Multitrait Quantitative Trait Loci Mapping for Milk Production Traits in Danish Holstein Cattle

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ABSTRACT

The aims of this study were (1) to confirm previously identified quantitative trait loci (QTL) on bovine chromosomes 6, 11, 14, and 23 in the Danish Holstein cattle population, (2) to assess the pleiotropic nature of each QTL on milk production traits by building multitrait and multi-QTL models, and (3) to include pedigree information on nongenotyped individuals to improve the estimation of genetic parameters underlying the random QTL model. Nineteen grandsire families were analyzed by single-trait (ST) and multitrait (MT) QTL mapping methods. The variance component-based QTL mapping model was implemented via restricted maximum likelihood (REML) to estimate QTL position and parameters. Segregation of the previously identified QTL was confirmed on bovine chromosomes 6, 11, and 14, but not on 23. A highly significant (1% chromosome-wise level) QTL was found on chromosome 6, between 37 and 73 cM. This QTL had a strong effect on protein percentage (PP) and fat percentage (FP) according to ST analyses, and effects on PP, FP, milk yield (MY), fat yield (FY), and protein yield (PY) in MT analyses. A QTL affecting PP was detected on chromosome 11 (at 70 cM) using ST analysis. The MT analysis revealed a second QTL (at 67 cM) approaching significance with an effect on MY. The ST analysis identified a QTL for MY and FP on chromosome 14, between 10 and 24 cM. The extended pedigree (nongenotyped animals) was included to estimate genetic parameters underlying the random QTL model; that is, additive polygenic and QTL variances. In general, the estimates of the QTL variance components were smaller but more precise when the extended pedigree was considered in the analysis.

Key words: QTL mapping, pleiotropy, multitrait analysis, milk production trait

INTRODUCTION

In dairy cattle, much effort has been undertaken to detect QTL and a number of chromosomes have been reported to harbor regions with significant effects for almost all the traits of economic importance. The QTL that map to regions containing likely candidate genes are of special interest. Candidate genes for milk production traits are found on *Bos taurus* autosome (BTA) 6, 11, 14, and 23 for κ -casein (**CSN3**), β -lactoglobulin (β -LG; Chung et al., 1998), diacylglycerol acyltransferase (**DGAT1**; Grisart et al., 2002), and prolactin (**PRL**; Chung et al., 1998), respectively. Quantitative trait loci have been identified on BTA6 and BTA14 affecting milk yield (**MY**), protein yield (**PY**), protein percentage (**PP**), fat yield (**FY**), and fat percentage (**FP**), and a few reports of QTL affecting mainly PP on BTA11 and BTA23 have been reported (reviewed by Khatkar et al., 2004).

The QTL are usually mapped for individual traits using single-trait (**ST**) analyses. Different traits, however, may be environmentally and genetically correlated. The genetic correlation may be due to the pleiotropic effect of a single QTL affecting more than one trait, or of linkage disequilibrium between 2 or more QTL, each affecting one trait only. When a QTL has a pleiotropic effect on 2 or more traits, a joint analysis involving both traits may result in a higher statistical power of detecting it, and in higher precision of the estimate of its map position (Jiang and Zeng, 1995; Knott and Haley, 2000; Sørensen et al., 2003). Multitrait (**MT**) QTL mapping allows decomposition of variances and covariances into polygenic and QTL components, which is relevant for selection purposes.

The results of previous QTL studies differ somewhat with respect to the number of QTL detected on a chromosome, their positions, and the extent to which the milk production traits are affected (Khatkar et al., 2004). Proper separation of variance components corresponding to QTL and polygenic parts is among the most important challenges in statistical analysis of data under a mixed inheritance model (Szyda et al., 2005). The granddaughter design, generally used for QTL mapping, is not suitable for estimation of variance com-

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ponents, due to confounding of genetic variance and residual variance within families. The only source of information available to separate the polygenic variance and residual variance is the differences in grandsires family means. Typically, only incomplete pedigree information is used in QTL; that is, only family links within grandsire families have been included in previous studies, leading to difficulties in separating polygenic variance from QTL variance in variance component-based methods. We expect that including more extensive pedigree information in the analyses will improve the estimates of parameters. The aims of this study were: 1) to test the hypothesis that QTL affecting milk production traits (MY, PY, FY, PP, and FP) are located on BTA 6, 11, 14, and 23, in the regions of candidate genes for κ -CN, β -LG, DGAT1, and PRL, respectively; 2) to build multitrait and multi-QTL models to assess the pleiotropic nature of each QTL and estimate QTL parameters relevant for selection purposes; and 3) to include the pedigree (nongenotyped) to estimate genetic parameters underlying the random QTL model; that is, additive polygenic and QTL variances.

