Interval and Composite Interval Mapping of Somatic Cell Score, Yield, and Components of Milk in Dairy Cattle

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ABSTRACT

Single-marker, interval-mapping (IM) and composite interval mapping (CIM) were used to detect quantitative trait loci (QTL) controlling milk, fat and protein yields, and somatic cell score (SCS). A granddaughter design was used to combine molecular genetic information with predicted transmitting abilities (PTA) and estimated daughter yield deviations (DYD) from eight Dairy Bull DNA Repository Holstein families. Models that included and excluded weights accounting for the uncertainty of the response variable were evaluated in each trait, family and phenotype (DYD and PTA) combination. The genotypic information consisted of 174 microsatellite markers along 29 *Bos taurus* autosomes. The average number of informative markers per autosome was three and the number of informative sons per family and marker varied between 21 and 173. Within-family results from the least squares single-marker analyses were used in expectation-maximization likelihood IM and CIM implemented with QTL Cartographer. Different CIM model specifications, offering complementary control on the background QTL outside the interval under study, were evaluated. Permutation techniques were used to calculate the genome-wide threshold test statistic values based on 1000 samples. Results from the DYD and PTA analyses were highly consistent across traits and families. The minor differences in the estimates from the models that accounted for or ignored the uncertainty of the DYD (variance) and PTA (inverse of reliability) may be associated to the elevated and consistent precision of the DYD and PTA among sons. The CIM model best supported by the data had 10 markers controlling for background effects. On autosome (BTA) three, a QTL at 32 cM influencing protein yield was located in family five and a QTL at 74 cM for fat yield was located in family eight. Two map positions associated with SCS were detected on BTA 21, one at 33 cM in family one and the other

at 84 cM in family three. A QTL for protein yield was detected between 26 and 36 cM on BTA six, family six, and a QTL for milk yield was detected at 116 cM on BTA seven in family three. The IM and CIM approaches detected a QTL at 3 cM on BTA 14 influencing fat yield in family four. Two map positions on BTA 29 were associated with significant variation of milk (0 cM) and fat yield (14 cM) in family seven. These results suggest the presence of one QTL with pleiotropic effects on multiple traits or multiple QTL within the marker interval. Findings from this study could be used in subsequent fine-mapping work and applied to marker-assisted selection of dairy production and health traits.

(Key words: composite interval mapping, outbred population, quantitative trait loci, weighted analysis)

Abbreviation key: BTA = *Bos Taurus* autosome, CMI05, CMI10, CMI20 = Composite interval mapping with five, ten and 20 background markers, DYD = Daughter yield deviation, IM = Interval mapping, PTA = Predicted transmitting ability, QTL = Quantitative trait loci.

INTRODUCTION

Many studies have identified quantitative trait loci (QTL) associated with the genetic variation of complex dairy traits (Georges et al., 1995; Spelman et al., 1996; Ashwell et al., 1998b; Zhang et al., 1998; Heyen et al., 1999). These studies have used different phenotypic and genetic data and a variety of statistical methods, from single marker to interval mapping and variance components within and across families.

The interval mapping (IM) approach uses information from consecutive informative markers simultaneously to estimate the QTL location and effect (Knott et al., 1996). A disadvantage of IM is that QTL outside the interval under consideration could lead to false positive or negative results. The composite interval mapping (CIM) approach can address these limitations, integrating interval mapping with information from multiple markers outside the interval (Jansen, 1993; Zeng, 1993). The additional markers account for phenotypic variation due to linked QTL outside the interval, hence

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reducing the residual variance and augmenting the precision of estimates. The CIM approach is not commonly used in outbred populations (i.e. dairy cattle) due to the variable information content along the genome and inconsistent linkage phase among families (Hoeschele et al., 1997). Furthermore, different specifications of the CIM model can lead to substantial changes in the number of false positive results and power of the analysis.

In addition to the molecular genetic information, the phenotypic indicators studied may influence the ability to detect QTL. In dairy cattle populations, two commonly used phenotypic indicators are the daughter yield deviation (**DYD**; Georges et al., 1995; Spelman et al., 1996; Ashwell et al., 1998b) and the predicted transmitting ability (**PTA**; Ashwell et al., 1998a; Zhang et al., 1998). The PTA includes the deviation of the daughter performance from the population mean adjusted for the genetic merit of the mate (i.e. DYD) and the genetic merit of the grandsire and granddam (VanRaden and Wiggans, 1991). The ability to detect QTL can be influenced by the indicator used and the associated uncertainty. Likewise, the genome-wide significance level and the consequent comparison-wide level used affect the number of QTL identified. The more stringent the significance probability threshold, the lower the probability to claim false positive results but the lower the probability to identify true positive (power) associations. The main goal of this study was to implement interval mapping and composite interval mapping in an outbred population. The supporting objectives were, 1) to evaluate the feasibility and adequacy of using readily available software for line crosses in outbred populations, 2) to assess the impact of using different phenotypic indicators in QTL mapping and, 3) to evaluate the influence of accounting for the uncertainty of the phenotypic indicators in QTL mapping. Single-marker, IM and CIM approaches were implemented using milk and composite production and somatic cell score indicators from a US Holstein population.

MATERIALS AND METHODS

Data

Eight Holstein grandsires from the Dairy Bull DNA Repository (Heyen et al., 1999) with phenotypic and molecular genetic data following a granddaughter design (Geldermann, 1975; Weller et al., 1990) were studied. The genetic marker information on grandsires and their sons was combined with the phenotypic indicators of the sons. The dams were assumed to have one progeny, to be a random sample from the population and unrelated to each other and to the males. Two of the

Table 1. Total number of sons, mean and standard deviation of daughter yield deviation (DYD) and predicted transmitting ability (PTA).

Trait and indicator	N ¹	Mean	SD ²
Milk yield DYD	1065	355.41	662.30
Fat yield DYD	1065	18.00	24.69
Protein yield DYD	1065	13.03	19.06
SCS DYD	1017	-0.01	0.22
Milk yield PTA	1065	391.47	605.54
Fat yield PTA	1065	19.39	22.36
Protein yield PTA	1065	14.68	17.32
SCS PTA	1065	3.18	0.17

¹Number of sons.

