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Quantitative Trait Loci Affecting Milk Production Traits in Finnish Ayrshire Dairy Cattle

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ABSTRACT

A whole genome scan of Finnish Ayrshire was conducted to map quantitative trait loci (QTL) affecting milk production. The analysis included 12 half-sib families containing a total of 494 bulls in a granddaughter design. The families were genotyped with 150 markers to construct a 2764 cM (Haldane) male linkage map. In this study interval mapping with multiple-marker regression approach was extended to analyse multiple chromosomes simultaneously. The method uses identified QTL on other chromosomes as cofactors to increase mapping power. The existence of multiple QTL on the same linkage group was also analyzed by fitting a two-QTL model to the analysis. Empirical values for chromosome-wise significance thresholds were determined using a permutation test. Two genome-wise significant QTL were identified when chromosomes were analyzed individually, one affecting fat percentage on chromosome (BTA) 14 and another affecting fat yield on BTA12. The cofactor analysis revealed in total 31 genome-wise significant QTL. The result of two-QTL analysis suggests the existence of two QTL for fat percentage on BTA3. In general, most of the identified QTL confirm results from previous studies of Holstein-Friesian cattle. A new QTL for all yield components was identified on BTA12 in Finnish Ayrshire.

(**Key words:** cofactor, dairy cattle, interval mapping, quantitative trait)

Abbreviation key: BTA = *Bos taurus* chromosome, **DYD** = daughter yield deviations, **FY** = fat yield, **F%** = fat percentage, **MY** = milk yield, **PIC** = polymorphic information content, **PY** = protein yield, **P%** = protein percentage.

INTRODUCTION

Detection of loci underlying the genetic variance for economically important traits in livestock has become feasible during the last decade. The availability of genetic linkage maps mainly composed of highly polymorphic microsatellite markers allows the genetic dissection of complex traits into QTL. Mapping QTL is the first step towards the understanding of genetic basis of economically important production and functional traits in livestock.

In dairy cattle, QTL mapping utilizes existing half-sib breeding populations, which are routinely produced by artificial insemination. Weller et al. (1990) proposed the granddaughter design to analyze linkage between a single marker and a QTL in an outbred half-sib data structure. The idea of interval mapping originally introduced by Lander and Botstein (1989) was implemented for half-sib designs by Georges et al. (1995) and Knott et al. (1996). Several QTL mapping efforts have been undertaken in different breeds of dairy cattle by using various mapping approaches (see Mosig et al., 2001). Having QTL mapping results from cattle with different origin improves the potential to implement fine mapping strategies that take advantage of historical recombinants. The improvement of mapping resolution is an essential step towards positional cloning of mapped QTL and marker-assisted breeding schemes.

The methods described by Georges et al. (1995) and Knott et al. (1996) assume only a single QTL on a linkage group and do not take possible QTL on other chromosomes into account. Jansen (1993) and Zeng (1994) proposed methods that account for the effects of linked and unlinked QTL but are only developed for inbred line cross experiments. Recently, methods for more complex pedigrees have been suggested (Jansen, 1996; Jansen et al., 1998; Kao et al., 1999). To increase the power and the precision of QTL mapping in outbred half-sib populations de Koning et al. (2001) developed a strategy for simultaneous analysis of multiple chromosomes. In this study, we applied this method to a range of milk production traits in our granddaughter design. We also ana-

lyzed the existence of multiple QTL on the same linkage group by fitting the two QTL model to the analysis (Spelman et al., 1996; Velmala et al., 1999). In this paper, we present the results of a whole genome scan for milk production QTL in Finnish Ayrshire dairy cattle.

MATERIALS AND METHODS

Families and Description of Phenotypic Data

Twelve half-sib families containing a total of 494 AI bulls from Finnish Ayrshire cattle were analyzed in a granddaughter design (Weller et al., 1990). The families were selected according to the availability of semen samples with the number of sons ranging from 21 to 82 per grandsire. In oldest families, bulls are not random samples of grandsires' sons because semen availability is influenced by results of progeny testing. This causes a selection bias in families in which semen is available only on the best bulls in that family.

