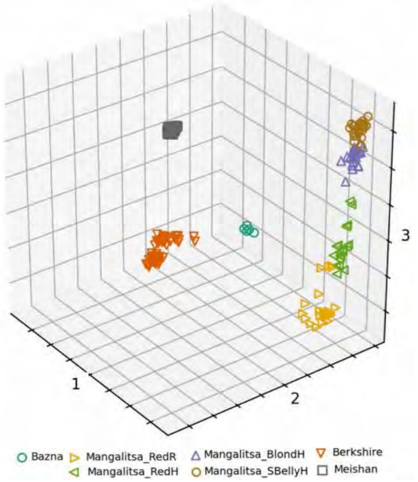

Fig. 4 Multidimensional scaling plot (MDS) generated using autosomal SNP data evidencing the genetic relationships between Bazna pigs *versus* presumed parental breeds (Mangalitza and Berkshire) and Chinese Meishan (Asian component)

This could be explained by the fact that there has been just a limited exchange of reproducers between these populations, which happened recently.

The most important observation is that the Bazna individuals have positioned between the Berkshire and Mangalitza breeds (RedMaRo and BloMaHu), at relatively equal distances. This could indicate a genetic contribution of both breeds to the formation of the Bazna breed.

The analysis of the genetic structure was performed at different values of K (Figure 5), in order to highlight the genetic components underlying the formation of the Bazna breed, in relation to the first possible contributing breeds (e.g. Mangalitza and Berkshire).

At K=2, two main clusters are observed: Asian cluster (dark blue), represented by the Meishan breed and European cluster (light blue), represented by Berkshire, Bazna and Mangalitza coloured varieties (Figure 5).

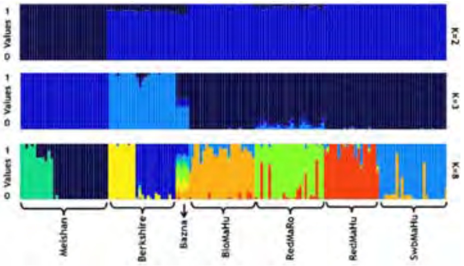

Fig. 5 Admixture analysis at different K values highlighting the genetic structure of the Bazna breed *versus* other possible contributing pig breeds to its formation (SNP data)

The K=2 results indicates that the Berkshire and Bazna breeds share a small proportion of Asian background with Meishan breed (dark blue). This is consistent with 18th-19th centuries introgression of British breeds (e.g. Berkshire) with Chinese pigs, which was subsequently spread to other European and American breeds [5, 7]. The presence of Asian pigs autosomal signature in Bazna pigs is expected, since Berkshire was probably a contributing breed to its genetic background. The presence Asian autosomal signature in Bazna pigs (Figure 5) is in agreement with the mitochondrial results, because several Asian maternal pig lineages were identified in Bazna pigs genetic background (Figure 2).

At K=3, the three breeds in the European cluster are divided into three subclusters, the first formed by the Berkshire breed (light blue), the second formed by the colour varieties of the Mangalitza breed (dark blue) and the third formed by the Bazna breed (mixed blue colours). The Asian Meishan breed grouped in a distinct cluster (blue), as at K=2. The analysed individuals within the Bazna breed have an admixed autosomal signature and share in a relatively equal ratio a common genetic background with the presumed parental breeds *i.e.* Mangalitza and Berkshire (Figure 5). The Asian genetic component (blue) in still visible in Bazna pigs at K=3.

At K=8, there is a marked differentiation of the four analysed pig breeds. At the same time both Meishan and Berkshire breeds split each into two different subpopulations. This might be explained probably by the fact that the genomic data used in this study come from different population that might had evolved differently (Figure 5).

The Mangalitza pigs (RedMaRo, RedMaHu, BloMaHu and SwbMaHu) split at K=8 in four distinct subpopulations (Figure 5), in agreement with the results highlighted in the PCA plot (Figure 4).

An important result, visible at K=8, refers to the admixed origin of the analysed individuals within the Bazna breed. These observations confirm the contribution of the parental breeds, Mangalitza (especially, RedMaRo and BloMaHu) and Berkshire, to the formation of the Bazna breed. The previously observed Asian genetic component (e.g. Meishan) is still visible.

The results obtained by the SNP autosomal data analysis are in agreement with MT-CYTB and support the contribution of the Mangalitza and British pigs gene pool (e.g. Berkshire) to Bazna pigs formation (at least in the analysed population), as it is mentioned by the historical records.

CONCLUSIONS

Comparative bioinformatic analyses of mitochondrial (MT-CYTB) and autosomal SNP genotyping data from Bazna pigs *versus* first presumed contributing breeds mentioned by historical records (Mangalitza and Berkshire), confirmed the contribution of both breeds to its current genetic background, in the analysed population. At the same time, the presence of Asian pigs mitochondrial (e.g. Vietnamese) and autosomal (e.g. Meishan) genetic signatures in Bazna pigs was noticed, which was probably introgressed *via* British breeds (e.g. Berkshire).

However, if we consider that historical records mention that the early populations originally formed in 1872 were later infused in different episodes with other British or German breeds to create the Bazna breed, it is very likely that its genetic background is much more complex. These aspects are investigated in on-going study aiming to have a broader understanding about the origin of Bazna pigs, which is essential for implementation of a reliable conservation - valorisation plan.

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