²Standard deviation.

grandsires were half-sibs but were assumed to be unrelated. The marker information is the same used by Heyen et al. (1999), and consisted of 174 microsatellite markers on 29 *Bos Taurus* autosomes (**BTA**) in 1065 sons. The number of sons with traceable allele inheritance varied with marker and ranged between 21 and 173. The average interval between markers, based on Kosambi's relative distances computed using CRIMAP 2.4 (Heyen et al., 1999) was 20 cM long and the number of markers per autosome varied between two to 11. The average length of the informative interval across the genome was 70 cM, and ranged from 52 to 81 cM. The phenotypic indicators (based on their daughter records and parental information) were the DYD and PTA (Animal Improvement Programs Laboratory, 2000) estimates for milk, fat and protein yields, somatic cell score ($SCS = \log_2(\text{cells}/100) + 3$), and associated number of daughters and reliability. The numbers of DYD and PTA records were 1016 and 1065, respectively, with missing DYD due to insufficient number of daughters to compute these estimates and corresponding PTA solely based on pedigree information. In the current study, 2000 genetic-evaluation based phenotypes were used. These records differ from the 1997 genetic-evaluation phenotypes studied by Heyen et al. (1999). The number of informative sons per marker and family ranged between 21 and 173. Statistical tests failed to reject the null hypothesis of normality for all the traits. The mean and standard deviation of DYD and PTA by trait and the number of informative sons are summarized in Table 1.

Models and Methods

In a granddaughter design, the within-family genotypic structure can be viewed as a backcross design in inbred line crosses. Assuming that the sons' dams are the backcross parental line, the grandsires represent the F1 generation at the heterozygous positions and the sons represent the backcross generation discriminated

according to allele inherited from the grandsire. In the granddaughters design, the dam population is assumed to carry the same two QTL alleles as the grandsire. If the frequency of both QTL alleles is equal, then the contrast between the two marker groups of sons [$a(1 - 2r)$] where a is the additive effect and r is the recombination rate between the marker and the QTL is similar to a backcross design (Weller, 2001). Consequently, the contrast between groups of granddaughters is half of the previous contrast. The use of an inbred line-cross design requires known linkage phase so that the recombinant events can be counted in the inherited grandsire autosomes. The unknown maternal allele does not provide additional information in this design, although it influences the estimated QTL frequency and magnitude (Weller, 2001). To overcome the phase-known restriction of the line-cross approach, the grandsire autosome-wide multi-loci linkage phase was elucidated based on the most frequent allelic combination found in the sons. This resulted in a multipoint phase determination.

The general expression of the interval and composite interval mapping models is:

$$y_{ij} = \beta_0 + \beta_1 M_j + \left(\sum_{i=2}^I \beta_i m_{ij} \right) + e_{ij} \quad [1]$$

where y_{ij} is the DYD or PTA of a trait recorded on the daughters of son j , β_0 is the intercept, β_1 is the regressor associated with the sons' probability of inheriting a marker (M_j) allele or QTL allele conditional on the genotype of the flanking markers (IM and CIM), β_i s ($i = 2$ to I) are the CIM regressors associated to the sons' probability of inheriting a background marker allele (m_{ij} , indexed from 2 to I) at positions other than that represented by M_j , and e_{ij} is the random residual assumed to be independent, identical, and normally distributed.

The interval and composite interval mapping analyses were implemented using QTL Cartographer (Basten et al., 2000) and the QTL allele probability was computed every two cM. Three sets of analyses were conducted to assess the impact of the number and position relative to the interval (window width) of the additional (background) markers controlling for the QTL background effects: 1) five background markers with a window width of ten cM (CIM05), 2) ten background markers with a window width of ten cM (CIM10), and 3) 20 background markers with a window width of 20 cM (CIM20). All (5, 10 or 20, respectively) background markers outside the window width (10 or 20 cM, respectively) around the interval studied were considered. These included markers on the autosome where the interval was located as well as any of the other 28

autosomes. The selected window width allowed the effect of QTL in the proximity to be accounted for, while minimizing the feasibility of the model to detect a QTL in the interval. Based on preliminary analyses and on the number and spacing of the informative markers in this study, single marker positions significant at $P < 0.2$ were considered to be potential background markers. Fewer background markers were fitted in some families and traits due to the high probability values ($P > 0.2$) of the remainder markers.

The estimated DYD and PTA have associated uncertainties. The analyses of these indicators should include these uncertainties so that less weight is given to the less precise estimates, compared with the more precise ones found in sons with large numbers of daughters with records. In its current implementation, QTL Cartographer cannot account for varying uncertainty. In order to incorporate the uncertainty, the DYD and PTA were transformed by analyzing $(\mathbf{L}')^{-1}\mathbf{y}$, where $\mathbf{y}$ is the vector of DYD or PTA and $\mathbf{L}'$ is the transpose of the Cholesky factorization of associated weight matrix such that $\text{Var}(\mathbf{y}) = \mathbf{L}'\mathbf{L}\sigma_e^2$. Since $\text{Var}[(\mathbf{L}')^{-1}\mathbf{y}] = (\mathbf{L}')^{-1}\text{Var}(\mathbf{y})\mathbf{L}^{-1} = (\mathbf{L}')^{-1}\mathbf{L}'\mathbf{L}\sigma_e^2\mathbf{L}^{-1} = \mathbf{I}\sigma_e^2$ (where $\mathbf{I}$ is the identity matrix), the transformation renders a variable with constant variance that is compatible with the QTL Cartographer implementation. In the present study, $\mathbf{L}$ is a diagonal matrix with entries equal to the uncertainty of $\mathbf{y}$. Matrices with off-diagonal entries, accounting for the correlation among estimates can be used. The measures of uncertainty used in this study were the variance of the DYD (Spelman et al., 1996) for the DYD model, and the inverse of the reliability for the PTA model (Georges et al., 1995).

Genome-wide Bonferroni and empirical (Churchill and Doerge, 1994) significance thresholds were computed within family and trait to account for the multiple and dependent testing along the genome, while providing complementary information. Results from preliminary interval mapping analyses using 1000 and 10,000 permutations of all markers across all families and traits provided a similar threshold. Hence, empirical thresholds corresponding to $P < 0.1$, 0.05, and 0.01 genome-wide significance levels were computed for interval and composite interval mapping likelihood ratio tests based on 1000 permutations. Thresholds for the analyses of adjusted and unadjusted PTA and DYD were consistent, and thus the most stringent set of thresholds were used in this study. Since the results from the analysis of PTA- and DYD-within-trait were highly correlated, PTA and DYD were assumed to represent the same trait, and testing was not adjusted within trait.

