Overlap in genomic variation associated with milk fat composition in Holstein Friesian and Dutch native dual-purpose breeds

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ABSTRACT

The aim of this study was to identify if genomic variations associated with fatty acid (FA) composition are similar between the Holstein-Friesian (HF) and native dual-purpose breeds used in the Dutch dairy industry. Phenotypic and genotypic information were available for the breeds Meuse-Rhine-Yssel (MRY), Dutch Friesian (DF), Groningen White Headed (GWH), and HF. First, the reliability of genomic breeding values of the native Dutch dual-purpose cattle breeds MRV, DF, and GWH was evaluated using single nucleotide polymorphism (SNP) effects estimated in HF, including all SNP or subsets with stronger associations in HF. Second, the genomic variation of the regions associated with FA composition in HF (regions on *Bos taurus* autosome 5, 14, and 26), were studied in the different breeds. Finally, similarities in genotype and allele frequencies between MRV, DF, GWH, and HF breeds were assessed for specific regions associated with FA composition. On average across the traits, the highest reliabilities of genomic prediction were estimated for GWH (0.158) and DF (0.116) when the 8 to 22 SNP with the strongest association in HF were included. With the same set of SNP, GEBV for MRV were the least reliable (0.022). This indicates that on average only 2 (MRV) to 16% (GWH) of the genomic variation in HF is shared with the native Dutch dual-purpose breeds. The comparison of predicted variances of different regions associated with milk and milk fat composition showed that breeds clearly differed in genomic variation within these regions. Finally, the correlations of allele frequencies between breeds across the 8 to 22 SNP with the strongest association in HF were around 0.8 between the Dutch native dual-purpose breeds, whereas the correlations between the native breeds and HF were clearly lower and around 0.5. There was no consistent relationship between the reliabilities of genomic prediction for a specific breed and the correlation between the allele

frequencies of this breed and HF. In conclusion, most of the genomic variation associated with FA composition in the Dutch dual-purpose breeds appears to be breed-specific. Furthermore, the minor allele frequencies of genes having an effect on the milk FA composition in HF were shown to be much smaller in the breeds MRV, DF, and GWH, especially for the MRV breed.

Key words: cattle breeds, milk, fatty acid, genomic variation

INTRODUCTION

Over the last few decades, a clear change in the dairy industry has taken place toward high production, especially in industrialized countries. High emphasis on selection for milk yield and the consolidation and globalization of breeding companies has led to professional breeding programs for specialized dairy cattle breeds (Brotherstone and Goddard, 2005; Shook, 2006). This development has led directly to a decline in use of native dual-purpose breeds, which have been used for milk and beef production. For example, in the Netherlands the percentage of dairy cows in dairy industry belonging to Dutch native dual-purpose breeds declined from 91.3 to 1.4% in the last 30 yr. This reduction in breed variability has led to a decrease of genetic variability in the total cattle population as individual cattle breeds comprise unique genetic variation (European Cattle Genetic Diversity Consortium, 2006). In this regard, safeguarding existing genetic variation can be important to be able, in the future, to exploit currently unknown genetic variation (Oldenbroek, 2007). In addition, from the perspective of breeding programs, it is important to know the magnitude of genetic variation between breeds and, more concretely, whether genomic prediction performed in Holstein-Friesian (HF) can be used in the numerically small native dual-purpose breeds. Therefore, it is of interest for the dairy cattle industry to know whether the genetic variation in native dual-purpose breeds differs from the HF breed. One trait, which is extensively studied in the HF breed and increasingly more important for the dairy industry, is the FA composition in milk (e.g., Soyeurt et al., 2006;

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Stoop et al., 2008). The interest of the dairy industry in the possibilities to modify FA composition in milk arises from supposed associations of milk FA composition with human health (e.g., Palmquist et al., 2006; Astrup et al., 2011) and milk processability (e.g., Smet et al., 2009). In addition to genetic variance for milk FA composition observed within breeds, differences in milk FA composition among dairy cattle breeds are also described in several studies (e.g., Soyeurt et al., 2011; Maurice-Van Eijndhoven et al., 2013a).

Studying the relation between the bovine genome and the FA composition in bovine milk is an important step toward understanding the genetic variability of milk FA composition. For example, on the bovine genome in 2 genes, namely diacylglycerol acyltransferase 1 (*DGAT1*) and stearoyl-CoA desaturase 1 (*SCD1*), polymorphisms are reported to have large associations with milk FA composition of HF cattle (e.g., Bouwman et al., 2011; Bernard et al., 2013). To investigate the genetic variability in native dual-purpose breeds, one possible approach is to compare whether different genomic regions have a similar contribution to trait variation in different cattle breeds. Allele frequencies in *DGAT1* and *SCD1* polymorphisms have been reported in several breeds (Spelman et al., 2002; Kgwatalala et al., 2007). For example, different allele frequencies of the *DGAT1* 232K allele in New Zealand HF (0.6), Ayrshire (0.22), and Jersey (0.88) were reported by Spelman et al. (2002) and of the *SCD1* 293A allele in Canadian HF (0.83) and Jersey (0.95) are reported by Kgwatalala et al. (2007). Therefore, an appropriate question is whether effects of genomic regions on the milk fat composition of HF cows are similar for the native dual-purpose breeds.

The aim of the current study is to investigate similarities in genomic variation associated with milk FA composition in the HF breed and native Dutch dual-purpose breeds. This will be achieved by evaluating the reliability of predicted genomic breeding values for fat composition in milk of the native Dutch dual-purpose cattle breeds Meuse-Rhine-Yssel (**MRY**), Dutch Friesian (**DF**), and Groningen White Headed (**GWH**) using SNP effects estimated in HF and by comparing the genomic variability in regions on the genome showing large effects on milk FA composition.