For every son the EBV were obtained from the national animal model evaluation of 1998, for five milk production traits: milk yield (**MY**), fat yield (**FY**), protein yield (**PY**), fat percentage (**F%**) and protein percentage (**P%**). Using daughter yield deviations (**DYD**) instead of EBV would have been more correct, because the EBV also contain information from relatives other than the bulls' daughters. However, the large number of daughters (from 105 to over 3000 per son) makes the difference between DYD and EBV negligible. In total, the analysis included records of ~140,000 daughters.

Genotyping and Map Construction

The sperm samples were provided by Finnish AI societies. DNA was extracted from sperm using phenol-chloroform protocol as described by Zadworny and Kuhnlein (1990).

For the genome scan, 147 microsatellite markers were selected using published bovine linkage maps (Barendse et al., 1994; Bishop et al., 1994; Ma et al., 1996; Barendse et al., 1997; Kappes et al., 1997). In addition, three candidate genes were included in the analysis. The haplotype for casein genes (α_{S1}-, β-, and κ-casein; BTA6) was considered as one marker (Velmala et al., 1995) and a growth hormone receptor (GHR; BTA20) 3′UTR polymorphism as one (Moisio et al., 1998). A single nucleotide polymorphism (snp) in the prolactin receptor gene (PRLR; BTA20) was also used (unpublished). The objective was to cover all 29 autosomes at ~20 cM intervals, from 2 to 14 markers per chromosome. Microsatellite amplifications were carried out using fluorescence-labeled primers. The PCR products were separated on 6% polyaclyamide gels using ALF or ALFexpress DNA sequencer (Amersham Pharmacia Biotech, Uppsala, Swe-

den) and actual allele sizes were determined using Fragment Manager 1.2 and ALFwin Fragment Analyzer 1.02 software (Amersham Pharmacia Biotech, Uppsala, Sweden).

Marker maps were established with maximum likelihood based programs (ANIMAP program package; Georges et al., 1995). First, the CHECKPED program was used to identify genotyping errors. Then the most likely recombination rates between adjacent markers were computed with LODTABLE. The linkage analyses were performed across families using the MAKEMAP program. This reveals the most likely order of markers. The Haldane mapping function was used to convert the recombination rates to map distances (cM).

Polymorphic information content (**PIC**) for each marker was calculated. Specific information about markers including PCR-conditions, primer sequences, number of alleles, PIC-values and linkage map locations are available in our web site (http://www.mtt.fi/julkaisut/cattleqtl/).

Statistical Methods

QTL mapping was performed using the multimarker regression approach (Knott et al., 1996) previously used by Spelman et al. (1996) and Vilkki et al. (1997). In short, the most likely linkage phases of the chromosomes of the grandsire were determined for every family. Then for every half-sib offspring, the probability of inheriting the alternative sire's haplotype was calculated at fixed intervals. This conditional probability provides an independent variable on which the phenotypic values can be regressed. A QTL with an additive effect was fitted at fixed intervals (1 cM) along the linkage group by regressing the trait score on the probability. The regression analysis was nested within families and weighted with the number of daughters. For every linkage group, the presence of QTL was calculated by comparing the pooled mean squares obtained from regression within families to the residual mean square. This analysis provides F-ratios along the linkage group with the maximum value being the most likely position of QTL. For more details see Vilkki et al. (1997).

The significance thresholds and the empirical P-values were estimated with the permutation test (Churchill and Doerge, 1994). Two different significance thresholds were calculated. The chromosome-wise significant levels (P_{chr}) for across-family analysis and within-family analysis were obtained by 10,000 permutations. For across-family analysis genome-wise (P_{genome}) significance thresholds were also established. The P_{genome} were obtained as follows: $P_{\mathrm{genome}} = 1 - (1 - P_{\mathrm{chr}})^c$, where c is the number of bovine autosomal chromosomes.

One of the benefits of the regression approach is the possibility to add cofactors to the analysis. This allows us to use the information of identified QTL to search for QTL with minor effects. The approach herein is identical to the method introduced by de Koning et al. (2001).

First, the candidate regions were identified from the initial interval mapping experiment. The QTL at 5% chromosome-wise threshold level were selected to serve as cofactors in the further analysis. The conditional probability of the QTL allele being inherited from sire to son was used as a "virtual marker." The benefit of using these virtual markers as cofactors is that any position on a linkage group can be included in the analysis in any family. Secondly, multiple linear regression was used to reestimate the combined effects of all cofactors. Third, the phenotypic data was adjusted for the effects of cofactors, separately for each linkage group, using unlinked cofactors. All linkage groups were reanalyzed with the adjusted phenotypes. Chromosome-wise significance thresholds were determined by permutations (Churchill and Doerge, 1994; Doerge and Churchill, 1996). When new significant candidate regions were identified a new set of cofactors was selected and interval mapping performed. The analysis was repeated until no new candidate regions were detected and the estimated locations of QTL became stable.