Table 2. Average likelihood ratio test permutation thresholds across all families for each trait and across all traits for the interval and composite interval mapping models.

Model ¹	Trait	Genome-wide significance level		
		$P < 0.1$	$P < 0.05$	$P < 0.01$
IM	Milk	11.0	12.4	15.7
IM	Fat	10.9	12.3	15.5
IM	Protein	10.8	12.2	15.7
IM	SCS	10.9	12.3	15.6
IM	Across all traits	13.5	14.9	18.1
CIM05	Milk	11.6	13.1	16.3
CIM05	Fat	11.5	12.9	16.3
CIM05	Protein	11.3	12.8	16.2
CIM05	SCS	11.6	13.3	16.3
CIM05	Across all traits	14.4	15.8	19.3
CIM10	Milk	12.1	13.9	17.5
CIM10	Fat	12.2	13.7	17.6
CIM10	Protein	11.9	13.6	17.2
CIM10	SCS	12.6	14.3	17.9
CIM10	Across all traits	15.3	17.0	20.9
CIM20	Milk	14.3	16.4	20.7
CIM20	Fat	15.4	17.4	22.3
CIM20	Protein	14.0	15.9	20.4
CIM20	SCS	16.2	18.3	23.4
CIM20	Across all traits	19.4	21.8	26.6

¹IM = interval mapping; CIM05, CIM10, CIM20 = composite interval mapping with five, ten and 20 background markers.

RESULTS AND DISCUSSION

The genome-wide permutation thresholds for DYD increased as the number of background markers included in the model increased (Table 2). This was most evident in families seven and eight, which had fewer sons than the rest of the families. Since the same background markers are included in the full (with QTL) and reduced (without QTL) model likelihood functions, the threshold increase could be explained by the number of informative sons contributing to the likelihood calculations in certain marker haplotypes. A low number of background markers in the model resulted in the analysis of more sons with genotypic information for all markers. For the data analyzed, 10 to 20 background markers were sufficient to reliably account for background QTL. In a few family-trait cases, twenty markers tended to give spurious likelihood ratio test peaks. This may be due to the limited number of sons informative for the background markers. In most cases, likelihood ratio peaks corresponding to the IM and CIM analyses suggested a QTL at the same or close positions although some analyses may have not surpassed the genome-wide threshold. Figure 1 depicts this situation for the analysis of protein DYD on BTA 27, in family five. In general, five background markers or no background marker (IM) resulted in higher residual variance and fewer results surpassing the genome-wide significance thresholds than using ten or twenty background markers.

The number of intervals tested was 71, 72, 52, 81, 80, 76, 69 and 56 for families one, two, three, four, five, six, seven and eight, respectively. Considering an average of 70 intervals, the Bonferroni adjusted comparison-wide significance values within family corresponding to genome-wide P significance levels of 0.1, 0.05 and 0.01 were 1.4×10^{-3} , 7.1×10^{-4} and 1.4×10^{-4} , respectively, for within trait and 3.6×10^{-4} , 1.8×10^{-4} , and

Figure 1. Likelihood ratio test values from the interval and composite interval mapping analyses of transformed protein DYD and associated 0.1, 0.05 and 0.01 permutation within-trait genome-wide P values for BTA 27 and family five. IM = interval mapping, CIM05, CIM10 and CIM20 = composite interval mapping with five, ten and 20 background markers. Informative marker positions are denoted by marks on the threshold lines.

Figure 2. Likelihood ratio test values from the composite interval analysis using ten background markers (CIM10) of (un)transformed protein daughter yield deviation (DYD) and predicted transmitted ability (PTA) and associated 0.1, 0.05 and 0.01 permutation genome-wide P values for BTA six and family three. Informative marker positions are denoted by marks on the threshold lines.

3.6×10^{-5} , respectively, across all traits. The associated probability significance values were marginally less stringent than those associated with the permutation results for both criteria (Table 2).

The results from the analysis of PTA and DYD records were consistent for most families and traits. Within family and trait, the untransformed DYD and PTA likelihood ratio test statistics from the IM analysis were highly (0.95 to 0.99) correlated. A few CIM DYD and PTA results differed on selected genome segments and traits. Evaluation of these cases showed that different background markers were included in the model. The DYD and PTA results were highly correlated when the same background markers were included. Due to the similarity between PTA and DYD results, only DYD results will be presented.

The results from the analysis of untransformed and transformed traits were typically very similar. The similarity of the results may be due to the large number of daughters available across sons, leading to similar transformation weights. This behavior is depicted in Figure 2, where the CIM10 likelihood ratio test estimates from the analysis of protein yield DYD and PTA likelihood ratio tests along BTA six in family three are plotted. Some variability across the different phenotypic indicators was expected, since the length of the first autosome interval was 64 cM. This suggests that in data sets with similar uncertainty level across observations, both phenotypic indicators can be used in QTL detection studies without weighting for the uncertainty. For SCS, the results from the untransformed DYD, transformed DYD, and untransformed PTA differed only slightly from untransformed PTA. This could be

due to our use of the inverse of the reliability of the son as an approximation of the variance of the PTA, the lower heritability of SCS (compared to yield traits), and to analysis of PTA that are solely based on pedigree information.

Other QTL detection studies have used PTA not weighted by the uncertainty (Vilkki et al., 1997; Thomsen et al., 2001), number of daughters (Velmalu et al., 1999), or reliability (Zhang et al., 1998). In other studies, de-regressed PTA were used to represent the daughter deviations adjusted for fixed effects (Schrooten et al., 2000; Thomsen et al., 2001). The de-regressed PTA should receive the same weight as DYD, since these studies consider that this indicator is similar to DYD. Thomsen et al. (2001) found that the de-regressed PTA and DYD gave similar results to, and more significant locations than, a nonweighted analysis of PTA. This difference is likely due to the different weighing factors used for both related measurements. The de-regressed PTA is directly proportional to PTA, and hence both measures should have a similar weighting factor.

