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## Use of meta-analyses and joint analyses to select variants in whole genome sequences for genomic evaluation: An application in milk production of French dairy cattle breeds

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### ABSTRACT

As a result of the 1000 Bull Genome Project, it has become possible to impute millions of variants, with many of these potentially causative for traits of interest, for thousands of animals that have been genotyped with medium-density chips. This enormous source of data opens up very interesting possibilities for the inclusion of these variants in genomic evaluations. However, for computational reasons, it is not possible to include all variants in genomic evaluation procedures. One potential approach could be to select the most relevant variants based on the results of genome-wide association studies (GWAS); however, the identification of causative mutations is still difficult with this method, partly because of weak imputation accuracy for rare variants. To address this problem, this study assesses the ability of different approaches based on multi-breed GWAS (joint and meta-analyses) to identify single-nucleotide polymorphisms (SNP) for use in genomic evaluation in the 3 main French dairy cattle breeds. A total of 6,262 Holstein bulls, 2,434 Montbéliarde bulls, and 2,175 Normande bulls with daughter yield deviations for 5 milk production traits were imputed for 27 million variants. Within-breed and joint (including all 3 breeds) GWAS were performed and 3 models of meta-analysis were tested: fixed effect, random effect, and Z-score. Comparison of the results of within- and multi-breed GWAS showed that most of the quantitative trait loci identified using within-breed approaches were also found with multi-breed methods. However, the most significant variants identified in each region differed depending on the method used. To determine which approach highlighted the most predictive SNP for each trait, we used a marker-assisted best unbiased linear prediction model to evaluate lists of SNP generated by

the different GWAS methods; each list contained between 25 and 2,000 candidate variants per trait, which were identified using a single within- or multi-breed GWAS approach. Among all the multi-breed methods tested in this study, variant selection based on meta-analysis (fixed effect) resulted in the most-accurate genomic evaluation (+1 to +3 points compared with other multi-breed approaches). However, the accuracies of genomic evaluation were always better when variants were selected using the results of within-breed GWAS. As has generally been found in studies of quantitative trait loci, these results suggest that part of the genetic variance of milk production traits is breed specific in Holstein, Montbéliarde, and Normande cattle.

**Key words:** multi-breed genomic evaluation, meta-analysis, sequence, quantitative trait locus detection

### INTRODUCTION

Around the world, the majority of routinely used procedures for genomic evaluation are based on chips containing tens of thousands of SNP. Several methodologies have been developed for genomic evaluation, of which the most commonly used are genomic BLUP (Meuwissen et al., 2001), which assumes that all SNP have a small effect on the trait, and BayesC (Kizilkaya et al., 2010) and BayesC $\pi$  (Habier et al., 2011), which assume a proportion ( $\pi$ ) of SNP have zero effect. Those methodologies do not take into account prior biological knowledge of traits, and estimate the effects of causative mutations only through SNP in linkage disequilibrium (LD) with them. Therefore, one way to improve the accuracy of genomic evaluations is through the identification and localization of causative mutations, which can then be directly included in the evaluation model. Unfortunately, extending genomic evaluation to the whole genome is not realistic due to the computational challenges involved, and selecting a reduced panel of SNP to include in the genomic evaluation is a very difficult challenge (VanRaden et al., 2017).

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Genome-wide association studies (**GWAS**) are widely used to study the genetic architecture of complex traits (Shi et al., 2012; Karlsson et al., 2013). They aim to screen the genome and detect associations between SNP and a disease or a QTL. However, the regions detected generally have large confidence intervals and contain many candidate genes, which makes it challenging to identify the causative mutation itself. As an example, despite the fact that a large number of regions have been associated with traits of economic importance in dairy cattle at the 50k or HD (800k) SNP genomic densities (Pryce et al., 2010; Meredith et al., 2012), very few causative mutations have thus far been validated.

As causative mutations are (generally) not included on SNP chips, GWAS highlight SNP in LD with a causative mutation, rather than the mutation itself. Instead, more accurate GWAS results can be obtained through the use of whole-genome sequences (**WGS**), because this approach enables the inclusion of millions of variants, including causative mutations. Toward this end, the 1000 Bull Genome Consortium aims to produce a large data set of sequenced animals (Daetwyler et al., 2014). For example, Run 4 contained WGS data of 1,147 bulls from 36 different breeds. This population is large enough to enable the imputation of WGS of all animals for which genotypes (e.g., 50k SNP) are available, which means that GWAS can be performed at the sequence level for all animals having both genotypes and phenotypes. However, the identification of causative mutations is still very difficult in a within-breed analysis, for 2 reasons: (1) the resolution is limited by the high level of LD between SNP in dairy cattle breeds, which leads to positive signals of association over large regions, and (2) high error rates of imputation have been observed for rare variants, which has led to false signals of association. Instead, an analysis that includes data from different breeds, each with its own pattern of LD, should address these shortcomings in 2 ways: first, it increases the population size with respect to a single-breed analysis, and second, it refines the locations of QTL shared across breeds (Raven et al., 2014). In addition, a false positive signal of association is unlikely to be present in multiple separate breeds. Furthermore, in a multi-breed (**MB**) approach, the long-range LD is expected to be lower than in a within-breed approach, and consequently, it should be possible to identify causative mutations with more accuracy.

A MB analysis can be performed 2 different ways: a MB GWAS on a joint data set, or a meta-analysis of results from multiple within-breed GWAS. Joint analyses are expected to yield the best resolution, as they minimize the effects of the long-range LD present within dairy cattle breeds. However, MB GWAS are

time consuming, and meta-analyses are a faster alternative. To this end, several methods of meta-analysis have been developed (e.g., fixed effect, random effect, or Z-score; Evangelou and Ioannidis, 2013) and have been used in dairy cattle (Buitenhuis et al., 2016; van den Berg et al., 2016).

In this study, we compared the ability of within-breed GWAS and MB analyses to detect QTL for milk production and milk composition in French Holstein (**HO**), Montbéliarde (**MO**), and Normande (**NO**) cattle. In the absence of functional analysis, it was not possible to know if a given method was able to highlight a causal mutation. However, by using genomic evaluation, we were able to measure the ability of a given approach to predict a phenotype. To this end, the lists of most significant QTL generated by the different methods were analyzed using marker-assisted best linear unbiased prediction (**MABLUP**).

## MATERIALS AND METHODS

### *Samples and Genotypes*

Reference populations for the association studies and genomic evaluations consisted of genotyped or sequenced bulls from the 3 main French dairy cattle breeds: HO, MO, and NO. The study population included 6,262 HO, 2,434 MO, and 2,175 NO bulls. For genomic evaluations, reference populations were split into 2 groups: (1) a training set containing 5,107 HO, 1,948 MO, and 1,740 NO bulls, in which performance, pedigrees, and genotypes were recorded and used to establish prediction equations, and (2) a validation set comprising younger bulls (1,155 HO, 486 MO, and 435 NO) from which only pedigrees and genotypes were known. To use the lists of QTL from GWAS in genomic evaluation, animals from the validation population were excluded from GWAS.