The existence of multiple QTL on the same linkage group was tested by fitting a two QTL model to the analysis (Spelman et al., 1996; Velmala et al., 1999). First, test statistics were calculated for one QTL vs. none, then for two QTL vs. none. The empirical thresholds were determined with permutation test (Churchill and Doerge, 1994; Doerge and Churchill, 1996) as previously. If the test statistics for two QTL vs. none were significant, an F-test for two QTL vs. one QTL was applied. This allows us to define whether the two QTL explain more variation than one QTL. The significance of the test statistics was determined by a standard F-table. In our data, the existence of two QTL were analyzed while fitting cofactors on other linkage groups.

RESULTS AND DISCUSSION

A total of 150 selected DNA markers were genotyped to construct a 2764 cM (Haldane) male genetic linkage map (Table 1). About 92% of the bovine genome was covered at ~20 cM intervals.

The mean heterozygosity for all markers within the 12 grandsires was 68%. The PIC of 147 microsatellite markers ranged from 0.22 (ILSTS090) to 1.0 (HEL9). For the casein haplotype, the PIC value was 0.68, for the GHR polymorphism 0.47, and for PRLR polymorphism 0.25. The average PIC of all markers was 0.65, thus the selected markers were fairly informative. To get better

understanding of the information content along the chromosomes the ratio of the actual variance of the conditional QTL probabilities found in the data and the expected variance under full information were calculated (Table 1). Most of the chromosomes were relatively informative with the range between markers being 0.37 to 0.68. The average information content at genome level was 0.53.

In the across-family analysis without cofactors 2 QTL exceeding 5% genome-wise significance thresholds and 14 QTL exceeding 5% chromosome-wise thresholds were identified (Table 2). The most striking results were found on *Bos taurus* chromosome (**BTA**) 14. A genome-wise significant QTL ($P_{genome} < 0.0029$) for F% was detected at the centromeric end of the chromosome. At the same position a QTL for FY ($P_{chr} = 0.0050$) was detected. The analysis within families suggests that the inheritance of ILSTS039 allele 14 (242 bp) correlates with an increase of F% in three families and of FY in one family. In family 9, an exceptionally high test statistic of 22.62 was observed for fat percentage (Figure 1). In this family, the size of the QTL effect for F% was 0.65%, which is almost 2.5 standard deviations (F% standard deviation for the unselected AI bull population in 1998 data is 0.27%). The QTL effect for F% in family 4 was 0.36% and in family 5 0.29%. In family 9, the effect for FY was 21 kg, which approximates more than one standard deviation. An effect for P% was also detected in family 9 and in family 6, but the position of the QTL was different. QTL affecting different milk constituents in BTA14 have been previously reported in several studies (Ashwell et al., 1998a; Ashwell et al., 1998b; Coppieters et al., 1998; Heyen et al., 1998; Ron et al., 1998; Heyen et al., 1999; Riquet et al., 1999; Moisio et al., 2000).

The second highest significance of test statistics in across-family analysis was found in BTA12. A genome-wise significant QTL ($P_{genome} < 0.0029$) for FY was detected between markers BM2507 and BM6404 with the latter being the closest marker. In family 7, the BM6404 allele 5 seems to be associated with higher production than other alleles. The estimated allele substitution effect was 0.72 standard deviation, about 11.7 kg (FY standard deviation for the latest young bull population is 16.2 kg). This was also seen in family 3, where the effect of BM6404 allele 5 for FY was nearly one standard deviation, 15.2 kg. In family 7, QTL effects for both milk yield and protein yield were also detected almost at the same position between BM2057 and BM6404. In this family, the same allele is associated with higher production in FY, MY, and PY. In family 7, the allele substitution effects for MY and PY were 343 and 9.3 kg, respectively.