Table 3 summarizes the 22 results that surpassed the within trait genome-wide $P < 0.1$ level for fat yield DYD. Thirty-two percent of these results surpassed the across-trait, genome-wide significance threshold ($P < 0.05$). All three CIM models supported the presence of three QTL on different families and autosomes. One or two QTL for fat DYD at position 60 cM on BTA three were detected in families two and eight. This location was also reported for families one and two by Heyen et al. (1999) by means of an IM approach. Ron et al. (1998), Zhang et al. (1998), and Heyen et al. (1999) indicated that a marker at 41 cM (TGLA 263) on BTA three was associated with variations on fat yield DYD and PTA. Ron et al. (2001) identified a QTL that influences fat percentage near position 55 cM on BTA six in two Israeli Holstein families. In our study, a QTL influencing fat DYD was located at 68 cM on BTA 12 in family four. This result is consistent with Heyen et al. (1999), who reported an association between the trait and marker BM4028 (68 cM) in the same family. The QTL detected with CIM10 at 0 cM on BTA 14 had an estimated effect of -6.8 kg on fat yield DYD in family four. This finding is consistent with Ron et al. (1998), Zhang et al. (1998), and Heyen et al. (1999), who reported QTL for the same trait and location in some of the same families considered in this study. Heyen et al. (1999) reported that marker ILSTS39, at the start of the linkage group on BTA 14, was associated with variations on fat DYD, and Ron et al. (1998) detected an association between marker CSSM66 (13 cM) and fat yield PTA. The CIM20 approach indicated the presence of a QTL 17 cM apart from that detected by CIM10 on the same family and

Table 3. Significant map positions for fat yield daughter yield deviation from the interval and composite interval mapping models.

BTA	Family	Model ¹	Location	Effect ²	Nom. P ³	Sig. ⁴	Sig.M.T. ⁵	Sig. Heyen ⁶
2	8	CIM10	0	9.6	2.6×10^{-04}	0.1	1	
2	8	CIM20	16	14.8	3.8×10^{-09}	0.01	0.05	
3	2	CIM05	55	7.9	5.0×10^{-04}	0.1	1	*
3	2	CIM10	63	8.8	8.4×10^{-05}	0.05	0.1	*
3	2	CIM20	55	9.8	1.4×10^{-06}	0.01	0.01	*
3	8	CIM05	72	-12.5	6.5×10^{-04}	0.1	1	
3	8	CIM10	74	-13.0	8.0×10^{-05}	0.05	1	
3	8	CIM20	76	-14.0	1.7×10^{-08}	0.05	0.1	
4	7	CIM20	24	15.2	7.6×10^{-12}	0.01	0.01	
6	8	CIM10	0	-11.9	8.0×10^{-07}	0.01	0.01	*
6	8	CIM20	0	-8.8	6.0×10^{-07}	0.1	1	*
12	4	CIM05	68	8.3	1.7×10^{-04}	0.05	1	*
12	4	CIM10	68	8.5	6.8×10^{-05}	0.05	1	*
12	4	CIM20	68	9.4	1.3×10^{-06}	0.01	0.05	*
14	4	CIM10	0	-6.8	3.9×10^{-04}	0.1	1	*
14	4	CIM20	17	10.9	1.2×10^{-04}	0.1	1	*
16	2	CIM20	37	-10.5	1.2×10^{-05}	0.01	0.05	
17	7	CIM20	52	-9.8	2.5×10^{-07}	0.1	1	*
21	7	CIM10	69	-10.2	2.3×10^{-05}	0.05	0.1	*
22	2	CIM20	18	-8.9	5.9×10^{-06}	0.01	0.05	
27	6	CIM10	20	-7.4	3.0×10^{-04}	0.1	1	
29	7	CIM10	14	-9.6	1.5×10^{-04}	0.1	1	

¹CIM05, CIM10, CIM20 = composite interval mapping with five, ten and 20 background markers.

²Estimated genetic effect, expressed as the trait difference associated with different QTL alleles (kg).

³Nominal *P* value. The position-wise *P* values corresponding to across trait within-family genome-wide of *P* < 0.05 and *P* < 0.1 Bonferroni thresholds are 0.000179 and 0.00036, respectively.

⁴Within-trait significance level 0.01 = *P* < 0.01; 0.05 = *P* < 0.05; 0.1 = *P* < 0.10; 1.0 = *P* > 0.1.

⁵Within family, across trait significance level 0.01 = *P* < 0.01; 0.05 = *P* < 0.05; 0.1 = *P* < 0.10; 1.0 = *P* > 0.1.

⁶Evidence of QTL in the same marker interval as reported by Heyen et al. (1999) based on single-marker and interval mapping models.

trait with an opposite effect (10.9 kg). Examination of the likelihood ratio test values along the autosome indicated that the peak between 10 and 20 cM was only significant at a genome-wide level for CIM20 (across trait genome-wide *P* < 0.05). The available marker data do not permit further elucidation of this map position. On BTA 22, a QTL located at 18 cM had a significant (across trait genome-wide *P* < 0.05) effect on fat yield DYD in family two.

The 15 results that surpassed the genome-wide *P* < 0.1 level for milk yield DYD are presented in Table 4. All three CIM models supported a QTL located at 115 to 117 cM on BTA seven in family three. Heyen et al. (1999) reported an association between marker ILSTS6 (115 cM) and milk yield DYD in the same family. We found that marker ILSTS96 (16 cM on BTA three) was also associated with milk DYD in family four.

Most positions that surpassed the genome-wide *P* < 0.1 level were found for protein yield DYD (Tables 5 and 6). Three distinct locations were significant (across trait genome wide *P* < 0.1) using all IM and CIM models. In family five a QTL was located at 32 cM on BTA three and in family three a QTL was located at 30 cM on BTA six. Heyen et al. (1999) found that marker ILSTS96 (16

cM on BTA three) was associated with protein DYD in families four and five. These results were partially confirmed in the present study, since only one QTL with effect on protein yield DYD in family five was detected between 28 and 47 cM. Zhang et al. (1998) indicated that marker TGLA37 located at 70 cM on BTA six was associated with fat and protein percentage DYD. Ron et al. (2001) reported a QTL that affected protein percentage near position 55 cM. In family one, CIM10 and CIM20 detected a QTL located between 53 and 63 cM on BTA 13. On BTA 14, IM and CIM located a QTL with effects on protein yield DYD between 91 and 103 cM in family six. Grisart et al. (2002), using positional candidate cloning techniques, identified a missense mutation in the centromeric end of BTA 14 that influences milk yield and composition. In addition, four other locations, 56 cM on BTA 21 in family three, 20 to 24 cM on BTA 27 in family five, 92 cM on BTA 11 in family two, and 48 cM on BTA 20 in family six, were consistently significant among CIM models (across-trait, genome-wide *P* < 0.05). Among these results, Heyen et al. (1999) detected associations between marker ILSTS103 (56 cM) on BTA three in family three and between marker

Table 4. Significant map positions for milk yield daughter yield deviation from the interval and composite interval mapping models.

