Understanding and utilizing genetic diversity in Dual-Purpose Blue: genome-wide association for type traits

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Abstract
Dual-Purpose Blue in Belgium and northern France is a local breed that is currently in the focus in order to understand its genetic distinctiveness and its use for local products. Its genomic evaluation systems are evolving, and its specific genetic distinctness was here studied for type traits. Results showed that throughout the 23 traits, there were two distinguishable genomic regions (BTA2 and BTA26) where BTA26 was associated with development traits and BTA2 was associated with muscularity traits, but also with associated traits as overall rump, udder depth, overall udder, and rear udder. Given these results future genomic evaluation systems should consider the segregating MSTN (myostatin), which is located on BTA2.

Introduction
Blue cattle in Belgium, but also in neighbouring northern France, originated from the crossbreeding between local dairy cattle and imported Durham (Shorthorn) bulls during the 1850-1880. Even if the presence of blue cattle in the low countries is attested by paintings from the 17th century, this crossbreeding mostly created the blue colour pattern. This pattern is a variant of the Shorthorn pattern where black replaces red leading to three colours: black (and white), white and blue roan. During the early 1900’s this breed evolved into a local dual-purpose breed. In Belgium, since the 1950’s, most breeders favoured the beefier type leading to the Beef Belgian Blue (BBB) breed. In France political circumstances let few farmers continue keeping blue cattle local known as the dual-purpose “Bleue du Nord”. For many years, French breeders have been joining forces with fellow dual-purpose breeders in the “Belgian Blue” Herdbook in Belgium who have recently changed the name of their Belgian Blue strain to “Dual Purpose Blue” (DPB), a name used hereafter for the whole population. Several projects have led to common genetic evaluations for several traits and a common understanding of the needs in this breed (BlueSter 2021). General efforts will be reported to exploit genomics as a tool and its use in the context of DPB cattle. The specific objective will be to illustrate this by estimating genomically enhanced estimated breeding values (GEBV) and by performing genome wide association studies (GWAS) for linear type traits.

Materials & Methods
DPB populations. The DPB are present in the southern Belgian (Walloon) part (~ 80%), as mentioned in France (~ 15%) and the remaining animals are in Flanders, around Brussels. This third population is not providing data to the joint DPB breeding program but selected young bulls are entering it, also based on genomic predictions. Flemish breeders can also access DPB AI sires. In total approximately 3,000 cows are (milk) performance recorded.
Genotyping. Extensive efforts are ongoing to genotype a large part of the currently active DPB population. This study, which used data available in May 2021, had access to 1,772 genotypes. The DPB population is rather unique as it has a tradition of testing for the MSTN (myostatin) mutation that is segregating in its population since it was discovered (Grobet et al. 1997). Also, given this fact, there is a large acceptance for genomic testing, it is expected that genotyping will cover nearly the whole population in a few years.

Performance recording and type data. Milk performance recording is well established, and individual data was available down to the 1970s in Wallonia. This is relevant as the DPB population was larger at that moment and semen of AI sires from this period is still being used. Recently, a transnational type recording program was initiated addressing the complexity of the needs for this dual-purpose breed. Therefore, the 23 traits scheme includes 18 individual traits: five udder traits, three muscularity traits, but also body development, including stature, rump, feet and legs and other traits and five additional overall traits that are scored and not computed from individual traits: udder, development, feet and legs, muscularity, and rump. These traits allowed the inclusion of less recent data from a simplified system previously used in France which handled only stature and these overall traits. Results reported in this study labelled udder, development, feet and legs, muscularity, and rump refer always to those 5 overall traits.

A total of 1,746 French and 10,701 Belgian records from 10,134 cows scored until the beginning of 2021 were included in this study. All traits are scored from 1 to 9 except stature which is measured. Normally, cows needed to be scored at least once in first or second lactation but could then be rescoring. For this reason, 50.7% and 37.6% of the records were scored in first or second lactation, respectively. The remaining were animals that were rescoring, mostly in the third lactation (10.0%), however late rescoring was also used because DPB breeding strives to obtain a high longevity.