The effect of adding cofactors to interval mapping can be seen in Table 3. In this study the selection criteria for a QTL to be included as a cofactor was 5% chromo-

Table 1. The genetic linkage map of Finnish Ayrshire cattle. Adjacent markers in a linkage group are connected with hyphens and the estimated distance from centromere are in parentheses. The number of markers (n) and the average information content (IC) are given. The distances are given in centimorgans (cM) calculated with Haldane mapping function. The total length of the map is 2764 cM and it covers about 92% of the bovine genome.

BTA	n	IC	
1	9	0.50	TGLA49(0)-ILSTS104(24)-TGLA57(67)-BM6506(86)-BM864(105)-CSSM032(119)-CSSM019(154)-MAF46(157)-BM3205(157)
2	8	0.59	ILSTS026(0)-INRA040(1)-TGLA61(17)-URB42(35)-BM4440(79)-TGLA226(101)-BM2113(147)- OARFCB11(167)
3	8	0.59	INRA006(0)-UWCA7(1)-FCGR2(15)-BL41(30)-INRA023(31)-HUJ246(63)-HUJ1177(97)-INRA197(130)
4	7	0.58	RM188(0)-HUJ673(22)-TGLA116(29)-BM6458(46)-BM1500(74)-BMS648(77)-BR6303(94)
5	7	0.56	BM6026(0)-BP1(13)-CSSM034(44)-ETH10(71)-BM1819(83)-ETH152(127)-BM2830(131)
6	14	0.62	ILSTS093(0)-INRA133(16)-ILSTS090(21)-URB016(39)-BM1329(41)-BM143(68)-ILSTS097(84)-BM4528(86)-RM028(89)-BM415(93)-CSN(104)-AFR227(107)-BP7(112)-BM2320(151)
7	6	0.50	BM7160(0)-BM6105(30)-BM6117(57)-INRA192(87)-ILSTS006(132)-BL1043(165)
8	4	0.52	IDVGA11(0)-INRAMTT180(42)-HEL9(81)-BMS2847(130)
9	6	0.55	INRA136(0)-ETH225(16)-CSSM025(64)-UWCA9(69)-TGLA73(102)-CSSM056(124)
10	4	0.37	CSSM038(0)-ILSTS053(44)-ILSTS070(100)-CSSM046(144)
11	7	0.52	BM716(0)-INRA177(28)-BM7169(45)-HELMTT41(62)-BMS1048(81)-TGLA438(113)-HEL13(131)
12	3	0.54	BMS2057(0)-BM6404(27)-BMS1316(73)
13	4	0.46	TGLA23(0)-BMS1352(27)-RM327(57)-BMS995(117)
14	5	0.60	ILSTS039(0)-BMS1747(16)-RM011(50)-BMS740(66)-BM4513(79)
15	6	0.50	MTGTG13B(0)-BR3510(14)-NCAM(40)-HEL1(52)-HBB(78)-RM4(115)
16	3	0.48	BM1311(0)-IDVGA49(34)-BM1706(62)
17	3	0.54	BMS941(0)-BM8125(39)-BM1233(62)
18	4	0.53	BMS1355(0)-BMS2213(26)-BMS2639(76)-TGLA227(110)
19	7	0.52	HEL10(0)-URB44(23)-BP20(33)-CSSM065(56)-MAP2C(65)-IOBT34(68)-ETH3(90)
20	7	0.56	BM3517(0)-TGLA304(19)-BM713(46)-GHR(57)-PRLR(64)-ILSTS072(65)-BM5004(97)
21	3	0.43	RM151(0)-INRA103(40)-TGLA122(68)
22	3	0.48	CSSM026(0)-BM1520(45)-OARFCB304(70)
23	6	0.68	CSSM005(0)-RM033(11)-BM1258(21)-BOLA-DRB1(31)-RM185(38)-CSSM024(53)
24	2	0.55	BMS2270(0)-BMS466(40)
25	4	0.50	BMC4216(0)-BMS130(13)-BMS1353(59)-AF5(70)
26	2	0.52	HEL11(0)-BMS2567(15)
27	2	0.48	BMS641(0)-INRA134(29)
28	2	0.58	BMS510(0)-BMS1714(18)
29	4	0.46	JAB5(0)-ILSTS057(4)-BMC8012(24)-BMC1206(77)

some-wise significance. The analysis of individual chromosomes revealed 14 such suggestive QTL ($P_{chr} < 0.05$; Table 2). When cofactors were used, the number of identified QTL for milk yield increased from 5 suggestive to 12 genome-wise significant QTL ($P_{genome} < 0.05$). Similarly, four new regions were identified for F% and for