MATERIALS AND METHODS

Data Collection

Phenotypic and genotypic data were collected in 3 different periods respectively on 2,500 and 1,867 cows belonging to 4 different breeds (Table 1). The complete data set contains 1 milk sample for each cow that was

analyzed using GC-MS or mid-infrared spectrometry (**MIR**), and genotyped animals are distributed differently across periods. In detail, milk samples of the first sample period were collected within the project of the Dutch Milk Genomics Initiative between May and June 2005. The GC-MS profiles were obtained from morning milk samples from 1,811 cows. Blood samples were also collected for genotyping. All cows belong to the HF breed and were sampled during the first parity between 97 and 355 DIM and each herd-test-day (**HTD**) comprises a minimum of 3 records. Detailed information on this data set is reported in previous studies, where it is referred to as the data representing summer milk (Rutten et al., 2009; Bouwman et al., 2012; Duchemin et al., 2013).

Data of the second sample period was collected during December 2008 and March 2009. The GC-MS profiles of morning milk samples were collected from 137 cows, and 79 of them had good-quality DNA samples available and were genotyped. Cows belonged to 3 breeds: MRY (50 samples from 3 herds), DF (44 samples from 3 herds), and GWH (43 samples from 3 herds). Parity of the cows varied between 1 and 9 and DIM varied between 5 and 535. Detailed information on these GC-MS data are reported in Maurice-Van Eijndhoven et al. (2011). An overview of the data is shown in Table 1.

Data of the third sample period was collected during the end of August 2011 and the beginning of November 2011. The GC-MS or MIR profiles of morning milk samples were collected from 552 cows and 280 of them were also genotyped (Table 1). All cows were purebreds belonging to the breeds MRY, DF, GWH, or HF (i.e., they were registered with at least 87.5% of the genes of any of the breeds mentioned). The data collected in 2011 contains 3 herds with milk samples of multiple breeds (one herd with 28 MRY and 15 HF cows; one herd with 6 GWH and 36 HF cows; and one herd with 5 MRY, 33 DF, and 4 GWH cows). The other 11 herds included in the study used a single breed: 242 MRY cows on 3 herds, 94 DF cows on 2 herds, 136 GWH cows on 3 herds, and 80 HF cows on 3 herds.

Measuring FA Composition

All milk samples were treated immediately with 0.03% (wt/wt) sodium azide to avoid microbiological growth. To obtain detailed FA compositions, all milk samples were analyzed at the laboratory of Qlip N.V. (Zutphen, the Netherlands) by GC-MS or by Fourier-transform interferogram machines (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark) to obtain MIR profiles. The GC-MS outputs were generated by analyzing methyl esters. Fatty acid methyl esters were prepared using fat fractions extracted from the milk,

Table 1. Overview of the number of animals per breed and fixed effects per sample period

Item	Data			
	Data 2005 ¹	Data 2008–2009 ¹	Data 2011 ¹	Data 2011 ²
Number of animals	1,811	137	319	233
Breed, n				
Meuse-Rhine-Yssel	0	50	144	98
Genotyped ³	0	39	106	0
Groningen Whited Headed	0	43	105	31
Genotyped ³	0	25	89	0
Dutch Friesian	0	44	70	24
Genotyped ³	0	15	56	0
Holstein-Friesian	1,811	0	0	80
Genotyped	1,811	0	0	0
Total with phenotypes	1,544	137	319	233
Total with genotypes	1,811	79	280	0
Fixed effects, average (SD)				
DIM	274 (42)	144 (88)	158 (94)	162 (98)
Parity ⁴	1.0 (0)	2.8 (2.0)	2.7 (1.8)	3.2 (1.9)
Age ⁵	26 (2)	52 (28)	50 (23)	56 (25)

¹FA composition based on GC-MS.²FA composition based on mid-infrared spectroscopy.³For all genotyped cows of the breeds, GC-MS phenotypes were also available.⁴Parity was divided in 4 classes for corresponding lactation numbers of parity 1, 2, and 3 and the 4th class included parity 4 – 10.⁵Age at calving in months.

as described in ISO Standard 15884 (ISO-IDF, 2002b). Methyl esters were analyzed, as described in ISO Standard 15885 (ISO-IDF, 2002a), according to the 100% FAME method with a 100-m × 0.25-mm polar column (Select Fame Varian CP 7420, Varian Inc., Palo Alto, CA).

For the final analyses, fat percentage, 1 group of FA, and 9 individual FA were chosen: short-chain FA (C4:0–C12:0); C12:1 *cis*-9; C14:0; C14:1 *cis*-9; C16:0; C18:0; C18:1 *trans*-6, 9, 11; C18:1 *cis*-9; C18:2 *cis*-9, 12 and C18:2 *cis*-9, *trans*-11. The group of FA and individual FA were analyzed as grams per deciliter of milk. The FA were chosen for (1) their reasonably high correlations between summer and winter milk (Duchemin et al., 2013), (2) their biological background (generated through de novo synthesis or by Δ^9 -desaturases; Schennink et al., 2008; Soyeurt et al., 2008), and (3) for the (lack of) relation with *DGAT1* or *SCD1* (Schennink et al., 2008).

Genotype Data

The breeds MRY, DF, and GWH were genotyped using the Illumina high-density (HD) SNP chip (Illumina, San Diego, CA) and the HF breed with a custom 50k SNP chip (Illumina Inc.), which was imputed to HD SNP data. For all breeds, genotypes were edited for call rates and minor allele frequency. Missing HD genotypes were imputed for MRY, DF, and GWH, and for HF all HD SNP not included on the 50k chip were

imputed. Additional editing steps were partly different, as explained below, because data were obtained from multiple studies.