MATERIALS AND METHODS

Experimental Data

Experimental Design. Nineteen Danish Holstein sires with 1,385 progeny-tested sons (ranging from 34 to 106 sons per sire) were analyzed using a granddaughter design (Weller et al., 1990). Each sire had between 70 and 100 daughters with records. Estimated breeding values were calculated using a BLUP ignoring family structure between sires. The EBV were obtained for 5 milk production traits (MY, PY, PP, FY, and FP) from the Danish Agricultural Advisory Service database. Details of methods and models used can be found on the World Wide Web (<http://www.lr.dk/kvaeg/diverse/principles.pdf>).

Genetic Markers. Sires and sons were genotyped for 5 markers on BTA6 (BM1329 at 35.5 cM; JMP36 at 52.4 cM; BM415 at 76.3 cM; BM4311 at 89.1 cM; and BL1038 at 122.3 cM), 7 markers on BTA11 (BM9067 at 9.5 cM; INRA131 at 38 cM; BM7169 at 41 cM; BMS710 at 67.5 cM; BMS989 at 85.4 cM; TGLA436 at 98.5 cM; and MB070 at 114.5 cM), 5 markers on BTA14 (RM180 at 19.1 cM; BM302 at 36.9 cM; BMS108 at 50.8 cM; BM4305 at 66.4 cM; and BL1036 at 78.7 cM), and 9 markers on BTA23 (CSSM005 at 7.2 cM; BM47 at 9.1 cM; UWCA1 at 22.1 cM; MB025 at 35.4 cM; MB019 at 36 cM; RM185 at 45.1 cM; BM1818 at 50.9 cM; BM1905 at 64.3 cM; and BM1443 at 67.1 cM). Marker positions were obtained from the USDA cattle marker map (<http://www.marc.usda.gov/genome>). Genotyping of sires and sons was carried out at the Danish Institute

of Agricultural Sciences, Research Center Foulum, Department of Genetics and Biotechnology.

Pedigree Information. We considered 2 types of pedigrees in the QTL analysis: restricted pedigree and extended pedigree. In the restricted pedigree, only the genotyped animals, that is, grandsires and sons, were considered and the grandsire families were assumed unrelated to each other. In the extended pedigree, all ancestors of grandsires and sons were traced back several generations until unknown parents were reached. This resulted in a pedigree with 6,571 animals (born as early as 1953) that were related to the genotyped grandsires and sons, and this whole pedigree of genotyped and nongenotyped animals was included in the extended pedigree analysis.

Statistical Analyses

Five milk production traits (i.e., MY, PY, PP, FY, and FP) were analyzed using both an ST and an MT QTL mapping procedure, based on the variance component method (Sørensen et al., 2003). In both QTL mapping procedures, traits were analyzed using linear mixed models that included the effects of multiple QTL. A detailed description of the ST and MT QTL mapping procedures is given below.

Linear Mixed Model. The traits were modeled using the following linear mixed model with n_q QTL:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\mu} + \mathbf{Z}\mathbf{u} + \sum_{i=1}^{n_q} \mathbf{W}_i \mathbf{q}_i + \mathbf{e},$$

where $\mathbf{y}$ is a vector of EBV calculated on t traits for each genotyped son, $\mathbf{X}$ is a known design matrix for the fixed effect that included the overall trait means ($\boldsymbol{\mu}$), $\mathbf{Z}$ is a known design matrix associating the observations with polygenic effects, $\mathbf{u}$ is a vector of polygenic effects, $\mathbf{W}$ is a known matrix associating the observations with the additive effects of the i th QTL represented by the vector $\mathbf{q}_i$, and $\mathbf{e}$ is a vector of random residual effects. The random variables $\mathbf{u}$, $\mathbf{q}_i$, and $\mathbf{e}$ are assumed to be multivariate normally distributed (MVN), and mutually uncorrelated. Specifically, $\mathbf{u}$ is MVN ($\mathbf{0}$, $\mathbf{G} \otimes \mathbf{A}$), $\mathbf{q}_i$ is MVN ($\mathbf{0}$, $\mathbf{K}_i \otimes \mathbf{IBD}_i$), and $\mathbf{e}$ is MVN ($\mathbf{0}$, $\mathbf{E} \otimes \mathbf{R}$). Matrices $\mathbf{G}$, $\mathbf{K}$, and $\mathbf{E}$ include variances and covariances among the traits due to polygenic effects, additive QTL effects and residuals effects. The symbol $\otimes$ represents the Kronecker product. The $\mathbf{A}$ is the additive relationship matrix that describe the covariance structure among the polygenic effects, $\mathbf{IBD}_i$ is the identity by descent (IBD) matrix that describes the covariance structure among the effects for the i th QTL, and $\mathbf{R}$ is a diagonal matrix with n_j^{-1} on the diagonals (n_j is the number of daughters of the son j).