P% and two for PY. For FY one additional QTL was identified at BTA18 but the effect was only significant at 5% chromosome-wise level. De Koning et al. (2001) also analyzed milk yield and, using cofactors, went from 5 suggestive to 8 significant QTL (BTA1, 5, 6, 12, 20, 21, 23, 29), using virtually the same data. In addition

Table 2. The results of the whole genome scan of Finnish Ayrshire with least-squares analysis across families. Significance thresholds were determined by permutation. The highest test statistics (F-ratio) and their position (cM) for all chromosome-wise significant effects ($P_{chr} < 0.05$) are shown. Two effects are genome-wise significant ($P_{genome} < 0.05$) when corrected by the number of analysed chromosomes.

Trait	BTA	F-ratio	Position (cM)	P_{chr}	P_{genome}
Milk yield	1	2.38	135	0.0290	0.5741
	5	2.54	98	0.0137	0.3297
	6	2.47	66	0.0250	0.5201
	12	2.65	21	0.0063	0.1675
	20	2.95	89	0.0019	0.0537
Fat %	14	5.83	0	< 0.0001	< 0.0029
Fat yield	12	3.81	28	< 0.0001	< 0.0029
	14	2.73	0	0.0050	0.1353
Protein %	6	2.74	68	0.0223	0.4849
	14	2.58	54	0.0205	0.4516
	23	2.41	21	0.0235	0.4982
Protein yield	5	2.32	131	0.0293	0.5779
	12	2.24	21	0.0218	0.4723
	25	2.87	44	0.0026	0.0727
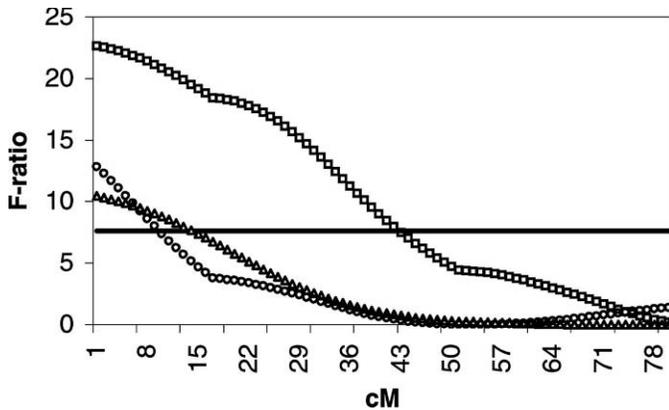
Figure 1. The within family results for fat percentage from regression analysis on BTA14. The test statistics (*F*-ratio) for three families (family 9, open squares; family 4, open circles; family 5, open triangles) are presented. The position (cM) of the QTL is at the centromeric end of the chromosome. The 5% chromosome-wise (solid line) risk level for family 9 is shown. The risk levels for families 4 and 5 are 6.31 and 7.24, respectively.

to the QTL reported by de Koning et al. (2001) we found four other significant QTL (Table 3). The difference between these two studies is that de Koning et al. (2001) analyzed 28 chromosomes instead of 29 because genotypes for BTA3 were not available at that time. In this study, BTA3 reveals a significant QTL for MY and is thus selected to serve as a cofactor.

The comparison between Tables 2 and 3 shows that the power of the analysis increases when mapping is augmented by cofactors. The test statistics increase because the residual variance decreases. The position of QTL seems to become more accurately estimated. The biggest change in test statistics was seen in the F% QTL at BTA14 where the *F*-ratio increased from 5.83 to 10.74 and the position remained the same. In BTA20 the test statistic for MY QTL was also noticeably increased but in this case the position was slightly changed. Figure 2 illustrates that addition of cofactors not only increases the test statistics and changes the best position of the QTL but also makes the *F*-ratio peak steeper.

The multimarker regression approach does not show the direction of the effect. To get a better understanding

Table 3. The result of the whole genome scan by combined analysis of multiple chromosomes. The highest test statistics (*F*-ratio) and their positions (cM) for all effects significant at genome-wise level ($P_{genome} <$ 0.05) are shown. The QTL from across-family analysis were selected to serve as cofactors using chromosome-wise 5% risk level as a selection criteria. The final number of cofactors used in the analysis for each trait are shown in the second column. P_{genome}-values are <0.0029 because only 10000 permutations were used, which gives the 0.0001 significance levels before Bonferroni correction for the number autosomes.