Five routinely collected production traits were analyzed: milk production (**MLK**), fat yield (**FY**), protein yield (**PY**), fat content (**FC**), and protein content (**PC**). For all traits, the phenotypes used in analyses were the daughter yield deviations (**DYD**) of each bull (VanRaden and Wiggans, 1991), defined as the average value of daughters' performance, adjusted for fixed and nongenetic random effects and for the additive genetic value of their respective dams. Each DYD was weighted by the effective daughter contribution (VanRaden and Wiggans, 1991). To limit the influence of bulls with higher numbers of daughters, weights were bound to a maximal number of daughters corresponding to a reliability of 0.9.

Bulls were genotyped at different densities. Key ancestors (i.e., bulls responsible for a considerable part

of the diversity of the different breeds) were genotyped at the high-density level (777k SNP, Illumina Bovine HD Beadchip, Illumina, San Diego, CA) or at the sequence level (1,000 Bull Genome Project, RUN4). All other bulls were genotyped at the 50k level (Illumina Bovine SNP50 BeadChip; Table 1). Imputations were performed with FIMPUTE software, which accurately and quickly analyzes large data sets (Sargolzaei et al., 2014). A 2-step imputation process was performed, from 54k SNP to 777k SNP and then from 777k SNP to sequence data, as recommended by van Binsbergen et al. (2014) to obtain accurate imputation. Following imputation, genotypic concordance rates were 89.7 and 93.7% in MO and HO, respectively (Sanchez et al., 2017). In the first step, the distance between SNP was not short enough to exploit LD at the population level (Berry et al., 2014). Therefore, imputations were performed independently in each breed, using the within-breed (**WB**) reference populations, which consisted of all animals genotyped at the 777k SNP level. For the next step (imputation from 777k to sequence level), 2 reports in the literature reported higher accuracy using a MB reference population (Bouwman and Veerkamp, 2014; Brøndum et al., 2014). Therefore, imputation at the whole genome sequence level was performed independently in each breed using all bulls from RUN4 of the 1000 Bull Genome Project as the reference population. Then, a quality control procedure based on minor allele frequencies (**MAF**) was performed to select the SNP to be used in different GWAS. Only SNP with a  $MAF > 0.01$  were kept for the WB GWAS. For joint analyses, we also retained only SNP with  $MAF > 0.01$ , but the MAF values were computed using data from the whole MB population. Meta-analyses were based on results from WB GWAS, and therefore contained only SNP shared among the 3 WB analyses. Table 2 summarizes the number of SNP used in each analysis.

### Within-Breed and Multi-Breed GWAS Approaches

A WB GWAS was performed for each of our study breeds; these were denoted as WB HO, WB MO,

and WB NO, respectively. Four different MB GWAS methods were used: a meta-analysis with fixed effects, denoted as fixed; a meta-analysis with random effects, denoted as random; a meta-analysis using the Z-score algorithm, denoted as Z-score; and a GWAS in which all HO, MO, and NO animals were included together, denoted as joint. All 4 methods are detailed in the next section.

**Within-Breed GWAS.** The GWAS were conducted independently in each breed (HO, MO, or NO) using imputed genotypes, pedigrees, DYD, and effective daughter contribution. The FASTA model (Chen and Abecasis, 2007), implemented in Wombat software (Meyer and Tier, 2012), estimated variance components with a polygenic model [1]. It is defined as

$$\mathbf{y} = \mu + \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}, \quad [1]$$

where  $\mathbf{y}$  is the vector of DYD,  $\mu$  the overall mean,  $\boldsymbol{\beta}$  is a vector of SNP effects, and  $\mathbf{u} \sim N(\mathbf{0}, \mathbf{A}\sigma_u^2)$  is a vector of random animal polygenic effects estimated using the  $\mathbf{A}$  relationship matrix calculated from the pedigree data and with  $\sigma_u$  representing genetic variance.  $\mathbf{X}$  and  $\mathbf{Z}$  are design matrices allocating DYD to SNP effects and animal polygenic effects, respectively, and  $\mathbf{e}$  is the vector of random residuals, normally distributed.

**Meta-Analyses.** The results of WB GWAS (HO, MO, and NO) were combined in meta-analyses. The fixed and random approaches were implemented in PLINK software (Purcell et al., 2007), and the Z-score approach was implemented in METAL software (Willer et al., 2010).

The fixed and random approaches estimate the overall SNP effect ( $\boldsymbol{\beta}$ ) by calculating a weighted mean. Weights are based on the inverse of the variance to assign more weight to breeds carrying more information.

In the fixed approach, the true  $\boldsymbol{\beta}$  is the same in all breeds. Therefore, the variance observed is only due to

**Table 1.** Number of bull genotypes available for the 3 main French dairy cattle breeds<sup>1</sup>

Breed	54k SNP	777k SNP	Sequenced	Total
Holstein	6,262	1,030	288	6,262
Montbéliarde	2,434	549	28	2,434
Normande	2,175	552	24	2,175

<sup>1</sup>Holstein, Montbéliarde, and Normande animals were sequenced or genotyped with 54k or 777k SNP chips.

**Table 2.** Number of SNP remaining after quality control filtering based on minor allele frequency for the different analyses: the 3 within-breed GWAS, the joint analysis, and the 3 meta-analyses (fixed effect, random effect, and Z-score)

Analysis	Number of SNP
Within-breed Holstein	12,315,091
Within-breed Montbéliarde	12,715,670
Within-breed Normande	12,436,528
Joint analysis	14,393,740
Meta-analysis: fixed effect approach	9,656,270
Meta-analysis: random effect approach	9,656,270
Meta-analysis: Z-score approach	9,656,270

WB random error. The weight for each breed  $i$  (for  $i = 1$  to  $k$ ) is calculated as

$$w_i = \frac{1}{\text{SE}_i^2}, \quad [2]$$

where  $w_i$  is weight for breed  $i$  and  $\text{SE}_i$  is standard error for breed  $i$ .

Then, overall  $\beta$  and  $\text{SE}$  are computed as

$$\beta = \frac{\sum_{i=1}^k w_i \times \beta_i}{\sum_{i=1}^k w_i}, \quad [3]$$

$$\text{SE} = \sqrt{\frac{1}{\sum_{i=1}^k w_i}}, \quad [4]$$

where  $k$  is the number of breeds and  $\beta_i$  is the SNP effect for the  $i$ th breed.