Table 1. Parameters in the 7 different models for traits 1, 2, and 3 in the two 3-trait combinations¹

Model	QTL parameters	Cov ₁₁ ²	Cov ₁₂	Cov ₁₃	Cov ₂₂	Cov ₂₃	Cov ₃₃
MT ₁₂₃	6	*	*	*	*	*	*
MT ₁₂	3	*	*		*		
MT ₁₃	3	*		*			*
MT ₂₃	3				*	*	*
MT ₁	1	*					
MT ₂	1				*		
MT ₃	1						*

¹MT = Multitrait model; subscripts indicate the traits affected by the QTL in the trivariate model.

²Cov_{ij} = Covariance between QTL effects on traits i and j.

IBD Matrices. The elements in the IBD matrix are a function of the marker data and the position (p) of a putative QTL on the chromosome. Here we used the most likely marker linkage phase in the sire and computed the IBD matrix using a recursive algorithm (Wang et al., 1995; Sørensen et al., 2003). The IBD matrices were computed for every 3 cM along the chromosomes and used in the subsequent variance component estimation procedure.

Estimation of Model Parameters. The variance components were estimated using the average information restricted maximum likelihood algorithm (AIREML; Jensen et al., 1997). The restricted likelihood was maximized with respect to the variance components associated with the random effects in the model. Maximizing a sequence of restricted likelihoods over a grid of specific positions yields a profile of the restricted likelihood of the QTL position (Sørensen et al., 2003). Parameters were estimated along the whole marked chromosome region every 3 cM with the restricted pedigree. For the extended pedigree, parameters were estimated for the most probable QTL location obtained by model using a restricted pedigree.

QTL Mapping Procedure. Single-trait and MT analyses were performed on the 5 production traits. Initial analyses using a two-dimensional scan for each chromosome-by-trait combination showed no evidence for 2 QTL segregating on any of the 4 chromosomes (results available on request). Therefore, all models used herein only fit one QTL on each chromosome. In the ST analyses, each chromosome was tested for the presence of a QTL affecting each of the 5 traits.

The MT QTL mapping procedure was based on 2 trivariate models. The first trivariate model included MY, PP, and FP, and the second trivariate model included MY, PY, and FY. For each trait combination, the following 3 steps were conducted:

Step 1. For each chromosome a sequence of models were fitted to assess if the QTL affected 1, 2, or 3 traits (as presented in Table 1). In the

fitted models, the QTL affected each trait separately, all combinations of 2 traits, or all 3 traits. Each model always included the fixed effect of mean and random polygenic and residual effects, but differed in which traits were affected by the QTL.

Step 2. Fix the selected model and position for the most significant QTL and run all models in Table 1 to search for the next QTL on the remaining chromosomes.

Step 3. Repeat step 2 until no new QTL is identified.

Test Statistics. Hypothesis tests for the presence of QTL were based on the asymptotic distribution of the likelihood ratio test (LRT) statistic, $LRT = -2\ln(L_{\text{reduced}} - L_{\text{full}})$, where L_{reduced} and L_{full} were the maximized likelihoods under the reduced and full models, respectively. The reduced model always excluded the QTL effect for the chromosome being analyzed. Thresholds were calculated using the method presented by Piepho (2001). This method is an alternative to permutation procedures and is applicable in complex situations. It requires the LRT from each of the putative QTL positions along the chromosome, the number of chromosomes, the degrees of freedom (df) for the LRT ($df = \text{number of parameters of } H_{\text{full}} - \text{number of parameters of } H_{\text{reduced}}$), and the chromosome-wise type I error rate (α). The test statistics were evaluated at 2 threshold levels of $\alpha = 0.01$ and 0.05 .

RESULTS

ST Analysis

Five significant QTL were detected by 1-trait–1-QTL analysis (Table 2), because QTL for FP were located on BTA6 and BTA14, for PP on BTA6 and BTA11, and for MY on BTA14. No QTL with significant effects on any of the 5 traits was observed on BTA23. Likelihood profiles for all traits and chromosomes are shown in Figure 1.

Table 2. The likelihood ratio tests (LRT) for QTL effects found by single-trait analysis on *Bos taurus* autosome (BTA) 6, 11, 14, and 23

Trait ¹	BTA6		BTA11		BTA14		BTA23	
	Position	LRT	Position	LRT	Position	LRT	Position	LRT
MY	40	4.3	67	0.1	15	7.7*	67	0.5
PY	31	2.2	106	0.1	15	3.8	34	0.4E-4
FY	31	0.7	10	0.6	42	1.6	22	0.7
PP	46	26.2**	70	6.0*	21	4.8	34	3.0
FP	46	15.5**	106	0.8	24	7.1*	67	0.9

¹MY = Milk yield, PY = protein yield, FY = fat yield, PP = protein percentage, FP = fat percentage.
P* < 0.05; *P* < 0.01.