BTA	Family	Model ¹	Location	Effect ²	Nom. P^3	Sig. ⁴	Sig.M.T. ⁵	Sig. Heyen ⁶
1	1	CIM20	124	133.6	5.3×10^{-04}	0.1	1	*
5	7	CIM20	56	-384.7	3.8×10^{-06}	0.1	1	
7	3	CIM05	117	-141.5	9.8×10^{-04}	0.1	1	*
7	3	CIM10	115	-176.4	3.4×10^{-05}	0.01	0.05	*
7	3	CIM20	117	-165.2	4.7×10^{-05}	0.01	0.05	*
9	8	CIM10	102	247.4	6.7×10^{-04}	0.1	1	*
9	8	CIM20	102	233.3	4.5×10^{-05}	0.1	1	*
14	6	CIM20	55	-463.1	1.1×10^{-04}	0.1	1	*
16	7	CIM20	83	376.4	1.4×10^{-05}	0.1	1	*
17	8	CIM20	52	-307.6	1.7×10^{-05}	0.05	1	
21	5	CIM05	10	275.4	3.8×10^{-04}	0.1	1	*
21	5	CIM10	8	288.8	5.8×10^{-05}	0.05	0.1	*
27	8	CIM10	1	237.1	4.2×10^{-04}	0.1	1	*
28	1	CIM20	35	-163.8	2.2×10^{-04}	0.05	1	*
29	7	CIM20	0	-248.0	9.1×10^{-06}	0.1	1	*

¹CIM05, CIM10, CIM20 = composite interval mapping with five, ten and 20 background markers.

²Estimated genetic effect, expressed as the trait difference associated with different QTL alleles (kg).

³Nominal P value. The position-wise P values corresponding to across trait within-family genome-wide of $P < 0.05$ and $P < 0.1$ Bonferroni thresholds are 0.000179 and 0.00036, respectively.

⁴Within-trait significance level $0.01 = P < 0.01$; $0.05 = P < 0.05$; $0.1 = P < 0.10$; $1.0 = P > 0.1$.

⁵Within family, across trait significance level $0.01 = P < 0.01$; $0.05 = P < 0.05$; $0.1 = P < 0.1$; $1.0 = P > 0.1$.

⁶Evidence of QTL in the same marker interval as reported by Heyen et al. (1999) based on single-marker and interval mapping models.

URB057 at 93 cM on BTA 11 and protein yield DYD in family two.

A total of 21 results surpassed the genome-wide $P < 0.1$ level for SCS (Table 7). On BTA 16, a QTL not detected by Heyen et al. (1999), was located at 51 cM in family eight. Also, on BTA 21 a QTL was detected at 33 cM in family one. This result is consistent with Heyen et al. (1999) who reported of an association between marker ETH131 (33 cM) and SCS DYD in the same family. The partially overlapping nature of the informative markers genotyped in either family prevents the evaluation of one versus multiple QTL associated with SCS on this autosome. A QTL with effects on SCS DYD was detected at 60 cM on BTA seven in family one and this finding has not been reported by previous studies.

Given the similarity of phenotypes and identity of the marker data used in this study and by Heyen et al. (1999), a one-to-one comparison of the results was conducted. Of the locations exceeding genome-wide or suggestive significance thresholds from the joint analysis of all families using the multimarker regression analysis reported by Heyen et al. (1999) the QTL for protein yield on autosome 3 at 16 cM (4 – 113cM) was confirmed but the QTL for fat yield on autosome 14 at 1 cM (0 – 31cM) was not verified. When the multimarker regression results of Heyen et al. (1999) that surpassed the 5% genome-wide threshold for autosomes three and 14 are considered, the current study confirmed substantial evidence for fat yield on BTA three, family two,

protein yield on BTA three, family 5, and fat yield on BTA14, family 4. The current study did not uncover sufficient evidence to confirm the findings of Heyen et al. (1999) for fat and milk yield on BTA3, family one, fat and milk yield on BTA three, family five, and milk yield on BTA14 on families four and five. Failure of the present analysis to identify locations reported by Heyen et al. (1999) could be due to the inability of the model to detect some QTL signals due to the effects of background markers or previously reported spurious false positive results.

Several studies have reported multiple QTL on the same autosome (Spelman et al., 1996; Heyen et al., 1999; Ron et al., 2001). While composite interval mapping can account for linked QTL in this study, none of the families showed evidence of multiple QTL on the same chromosome influencing the same trait. Although in family eight the scan on BTA three indicated multiple likelihood ratio test peaks, these did not reach the significance threshold for different CIM models (Figure 3). Two QTL locations (26 to 36 cM and 80 cM) on BTA six were identified in two families (families 3 and 2, respectively) associated with protein yield DYD (Table 4). This suggests that some of the families studied could have been homozygous for at least one QTL and that insufficient marker information was available to account for multiple QTL. The incorporation of more marker information in the promising intervals and in intervals over 20 cM long will permit verification of

Table 5. Significant map positions for protein yield daughter yield deviation from the interval and composite interval mapping models, BTA one to 14.