In the random approach, the true  $\beta$  may differ among breeds. Two components of variation are taken into account to calculate the weight of each breed: the first due to WB random error, as in the fixed approach, whereas the second component is random variation arising from the assumption that overall  $\beta$  values are sampled from a population of  $\beta$  (between-breed random error). The weight for each breed  $i$  (for  $i = 1$  to  $k$ ) is calculated as

$$\alpha_i = \frac{1}{\text{SE}_i^2 + \tau^2}, \quad [5]$$

$$\text{with } \tau^2 = \frac{[Q - (k - 1)]}{\left[ \sum_{i=1}^k w_i - \frac{\left( \sum_{i=1}^k w_i^2 \right)}{\left( \sum_{i=1}^k w_i \right)} \right]}, \quad [6]$$

$$\text{and } Q = \sum_{i=1}^k w_i \times (\beta_i - \beta), \quad [7]$$

where  $\alpha_i$  is the weight defined in the random approach for the  $i$ th breed,  $w_i$  is the weight defined in the fixed approach for the  $i$ th breed,  $k$  is the number of breeds, and  $\beta$  is the estimation of SNP effect in the fixed approach. Then, overall  $\beta$  and  $\text{SE}$  are computed as

$$\beta = \frac{\sum_{i=1}^k \alpha_i \times \beta_i}{\sum_{i=1}^k \alpha_i}, \quad [8]$$

$$\text{SE} = \sqrt{\frac{1}{\sum_{i=1}^k \alpha_i}}. \quad [9]$$

Finally, the statistical test  $t_{n-1,1} = \beta_s / \text{SE}_s$  was computed, with  $\beta_s$  and  $\text{SE}_s$  the SNP effect and the standard error for the SNP. Associated  $P$ -values were obtained using a Student's test with  $(n - 1)$  degrees of freedom ( $n$  being the number of animals in the analysis).

In contrast with these 2 methods, the Z-score approach did not use the estimation of  $\beta$ , but rather a nominal  $P$ -value ( $p_i$ ) for the  $i$ th breed and effect direction of  $\beta$  ( $\Delta_i$ ) to calculate an overall  $P$ -value using the following statistical test:

$$Z = \frac{\sum_{i=1}^k Z_i \times \delta_i}{\sqrt{\sum_{i=1}^k \delta_i^2}}, \quad [10]$$

with  $Z_i = \phi^{-1} \left( \frac{p_i}{2} \right) \times \Delta_i$ ;  $\delta_i = \sqrt{n_i}$  where  $\phi^{-1}$  is the inverse of the normal cumulative distribution function and  $n_i$  is the number of animals in breed  $i$ .

**Joint Analyses.** In the joint analysis, pedigrees, genotypes, and phenotypes of HO, MO, and NO bulls were merged into a single data set to be jointly analyzed. The model was similar to the previous one [1], except that it included a breed-specific fixed effect:

$$\mathbf{y} = \mathbf{W}\mathbf{a} + \mathbf{X}\beta + \mathbf{Z}\mathbf{u} + \mathbf{e}, \quad [11]$$

where  $\mathbf{a}$  is the vector of the breed effects (3 levels) and  $\mathbf{W}$  is the design matrix allocating phenotypes to breed effects. The FASTA model [11] assumed that genetic and residual variances are common to the 3 breeds for each of the considered traits. This assumption is possible because, for each trait, genetic and residual variances have the same range of values in the 3 breeds (same heritability).

### QTL Detection

Association tests were performed on SNP recovered with the different approaches (fixed, random, and Z-score for each breed, together with the joint analysis) for each trait, and we then evaluated the results to compare the number of QTL detected.

A SNP was considered to be significantly associated with a trait if its  $-\log_{10}(P\text{-value})$  was higher than a significance threshold. Thresholds were fixed at 9, which corresponded to a Bonferroni correction with a type 1 error rate of 5%. At sequence-level density, neighboring

SNP are in strong LD. The QTL were thus defined as the most significant SNP in a sliding window of 1 Mb, an approach referred to hereafter as QTL exploration by sliding window (QES). The number and locations of detected QTL were compared between WB and MB approaches.

### Post-GWAS Genomic Predictions (MABLUP)

To assess the ability of the 4 different MB GWAS to pinpoint QTL, lists of QTL detected by the 4 different MB GWAS were tested using a MABLUP model (Fernando and Grossman, 1989; Boichard et al., 2012). Each MABLUP was performed independently in each breed and with different lists of QTL, obtained from either WB or MB GWAS. The following MABLUP model was used:

$$\mathbf{y} = \mu + \sum_{j=1}^n \mathbf{X}_j \beta_j + \mathbf{Z}\mathbf{u} + \mathbf{e}, \quad [12]$$

where  $\mathbf{y}$  is the vector of the bulls' DYD,  $\mu$  is the overall mean,  $\sum_{j=1}^n \mathbf{X}_j \beta_j$  is the sum of QTL effects considered in the model,  $\mathbf{u}$  is a random vector of animal polygenic effects [distributed as  $\mathbf{u} \sim N(0, \mathbf{A}\sigma_u^2)$ ], and  $\mathbf{e}$  is a vector of random residuals, normally distributed, and  $\sigma_u$  the genetic variance. The  $\mathbf{A}$  matrix was the relationship matrix calculated from the pedigrees.

**Lists of QTL.** The SNP included in the model [12] were selected from the results obtained with the different GWAS. SNP were defined using the QES method applied without any constraint on the  $-\log(P\text{-value})$ . For each trait, the 25, 50, 100, 250, 500, 1,000, and 2,000 most significant SNP were tested (the scenario with 2,000 QTL corresponds to a scenario with approximately one QTL per Mb all over the genome). For some traits, despite our constraint of only one QTL in a 1-Mb interval, all the QTL on the list were located in a limited number of chromosomal segments.

To avoid this problem, we tested another list of SNP that were more homogeneously distributed along the whole genome: 17 SNP on each chromosome, for a total of 493 SNP.

**Accuracies of Genomic Evaluation.** The QTL effects were estimated from bulls of the training populations to predict the genomic estimated breeding values (GEBV) of young bulls in the validation set. The accuracy of genomic evaluation was assessed as the Pearson correlation between predicted DYD (GEBV) and true DYD in the validation population.

**Locations of QTL Selected for Genomic Evaluations.** The locations of QTL included in the genomic evaluation model were also compared based on the lists of QTL used (WB- or MB-meta or joint GWAS analyses). Pairwise comparisons were carried out and QTL were classified in 3 categories: (1) common QTL, which were identical and detected by both methods; (2) neighboring QTL, which were located in the same 100-kb interval in both methods; and (3) different QTL, which were present in one list but with no common or neighboring QTL in the second list. Sargolzaei et al. (2008) observed high LD values for SNP located within 100 kb of each other. Consequently, SNP detected in this interval have a high probability of being in linkage disequilibrium with the causal mutation.

## RESULTS

The results of all WB and MB GWAS analyses were first compared with regard to the number of QTL detected, QTL locations, and the accuracies of genomic predictions.