MT Analysis

Three-Trait Model with MY, PP, and FP. In step 1 of the mapping procedure using a 3-trait–1-QTL model, BTA6 showed the most significant results (see Table 3

and Figure 2). Three different models had significant QTL, rejecting the null hypothesis of no QTL segregating. The effect was significant at the 5% level when the QTL was modeled only to affect PP, but increased to 1% significance when the QTL was assumed to affect

Figure 1. Quantitative trait loci results on *Bos taurus* autosome (BTA) 6, 11, 14, and 23 from single-trait analysis for fat yield (—), fat percentage (■), milk yield (▲), protein yield (●), and protein percentage (×), and 5% thresholds for protein percentage (----), fat percentage (—), and milk yield (— · · —). The x-axis indicates the chromosomal position in morgans and the y-axis shows the likelihood ratio test (LRT) statistics.

Table 3. Position estimates and likelihood ratio test statistics (LRT) for 7 multitrait models including milk yield (MY), protein percentage (PP), and fat percentage (FP) for each of the 4 *Bos taurus* autosome (BTA) for percentage traits

Trait	BTA6		BTA11		BTA14		BTA23	
	Position	LRT	Position	LRT	Position	LRT	Position	LRT
MY	34	1.8	10	0.2	24	1.7	34	0.1
PP	70	10.6*	67	7.1*	27	1.3	34	1.2
FP	109	1.8	10	0.3	30	2.6	10	2
MY+PP	70	11.0	67	9.6	18	3.1	34	1.2
MY+FP	28	2.6	10	0.6	15	6.0	10	1.7
PP+FP	67	17.6**	NC ¹	—	42	4.6	NC	—
MY+PP+FP	58	28.2**	67	8.3	15	11.3	NC	—

¹Likelihood did not converge to a maximum.

* $P < 0.05$; ** $P < 0.01$.

PP and FP, or PP, FP, and MY. One cannot make a proper LRT to compare these 3 competing models, but the QTL likely affected all 3 traits, which was the assumption underlying the subsequent models.

The MT likelihood profile (Figure 2) is very similar to the ST profile for PP (Figure 1) and the LRT reached a maximum between the markers BM1329 (35.5 cM) and BM4311 (89.1 cM). In step 1, the QTL on BTA11 was significant ($P < 0.05$) only for PP (Table 3). This

QTL was located between the markers BM7169 (42 cM) and BMS989 (85.4 cM), and the highest LRT was observed at 70 cM, close to the marker BMS710 (67.5 cM). The step 1 analyses did not reveal a significant QTL on either BTA14 or 23.

In step 2 of the mapping procedure, a 3-trait–2-locus model was built based on the results from the 3-trait–1-QTL model. The most significant QTL position on BTA6 was fixed at 58 cM and the analysis searched for

Figure 2. Three-trait [milk yield (MY), fat percentage (FP), and protein percentage (PP)]–1-QTL model on *Bos taurus* autosome (BTA) 6. Quantitative trait loci with effects on FP (□), MY (△), PP (×), FP and MY (○), PP and MY (■), PP and FP (●), and MY, PP, and FP (▲) and 5% thresholds for model with effects on PP (—), for model with effect on PP+FP (— · —), and for model with effect on all 3 traits (····). The x-axis indicates the chromosomal position in morgans and the y-axis shows the likelihood ratio test (LRT) statistics.

Table 4. Position estimates and likelihood ratio test statistics (LRT) for the 7 multitrait models including milk yield (MY), protein percentage (PP), and fat percentage (FP) for *Bos taurus* autosome (BTA) 11 and 14 when a QTL at 58 cM on BTA6 was assumed to affect MY, PP, and FP

Trait	BTA11		BTA14	
	Position	LRT	Position	LRT
MY	117	0.4	22	2.1
PP	67	8.8*	30	2.0
FP	117	0.7	30	2.6
MY+PP	NC ¹	—	NC	—
MY+FP	117	0.4	15	5.9
PP+FP	NC	—	NC	—
MY+PP+FP	NC	—	18	11.8

¹Likelihood did not converge to a maximum.

* $P < 0.05$.

QTL on each of the remaining chromosomes (Table 4 and Figure 3). The QTL on BTA11 was observed at the same position (67 cM) for PP, with LRT = 8.8, which

was significant at the 5% threshold level. However, the putative QTL on BTA14 was not significant in the 3-trait–2-locus model. In some cases, convergence problems were observed when the QTL was assumed to affect more than one trait.

Three-Trait Model with MY, PY, and FY. In step 1 of the mapping procedure, BTA6 showed the most significant results for MY, FY, and PY with the 3-trait–1-QTL model analysis (Table 5 and Figure 4). A QTL with significant effects on all 3 yield traits was found between markers BM1329 and BM4311. Six different models had significant QTL effects. The most significant model in which the QTL was assumed to affect a single trait was for MY. The greatest LRT was obtained by assuming that the QTL affected both MY and FY. In contrast, the LRT increased only slightly when PY was considered with MY. Therefore, the subsequent models were based on the assumption that the QTL directly affected MY and FY.