Trait	Number of cofactors	BTA	*F*-ratio	Position (cM)	P_{genome}
Milk yield	12	1	4.76	137	< 0.0029
		2	3.58	35	0.0029
		3	5.05	62	< 0.0029
		5	4.56	94	< 0.0029
		6	7.44	66	< 0.0029
		12	7.56	12	< 0.0029
		20	7.73	82	< 0.0029
		21	7.39	24	< 0.0029
		23	3.61	4	< 0.0029
		25	3.84	70	< 0.0029
		27	4.18	29	< 0.0029
		29	5.37	34	< 0.0029
Fat %	5	3	3.21	1	0.0464
		6	3.22	95	0.0290
		14	10.74	0	< 0.0029
		19	3.84	67	< 0.0029
		26	3.1	15	0.0116
Fat yield	2	12	4.03	27	< 0.0029
		14	3.23	5	0.0398
Protein %	7	3	4.88	1	< 0.0029
		6	5.58	66	< 0.0029
		12	4.32	10	0.0029
		14	5.70	50	< 0.0029
		20	3.22	68	0.0144
		23	3.31	21	0.0144
		25	3.48	0	0.0058
Protein yield	5	5	3.49	77	0.0144
		12	3.33	16	0.0029
		25	3.61	66	< 0.0029
		27	2.65	29	0.0399
		29	2.86	28	0.0342
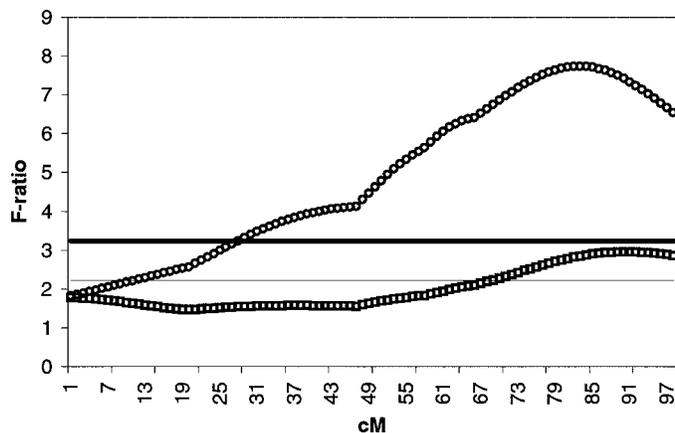
Figure 2. The comparison between single chromosome analysis and multiple chromosome analysis for milk yield on BTA20. The test statistics (*F*-ratio) for initial across-family analysis (open squares) and cofactor analysis (open circles) are shown. The QTL from across-family analysis was selected to serve as a cofactor using 5% chromosome-wise risk level (thin solid line) as a selection criterion. The 5% genome-wide significant threshold (thick solid line) for cofactor analysis on BTA20 is shown.

of the QTL found within families, results were checked individually after cofactor analysis. As seen in Table 3, it is quite common that a QTL detected in one milk production trait reappears for another trait. Some caution should be taken before drawing any final conclusions about the detected milk production QTL, especially F% and P%, without fully understanding the true nature of the trait.

Regarding chromosome areas with QTL for several milk traits at the same position, the effect on MY and F% or P% (or both) are usually opposite (data not shown). This means that either the high QTL allele for MY is on the same chromosome phase with the low F% or P% (or both) alleles and vice versa, or it reflects the "pleiotropic" effect of one gene. We assume that the percentage traits are most often reflecting the amount of water in milk as the increase in milk water content decreases the proportion of milk solids. We would like to point out, that depending on the power of the analysis, in several cases the true nature of the effect could be easily missed by missing one of the correlated effects. The cofactor analysis increases the power of detection of QTL noticeably. Interestingly, in our data the effect seen in MY and F% or P% (or both) is never reflected in fat and protein yields except in BTA25, where QTL for MY, P% and PY were detected.

