# 203. Is it possible to differentiate meat products of a local breed from those of its sister breed based on genotypes?

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## Abstract

To maintain endangered breeds and preserve their intrinsic diversity, it is often advocated to develop derived labelled products and the related certification process. However, this certification process is not always easily implemented if the endangered breed is closely related to another one. The breed traceability is only possible if the genetic diversity between those breeds is high enough. In this study, it was determined if meat of Dual-Purpose Blue (DPB) animals can be differentiated from meat of its sister breed, the Beef Belgian Blue (BBB), by the application of a genomic test. The results showed that the meat of DPB was completely distinguished from the meat of BBB, and this, with a probability of one for all meat samples. It therefore seemed possible to differentiate the DPB derived products from those of BBB with a high accuracy, meaning the implementation in routine of a certification process seemed possible.

## Introduction

The Dual-Purpose Blue (DPB) breed can be considered as the old type of the previously called Mid and Upper Belgium Breed, from which the well-known Beef Belgian Blue (BBB) also derived. Both branches (DPB and BBB) separated during the seventies (Mota *et al.*, 2017). However, from this point, the BBB gained popularity while the number of DPB decreased to the point it is now considered an endangered breed. The BlueSter project (BlueSter, 2021) aimed to maintain this breed by, among other measures, *in situ* conservation and the development of local products. The consumer confidence can be ensured through a certification process using a genomic breed assignment tool for breed derived-products (e.g. Judge *et al.*, 2017). However, implementing this kind of certification is not always easy if the endangered breed is closely related to another one, as it is in our case. The objective of this study was therefore to determine if it was possible to differentiate meat products of the endangered DPB breed from those of its mainstream sister breed, the BBB, by the use of a genomic breed assignment test previously developed.

## Materials & methods

All computations were run on R v.4.1.2 (R Core Team, Vienna, Austria, 2021) and visualized on R Studio (R Studio Team, Boston, USA, 2020).

**Breeds.** The two main breeds of interest in this study were the DPB, a transboundary dual-purpose breed of Southern Belgium and the North-East of France, and the BBB, a beef breed mostly located in Belgium. Whereas all BBB animals are homozygous for a partially recessive allele 'mh' resulting in muscular hypertrophy, a disparity is found in the DPB population where the wild type of the allele '+' is still existing (Mota *et al.*, 2017). In other words, depending on the selection objective of the breeder, DPB animals can be 'mh/mh' (in farms with a more meat-orientated breeding goal, mostly found in Belgium), 'mh/+' or '+/+' (in farms with a more dairy-orientated breeding goal, as most French breeders), and two or three genotypes can be coexisting in the same farm.

**Datasets.** Two different datasets were used in this study: the first one was used for the principal component analysis, aiming to determine if the breeds of interest can be differentiated, and the second one was used by the breed assignment model. For the principal component analysis, 60 DPB and 60 BBB, considered as the reference population of their respective breed, were studied. Both Belgian (n=52) and French animals (n=8) were represented in the DPB population. For the breed assignment model, a total of 32 samples of meat pieces, coming from three breeds, were genotyped with the EuroG MD v2 (Illumina, San Diego, CA, USA). The three breeds included were the BBB (n=8), the DPB (n=16) and the Holstein (HOL, n=8). The BBB animals were born in four different farms but all fattened at the same place. The DPB animals came from two different breeders in a balanced manner (n=8 for each breeder). One of the breeders was located in Belgium and only provided 'mh/mh' animals to be slaughtered. The other one was located in France and raised both 'mh/+' and '+/+' animals. It was thus considered that the DPB breed was well represented as animals from the three genotypes were sampled. The HOL animals were also raised by different breeders and were considered as a 'control' for the study.

**Principal component analysis.** The PCA was based on a total of 17,667 SNPs as in a previous study (Wilmot *et al.*, 2022) and on the first dataset. Missing values per individual were imputed following a procedure of the missMDA v1.18 R package (Josse and Husson, 2012), related to PCA, and described in Wilmot *et al.* (2022). The FactomineR v.2.4 R package (Lê *et al.*, 2008) was used to perform the PCA on the matrix of correlations.

**Breed assignment model.** The breed assignment model used in this study to determine the breed of origin of meat samples for each of the 32 slaughtered animals was previously described in Wilmot *et al.* (2021) as the second best model to be used for breed assignment. Because the second best model used less SNPs than the first one (2,005 vs 7,153), it was preferred for routine analyses. In brief, the chosen model was based on 2,005 SNPs and used the Nearest Shrunken Centroids method to classify each individual. Twelve breeds can be detected with this model of which the BBB, the DPB and the HOL. Each meat sample was assigned to the breed for which it had the highest probability to belong to.

## Results
**Principal component analysis.** The first two dimensions of the PCA are illustrated in Figure 1. There was a gap between animals from both breeds, even if one BBB animal was closely related to the DPB cluster. Therefore, it seemed possible to differentiate DPB from BBB. Moreover, as expected because of the different selection objectives and the three muscular genotypes existing in the DPB breed, there was a greater diversity in the DPB population compared to BBB animals i.e. DPB animals were more widespread than BBB animals in Figure 1.