Figure 3. Three-trait [milk yield (MY), fat percentage (FP), and protein percentage (PP)]–2-QTL (fixing the QTL on BTA6) model on BTA11. Quantitative trait loci effects on FP (□), MY (△), PP (×), FP and MY (■) and the 5% threshold for model with effect on PP (—). The x-axis indicates the chromosomal position in morgans and the y-axis shows the likelihood ratio test (LRT) statistics.

Table 5. Position estimates and likelihood ratio test statistics (LRT) for 7 multitrait models including milk yield (MY), protein yield (PY), and fat yield (FY) for each of the 4 *Bos taurus* autosomes (BTA) for yield traits

Trait	BTA6		BTA11		BTA14		BTA23	
	Position	LRT	Position	LRT	Position	LRT	Position	LRT
MY	58	22.5**	67	9.1*	15	3.3	34	3.2
PY	70	10.3*	67	7.2	24	1.3	34	1.3
FY	112	1.9	10	0.4	30	2.3	10	1.9
MY+PY	58	23.6**	67	9.8	15	10.7	34	3.2
MY+FY	58	27.9**	76	6.0	24	6.8	34	3.6
PY+FY	67	17.4*	67	7.2	15	4.8	34	3.8
MY+PY+FY	58	28.3**	67	9.9	21	10.9	34	4.3

* $P < 0.05$; ** $P < 0.01$.

A significant ($P < 0.05$) QTL for MY was detected at position 67 cM of BTA11 with this model (Table 5). The QTL on BTA11 showed an additional effect on PY, which approached the 5% significance threshold. The QTL affecting MY and PY on BTA14 was just below the 5% significance threshold.

In step 2 of the mapping procedure, a 3-trait–2-locus model was built based on the results from the 3-trait–1-QTL model. The resulting 3-trait–2-locus model (with

a fixed QTL at 58 cM on BTA6) detected a significant QTL for MY on BTA11 (Table 6 and Figure 5). The QTL was mapped between BM7169 (41 cM) and BMS989 (85.4 cM) with the peak at 67 cM.

In step 3, a 3-trait–3-QTL model with fixed positions for the QTL on BTA6 and BTA11 was used to scan for QTL on BTA14. A pleiotropic QTL effecting MY and PY on BTA14 approached significance (LRT statistics of 10.52 vs. threshold 11.44 at the 5% significance level).

Figure 4. Three-trait [milk yield (MY), fat yield (FY), and protein yield (PY)]–1-QTL model on BTA6. Quantitative trait loci effects on FY (○), PY (■), MY (△), FY and PY (●), MY and PY (□), MY and FY (▲), and all 3 traits (×) and 5% thresholds for model with effect on MY (—), for model with effect on MY+FY (— · — · —), and for model with effect on all 3 traits (----). The x-axis indicates the chromosomal position in morgans and the y-axis shows the likelihood ratio test (LRT) statistics.

Table 6. Position estimates and likelihood ratio test statistics (LRT) for 7 multitrait models including milk yield (MY), protein yield (PY), and fat yield (FY) for *Bos taurus* autosome (BTA) 11 and 14 when a QTL at 58 cM on BTA6 is assumed to affect MY and FY

Trait	BTA11		BTA14	
	Position	LRT	Position	LRT
MY	65	11.3*	18	4.0
PY	69	8.3	30	1.6
FY	77	0.1	30	1.9
MY+PY	65	11.7	10	11.1
MY+FY	65	11.3	26	7.1
PY+FY	69	8.3	42	5.4
MY+PY+FY	41	0.3	22	11.3

* $P < 0.05$.

Estimation of Variance Components

Estimates (based on REML) of QTL, polygenic, and residual variances and covariances were obtained at the most likely QTL positions of all the significant QTL. Table 7 shows that the significant QTL generally explained a greater proportion of the genetic variance with the restricted pedigree (10 to 40%) than with the

extended pedigree (2.4 to 13.8%) in the 1-trait–1-QTL analysis. For all traits, the polygenic variance increased when the extended pedigree was used, but the residual variance decreased. The standard errors of the estimates were generally lower when the extended pedigree was used. The QTL on BTA6 explained 27% of the total genetic variance for FP and 40% of PP when the restricted pedigree was used. With the extended pedigree, the genetic variance explained by this QTL decreased to 11% for FP and 13.8% for PP. The QTL on BTA11 explained 11.4% of the total genetic variance on PP with the restricted pedigree, but only 2.4% with the extended pedigree. The QTL on BTA14 explained 18 and 10% for MY and FP, respectively, using the restricted pedigree, but these values dropped to 4.3 and 4.5% with the extended pedigree.