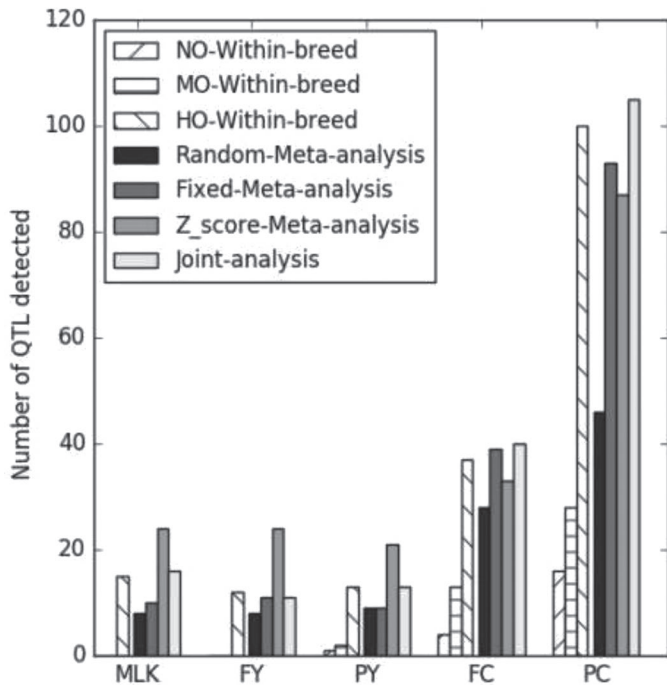
### Number of QTL Detected

The number of QTL detected for each trait varied according to the GWAS method used (Figure 1).

In WB GWAS analyses, regardless of the trait analyzed, a larger number of QTL was detected in HO than in MO or NO bulls. In the 3 breeds, the largest number of QTL was found for the PC trait, with 100 QTL in HO compared with only 28 and 16 in MO and NO, respectively.

The number of QTL detected differed also depending on the MB GWAS analysis used. For yield traits (MLK, FY, and PY), the average number of QTL detected per trait was similar between the random (8 QTL) and the fixed (10 QTL) analyses. The numbers of QTL recovered by the joint and Z-score analyses were higher, 13 and 23 QTL, respectively. A different pattern was found with content traits (FC and PC), for which a lower number of QTL was found with the random MB method (28 and 46 QTL for FC and PC, respectively) than with the other 3 MB methods (all more than 33 and 87 QTL for FC and PC, respectively).

Regardless of the trait analyzed, MB analyses detected a higher number of QTL than WB analyses did. However, depending on the nature of the trait under investigation, the optimal MB method, which maximized the number of QTL detected, was different. For yield traits, the highest numbers of QTL were found with the Z-score method, whereas for content traits, the joint analysis performed better. In addition, differences



**Figure 1.** Number of QTL detected by each analysis [within-breed genome-wide association studies (GWAS), 3 types of meta-analysis, or joint analysis]. Analyzed traits are milk production (MLK), fat yield (FY), protein yield (PY), fat content (FC), and protein content (PC). Traits were first analyzed using within-breed GWAS in 3 separate data sets representing Normande (NO), Montbéliarde (MO), or Holstein (HO) bulls. Results of the 3 within-breed GWAS were combined to perform meta-analyses based on 1 of 3 models (fixed, random, or Z-score). Joint analysis was performed considering all animals together.

between WB and MB analyses were greater for yield traits than for content traits.

### QTL Locations

We then compared the locations of QTL detected by within- and MB GWAS for the 5 production traits. The comparisons for all traits are presented in Supplemental Figures S1 to S5 (<https://doi.org/10.3168/jds.2017-13587>). Figure 2 focuses on the PC trait, which had the highest number of QTL among the studied traits.

For this trait, QTL detected in HO were located on chromosomes 3, 5, 6, 14, 15, 20, and 29. Of these, only one QTL was shared among all 3 breeds (on chromosome 6), while another was shared between HO and NO (on chromosome 5). An additional breed-specific QTL was observed in MO on chromosome 21. All the QTL detected by the WB GWAS, with the exception of those located on chromosomes 15 and 29 in HO, were also found with at least one MB GWAS method. Furthermore, all the chromosomal regions identified with meta-analyses were also found with joint analyses,

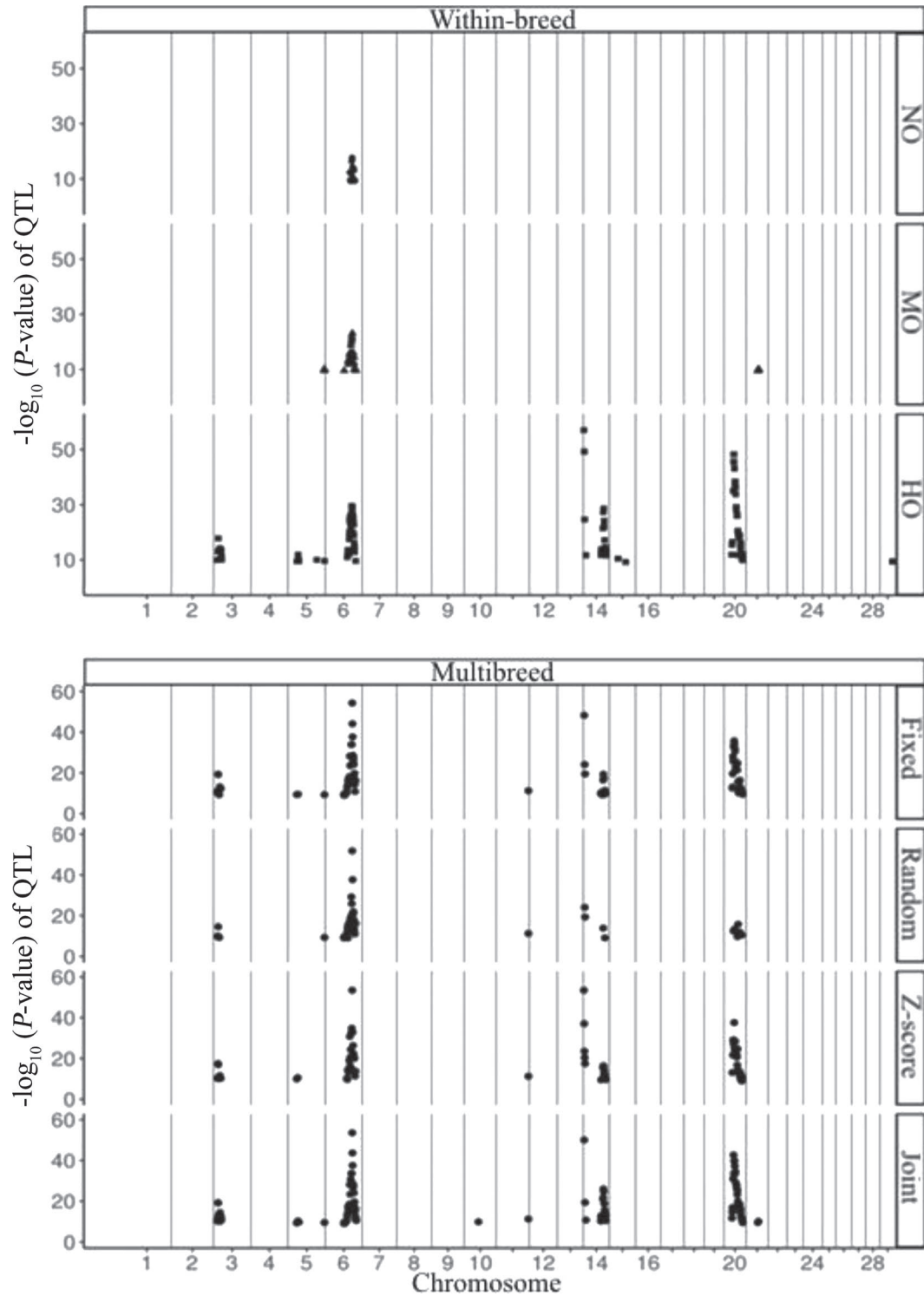
but joint analyses revealed 2 additional QTL, located on chromosomes 10 and 21, that were not detected in meta-analyses. Finally, all 4 MB analyses detected a QTL, located at the end of chromosome 11, that was not found in any WB GWAS analysis.