In total, 359 animals were genotyped for the breeds MRY, DF, and GWH. Genotypes with a GC-MS score <0.2 were set to missing. After this initial editing step, we removed SNP with a call rate <85%, a minor allele frequency <0.02, unknown map position, known position on the sex chromosomes, or when not all 3 genotypes were observed. Removing SNP for which 3 genotypes were not observed is similar to removing SNP based on a commonly applied Hardy-Weinberg test aiming to remove only the most extreme outlier SNP and not those that depart from Hardy-Weinberg equilibrium due to selection (Wiggans et al., 2009). Further edits included removing animals with a call rate <85% (n = 8), parent-offspring inconsistencies (n = 2), and because, based on principal component analysis of the genotype data, animals clustered with a different breed than registered by the herd book registration (n = 1). Finally, missing genotypes were imputed using Beagle (Browning and Browning, 2007). After these edits, 619,056 segregating SNP were available.

The HF data contained 1,684 animals with imputed HD genotypes for 734,393 SNP. Those animals were initially genotyped with a custom 50k SNP chip. The HD genotypes were imputed using HD genotypes of all 55 sires of the HF cows, combined with HD genotypes of another 1,278 HF bulls available at CRV (Arnhem, the Netherlands). More details on these (imputed)

genotypes are provided by Bouwman et al. (2014). Before combining them with the other data set, SNP with a minor allele frequency <0.02 or when not all 3 genotypes were observed were removed, leaving 602,445 SNP.

The imputed HD SNP data for the HF animals was combined with the HD SNP data of the other breeds, yielding a combined data set consisting of 571,275 SNP, which met the editing criteria in both data sets. In the combined data set, from each pair of neighboring SNP with an r^2 (linkage disequilibrium) value of 1.0, one of the SNP was removed. After this final step, 350,207 SNP were left in the data set used for analysis.

Statistical Analysis: Correcting Phenotypes for Fixed Effects

Before estimating SNP effects, the record of each cow was corrected for the fixed effects of DIM, parity, age at calving, HTD, breed, and method of measuring the FA using the following model (using ASReml 3.0, Gilmour et al., 2009):

$$y_{ijklmno} = \mu + b_1 \times \text{DIM}_i + b_2 \times \exp^{-0.05 \times \text{DIM}} + \text{parity}_j + b_3 \times \text{age}_k (\text{parity}_j) + \text{HTD}_l + \text{breed}_m + \text{method}_n + e_{ijklmno},$$

where $y_{ijklmno}$ was the dependent variable for cow o in DIM i , with parity j , age at calving k , producing at HTD l , belonging to breed m , and with FA composition obtained using method n . The μ was the overall mean of the model; b_1 and b_2 were the fixed regression coefficients on DIM _{i} modeled with a Wilmink curve

(Wilmink, 1987); parity _{j} was the fixed effect with 4 classes for corresponding lactation numbers of parity 1, 2, and 3 and the fourth class included parity 4 to 10; b_3 was the fixed regression coefficient on age at calving (age _{k} , days) within the j th parity; HTD _{l} was the effect defining groups of cows sampled in the same herd on the same sample date; breed _{m} was the effect defining groups of cows belonging to the same breed (MRY, GWH, DF, or HF); method _{n} was the effect with 2 classes corresponding for the method used to obtain the FA composition (GC-MS or MIR); and $e_{ijklmno}$ is the random residual effect. Predicted FA composition based on MIR profiles was included for 272 animals without genotypes as y variate in the analysis to obtain more precise correction of the phenotypes for the fixed effects. Using MIR the majority of FA can be predicted with moderate ($R^2 = 0.60$ to 0.80 for C14:0 *cis*-9, C16:0 *cis*-9, and C18:0) to high ($R^2 \geq 0.80$ for the individual FA C4:0–C16:0 and groups of FA) accuracy (Maurice-Van Eijndhoven et al., 2013b). The residuals of this model for the records obtained with GC-MS were used as corrected phenotypes in the subsequent genomic analyses. Table 2 shows an overview of the corrected phenotypes per sample period.

Statistical Analysis: Estimation of SNP Effects

To identify if similarities existed in genomic variation associated with milk FA composition, the fat composition in milk of Dutch cattle breeds MRV, DF, and GWH was predicted using SNP effects estimated based on the 1,544 HF cows. For this part of the analysis, genotype data and the residuals obtained from the model to precorrect the phenotypes were used. The

Table 2. Overview of the corrected phenotypes per sample period

Item	Year(s)			
	2005 ¹	2008–2009 ¹	2011 ¹	2011 ²
Number of phenotypes	1,811	137	319	233
Traits, ³ average (SD)				
Fat%	4.26 (1.25)	4.56 (1.29)	4.55 (1.39)	4.36 (1.54)
C4:0–C10:0	0.4219 (0.1498)	0.5085 (0.1698)	0.4667 (0.1470)	0.4349 (0.1556)
C12:1	0.0046 (0.0024)	0.0043 (0.0029)	0.0047 (0.0031)	0.0056 (0.0024)
C14:0	0.4738 (0.1447)	0.5651 (0.1895)	0.5292 (0.1926)	0.4419 (0.1542)
C14:1 <i>cis</i> -9	0.0491 (0.0233)	0.0464 (0.0245)	0.0489 (0.0253)	0.0601 (0.0273)
C16:0	1.2494 (0.4813)	1.3812 (0.4852)	1.3336 (0.4852)	0.9825 (0.6584)
C18:0	0.4234 (0.1903)	0.4782 (0.1877)	0.4406 (0.2314)	0.3446 (0.1891)
C18:1 <i>cis</i> -9	0.0680 (0.1333)	0.0589 (0.1399)	0.0755 (0.1889)	0.0873 (0.1807)
C18:1 <i>trans</i> -6,9,11	0.8596 (0.1783)	0.7596 (0.1739)	0.8732 (0.2328)	0.9456 (0.2413)
C18:2 <i>cis</i> -9,12	0.0472 (0.0170)	0.0593 (0.0264)	0.0538 (0.0259)	0.0591 (0.0206)
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.0232 (0.0142)	0.0182 (0.0086)	0.0273 (0.0228)	0.0350 (0.0161)

¹Milk samples analyzed using GC-MS.