BTA	Family	Model ¹	Location	Effect ²	Nom. P^3	Sig. ⁴	Sig.M.T. ⁵	Sig. Heyen ⁶
1	1	CIM10	109	4.4	5.7×10^{-04}	0.1	1	*
1	6	CIM20	46	6.2	9.4×10^{-05}	0.1	1	*
1	8	CIM20	61	-7.6	1.6×10^{-05}	0.1	1	*
3	4	CIM20	122	-5.5	9.1×10^{-05}	0.1	1	*
3	5	CIM05	32	-8.8	3.9×10^{-08}	0.01	0.01	*
3	5	CIM10	32	-9.9	2.4×10^{-10}	0.01	0.01	*
3	5	CIM20	47	-10.3	6.6×10^{-11}	0.01	0.01	*
3	5	IM	28	-7.6	6.8×10^{-06}	0.01	0.01	*
5	5	CIM05	101	-8.2	1.5×10^{-04}	0.05	0.1	
5	5	CIM10	95	-9.1	2.9×10^{-05}	0.01	0.05	
5	5	CIM20	91	-11.9	1.9×10^{-07}	0.01	0.01	
6	2	CIM10	80	9.4	2.3×10^{-04}	0.1	1	*
6	3	CIM05	28	-6.9	2.1×10^{-04}	0.05	0.1	*
6	3	CIM10	30	-6.7	1.7×10^{-04}	0.05	0.1	*
6	3	CIM20	36	-6.6	1.1×10^{-04}	0.05	0.1	*
6	3	IM	26	-7.1	2.7×10^{-04}	0.05	0.1	*
11	2	CIM05	92	-5.9	3.0×10^{-04}	0.1	1	*
11	2	CIM10	92	-6.3	1.4×10^{-04}	0.05	1	*
11	2	CIM20	92	-6.4	4.4×10^{-05}	0.05	1	*
12	1	CIM20	0	-5.5	2.5×10^{-04}	0.1	1	*
13	1	CIM05	57	5.2	3.2×10^{-04}	0.05	1	*
13	1	CIM10	61	5.4	9.4×10^{-05}	0.05	0.1	*
13	1	CIM20	53	5.4	3.1×10^{-05}	0.01	0.05	*
13	1	IM	63	4.9	7.3×10^{-04}	0.1	1	*
14	6	CIM05	91	-6.4	3.3×10^{-04}	0.1	1	*
14	6	IM	103	-6.6	3.6×10^{-04}	0.05	1	*

¹CIM05, CIM10, CIM20 = composite interval mapping with five, ten and 20 background markers.

²Estimated genetic effect, expressed as the trait difference associated with different QTL alleles (kg).

³Nominal P value. The position-wise P values corresponding to across trait within-family genome-wide of $P < 0.05$ and $P < 0.1$ Bonferroni thresholds are 0.000179 and 0.00036, respectively.

⁴Within-trait significance level $0.01 = P < 0.01$; $0.05 = P < 0.05$; $0.1 = P < 0.10$; $1.0 = P > 0.1$.

⁵Within family, across trait significance level $0.01 = P < 0.01$; $0.05 = P < 0.05$; $0.1 = P < 0.1$; $1.0 = P > 0.1$.

⁶Evidence of QTL in the same marker interval as reported by Heyen et al. (1999) based on single-marker and interval mapping models.

some of the findings and reduce the uncertainty in other regions.

A number of genome positions reported in the literature and in this study appear to contain one QTL with pleiotropic effects or multiple QTL within a chromosomal region influencing different traits. On BTA 27, a QTL was detected at 20 cM influencing protein (-8.4 kg) and fat yield (-7.4 kg) in families five and six, respectively. At approximately 40 cM on BTA 28 a QTL was identified influencing milk yield (-164 kg) and protein yield (-5 kg) in families one and six, respectively. A multivariate interval mapping approach would permit the evaluation of the one versus multiple QTL hypotheses. Figure 3 summarizes the evidence for one or more QTL located near 60 cM on BTA three influencing fat yield DYD in families two and eight ($P < 0.05$) and protein yield DYD in family five, although this peak does not surpass the genome-wide threshold. A similar situation is depicted in Figure 4. BTA 21 appears to harbor QTL with different relationships across families and traits. In family five a QTL for milk yield DYD was located at 10 cM but a QTL for SCS DYD was detected

at approximately 60 cM in family seven and for protein DYD in family three. In addition, a QTL for SCS DYD was detected at 84 cM on the same autosome. Heyen et al. (1999) reported that marker ILST103 located at 56 cM on BTA 21 was associated with variations on milk and protein DYD in family three.

The advantage of CIM over IM is that, by including background markers in the model, additional QTL that may be present in the genome can be accounted for. The relatively fewer locations that surpassed the genome-wide threshold in the IM approach, when compared with the three CIM specifications (Tables 3 to 7 and Figure 1), suggest that CIM has enhanced sensitivity and power over IM. One disadvantage of CIM is the required selection of the number and relative position of background markers. Too many markers will artificially augment the number of positions that surpass the significance threshold due to underestimated residual variance. On the other hand, a limited number of background markers may reduce the power of the analysis (Zeng, 1994). In this study, the lack of informative sons for all markers illustrated the challenges of using line-

Table 6. Significant map positions for protein yield daughter yield deviation from the interval and composite interval mapping models, BTA 15 to 29.

BTA	Family	Model ¹	Location	Effect ²	Nom. P^3	Sig. ⁴	Sig.M.T. ⁵	Sig. Heyen ⁶
16	4	CIM20	3	-5.9	3.9×10^{-05}	0.05	1	*
16	5	CIM10	89	7.0	3.8×10^{-04}	0.1	1	
16	6	CIM20	26	-6.6	1.9×10^{-05}	0.05	0.1	*
17	1	CIM20	0	-4.9	4.2×10^{-04}	0.1	1	
17	4	CIM20	0	-5.6	9.6×10^{-05}	0.1	1	*
20	6	CIM05	48	-8.9	5.8×10^{-06}	0.01	0.01	*
20	6	CIM10	48	-8.4	6.5×10^{-06}	0.01	0.05	*
20	6	CIM20	48	-7.9	1.3×10^{-06}	0.01	0.05	*
21	3	CIM05	56	4.9	2.6×10^{-04}	0.05	1	*
21	3	IM	56	5.3	2.5×10^{-04}	0.05	0.1	*
22	7	CIM05	0	-6.5	6.7×10^{-04}	0.1	1	*
24	8	CIM20	26	11.6	2.7×10^{-06}	0.05	1	
27	4	CIM10	0	-5.8	1.2×10^{-04}	0.05	1	*
27	4	CIM20	0	-6.2	4.8×10^{-06}	0.01	0.1	*
27	5	CIM05	24	-7.6	1.4×10^{-04}	0.05	0.1	*
27	5	CIM10	20	-8.4	2.0×10^{-05}	0.01	0.05	*
27	5	CIM20	18	-8.6	3.8×10^{-05}	0.05	0.1	*
28	6	CIM05	43	-5.0	6.5×10^{-04}	0.1	1	*

¹CIM05, CIM10, CIM20 = composite interval mapping with five, ten and 20 background markers.