### Accuracies of Genomic Predictions

Genomic estimated breeding values were computed within each breed using lists that contained between 25 and 2,000 variants, which were selected from each breed's respective WB GWAS and the 4-MB analyses of the training population. The lists of variants selected from the HO WB GWAS were also used to estimate GEBV in the NO and MO data sets. Accuracies of GEBV were estimated for the 5 production traits by calculating correlations between GEBV and DYD in the validation population. Because similar tendencies were observed for the 5 traits (Supplemental Figure S6; <https://doi.org/10.3168/jds.2017-13587>), mean accuracies of GEBV were presented in Figure 3.

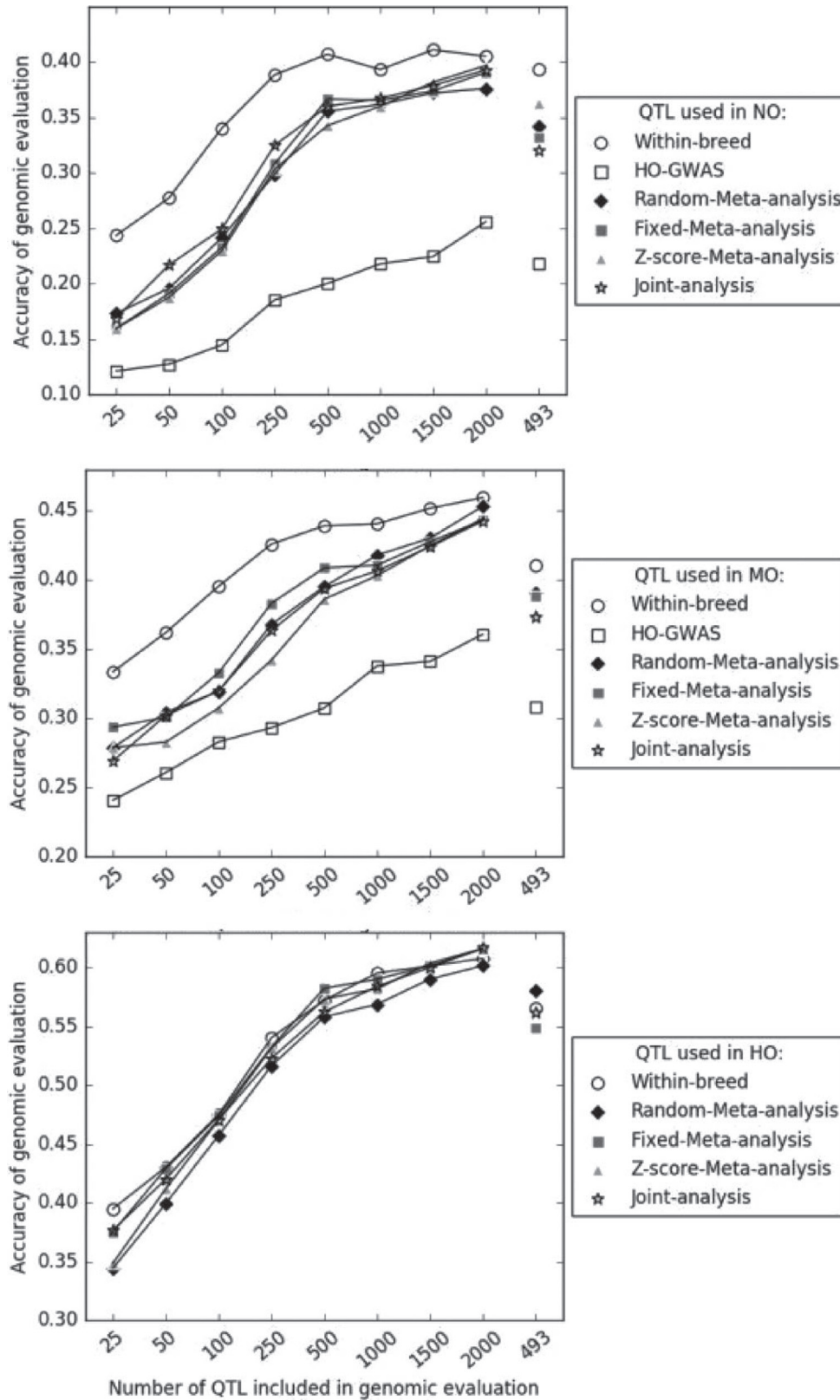
In NO and MO, regardless of the number of QTL selected from GWAS results or their distribution on the genome, correlations between GEBV and DYD were, on average, higher when we used variants selected from the corresponding WB GWAS than when we used variants found with MB approaches (+3 to +8 points in MO and +3 to +11 points in NO). In HO, the accuracies of GEBV were higher when they were based on the lists of variants from the HO GWAS and MB GWAS; in addition, values for this breed were more accurate than those found in NO or MO. The accuracies of GEBV resulting from the HO GWAS differed from those of the 4 MB GWAS by -1 to +5 points. Depending both on the breed and on the number of variants selected from GWAS, the accuracies of GEBV based on MB GWAS results varied. In NO, the accuracy of genomic predictions was dependent on the number of variants included in the model. For example, when the model included 25 variants, all methods yielded GEBV of similar accuracy (ranging from 0.16 to 0.17). The GEBV were more accurate in joint analyses that included 50 (0.22), 100 (0.25), 250 (0.32), or 1,000 (0.37) variants; together, they were +1 to +3 points more accurate than other MB analyses. With 500 variants, the Z-score method led to less accurate predictions than other MB methods did (0.34); for all other Z-score models, though, accuracies ranged between 0.36 and 0.37. Of all random models, those containing 1,500 (0.37) and 2,000 (0.38) variants were less accurate.