²Milk samples analyzed using mid-infrared spectroscopy.

³Corrected phenotypes for fat percentage (the production trait fat percentage was analyzed using mid-infrared spectroscopy for all data) and individual FA and groups of FA in grams per deciliter of milk.

model used is commonly known as BayesC (Habier et al., 2011), and is described by

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{Z}\mathbf{u} + \mathbf{X}\boldsymbol{\alpha} + \mathbf{e},$$

where $\mathbf{y}$ contains precorrected phenotypes, $\mathbf{1}$ is a vector of ones, μ is the overall mean, $\mathbf{Z}$ is an incidence matrix that links records to individuals, $\mathbf{u}$ contains random polygenic effects of all individuals, $\mathbf{X}$ is a matrix that contains the scaled and centered genotypes of all individuals, $\boldsymbol{\alpha}$ contains the (random) allele substitution effects for all loci, and $\mathbf{e}$ contains the random residuals (see Technical Appendix; <http://dx.doi.org/10.3168/jds.2014-9196>). Polygenic effects were included in the model because the HF data contained only a limited number of sires with relative large groups of daughters. Omitting a polygenic effect with such a data structure is expected to lead to spurious associations (e.g., Balding, 2006) that will not have any predictive value for the other breeds.

From the output of the BayesC model, a Bayes Factor (BF) was calculated for each SNP as

$$\text{BF} = \frac{\Pr(H_1 | y)}{1 - \Pr(H_1 | y)} \div \frac{\Pr(H_1)}{1 - \Pr(H_1)},$$

where H_1 is the hypothesis that the marker has a non-zero effect; $\Pr(H_1 | y)$ is the posterior probability of the hypothesis and $\Pr(H_1)$ is the prior probability of the hypothesis; and $1 - \Pr(H_1 | y)$ and $1 - \Pr(H_1)$ represent the posterior and prior probability for the alternative hypothesis, respectively. Bayes Factors are an indication of the size of the effects SNP have on the trait analyzed. High BF indicates that a SNP is likely to be associated with a QTL affecting the trait. To give an overview of SNP having an effect on the different milk traits in HF, a Manhattan plot for the BF for each SNP was produced. Plotted BF were smoothed by calculating for each SNP the average of its own BF and those of the 4 SNP located on either side.

Statistical Analysis: Estimation of Genomic Estimated Breeding Values

The SNP effects estimated using the data set of the HF population sampled in 2005 were used to calculate genomic estimated breeding values (GEBV) for the genotyped cows of the breeds MRY, DF, and GWH sampled in 2008 to 2009 and 2011. To check how valid the GEBV are using SNP effects estimated in HF, reliabilities (i.e., accuracies squared) of the predicted breeding values were calculated. The reliability can be expressed as:

$$r_{gg}^2 = r_{yg}^2 / h^2,$$

which follows Verbyla et al. (2010), where r_{yg}^2 is the squared correlation between the observed phenotype in MRY, DF, or GWH and their GEBV, and h^2 is the heritability of the trait. The h^2 used were estimated for a Dutch MRY population (Maurice-Van Eijndhoven et al., 2014; Supplementary Table S1; <http://dx.doi.org/10.3168/jds.2014-9196>) and used for all 3 breeds (MRY, DF, and GWH) because no h^2 estimates were available for DF and GWH. Heritabilities for MRY for the individual FA C12:1 to C18:0 ranged from 0.23 to 0.34, for the individual FA C14:1 *cis*-9 was 0.27, for the individual C18 unsaturated FA ranged from 0.10 to 0.26, and for the short-chain group of FA C4:0 to C10:0 was 0.27. For C12:1 and C18:1 *trans*-6, 9, 11 no estimates were available for h^2 for MRY. To enable approximation of the accuracies for those 2 FA, values of respectively 0.3 and 0.16 were used, resembling to h^2 estimates obtained in MRY for FA that were shown to have similar variance as for C12:1 and C18:1 *trans*-6, 9, 11 in HF. Because the same h^2 estimates were used for all breeds, this only allowed to approximate accuracies of GEBV instead of predicted phenotypes, although it did not allow us to differentiate between the breeds. The GEBV were calculated using all SNP or only SNP having an estimated effect in HF with different thresholds of BF (greater than 10, 100, or 1,000). This allows us to investigate the effect of regions with greater effects on FA composition in HF on the accuracy of prediction in the other breeds. To further evaluate similarities between breeds in those genomic regions, allele frequencies were compared, as well as variance explained per SNP. Finally, the percentage of the genetic variation explained by the SNP for each breed was computed as $2pq\hat{\alpha}^2 / \text{var}(a)$, where $\text{var}(a)$ was the genetic variance for HF calculated by $\text{var}(a) = h^2 \times \text{var}(y)$, where y were precorrected phenotypes and the h^2 for HF were obtained from Maurice-Van Eijndhoven et al. (2014). For trait C18:2 *cis*-9, *trans*-11, no SNP were found with a BF >1,000; therefore, this trait was not included in this last evaluation. To further evaluate similarities between breeds in those genomic regions, allele frequencies for the different scenarios were compared.

To allow comparison of the reliabilities obtained for the other breeds, reliabilities within HF were computed as well. This involved a random 10-fold cross validation, where all HF animals were randomly allocated to 10 equally sized folds. For each of the folds, GEBV were predicted using the other 9 folds as training data. Here, the same BayesC model including polygenic effects was applied and polygenic effects were not used for the

prediction, similar as for other breeds. Heritabilities, required to compute the reliabilities, were obtained from (Maurice-Van Eijndhoven et al., 2014).