Generating genomically enhanced estimated breeding values (GEBV). A transnational genomic evaluation system is currently under development for type traits. This system is based on two steps. In a first step, as there were missing traits, it was preferable to use a multiple-trait model allowing missing values. Moreover, tests showed that a full multiple-trait model had convergence issues. However, the use of a canonical transformation adopted for multiple diagonalization (Misztal et al. 1995) was able to handle easily the required 23 trait repeatability model. Associated (co)variance components were estimated as outlined by Misztal et al. (1995) on a subset with no missing values. These computations were based on 9,777 Walloon and 934 French records of 7,514 DPB cows. The used model was very close to the model used for Holstein cattle in the Walloon region of Belgium (Croquet et al. 2006) but without heterogeneous variance adjustments. The model had five fixed effects with a herd-year-month of classification x classifier-system contemporary group effect where classifier-system distinguished between classifiers working in the three recognized systems (Walloon, France-old and France-new). Also included were age and lactation stage effects distinguishing between fine overall effects and larger classifier-system specific effects. A direct implementation of a genomic single-step model in this setting was however not available. Therefore, in a second step, using estimated breeding values (EBV) and associated reliabilities (REL) generated in the first step, the Bayesian approach as proposed by Vandenplas et al. (2014) and used by Colinet et al. (2018), but only limited to local data, was used. Conceptually, this is equivalent to replacing the pedigree-based relationship matrix $A$ by a combined matrix $H$ transforming EBV into GEBV and REL into genomically enhanced REL (GREL) thus approximating closely an ssGBLUP.
**Genome wide association study (GWAS).**
The SNP effects were estimated individually for each of the 23 traits using the postGSf90 software (Aguilar et al. 2014). The vector animal effects (GEBV) was separated in genotyped ($a_g$) and ungenotyped animals ($a_u$). The GEBV of genotyped animals were considered being a function of the SNP effects, $a_g = Zu$, where $Z$ is a matrix relating genotypes of each locus to SNP marker effect and $u$ is a vector of the SNP marker effect. The variance of animal effects was assumed as: $\text{Var}(a_g) = \text{Var}(Zu) = ZDZ'\sigma_u^2 = G\sigma_a^2$ where $D$ is a diagonal matrix of weights for variances of markers ($D = I$), $\sigma_u^2$ is the genetic additive genetic variance captured by each SNP marker when the weighted relationship matrix ($G$) was built with equal weights. The SNP effects were obtained using the following equation: $\hat{u} = \lambda DZ'G^{-1}a_g = DZ'[ZDZ']^{-1}a_g$ where $\lambda$ was defined as a normalizing constant (VanRaden 2008) and the percentage of genetic variance explained by the $i$th genomic region was estimated as: $\left(\text{Var}(a_i)/\sigma_{a_i}^2\right)\times100 = \left(\text{Var}\left(\sum_{j=1}^{25} Z_j \hat{u}_j\right)/\sigma_{a_i}^2\right)\times100$ where $a_i$ is the genetic value of the $i$th region that consists of 25 adjacent SNPs, $\sigma_{a_i}^2$ is the total genetic variance, $Z_j$ is the vector of the SNP content of the $j$th SNP for all individuals, and $\hat{u}_j$ is the marker effect of the $j$th SNP within the $i$th region. The results were presented by the proportion of variance explained by each window of 25 adjacent SNPs and windows explaining for at least 1.0 % of the total additive genetic variance were identified.

**Results**

**Heritabilities, repeatabilities and correlations.** Heritabilities across all 23 traits ranged from 0.15 for foot angle to 0.74 for stature. Repeatability ranged from 0.28 for foot angle to a very high 0.84 for stature. Table 1 shows heritabilities and correlations among the 5 overall traits.

**Table 1. Genetic (above diagonal) and phenotypic (below diagonal) correlations, and heritabilities (diagonal) for overall traits.**

<table>
<thead>
<tr>
<th></th>
<th>Udder</th>
<th>Development</th>
<th>Feet and Legs</th>
<th>Muscularity</th>
<th>Rump</th>
</tr>
</thead>
<tbody>
<tr>
<td>Udder</td>
<td>0.37</td>
<td>0.15</td>
<td>0.10</td>
<td>-0.66</td>
<td>0.48</td>
</tr>
<tr>
<td>Develop</td>
<td>0.08</td>
<td>0.63</td>
<td>0.26</td>
<td>-0.19</td>
<td>0.47</td>
</tr>
<tr>
<td>Feet and Legs</td>
<td>0.10</td>
<td>0.15</td>
<td>0.19</td>
<td>0.00</td>
<td>0.12</td>
</tr>
<tr>
<td>Muscularity</td>
<td>-0.36</td>
<td>-0.08</td>
<td>0.01</td>
<td>0.60</td>
<td>-0.65</td>
</tr>
<tr>
<td>Rump</td>
<td>0.28</td>
<td>0.28</td>
<td>0.06</td>
<td>-0.39</td>
<td>0.42</td>
</tr>
</tbody>
</table>

**Figure 1.** Additive genetic variance explained by windows of 25 adjacent SNP across chromosomes for the 4 overall traits udder, development, muscularity, and rump in Dual-Purpose Blue cows.
**Genome wide association studies (GWAS).** Throughout the 23 traits, there were two important genomic regions. The genomic region located between 20.8 to 22.9 Mb on BTA26 was associated with traits including chest width, overall development (Figure 1), and stature. A region located between 5.3 to 7.3 Mb on BTA2 was associated with muscularity traits, but also with overall rump, overall udder (Figure 1), udder depth, and rear udder.

**Discussion**

**Heritabilities, repeatabilities and correlations.** Heritabilities were, as expected, with a tendency on the upper bound compared to those reported in literature. In general, repeatability tended to be high too. On a genetic level, an expected strong opposition between udder and muscularity appeared clearly, underlying the importance to include both in an adapted breeding objective for this dual-purpose breed.

**Genome wide association studies (GWAS).** At least one study in a completely different breed (Holstein) has associated BTA26 with an overall development trait (body form composite index) (Ashwell *et al.* 2005). Previous studies showed that SNPs inside the region on BTA2 position 5.3 to 7.3 Mb in various breeds of dairy and beef cattle were linked to mutations in MSTN (myostatin), the cause of the double-muscled phenotype in cattle, in general considered as the most important functional gene in the region found on BTA2. A visible distinct muscular hypertrophy (mh) occurs with high frequency in the DPB breed, linked to a specific deletion (Grobet *et al.* 1997), without being homozygous as in BBB. As explained previously, a large part of the DPB population is genotyped for this mutation. Given the large variance on BTA2, strategies may be useful to include mh in the future genomic evaluation system as already proposed for calving difficulty and associated traits (Mota *et al.* 2017).

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**References**