In analyses of the MO data set, the fixed method outperformed other MB methods when 25, 100, 250, or 500 variants were present in the models (+1 to +2 points more accurate). Instead, the Z-score approach



**Figure 2.** Top panel: The locations of QTL associated with milk protein content along the whole genome, according to within-breed analyses of Normande (NO), Montbeliarde (MO), and Holstein (HO) data sets. Bottom panel: QTL locations as detected by a fixed-effect model, random-effect model, or Z-score model of meta-analysis and multi-breed joint analysis.





**Figure 3.** Mean accuracy over the 5 traits of genomic evaluation in Normande (NO), Montbeliarde (MO), and Holstein (HO), based on lists of SNP used to estimate genomic estimated breeding values of young animals. These SNP were selected from the results of within-breed genome-wide association studies (GWAS), joint GWAS, or meta-analysis GWAS (fixed effect, random effect, or Z-score).

was generally the least accurate MB method when 500 or fewer variants were present in the model (−1 to −4 points compared with other MB methods); the only exception was for the models containing 25 variants (0.29 for fixed, 0.28 for random, 0.28 for Z-score, and 0.27 for joint). The fixed and joint models yielded similar results when they contained 1,000 (0.41), 1,500 (0.42), or 2,000 (0.44) variants, but the random approach was more accurate than either of these when models contained 1,000 (0.42), 1,500 (0.43), and 2,000 (0.45) variants.

The accuracies of genomic predictions generated from HO data were always higher than those obtained using the MO or NO data sets for a given number of variants, and all GWAS methods produced similar results. Regardless of the number of variants included, the least accurate models were those that used the random MB method. The fixed MB GWAS was the most predictive MB method and even slightly outperformed the HO WB GWAS when 500 (0.58 versus 0.56) variants were considered. Other MB methods yielded different results depending on the number of variants selected. The joint analysis was more accurate than Z-score for models containing 25 (0.38 versus 0.35) or 50 (0.42 versus 0.41) variants. However, the 2 methods yielded similar results using lists of 1,000 (0.58), 1,500 (0.60), or 2,000 (0.61) variants, and Z-score was more accurate than the joint approach when 250 (0.53 vs. 0.52) or 500 (0.57 vs. 0.56) variants were included.

When we specifically selected 493 variants to be more evenly distributed across the genome, we found similar results to those previously obtained with lists of 500 variants. In NO, joint GWAS led to more accurate genomic predictions (0.36) than the fixed (0.34), random (0.33), or Z-score (0.32) methods. In MO, analysis of lists with the fixed and random approaches yielded higher prediction accuracies (0.39) than those obtained by joint and Z-score (both 0.37). In HO, fixed was the most accurate method (0.58), followed by joint and Z-score (0.56), and then random (0.55).

In all cases, the use of the WB HO GWAS to predict GEBV in the NO and MO validation populations led to a decrease in accuracies compared with the values obtained with the WB MO or NO GWAS (−9 points to −13 points for MO and −12 to −20 points for NO).

### **Locations of Variants Selected for Genomic Predictions**

We next performed pairwise comparisons of the lists of 493 QTL that were generated by each WB and MB method; these lists contained the 17 most significant SNP from each chromosome (Figure 4). As described in the Materials and Methods section, QTL were consid-

ered to be common, neighboring, or different between analyses according to their location(s).

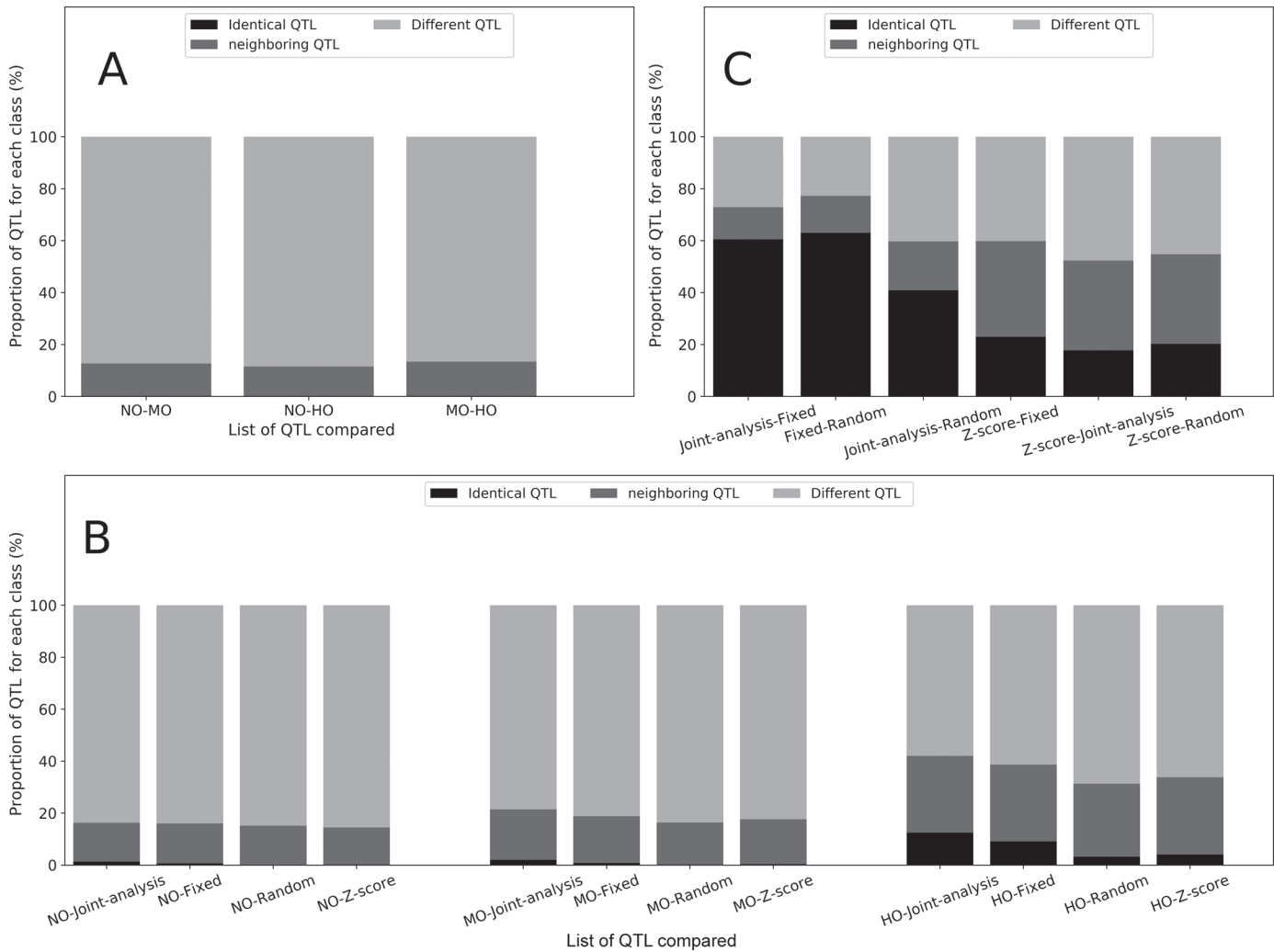
No QTL were shared between the 3 WB analyses (Figure 4A), and only one was shared between NO and HO for FY, PY, and FC. Among the 493 QTL retained in each WB analysis, only a small number were classified as neighboring in the 3 pairwise comparisons (12.5% on average). Most of the QTL selected from the WB GWAS (87.4% on average) were therefore breed specific and located in different regions in each breed.