RESULTS

Corrected Data

Cows sampled in 2005 were on average 25 mo old at calving, sampled at 274 DIM, and were all in their first parity (Table 1). Cows sampled in 2008 to 2009 and 2011 were older, being on average 49 to 55 mo old at calving, sampled at 144 to 162 DIM, and in their third parity. An overview of the data, after correction for fixed effects, is given in Table 2. The data are grouped per sample period to enable comparison of properties across the underlying data sets. Fat percentage was, on average, highest for data collected in 2008 to 2009 (4.56%), lowest for data collected in 2005 (4.26%), and most variable for data collected in 2011 ($SD = 1.39$ – 1.54). The average contents in milk of all saturated FA (C4:0–C10:0; C14:0; C16:0; and C18:0) of the samples collected in 2008 to 2009 were higher and all unsaturated FA contents, except C18:2 *cis*-9, 12, were lower compared with the samples collected in 2005 and 2011. The average contents of unsaturated FA, except C18:2 *cis*-9, 12, were generally lowest in the data collected in 2011, which was analyzed using MIR. Nevertheless, the standard deviations were comparable for all periods.

SNP Effects: Reliabilities of GEBV

Figure 1 gives an overview of the BF of all SNP across the genome for each of the considered traits. Several peaks are observed for the BF across multiple traits, indicating several QTL that are associated with the FA traits included in our study. As shown in Table 3, between 2,405 and 4,734 SNP showed a BF above the threshold of 10, between 46 and 311 SNP above 100, and between 0 and 22 SNP above 1,000.

The reliabilities of GEBV of fat content ranged from 0.158 to 0.193 for GWH and from 0.237 to 0.256 for DF when SNP effects were used with a BF >10; no clear improvement was observed when using SNP with a BF >100 or >1,000 (Table 4). For MRY, the reliabilities for total fat percentage were clearly lower (between 0.034 and 0.046) and no clear improvement was observed when using SNP with a BF >100 or >1,000. The GEBV of the FA C12:1 showed relatively high reliabilities for GWH (0.397–0.492) and for DF around 0.150 when considering only SNP with a BF >10. For the FA C14:0 and C16:0, the reliabilities of the GEBV for GWH were all above 0.2 when consider-

ing only SNP with a BF >10. The highest reliability (0.758) was observed when using all SNP for GWH and C14:1 *cis*-9. However, the reliability dropped almost to zero when only SNP were considered with a BF >10. For the FA C18:1 *cis*-9, the reliability of the GEBV for DF were relatively high (0.182–0.185), regardless the possible threshold based on BF, whereas for MRY and GWH the reliabilities were almost zero. The reliabilities of the GEBV for MRY were below 0.1 for all traits, except for C14:1 *cis*-9 using all 305,207 SNP effects to calculate the GEBV (0.178); however, when considering only SNP with a BF >10, this reliability dropped toward zero.

Of all 33 traits by breed combinations, 10, 11, and 12 had reliabilities greater than 0.05 when using SNP with BF >10, >100, or >1,000, respectively (Table 4). Reliabilities of the GEBV for HF were in general considerably higher than for the other breeds, only in a few cases the reliabilities for the other breeds were higher than the HF reliabilities.

Overall, the reliabilities of GEBV clearly differed between using all 350,207 SNP and when considering only SNP with a BF >10, >100, or >1,000, whereas between subsets of SNP with the different BF thresholds the reliabilities of the GEBV were very similar. The estimation of SNP effects in HF have very low predictive ability in MRY (on average 0.022 with a BF >1,000) and some clear predictive ability in DF and GWH, although the realized reliabilities were on average low, being 0.116 for DF and 0.158 for GWH.

SNP Variances

Table 5 shows the predicted variances explained per SNP for the SNP whose variance in at least 1 of the breeds was $\geq 1\%$ relative to the total genetic variance in the HF breed. The region of *DGAT1* on BTA14 was found to have the largest effect on most of the traits. This region explained large parts of the genetic variance in HF for the traits fat percentage, with C4:0 to C10:0, C12:1, C14:0, C14:1 *cis*-9, C16:0, C18:0, and C18:2 *cis*-9, 12 ranging from 1.1 (C18:2 *cis*-9, 12) to 19.0% (C16:0). For the breeds DF and GWH, the region of *DGAT1* was also found having an effect for several traits (ranging from 0.2% for C18:2 *cis*-9, 12 to 3.3% for C16:0 for GWH and from zero effect for 6 traits to 2.2% for C16:0 for DF), although heavily reduced in comparison with HF. Almost no effect of the genomic region on BTA14 was found for the traits in MRY. On BTA26, a genomic region was found having an effect on the genetic variance of the traits C12:1 and C14:1 *cis*-9 for all breeds, which was largest for HF and DF (explaining 2.8% of C12:1 in HF milk; 8.0% of C12:1 in DF; 2.6% of C14:1 *cis*-9 in HF; 7.4% of C14:1

Figure 1. Manhattan plot showing the Bayes Factors for each SNP having an effect on the bovine milk FA in Holstein-Friesians. Plotted Bayes Factors were the average of the SNP itself and those of the 4 SNP located on either side. C18:1 *trans* = sum of C18:1 *trans*-6, C18:1 *trans*-9, and C18:1 *trans*-11; C18:2 *cis* = C18:2 *cis*-9,12; CLA = C18:2 *cis*-9,*trans*-11.

cis-9 in DF of the genetic variance for the whole region, sum of both SNP, respectively). For GWH, this region explained 1.8% of the variance of the trait C12:1 and 5.1% of C14:1 *cis*-9 and for MRY 1.5% of C12:1 and 4.2% of C14:1 *cis*-9. On BTA5, one SNP was found with an effect on the genetic variance of the traits fat percentage, for C4:0 to C10:0, C12:1, C14:0, and C16:0 this effect was estimated to explain between 1 and 2%

of the genetic variance for HF, between 0.3 and 0.6% for DF and MRY, and almost negligible for GWH.