The closer study of within-family results supports the idea that many of the QTL affect the liquid component of milk. The tendency can be seen in chromosomes 1, 3, 6, 20, 21, and 23. Similar observations have been made in the Holstein-Friesian population for chromosomes 6,

9, 14, 20, and 26 (Zhang et al., 1998). Because lactose is the major osmotic molecule in milk it is tempting to suggest that many QTL detected for milk yield are due to the genetic factors related to lactose synthesis and secretion. However, it should not be forgotten that lactose is not the only osmotic component of milk. Some minerals, especially calcium and phosphorus, affect the osmotic potential, too. They are usually complexed with the main protein components of milk, casein and casein micelles.

On BTA12 and BTA14, genome-wise significant QTL clearly affecting yield were found. The QTL effects for MY, FY, and PY in BTA12 are parallel. The single chromosome analysis of BTA12 revealed QTL for FY in two families (3 and 7) and also QTL for MY and PY in family 7. After cofactor analysis the latter two were also seen in family 3 ($P_{chr} < 0.05$). The allele substitution effects in this family for MY and PY was, respectively, 192 and 5.2 kg. This is the only QTL detected in Finnish Ayrshires that has an influence on the overall yield. The cofactor analysis did not reveal any additional information at family level for fat traits in BTA14.

The distribution of detected QTL among different chromosomes and families (Figure 3) shows a rather disappointing picture considering the practical applications of the results. First, the assumption that the majority of the QTL affect the liquid component of milk seems to be supported by the large number milk yield and percentage QTL compared with fat and protein yield QTL. Second, for the genuine yield QTL, only few families share each QTL, hampering both further fine mapping and use of marker information in breeding.

A detailed literature survey concerning observed milk production QTL in different breeds of dairy cattle has recently been published (Mosig et al., 2001). In our study, many results reported in the literature were confirmed, particularly those in chromosomes 1, 2, 3, 6, 14, 20, 21, 23, 26, 27, and 29. In fact, only BTA12 revealed QTL specific for Finnish Ayrshire. In cofactor analysis the QTL for MY and for PY in BTA5 were highly significant. Heyen et al. (1999) reported a QTL for F% on the same chromosome. These are the only QTL reported for BTA5 so far. Similarly, QTL on BTA18 and BTA25 have been reported in only a few studies. Ashwell et al. (1997) reported a significant marker association for FY in BTA18. This was confirmed in our study. Mosig et al. (2001) reported significant effect for P% in BTA25. In our study, a genome-wise significant QTL for MY, P%, and PY were observed in BTA25, too.

Several QTL were detected in family 9, some with very high test statistics. Some caution should be taken regarding results from such families, particularly when the family size is small. In this family, the allele substitution effect in BTA14 is similar to other segregating fami-

a.)

b.)

c.)
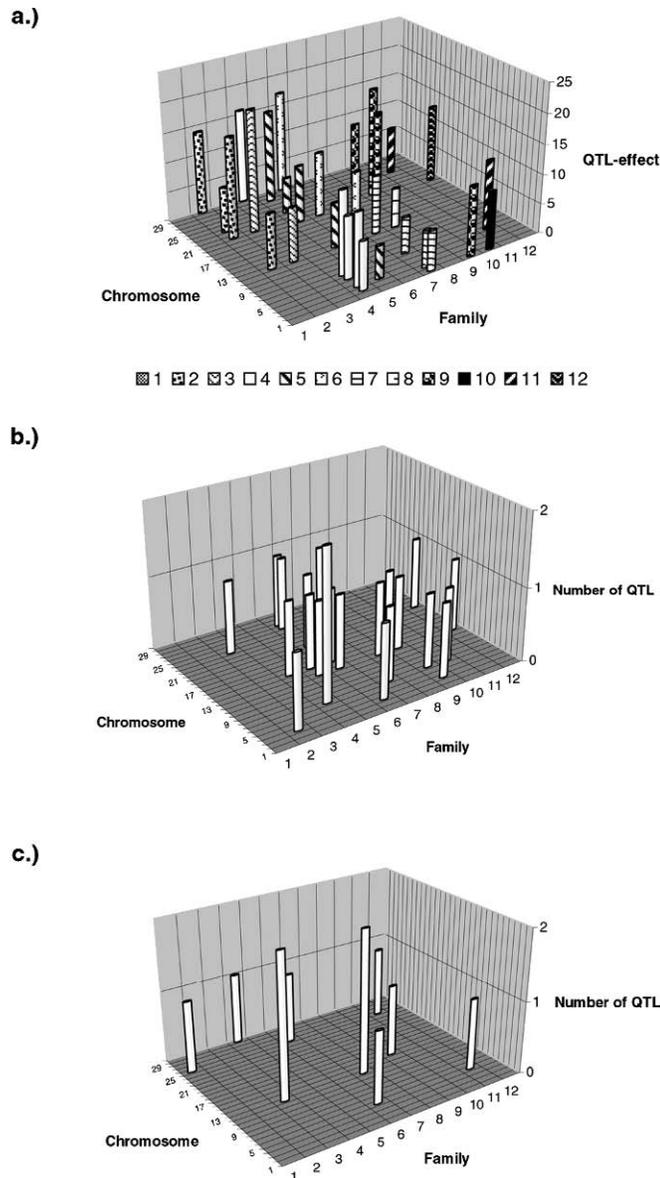
Figure 3. The within-family results from the whole genome scan with cofactors for each trait. All effects significant at the 5% chromosome-wise level after Bonferroni correction for the number of families (12) are shown. The families are numbered according to age. a) The distribution of milk yield QTL across families and the genome. The size of the QTL effect is shown on the z-axis in EBV indices, ten points corresponding to one standard deviation. Different patterns for columns are used to separate the families. b) The distribution of the percentage trait QTL (both fat and protein). The number of QTL at each chromosome in each family is shown on the z-axis. c) The distribution of fat yield and protein yield QTL. The number of QTL at each chromosome in each family is shown on the z-axis.