**Breed assignment of meat samples.** A model based on 2,005 SNPs and on the Nearest Shrunken Centroids method was used to classify all the meat samples in the breed they were supposed to belong to. From the 2,005 SNPs, 167 were used to differentiate DPB from the other breeds existing in the reference population and 173 were used to differentiate BBB from the other reference breeds. An overlap of seven SNPs was observed between both SNP panels. The confusion matrix of the breed assignment test is available in Table 1. All meat samples were correctly attributed to their breed of origin, and this, with the maximum probability.
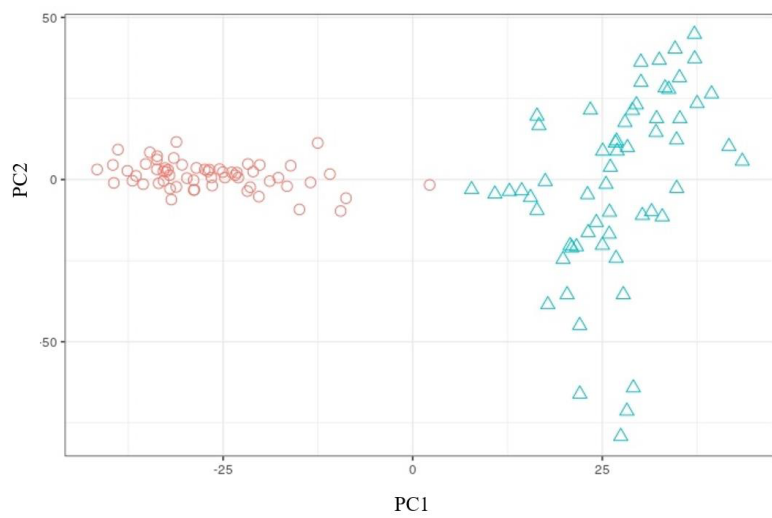
**Figure 1.** First two dimensions of the principal component analysis based on genotypes of reference Dual-Purpose Blue and Beef Belgian Blue. O = Beef Belgian Blue; Δ = Dual-Purpose Blue.

**Table 1.** Confusion matrix of meat samples based on the breed assignment test.

| Predicted breed | Breed of origin | | |
|---|---|---|---|
| | Beef Belgian Blue | Dual-Purpose Blue | Holstein |
| Beef Belgian Blue | 8 | 0 | 0 |
| Dual-Purpose Blue | 0 | 16 | 0 |
| Holstein | 0 | 0 | 8 |

## Discussion

The possibility to distinguish local meat products of an endangered breed, the DPB, from a mainstream sister breed, the BBB, based on genotypes, was studied. The breed assignment test allowed to perfectly differentiate the DPB meat samples from the BBB meat samples, with a probability of one for all meat samples, even those of DPB animals carrying the 'mh/mh' genotype, highlighting the high accuracy of the test.

This high accuracy can be partially explained by the fact that the SNPs used by the model were selected to differentiate each breed in the reference population from the others. Therefore, the panel comprised SNPs dedicated to differentiate DPB animals from BBB animals. It is then expected that significant phenotypic and genetic differences have arisen between both breeds since their divergence in the seventies, even for 'mh/mh' DPB animals. It has for example been suggested that the dairy performances were not necessarily inconsistent with the 'mh/mh' genotype as the more dairy DPB cows ('+/+') were producing on average 5,000 litres whereas the meat producing-DPB cows ('mh/mh') were still able to produce 4,000 litres (BlueSter, 2021). Moreover, it has been reported that, even when DPB animals carry the 'mh/mh' genotype, caesarean sections were more frequent in BBB than in DPB. It seemed that DPB breeders selected more for decreased birth weight and decreased calving difficulties, even within the 'mh/mh' animals, than BBB breeders (Mota *et al.*, 2017).

The fact that the breed assignment test can make the difference between genotypes of these two very closely related breeds, that diverged only from each other less than 50 years ago, is not trivial. It means that the genetic diversity between DPB and BBB was sufficiently high to differentiate these breeds. As a counter-example, the Angus and Red Angus cattle could not be effectively assigned to their respective breed in the study of Kuehn *et al.* (2011), even if both breeds started to separate much earlier (Márquez *et al.*, 2010) than DPB and BBB breeds. Besides, whereas the same three colour patterns (black-pied, blue-pied and white) are co-existing both in DPB and BBB, Angus and Red Angus clearly segregate from this point of view. However, the method chosen by Kuehn *et al.* (2011) was based on a regression of allelic frequencies and their objective was slightly different: determining breed composition instead of assigning animals to a breed.

Finally, the results showed that it would be possible to use the studied breed assignment test in routine as one step of a certification process of local products, especially if a SNP chip containing all the selected SNPs is used.

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