Correlations of Allele Frequencies

To assess similarities between the breeds across the whole genome, versus in regions associated with FA composition, the correlation of allele frequencies be-

Table 3. Number of SNP¹ with a Bayes Factor (BF) greater than 10, 100, or 1,000

Item	BF >10	BF >100	BF >1,000
Fat% ²	3,292	211	14
C4:0-C10:0	3,942	301	13
C12:1	3,527	255	15
C14:0	3,852	260	22
C14:1 <i>cis</i> -9	3,140	198	16
C16:0	3,328	172	14
C18:0	3,622	170	9
C18:1 <i>trans</i> -6,9,11	3,593	132	14
C18:1 <i>cis</i> -9	2,405	201	13
C18:2 <i>cis</i> -9,12	2,552	80	8
C18:2 <i>cis</i> -9, <i>trans</i> -11	2,707	46	0

¹Number of SNP that exceeded the BF threshold and segregated in Meuse-Rhine-Yssel, Dutch Friesian, or Groningen White Headed.

²Fat as percentage and individual FA and groups of FA in grams per deciliter of milk.

tween the breeds MRY, DF, GWH, and HF were calculated (Table 6). Across all 350,207 SNP, the correlation was between 0.64 (DF and GWH) and 0.71 (DF and HF as well as MRY and HF; Table 6). When including only SNP with a BF above one of the thresholds for a particular trait, correlations ranged from 0.63 (DF and GWH) to 0.70 (DF and HF as well as MRY and HF) for BF >10; from 0.68 (DF and GWH) to 0.72 (DF and HF, MRY and HF, and MRY and DF) for BF >100; and from 0.50 (MRY and HF) to 0.83 (MRY and DF) for BF >1,000. For the FA C18:1 *trans*-6, 9, 11, the correlations of the allele frequencies between the breeds were relatively high (on average 0.80) when considering only SNP with a BF >100; however, the reliabilities of the GEBV for this trait were low. A similar trend was found for the FA C14:1 *cis*-9 showing allele frequencies of on average 0.79 when considering only SNP with a BF >1,000. When considering only SNP with a threshold based on the BF >1,000, it was remarkable that the correlations of the allele frequencies between HF and the other Dutch breeds were relatively low (on average 0.50–0.53), whereas between the other Dutch breeds MRY, DF, and GWH the correlation was relatively high (on average 0.80–0.83). Considering only SNP with BF >10 or >100, yielded correlations between allele frequencies that were very similar to the correlations across all SNP (results not shown). In summary, both the GEBV reliabilities and the correlations between allele frequencies clearly showed differences between the breeds, albeit no consistent relationship between both statistics was noted.

DISCUSSION

The aim of our study was to identify similarities in genomic variation associated with detailed milk fat composition between the HF breed and native dual-

Table 4. The reliabilities¹ of the GEBV² in native Dutch dual-purpose breeds

Item ³	MRY, ⁴ BF ⁵			DF, ⁴ BF			GWH, ⁴ BF			HF, ⁴ BF	
	All 350,207 SNP	>10	>100	>1,000	All 350,207 SNP	>10	>100	>1,000	All 350,207 SNP	>10	>1,000
Fat%	0.041	0.046	0.046	0.034	0.072	0.245	0.256	0.237	0.050	0.193	0.158
C4:0-C10:0	0.077	0.000	0.000	0.000	0.121	0.043	0.042	0.068	0.000*	0.060	0.056
C12:1	0.001	0.002	0.003	0.000*	0.030	0.152	0.162	0.147	0.492	0.397	0.445
C14:0	0.006	0.000*	0.000*	0.000*	0.005	0.045	0.051	0.082	0.049	0.265	0.240
C14:1 <i>cis</i> -9	0.178	0.000*	0.000*	0.000*	0.041	0.000*	0.000*	0.000*	0.758	0.000*	0.000*
C16:0	0.046	0.000*	0.000*	0.000*	0.143	0.075	0.082	0.081	0.098	0.244	0.203
C18:0	0.062	0.001	0.001	0.000	0.000*	0.033	0.036	0.018	0.006	0.000*	0.001
C18:1 <i>trans</i> -6,9,11	0.000*	0.064	0.064	0.076	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
C18:1 <i>cis</i> -9	0.000*	0.005	0.002	0.000	0.000*	0.182	0.255	0.285	0.000*	0.000*	0.001
C18:2 <i>cis</i> -9,12	0.000*	0.000*	0.000*	0.000*	0.005	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
C18:2 <i>cis</i> -9, <i>trans</i> -11	0.000*	0.000*	0.000*	0	0.011	0.000*	0.000*	0	0.000*	0.000*	0

¹Reliabilities, defined as r_{gg}^2 , of the genomic estimated breeding values (GEBV).

²GEBV of cows of Dutch native dual-purpose breed origin were estimated using SNP effects estimated in a Dutch Holstein-Friesian population.

³Fat as percentage and individual FA and groups of FA in grams per deciliter of milk.

⁴MRY = Meuse-Rhine-Yssel, DF = Dutch Friesian, GWH = Groningen White Headed, and HF = Holstein-Friesian.

⁵BF = Bayes Factors.

*The underlying correlation (r_{gg}), between the predicted breeding values ($\hat{g}$) and the phenotypes (y), was negative and therefore set to zero.