²Estimated genetic effect, expressed as the trait difference associated with different QTL alleles (kg).

³Nominal P value. The position-wise P values corresponding to across trait within-family genome-wide of $P < 0.05$ and $P < 0.1$ Bonferroni thresholds are 0.000179 and 0.00036, respectively.

⁴Within-trait significance level $0.01 = P < 0.01$; $0.05 = P < 0.05$; $0.1 = P < 0.10$; $1.0 = P > 0.1$.

⁵Within family, across trait significance level $0.01 = P < 0.01$; $0.05 = P < 0.05$; $0.1 = P < 0.1$; $1.0 = P > 0.1$.

⁶Evidence of QTL in the same marker interval as reported by Heyen et al. (1999) based on single-marker and interval mapping models.

Table 7. Significant map positions for SCS daughter yield deviation from the interval and composite interval mapping models.

BTA	Family	Model ¹	Location	Effect ²	Nom. P^3	Sig. ⁴	Sig.M.T. ⁵	Sig. Heyen ⁶
1	2	CIM20	80	-0.2	9.7×10^{-05}	0.1	1	
1	5	CIM20	126	-0.2	3.0×10^{-05}	0.05	0.1	*
5	8	CIM10	36	-0.2	5.0×10^{-06}	0.01	0.05	
7	1	CIM20	60	-0.3	3.6×10^{-05}	0.01	0.05	*
11	8	CIM20	38	0.2	9.7×10^{-08}	0.1	1	*
12	8	CIM10	52	0.2	4.7×10^{-04}	0.1	1	
13	2	CIM20	0	-0.2	1.4×10^{-05}	0.01	0.05	
15	4	CIM05	41	-0.1	1.4×10^{-04}	0.1	1	*
15	4	CIM10	43	-0.1	2.0×10^{-04}	0.05	1	*
16	8	CIM05	51	-0.2	4.9×10^{-04}	0.1	1	
16	8	CIM10	51	-0.2	3.7×10^{-06}	0.01	0.05	
16	8	CIM20	51	-0.2	3.1×10^{-08}	0.1	0.1	
18	6	CIM20	80	0.3	2.2×10^{-05}	0.05	0.1	
20	4	CIM20	82	-0.2	9.8×10^{-06}	0.1	0.1	*
20	5	CIM20	0	0.1	9.0×10^{-05}	0.05	1	*
21	1	CIM05	33	-0.2	6.5×10^{-04}	0.1	1	*
21	1	CIM10	33	-0.2	3.5×10^{-04}	0.1	1	*
21	1	CIM20	33	-0.2	1.2×10^{-04}	0.05	0.1	*
21	3	CIM10	84	0.2	1.8×10^{-04}	0.05	0.1	*
21	3	CIM20	84	0.2	1.8×10^{-04}	0.05	0.1	*
27	8	CIM20	20	0.3	5.0×10^{-08}	0.1	0.1	

¹CIM05, CIM10, CIM20 = composite interval mapping with five, ten and 20 background markers.

²Estimated genetic effect, expressed as the trait difference associated with different QTL alleles ($\log_2(\text{SCS}/100+3)$).

³Nominal P value. The position-wise P values corresponding to across trait within-family genome-wide of $P < 0.05$ and $P < 0.1$ Bonferroni thresholds are 0.000179 and 0.00036, respectively.

⁴Within-trait significance level $0.01 = P < 0.01$; $0.05 = P < 0.05$; $0.1 = P < 0.10$; $1.0 = P > 0.1$.

⁵Within family, across trait significance level $0.01 = P < 0.01$; $0.05 = P < 0.05$; $0.1 = P < 0.1$; $1.0 = P > 0.1$.

⁶Evidence of QTL in the same marker interval as reported by Heyen et al. (1999) based on single-marker and interval mapping models.

Figure 3. Likelihood ratio test values from the composite interval analysis using ten background markers (CIM10) for fat yield daughter yield deviation (DYD) in families two and eight and protein yield DYD in family five on BTA three and associated 0.1, 0.05 and 0.01 permutation genome-wide P values. Informative marker positions are denoted by marks on the threshold lines.

cross QTL detection programs in outbred populations. In some cases, few sons with sufficient informative markers analyzed on CIM models with large number of background markers resulted in spurious results. The choice of background markers must balance a reduced number of background markers that may hinder the control for QTL outside the interval studied, and a large number of background markers that increases the number of model degrees of freedom and may artificially decrease the residual variance estimate, resulting in false positiveness. Also, selection of background markers near the interval under study (small window width)

Figure 4. Likelihood ratio test values from the composite interval analysis using ten background markers (CIM10) for milk yield daughter yield deviation (DYD) in families seven, protein yield and SCS DYD in family three on BTA 21 and associated 0.1, 0.05 and 0.01 permutation genome-wide P values. Informative marker positions are denoted by marks on the threshold lines.

could account for (absorb) the effect of the QTL in the interval, thus hindering the chances of detection and power. In this study, multiple CIM specifications were considered to allow the confirmation of results. However, direct comparison of the CIM models was difficult, since the background markers vary across model specifications. In general, the results from the CIM10 and CIM20 models were consistent, indicating that in the present study ten to 20 background markers provided a balance between model complexity, available information and background QTL adjustment. An additional limitation of QTL Cartographer is that the single marker model implementation predicts the missing marker genotypes (Basten et al., 2000), and this could lead to incorrect allele assignment and biased results.

CONCLUSIONS

Interval mapping and composite interval mapping approaches developed for line crosses were extended to detect QTL in an outbred dairy cattle population. A granddaughter design was modeled as a backcross design and the linkage phases were based on the most frequent haplotypes found in the sons. The approach was successful in identifying QTL influencing production and health traits. Some estimated QTL positions were consistent with previous studies and new locations were uncovered. Few reported locations were not corroborated in this study. These could be due to the inability of the model to detect some QTL signals due to background markers effects or false positive results reported in previous studies. Virtually identical location estimates were obtained from the analysis of DYD and PTA records regardless of the presence or absence of adjustment for the uncertainty of the estimator. This was attributed to similar weights between sons due to a large number of daughters. In general, the information from the sparse map was insufficient to detect significant (across trait genome-wide $P < 0.05$) interval mapping positions but the inclusion of background markers in the composite interval mapping approach enhanced the ability to detect QTL. Information from more informative markers, more families, and more sons per family will further enhance the power of the study to detect QTL. A few composite interval mapping results may be spurious since few sons were genotyped for all the markers included in the model. A number of map locations were associated with more than one trait. These positions may stem from QTL with pleiotropic effects or multiple QTL in the region. The utilization of multivariate composite interval mapping models may enhance the power to detect QTL, the precision of the estimates and permit to test pleiotropic versus multiple QTL models. The estimates of QTL location and effect

can be used to further fine mapping and positional candidate gene studies and ultimately applied in marker-assisted selection programs to enhance dairy cattle production and health traits.