MT Analysis for Percentage Traits. The QTL located on BTA6 explained 17% of genetic variance share for MY, 20% for FP, and 31% for PP with the restricted pedigree (Table 8). However, with the extended pedigree, these proportions decreased to 4, 10, and 14%, respectively (Table 9). The best model fit was observed

Figure 5. Three-trait [milk yield (MY), fat yield (FY), and protein yield (PY)]–2-QTL (fixing the QTL on BTA6) model on BTA11. Quantitative trait loci effects on FY (○), PY (■), MY (△), FY and PY (●), MY and PY (□), MY and FY (▲), and all 3 traits (×) and 5% threshold for model MY (----). The x-axis indicates the chromosomal position in morgans and the y-axis shows the likelihood ratio test (LRT) statistics.

Table 7. Variance component estimate in the 1-trait–1-QTL model including genotyped animals (restricted pedigree) and both genotyped and nongenotyped animals in the pedigree (extended pedigree)

BTA ¹	Trait ²	With restricted pedigree			With extended pedigree		
		QTL	Polygene	Residual	QTL	Polygene	Residual
6	FP	0.0047 ± 0.0023	0.0125 ± 0.0028	0.0028 ± 0.0001	0.0021 ± 0.001	0.0167 ± 0.0016	0.0017 ± 0.0008
	PP	0.00105 ± 0.00044	0.00156 ± 0.00071	0.00098 ± 0.00043	0.00049 ± 0.00021	0.0031 ± 0.0031	0.00028 ± 0.00013
11	PP	0.00034 ± 0.00022	0.0026 ± 0.0005	0.00074 ± 0.00034	0.00009 ± 0.00011	0.0036 ± 0.0003	0.00026 ± 0.00013
14	FP	0.0017 ± 0.0011	0.0156 ± 0.0022	0.0026 ± 0.0013	0.00084 ± 0.00067	0.0179 ± 0.0016	0.0019 ± 0.0008
	MY	6,529 ± 4,370	25,101 ± 7,758	29,804 ± 4,977	2,225 ± 1,892	50,177 ± 5,229	13,197 ± 2,990

¹BTA = *Bos taurus* autosome²MY = Milk yield, PP = protein percentage, FP = fat percentage.

when a QTL affecting all 3 traits (MY, FP, and PP) was considered in the model (Table 3).

MT Analysis for Yield Traits. In step 1 for the trivariate model with MY, FY, and PY, the final model chosen was one for which QTL for MY and FY and for MY were assumed to be on BTA6 and 11, respectively. The parameters for this model were estimated with a restricted pedigree (Table 10) and with an extended pedigree (Table 11). The trends in parameter estimates with the 2 types of pedigree were similar to those observed earlier with the trivariate model for percentage traits. For example, the QTL on BTA6 explained 17% of the total genetic variance of MY with the restricted pedigree, which dropped to 5% with extended pedigree. The genetic proportion of the total variance increased for all 3 traits when the extended pedigree was used and the residual (co)variances decreased markedly. The proportion of genetic variance explained by the QTL was always lower. Sampling variances of parameter estimates were generally smaller with extended pedigree analyses.

DISCUSSION

Quantitative trait loci affecting milk production traits were found on BTA6, BTA11, and BTA14, but not on BTA23 in the Danish Holstein cattle population. The results were generally in good agreement with the literature, but only the QTL on BTA14 was located near

the corresponding candidate gene (DGAT1) and showed similar effects. The QTL effects identified in ST analyses on BTA6 (effect on PP and FP) and BTA11 (effect on PP) were confirmed in MT analysis with a greater value for the test statistic. Moreover, additional QTL effects on BTA6 (MY and FY) and BTA11 (MY) were detected due to the advantage of MT analysis. The QTL effects found in ST analysis on BTA14 were not confirmed in MT analysis. The candidate gene, DGAT1 (affecting FP and MY), is located in the proximal region of BTA14. However, in the present study the first marker genotyped on BTA14 was at 19.1 cM. This may be the reason for not detecting a significant QTL effect on this chromosome in MT analysis.