Table 5. The predicted variances explained per SNP with position on the genome for the SNP which explained $\geq 1\%$ of the genetic variance in at least 1 of the breeds

Trait	SNP name	BTA ¹	Position (bp)	SNP var ²	HF ^{3,4}	DF ^{3,4}	GWH ^{3,4}	MRY ^{3,4}
Fat%	ARS-BFGL-NGS-4939	14	1,801,116	0.48567	13.3	1.5	2.3	0.0
Fat%	BovineHD1400000206	14	1,679,844	0.04544	1.2	0.0	0.3	0.2
C4:0-C10:0	ARS-BFGL-NGS-4939	14	1,801,116	0.06546	13.5	1.5	2.4	0.0
C4:0-C10:0	BovineHD1400000206	14	1,679,844	0.00654	1.3	0.0	0.3	0.2
C12:1	ARS-BFGL-NGS-4939	14	1,801,116	0.00082	10.8	1.2	1.9	0.0
C14:0	ARS-BFGL-NGS-4939	14	1,801,116	0.04398	9.0	1.0	1.6	0.0
C14:1 <i>cis</i> -9	ARS-BFGL-NGS-4939	14	1,801,116	0.00399	3.4	0.4	0.6	0.0
C14:1 <i>cis</i> -9	BovineHD1400000206	14	1,679,844	0.00451	3.8	0.0	0.9	0.6
C14:1 <i>cis</i> -9	BovineHD1400000199	14	1,638,045	0.00192	1.6	0.2	0.9	0.3
C16:0	ARS-BFGL-NGS-4939	14	1,801,116	0.99652	19.0	2.2	3.3	0.0
C18:0	ARS-BFGL-NGS-4939	14	1,801,116	0.03338	7.5	0.9	1.3	0.0
C18:0	BovineHD1400000206	14	1,679,844	0.00751	1.7	0.0	0.4	0.3
C18:0	BovineHD1400000204	14	1,667,797	0.00586	1.3	0.0	0.3	0.2
C18:2 <i>cis</i> -9-12	ARS-BFGL-NGS-4939	14	1,801,116	0.00030	1.1	0.1	0.2	0.0
C12:1	BovineHD2600005462 ⁵	26	21,141,280	0.00026	2.8	2.6	1.8	1.4
C14:1 <i>cis</i> -9	BovineHD2600005462 ⁵	26	21,141,208	0.01150	8.0	7.3	5.1	4.1
Fat%	BovineHD0500026666	5	93,951,064	0.05577	1.5	0.4	0.2	0.6
C4:0-C10:0	BovineHD0500026666	5	93,951,064	0.00761	1.5	0.4	0.2	0.6
C12:1	BovineHD0500026666	5	93,951,064	0.00010	1.3	0.4	0.2	0.6
C14:0	BovineHD0500026666	5	93,951,064	0.00540	1.1	0.3	0.2	0.4
C16:0	BovineHD0500026666	5	93,951,064	0.06288	1.2	0.3	0.2	0.5

¹*Bos taurus* autosome where SNP is located.²The genetic variance (var) explained by the SNP.³MRY = Meuse-Rhine-Yssel, DF = Dutch Friesian, GWH = Groningen White Headed, and HF = Holstein Friesian.⁴The percentage of the total genetic variance explained by the SNP for each breed; that is, $(2pq\hat{\alpha}^2) / \text{var}(a)$, where $\text{var}(a)$ is the variance estimated for HF.⁵SNP was in complete linkage disequilibrium with the SNP located on the base pair positions 21,140,458 and 21,146,794, thus published SNP effect is the accumulated effect of those three SNP in and around the *SCD1* gene.

purpose breeds in the Dutch dairy industry. The extent of the overlap in genomic variation between HF and Dutch dual-purpose breeds was investigated by calculating GEBV for fat composition in the native dual-purpose breeds using SNP effects estimated for HF and by evaluating the underlying SNP effects and variation on the genome within the associated regions. Reli-

abilities of GEBV across breeds are reported to be low because linkage disequilibrium (**LD**) observed within breeds is only partly conserved across breeds (Goddard and Hayes, 2009; Hayes et al., 2009; Hozé et al., 2014). The high-density SNP-chip (Illumina) used in the current study is characterized by short-distance LD that was expected to be maintained across breeds (de Roos

Table 6. The correlations of allele frequencies between breeds¹ across all SNP, or based only on SNP with a Bayes Factor $>1,000$

Trait ²	No. of SNP ³	HF and MRY	HF and DF	HF and GWH	MRY and DF	MRY and GWH	DF and GWH
—	350,207	0.71	0.71	0.66	0.68	0.65	0.64
Fat%	14	0.67	0.50	0.66	0.88	0.88	0.87
C4:0-C10:0	13	-0.21	0.29	0.44	0.79	0.63	0.94
C12:1	15	0.62	0.22	0.22	0.83	0.86	0.90
C14:0	22	0.40	0.57	0.41	0.65	0.62	0.76
C14:1 <i>cis</i> -9	16	0.63	0.81	0.64	0.91	0.91	0.84
C16:0	14	0.49	0.36	0.41	0.76	0.88	0.85
C18:0	9	0.34	0.61	0.54	0.74	0.83	0.55
C18:1 <i>trans</i> -6, 9, 11	14	0.39	0.42	0.54	0.90	0.94	0.89
C18:1 <i>cis</i> -9	13	0.63	0.40	0.54	0.87	0.59	0.54
C18:2 <i>cis</i> -9, 12	8	0.60	0.71	0.46	0.97	0.82	0.71
C18:2 <i>cis</i> -9, <i>trans</i> -11	—	—	—	—	—	—	—
Average correlation	—	0.50	0.52	0.53	0.83	0.80	0.80

¹MRY = Meuse-Rhine-Yssel, DF = Dutch Friesian, and GWH = Groningen White Headed.²For the traits fat (expressed in percentage) and individual FA and groups of FA (expressed in g/dL milk) the correlations between breeds are given of allele frequencies of SNP with an effect above the thresholds of a Bayes Factor of 1,000.³Number of SNP used to estimate the EBV depends on if the SNP are segregating in the breeds MRY, DF, or GWH.

et al., 2008). The LD between SNP on the HD SNP chip is indeed being maintained across breeds. However, several empirical studies have shown that the accuracy of across-breed genomic prediction, even when using the HD chip is poor for most traits (Harris et al., 2011; Erbe et al., 2012). Nevertheless, the GEBV reliabilities based on across-breed genomic prediction are expected to be higher for traits with few QTL with large effects, such as fat percentage (e.g., Hayes et al., 2009), and is likely to be higher for FA composition. Thus, if genomic variation associated with detailed milk fat composition in HF were similar to the genomic variation of the native dual-purpose breeds, GEBV reliabilities based on SNP effects estimated in HF were expected to be high. On the contrary, for traits where the genomic variation in HF was very different compared with the other breeds, GEBV reliabilities were expected to be low.