When we compared the lists of QTL obtained with WB or MB GWAS (Figure 4B), the average proportions of common or neighboring QTL were higher. However, results differed depending on the breed. For example, there were more common and neighboring QTL in the comparison of MB and HO GWAS (36.5% on average) than in comparisons with MO (18.6% on average) or NO (15.4% on average). Likewise, results also differed depending on the method used, with comparisons involving the joint method yielding the highest proportions of common or neighboring QTL. Among the meta-analysis methods, the highest and lowest proportions of common or neighboring QTL were obtained with the fixed and random approaches, respectively, whereas Z-score gave intermediate results.

Finally, we performed pairwise comparisons of all MB GWAS methods (Figure 4C). The proportion of common or neighboring QTL was higher in comparisons of QTL lists generated by different MB approaches than in comparisons of lists generated by MB and WB methods. The comparisons that shared the highest proportion of common and neighboring QTL were of fixed and random (77.2%), followed by joint and fixed (72.9%). In comparisons of joint and random, as well as of Z-score and fixed, almost 60% of QTL were common or neighboring, but in the former case, we also found a higher proportion of identical variants (40.9%) compared with the latter (23.0%). Finally, 52.4% of variants in the Z-score lists were common and neighboring with joint variants, whereas 54.8% were common or neighboring with the random lists.

## **DISCUSSION**

The amount of high-throughput sequencing data available for livestock species is constantly increasing, and this is particularly true in bovines thanks to the efforts of the 1000 Bull Genomes Consortium. However, because of computational limitations, it is simply not feasible to include information for millions of variants in routine genomic evaluations. It therefore becomes necessary to select variants relevant to traits of interest to predict the performance of candidate animals. In this study, we investigated the selection of variants in



**Figure 4.** Identification of common, neighboring, and different QTL based on pairwise comparisons of lists of 493 SNP used for genomic evaluation (means for the 5 traits). Comparisons were made of different within-breed lists (A), of within-breed and multi-breed lists (B), and of different multi-breed lists (C). NO = Normande; MO = Montbéliarde; HO = Holstein.

a MB population to maximize the size of the reference population and to determine as precisely as possible the locations of QTL shared between breeds. Different methods of meta-analysis and joint analysis were evaluated for their ability to select candidate variants and define a panel of SNP to be included in genomic evaluation models.

First, we compared QTL detection results among all GWAS methods. Previous studies have shown the effect of sample size on the significance of statistical tests (Royall, 1986; Sullivan and Feinn, 2012; Korte and Farlow, 2013). In our study, the HO population was almost 3 times larger than either the MO or NO population. The power of detection was thus higher in HO than in the other 2 breeds, which could explain why we detected more QTL in HO than in MO or NO.

Under the hypothesis that most QTL are shared across breeds (MB QTL), merging the 3 populations to conduct a joint analysis should help to increase the power to detect MB QTL. In practice, though, the numbers of QTL detected using joint analyses and the WB HO GWAS were similar. One possible explanation for this could be that the 3 breeds in our study, which are the 3 main cattle breeds in France, have been subjected to directed selection for many years, with specific breeding objectives for each breed (Boichard et al., 2012). The resulting genetic divergence between these breeds could thus be partly responsible for the relatively low proportions of MB QTL found here.

Compared with joint analyses, meta-analysis approaches are easier to implement as they are less computationally demanding, do not require the complete

original data sets, and can account for differences in population sizes. We tested 3 meta-analysis methods commonly used in QTL studies: fixed, random, and Z-score. In general, the fixed and Z-score approaches detected an equivalent number of QTL compared with the joint and WB HO analyses. In contrast, the random method failed to find all the QTL that had been detected by the joint and WB HO methods. As shown by Hedges and Vevea (1998), the reduced power of the random method could be due to the inclusion of heterogeneity in the meta-analysis. High heterogeneity between breeds decreases the weight given to each breed ( $\alpha_i$ ), resulting in a decrease in the SNP effect ( $\beta_i$ ).

Even if the number of QTL detected with Z-score, fixed, joint, and WB HO analyses was equivalent, the locations of the detected QTL differed substantially among analyses. Some QTL were identified at the same location by the different WB (e.g., a QTL on chromosome 6) or MB analyses (e.g., QTL on chromosomes 3, 6, 11, 14, and 20). However, the different MB approaches also yielded information for distinct genomic regions. For example, QTL were found on chromosomes 10 and 21 only with joint analyses and a QTL was identified on chromosome 5 in all MB analyses except random.

The QTL results obtained in our study are consistent with those previously reported in dairy cattle for milk production and composition traits. For example, we detected QTL on chromosomes 6, 11, 14, and 20, where genes affecting milk have already been identified: the casein genes on chromosome 6 (Caroli et al., 2009), the *PAEP* gene, which encodes the  $\beta$ -LG protein, on chromosome 11 (Ganai et al., 2009), the *DGAT1* gene on chromosome 14 (Grisart, 2002), and the *GHR* gene on chromosome 20 (Blott et al., 2003).

To compare the resolution of the different methods and determine if QTL detected in MB analyses were shared across breeds or specific to HO, we selected variants based on the results of different GWAS methods and included them in a marker-assisted BLUP analysis. Because only these SNP were included in the model, we did not expect to maximize the correlation between GEBV and DYD in the validation population. Our aim was only to measure the relative abilities of the methods tested here to generate lists of QTL to predict performance.

However, as a reference, a MABLUP using 50k SNP provided mean accuracies of genomic prediction over the 5 traits of 0.534 for MO, 0.475 for NO, and 0.775 for HO. These values were obviously much higher than accuracies obtained in our study because lists we tested contained much less SNP than the 50k (Weller et al., 2014). Moreover, bias values, reported in Supplemental Figure S7 (<https://doi.org/10.3168/jds.2017-13587>),

were higher than values obtained with a MABLUP using the 50k SNP (0.17 in MO, 0.17 in NO, and 0.08 in HO) but equivalent for all the methods and scenarios tested.