In this study we fitted multivariate models with 3 traits (MY, PP, and FP; and MY, PY, and FY). We expected to find pleiotropic QTL affecting these traits based on QTL effects and candidate gene effects previously found in other studies on chromosomes 6, 11, 14, and 23. An MT model with all 5 traits was not applied because of possible convergence difficulties and high computational demand associated with estimating the covariances among the traits. Even with the 3-trait models some convergence problems were observed when the QTL was assumed to affect more than one trait. The problems occurred when a QTL was assumed to have a pleiotropic effect on a trait for which it explained very little variance, which resulted in parameters near the boundary of the parameter space, like

Table 8. Variances and covariances of final model for yield traits with restricted pedigree QTL on *Bos taurus* autosome (BTA) 6 affecting milk yield (MY), fat percentage (FP), and protein percentage (PP)

Trait	QTL _{BTA6}			Polygenic			Residual		
	FP	MY	PP	FP	MY	PP	FP	MY	PP
FY	0.0038 ± 0.0017			0.0149 ± 0.0019			0.0016 ± 0.0007		
MY	−4.71 ± 2.09	6,145 ± 3,356		−11.08 ± 2.66	30,038 ± 5,596		−4.55 ± 1.68	26,570 ± 4,974	
PP	0.0018 ± 0.0008	−2.49 ± 1.04	0.001 ± 0.0004	0.0038 ± 0.0008	−4.19 ± 1.32	0.0022 ± 0.0004	0.0005 ± 0.0003	−2.70 ± 0.90	0.0005 ± 0.0002

Table 9. Estimates of variances and covariances from the final model for dairy production traits with extended pedigree and QTL on chromosome 6 affecting milk yield (MY), fat percentage (FP), and protein percentage (PP)

Trait	QTL			Polygenic			Residual		
	FP	MY	PP	FP	MY	PP	FP	MY	PP
FP	0.002 ± 0.001			0.017 ± 0.002			0.0014 ± 0.0007		
MY	-2.05 ± 1.38	2,297 ± 2,744		-16.61 ± 2.74	51,922 ± 8,013		-1.83 ± 1.29	12,084 ± 4,404	
PP	0.0009 ± 0.0004	-1.04 ± 0.66	0.0005 ± 0.0002	0.0052 ± 0.0006	-7.79 ± 1.34	0.0032 ± 0.0003	0.0001 ± 0.0002	-0.89 ± 0.63	0.0002 ± 0.0001

QTL variances near zero in combination with QTL correlations near unity. Because the convergence problems were usually experienced when estimates of QTL were extremely small, they were unlikely to have affected the conclusions. The QTL on BTA6 showed significant effects on almost all milk production traits. Strong effects on PP and FP were found in ST analyses and subsequently confirmed in the MT analyses. In comparison, the MT analysis showed an increase in the fraction of the overall genetic variance explained by the QTL for MY, but a lower proportion of the genetic variance explained for PP and FP. Khatkar et al. (2004), using meta-analysis, reported the presence of 2 QTL for MY on BTA6, one at 49 ± 5 cM (within the QTL peak of our finding) and another at 87 ± 7.9 cM. With ST analyses we found strong QTL effects on PP and FP at 45 cM and with MT analysis, the peak moved to 58 cM. Freyer et al. (2002) also detected QTL with an effect on PP at similar position 46 cM in German Holstein-Friesian cattle. Olsen et al. (2005) mapped a QTL on BTA6 affecting milk production traits within a distance of 420 kb and the QTL was located at 48.3 cM (as per MARC, USDA map). Schnabel et al. (2005) concluded that there are several milk-trait QTL located at 57 to 68 cM on BTA6 affecting MY, PY, PP, and FP. They identified the OPN3907 mutation in the osteopontin gene as the causal mutation underlying the PP QTL located in this region. In the present study, ST analyses were highly significant in the same region. For PP, LRT was 26.2 and for FP, it was 15.5.

The MT analyses, which take advantage of the correlation structure of the traits (Jiang and Zeng, 1995; Sørensen et al., 2003), helped to detect QTL effects on BTA6 that were not detected in ST analyses. When the QTL was modeled to affect only MY in the MT analysis including MY, FP, and PP, its effect was not significant, but its effect became significant when it was assumed to affect all 3 traits. This result could be caused by the influence of FP and PP, which are negatively correlated with MY and might cause the partitioning of part of QTL variance of MY into the polygenic effect. This phenomenon of MT analysis was also noticed in the evaluation of the other chromosomes. The QTL on BTA6 was located to a wider area (37 to 73 cM) and the peaks of most QTL effects on traits and trait combinations were concentrated within 57 to 65 cM, which implies the possibility of one pleiotropic QTL or closely linked pleiotropic QTL. Similarly, Freyer et al. (2003) reported a pleiotropic QTL at 68 cM with effects on FY and PY. Although we found a QTL with similar effects as the candidate gene for CSN3, the QTL was not situated in the area of this candidate gene. However, consistent with van Tassel et al. (2000), BTA6 provides strong evidence for QTL effects on multiple yield traits.