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REFERENCES

- Animal Improvement Programs Laboratory. 2000. <http://aipl.arsusda.gov/index.html>. Accessed November, 2000.
- Ashwell, M. S., Y. Da, P. M. VanRaden, C. E. Rexroad Jr., and R. H. Miller. 1998a. Detection of putative loci affecting conformational type traits in an elite population of United States Holsteins using microsatellite markers. *J. Dairy Sci.* 81:1120–1125.
- Ashwell, M. S., Y. Da, C. P. Van Tassel, P. M. VanRaden, R. H. Miller and C. E. Rexroad. 1998b. Detection of putative loci affecting milk production and composition, health, and type traits in a United States Holstein population. *J. Dairy Sci.* 81:3309–3314.
- Basten, C. J., B. S. Weir, and Z. B. Zeng. 2000. QTL Cartographer, Version 1.14. North Carolina State University, Raleigh.
- Geldermann, H. 1975. Investigations on inheritance of quantitative characters in animals by gene markers. *Theor. Appl. Genet.* 46:319–330.
- Georges, M., D. Nielsen, M. Mackinnon, A. Mishra, R. Okimoto, A. T. Pasquino, L. S. Sargeant, A. Sorensen, M. R. Steele, X. Zhao, J. E. Womack, and I. Hoeschele. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics* 139:907–920.
- Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P. Berzi, N. Cambisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges, and R. Snell. 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Res.* 12:222–231.
- Heyen, D. W., J. I. Weller, M. Ron, M. Band, J. E. Beever, E. Feldmesser, Y. Da, G. R. Wiggans, P. M. VanRaden, and H. A. Lewin. 1999. A genome scan for QTL influencing milk production and health traits in dairy cattle. *Physiol. Genomics* 1:165–175. Appendix available: <http://cagst.animal.uiuc.edu/genemap/WEB/intro.html>. Accessed March, 2002.
- Hoeschele, I., P. Uimari, F. E. Grignola, Q. Zhang, and K. M. Gage. 1997. Advances in statistical methods to map quantitative trait loci in outbred populations. *Genetics* 147:1445–1457.
- Jansen, R. C. 1993. Interval mapping of multiple quantitative trait loci. *Genetics* 135:205–211.
- Knott, S. A., J. M. Elsen, and C. S. Haley. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *Theor. Appl. Genet.* 93:71–80.
- Ron, M., D. W. Heyen, J. I. Weller, M. Band, E. Feldmesser, H. Pasternak, Y. Da, G. R. Wiggans, P. M. VanRaden, E. Ezra, and H. A. Lewin. 1998. Detection and analysis of a locus affecting milk concentration in the US and Israeli dairy cattle populations. *Proc. 6th World Congr. Genet. Appl. Livest. Prod.* 26:422–425.
- Ron, M., D. Kliger, E. Feldmesser, E. Seroussi, E. Ezra and J. I. Weller. 2001. Multiple Quantitative trait locus analysis of bovine autosome 6 in the Israeli Holstein population by a daughter design. *Genetics* 159:727–735.
- Schrooten, C., H. Bovenhuis, W. Coppieters, and J. A. M. Van Arendonk. 2000. Whole genome scan to detect quantitative trait loci for conformation and functional traits in dairy cattle. *J. Dairy Sci.* 83:795–806.
- Spelman, R. J., W. Coppieters, L. Karim, J. A. M. van Arendonk, and H. Bovenhuis. 1996. Quantitative trait loci analysis for five milk production traits on autosome six in the Dutch Holstein-Friesian population. *Genetics* 144:1799–1808.
- Thomsen, H., N. Reinsch, N. Xu, C. Looft, S. Grupe, C. Kuhn, G. A. Brockmann, M. Schwerin, B. Leyhe-Horn, S. Hiendleder, G. Erhard, I. Medjugorac, I. Russ, M. Forster, B. Brening, F. Reinhardt, R. Reents, J. Blumel, G. Averdunk, E. Kalm. 2001. Comparison of estimated breeding values, daughter yield deviations and de-regressed proofs within a whole genome scan for QTL. *J. Anim. Breed. Genet.* 118:357–370.
- VanRaden, P. M., and G. R. Wiggans. 1991. Derivation, calculation, and use of national animal model information. *J. Dairy Sci.* 74:2737–2746.
- Velmalä, R. J., H. J. Vilkkilä, K. T. Elo, D. J. de Koning, and A. V. Mäki-Tanila. 1999. A search for quantitative trait loci for milk production traits on autosome 6 in Finnish Ayrshire cattle. *Anim. Genetics* 30:136–143.
- Vilkkilä, H. J., D. J. de Koning, K. Elo, R. Velmalä, and A. V. Mäki-Tanila. 1997. Multiple marker mapping of quantitative trait loci of Finnish dairy cattle by regression. *Genetics* 80:198–204.
- Weller, J. I. Quantitative trait loci analysis in animals. 2001. CABI Publishing, UK.
- Weller, J. I., Y. Kashi, and M. Soller. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *J. Dairy Sci.* 73:2525–2537.
- Zhang, Q., D. Boichard, I. Hoeschele, C. Ernst, A. Eggen, B. Murkve, M. Pfister-Genskow, L. A. Witte, F. E. Grignola, P. Uimari, G. Thaller, and M. D. Bishop. 1998. Mapping quantitative trait loci for milk production and health traits of dairy cattle in a large outbred pedigree. *Genetics* 149:1959–1973.
- Zeng, Z. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proc. Natl. Acad. Sci.* 90:10972–10976.
- Zeng, Z. 1994. Precision mapping of quantitative trait loci. *Genetics* 136:1457–1468.