lies and thus the QTL seems to be genuine. However, in some chromosomes the observed QTL effect may be due to having information only on few individuals.

We also analyzed the possible presence of two QTL on the same linkage group, while correcting for QTL on other linkage groups. Suggestive evidence for the presence of two QTL was found only on chromosome 3 (F = 2.08). One QTL was located at the centromeric end of the chromosome (1 cM) and the other at the distal end of the chromosome (106 cM). In our previous study, two QTL were detected in BTA6 for P% and MY ($P_{chr} < 0.1$) and for F% ($P_{chr} < 0.05$; Velmala et al., 1999). In this study no significant support for our previous findings was observed. One difference between these two studies is that Velmala et al. (1999) used EBV from the national animal model evaluation of 1996, whereas the EBV in this study are from the evaluation of 1998. Another difference between these two studies is that here the existence of two QTL was jointly analyzed with the cofactors. Possibly, the earlier result was due to linkage disequilibrium extending over different chromosomes, creating spurious association, which was removed by cofactors.

In Finnish Ayrshires, several loci affecting milk production traits are still segregating. Most of the QTL studies in dairy cattle have been carried out in different Holstein-Friesian populations (see Mosig et al., 2001). Interestingly, the analysis of the red and white cattle breed reveals only one QTL not detected in Holstein-Friesians or other dairy populations. Recent studies on genetic relationships among cattle breeds have suggested a common ancestry for European Ayrshire and Friesian breeds (Kantanen et al., 2000). An interesting question is whether the QTL detected so far have the same origin in different breeds of cattle. However, it is difficult to compare studies to acquire comprehensive results because different studies have used different designs, methods, traits, and levels of statistical significance. Moreover, the physiological nature of milk production traits makes comparative analysis even more complicated because some of the studies cover only one or two traits.

Drawing conclusions about the detected QTL should be done with caution because some effects may have remained undetected. This is probably due to the relatively low power of analyses used for QTL detection so far. The augmentation of cofactors not only increases the power to detect QTL but also clarifies the nature of milk production QTL found. A further improvement of mapping precision and power would be gained by taking into account effects of the putative QTL on several traits simultaneously (Korol et al., 2001).

CONCLUSIONS

In this study, many QTL reported in previous mapping experiments were confirmed in Finnish Ayrshire. Apparently, only a few of the identified loci truly affect yield. The cofactor analysis results suggest that most of the QTL observed affect the amount of water in milk. This

is only a hypothesis, as other likely explanations exist. Fine mapping or multitrait QTL mapping would improve mapping resolution and the estimation accuracy of the QTL. The importance of understanding the nature of QTL (e.g., to distinguish between linkage and pleiotropy or genetic interactions between QTL) should not be bypassed when such applications as marker-assisted selection are under consideration.

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