The QTL effect on PP was detected on BTA11 in ST analysis and subsequently confirmed with a higher value of the test statistic in MT analysis. Location of this QTL was very stable (between 65 and 69 cM). The QTL on BTA11 was not situated in the area of the candidate gene for LGB, but it showed a significant

Table 10. Estimates of variances and covariances from the final model for yield traits with restricted pedigree and QTL on chromosome 6 affecting milk yield (MY) and fat yield (FY) and a QTL on chromosome 11 affecting MY

Trait	QTL ₆		QTL ₁₁	Polygenic			Residual		
	FY	MY	MY	FY	MY	PY	FY	MY	PY
FY	4.02 ± 2.25			72.40 ± 6.45			24.34 ± 4.23		
MY	-106.63 ± 50.67	6,047.34 ± 1,812.58	2,996.16 ± 1,149.43	291.48 ± 138.50	26,883.49 ± 4,792.14		703.17 ± 97.07	26,860.50 ± 3,279.78	
PY ¹	—	—	—	21.58 ± 3.45	655.10 ± 101.42	25.04 ± 2.84	17.09 ± 2.50	660.49 ± 76.65	17.36 ± 2.12

¹PY = Protein yield.

Table 11. Estimates of variances and covariances from the final model for yield traits with an extended pedigree and QTL on chromosome 6 affecting milk yield (MY) and fat yield (FY) and a QTL on chromosome 11 affecting MY

	QTL ₆		QTL ₁₁	Polygenic			Residual		
Trait	FY	MY	MY	FY	MY	PY	FY	MY	PY
FY	3.22			87.29			17.47		
	± 2.53			± 9.24			± 5.18		
MY	−69.41	2,826.75	813.34	768.60	50,447.80		330.25	12,113.2	
	± 42.52	± 1,207.08	± 684.03	± 163.09	± 4,906.68		± 93.60	± 2,750.22	
PY ¹	—	—	—	33.85	1,084.84	36.01	8.85	325.51	9.98
				± 4.53	± 117.36	± 3.43	± 2.65	± 69.32	± 2.03

¹PY = Protein yield.

effect on PP consistent with QTL findings of Mosig et al. (2001) in the Israeli Holstein cattle population. The effect on MY was significant only in MT analysis including yield traits (i.e., FY and PY along with MY), but was not significant in ST analysis and in MT analysis including MY, PP, and FP. This result shows that the advantage of MT analyses may vary with the correlation structure between the traits, as previously observed by Evans (2002) and Sørensen et al. (2003). When the QTL effect on one trait is high, MT analysis may have only a limited advantage.

The QTL on BTA14 showed a significant effect on FP and MY in a 1-trait–1-QTL analysis. It corresponded in location and effect to the candidate gene DGAT1 (Grisart et al., 2002; Winter et al., 2002). Contrary to our expectation, no effect of this QTL was significant in MT analyses. Only the joint QTL effect on MY and PY showed significance at 10%. Coppieters et al. (1998) also detected a QTL close to the centromere with effects on MY, FP, and PP in the Holstein-Friesian population whereas Ashwell et al. (2001) reported pleiotropic QTL affecting FY and FP in the US Holstein population.

The extended pedigree was used for QTL analysis to get more precise parameter estimates. The estimated proportion of genetic variance increased for all traits with the extended pedigree. This increase may be due to more precise partitioning of polygenic variance components, because the relationship among grandsires and sons in different grandsire families was accounted for. The restricted pedigree approach assumed the grandsires were not related, which was true in most cases. The residual variances decreased with the extended pedigree. However, the QTL contribution to genetic variance also decreased when the extended pedigree was used. The estimate of the QTL effect may be inflated with the restricted pedigree because of poor separation of polygenic and QTL variances. The QTL component in the total genetic variance decreased with increasing distance from the most probable QTL position. In contrast, the residual component was not confounded with genetic components, because its estimates

remained stable along the chromosome. The estimates of variance components were more reliable (smaller sampling variance) when the extended pedigree was used. Szyda et al. (2005) observed that only 4% of the genetic variance of first-lactation MY was explained by the QTL located on BTA6 in the German Holstein population when using the full pedigree (including both genotyped and nongenotyped animals in the analysis). They also reported that 6 and 4% of the genetic variance of FY and PY, respectively, were explained by this QTL. Their low estimates for QTL variance for BTA6 when using a full pedigree provides additional evidence that our estimates with the restricted pedigrees may have been inflated.

CONCLUSIONS

Highly significant pleiotropic QTL affecting PP, FP, MY, and FY in a Danish Holstein cattle population were found on BTA6. A significant QTL with effects on PP and MY was detected on BTA11, and a putative QTL affecting FP, MY, and PY was found on BTA14. When including an extended pedigree in the QTL analyses we observed that (1) the genetic variance explained by the QTL was lower compared with analysis with a restricted pedigree, and (2) sampling variances of parameter estimates were smaller. The detection of pleiotropic QTL affecting milk production traits as well as better understanding of the background of the genetic correlations enable one to improve breeding procedures. The results provide information that can be used for improved MT evaluation of breeding values that account for QTL effects.

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