To get a better indication of the effect of individual SNP that have an increasingly larger effect on the traits in HF, GEBV were calculated using different subsets of SNP based on their BF. The highest BF used in this study were >1,000, which resulted in a subset of SNP that are very likely to have a strong association with the milk fat composition in HF. The reliabilities of GEBV in our study ranged from 0.000 to 0.758; however, reliabilities for the majority of traits were just above zero, indicating large differences in genomic variation associated with detailed milk fat composition between HF and native Dutch dual-purpose breeds. Comparing the native Dutch dual-purpose breeds, the GEBV calculated for the MRY breed were clearly the least reliable whereas for GWH they were most reliable, on average. This indicates that the genetic variation associated with milk fat composition was most different between MRY and HF and least different between GWH and HF.

SNP Effects

We identified 4 regions having a large effect on the FA composition and explaining more than 1% of the total genetic variation in the HF breed. Largest effect was found on BTA14 in the region of the *DGAT1* gene. Several genome-wide association studies and QTL-mapping studies based on the HF breed reported clear associations of the region of *DGAT1* on BTA14 with fat percentage and FA composition in the milk of HF cows (e.g., Grisart et al., 2002; Bouwman et al., 2011). The BTA26 harbors the *SCD1* gene, which is also reported having an effect on the milk FA composition (e.g., Mele et al., 2007; Schennink et al., 2008; Bouwman et al., 2011). This region was also identified as having an effect on the variability of the content of the FA C12:1 and C14:1 *cis*-9 in current study. A third region with a clear

effect on fat percentage, the group of FA C4:0 to C10:0, and some individual FA was found on BTA 5, where a peak on the Manhattan plot was shown at an SNP at base pair position 93,951,064. This position on BTA5 is close to the microsomal glutathione S-transferase 1 (*MGST1*) gene, which was found by Wang et al. (2012) to be associated with milk fat percentage.

GEBV, Allele Frequencies, and Genotypes

In our study, we hypothesized that the allele substitution effects estimated in HF can also be used for the other breeds. As for several traits in the other breeds, the reliability is clearly above 0 when only a small number of SNP are used with high BF; this hypothesis is likely to be valid at least in those cases. In the context of genomic prediction, the analyses performed can be regarded as across-breed genomic prediction, in the sense that the predicted breeds were not included in the reference population used to estimate the SNP effects. Our results suggest that across-breed genomic prediction for some of the traits studied is beneficial. In the literature, empirical studies on across-breed genomic prediction also reported greater-than-zero reliabilities for fat percentage, but generally close to zero reliabilities for other traits (Hayes et al., 2009; Pryce et al., 2011). Reliabilities in the current study based on SNP using BF >10, >100, or >1,000 were generally close to those using all SNP. This indicates that adding SNP with, for example, BF <10, merely adds noise rather than a signal with predictive ability. The sampling variance caused by this apparently lead to a few cases where the reliabilities with all SNP were considerably higher than using any of the subsets, and even higher than the HF reliabilities. As indicated, the nonzero reliabilities with high BF mostly resulted from 2 genes with large effect, *SCD1* and *DGAT1*. Therefore, our study provides an example where across-breed genomic prediction is beneficial. However, it should be noted that the results observed here are not generalizable. The traits studied here are partly affected by relatively few QTL with large effect, whereas traits included in current breeding goals are mostly polygenic by nature (Kemper and Goddard, 2012).

No relationship was observed between the correlations of allele frequencies and reliabilities of GEBV. However, we hypothesized that minor allele frequency at regions associated with milk FA composition have an effect on genetic variance explained by SNP and GEBV reliabilities. The minor allele frequencies of genes having an effect on the detailed milk FA composition in HF were shown to be much smaller in the breeds MRY, DF, and GWH, which is especially the case for the MRY breed. These lower minor allele frequencies for

MRY are the most likely explanation for the near zero observed reliabilities for MRY. This is also consistent with the earlier finding by Maurice-Van Eijndhoven et al. (2014) that the heritabilities for milk FA composition of the MRY breed were clearly smaller than those estimated for HF.

A reasonable explanation for these results could be the small variation in genotypes in the regions where QTL with large effect were found in HF (results are shown in Supplemental Tables S2 and S3; <http://dx.doi.org/10.3168/jds.2014-9196>). Despite the limited numbers of genotyped animals of the other breeds, a clear and consistent difference in the frequencies of genotypes was observed in the *DGAT1* region for the other breeds compared with HF. High frequencies of the major genotype and almost no cases of the minor genotype were also found in other populations of dual-purpose breeds (e.g., Thaller et al., 2003). Another explanation could be that the genetic variance of fat composition in milk of the Dutch dual-purpose cattle breeds is largely regulated by other and still unknown genomic regions.

CONCLUSIONS

Estimated SNP effects for FA composition based on HF data had low to no predictive ability in MRY, whereas they did have clear predictive ability for several traits for DF and GWH. The lack of predictive ability in MRY indicates that SNP effects estimated in HF explain less of the genetic variation of the FA composition in milk of MRY compared with the genetic variation of the milk fat composition in DF and GWH. Overall, most of the genomic variation associated with FA composition in the Dutch dual-purpose breeds appears to be breed-specific; however, overlap with variation in HF on average ranges from very small (MRY) up to ~16% (GWH) in terms of realized reliabilities using SNP effects estimated in HF. Furthermore, the minor allele frequencies of genes having an effect on the detailed milk FA composition in HF were shown to be much smaller in the breeds MRY, DF, and GWH, which is especially the case for the MRY breed.

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