In MO and NO, the GEBV of animals in the validation set were more accurate when QTL were selected from WB than from MB GWAS. This could be due to the fact that a large part of the QTL are breed specific, as observed by Raven et al. (2014).

Of the MB approaches, fixed led to more accurate GEBV, whereas Z-score gave the least accurate results. This contradicts the report of van den Berg et al. (2016), who found that Z-score was the best method for detecting QTL. However, their study compared meta-analysis results with those of joint analyses only in a GWAS context, with the assumption that joint analyses represented the optimal method. Here, our objective was quite different, as each method was tested for its ability to select the best lists of variants for genomic evaluations.

The fact that the results obtained with HO WB and MB analyses were similar suggests that the QTL selected with both approaches could be identical. However, in NO and MO, the use of QTL selected from HO GWAS led to less accurate GEBV than the use of QTL from MB GWAS.

We were also able to determine that selecting QTL to maximize the homogeneity of their distribution across the genome did not improve the accuracy of genomic predictions.

Detection of QTL among the different MB approaches was generally quite similar; however, the genomic predictions based on the lists of selected QTL from each method varied markedly in accuracy. To explain this result, we calculated the proportion of common, neighboring, and different QTL in pairwise comparisons of all methods.

First, very few QTL were common or neighboring between WB analyses. As was previously found in a comparison of Holstein and Jersey cattle by Raven et al. (2014), this result suggests that relatively few of the QTL detected in the MO, NO, and HO data sets are shared between breeds. This could also explain why the lists of QTL generated by a given WB analysis poorly predicted the performance of animals from other breeds. In this regard, MB approaches have more potential for use in pinpointing regions shared between different breeds. Additionally, in a MB population, LD is conserved over short distances, so QTL mapping can be more precise with these approaches even for QTL that do not segregate in all breeds. Alternatively, the small number of common or neighboring QTL could reflect a lack of power to detect QTL in NO and MO data due to the small sample size for these breeds.

Proportions of common and neighboring QTL were higher in comparisons of lists generated by WB and MB approaches than of different WB lists. As MB approaches should summarize information from the entire population, we would expect that a list of QTL selected from a given WB analysis would be more similar to that generated using a MB approach than to another WB list. Here, despite differences in the numbers of animals among breeds, MB approaches were able to merge information from the 3 breeds to detect common QTL associated with milk production traits.

Between the fixed and random approaches, the proportion of common or neighboring QTL was very high (80%). However, the fixed approach was best for predicting the performance of young animals. Because it took heterogeneity into account in estimating SNP effects, the random method was apparently less efficient in the detection of QTL. This may be due to the small number of breeds (here, only 3) used to estimate heterogeneity, which could introduce bias in estimation (Ioannidis et al., 2007).

Among all the MB methods tested in our study, Z-score appeared to be the least useful, as it was the least accurate in its genomic evaluation. The proportions of common and neighboring QTL between Z-score and other MB approaches were relatively low (around 60%). This result differs from those obtained in a GWAS context by van den Berg et al. (2016), who observed a strong correlation between the *P*-values from Z-score and the *P*-values from joint analyses.

The highest accuracies for genomic evaluation were obtained with fixed and joint analyses, which appear to be the most promising methods for performing MB GWAS and for selecting QTL for genomic evaluation. However, because the joint method is computationally more intensive than the fixed method, the latter represents a good alternative for MB GWAS. With increasing access to high-quality sequence data, the inclusion of causative mutations for traits of interest in official genomic assessments will become easier and more common. In addition, the customization of low- or medium-density SNP chips to include candidate causative variants also represents a powerful tool. In either case, the use of meta-analyses to identify these causative mutations appears to be a promising strategy.

Low imputation quality for NO and MO could be a factor explaining the lower number of detected QTL in these breeds. Imputation from HD SNP to sequence level was performed with the MB reference population from RUN4 of the 1000 Bull Genome Project. However, this reference population contains only 28 MO and 24 NO bulls, whereas 288 HO bulls were available. Using this data set, Sanchez et al. (2017) showed that imputation accuracy, especially for variants with low

MAF, was lower in MO than in HO, with genotypic concordance rates of 89.7 and 93.7%, respectively. Because MO and NO have similar population structures and numbers of whole genome sequences of major ancestors, imputation accuracies in the 2 breeds would likely be equivalent. RUN6 of the 1000 Bull Genome population contains 2,333 animals, including 54 MO and 44 NO bulls; the availability of these new data will undoubtedly improve the quality of whole genome sequence imputations. In the future, it would be interesting to compare the results reported here with those generated with the updated data set. In addition, the customization of low- or medium-density SNP chips to include candidate causative variants also represents a powerful tool. In either case, the use of meta-analyses to identify these causative mutations appears to be a promising strategy. Nevertheless,

- (1) because accuracies of GEBV were lower than those obtained with a GBLUP based on 50K SNP (probably due to a good cover of the genome) and
- (2) because significant segregating QTL change over time (due to fixation of positive alleles with high frequencies, and increase of frequency of positive alleles with low frequencies (Glick et al., 2012),

a model that combines a genomic relationship matrix based on a classical 50K chip and a list of selected putative QTL, as proposed by Van Raden et al. (2017), could be investigated.

Instead of sharing GEBV data, as is widespread now, this study demonstrates the possibility of exchanging data on SNP effects. As reference population size is a key parameter for accuracy in imputation methods, GWAS methods, and genomic prediction approaches, the use of MB methods, which are inherently based on larger populations, could be one way to improve future analyses. Another possibility could be to exchange WB data from different countries. International genomic evaluation methods for dairy cattle have been developed (VanRaden and Sullivan, 2010), which are based on GEBV computed in each country. Instead of sharing GEBV, approaches could be developed to share marker-effect estimations. In this case, meta-analysis could become a method of choice, particularly the fixed-effect model that showed the most promise here.

## CONCLUSIONS

This study investigated the ability of WB and MB GWAS to detect QTL and to select whole-genome variants for genomic evaluations of the 3 most economically important cattle breeds in France: HO, MO, and NO.

Multi-breed GWAS outperformed WB GWAS for QTL detection. Most of the QTL regions identified using WB GWAS were also found with MB approaches. In addition, MB GWAS led to the detection of new QTL that were not identified with WB GWAS. Among all of the MB methods tested in this study, the selection of SNP with meta-analyses (fixed-effect model) resulted in the highest accuracies of genomic evaluation. However, accuracies of genomic evaluations were always higher when variants were selected from WB GWAS results. As is generally found in QTL studies, these results suggest that part of the genetic variance of milk production traits is breed specific in the 3 breeds studied here.

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