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Whole-genome scan to detect QTL for milk production, conformation, fertility and functional traits in two US Holstein families

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Summary

A genome scan was conducted in two US Holstein half-sib families to identify quantitative trait loci (QTL) affecting milk production and conformation traits using the granddaughter design. The sires of the two studied families were related as sire and son and had 96 and 212 sons respectively. A total of 221 microsatellite loci were scored in both families. Statistical analysis was performed using two different analytical methods; half-sib least squares regression and Bayesian Monte Carlo Markov Chain. Traits analysed included five traditional milk production traits, somatic cell count, daughter pregnancy rate, male fertility and 20 conformation traits. A total of 47 tests achieved at least genome-wise significance. However, results from the two methods of analysis were only concordant for QTL location and level of significance in eight instances.

Keywords conformation, fertility, milk production, quantitative trait loci.

Introduction

Since the first whole-genome scan in dairy cattle by Georges *et al.* (1995), numerous genome scans and subsequent fine-mapping projects have been undertaken to identify the genomic regions harbouring genes that underlie phenotypic variation in dairy production traits. To date, most quantitative trait loci (QTL) mapping studies in dairy populations have focused on milk production traits. Bovenhuis & Schrooten (2002) provided a review of published reports on QTL mapping in dairy cattle, from which it is evident that there are many QTL influencing each trait. Some QTL have been confirmed in several studies, such as those on *Bos taurus* autosomes (BTA) 3, 6, 14 and 20, while others have yet to be resolved.

Recently, mapping studies have begun to focus on traits such as conformation, health, fertility and calving ease (Schrooten *et al.* 2000; Klungland *et al.* 2001; Kühn *et al.* 2003; Ashwell *et al.* 2004). Genetic progress by way of conventional breeding for these traits has been hampered by low heritabilities as well as by difficulty in defining the phenotypes. However, if QTL with large effects underlie the available additive genetic variation in these traits, marker-assisted selection for desirable genotypes could lead to significant genetic improvement in these animal health and well-being traits. Because there has been difficulty in defining several of the phenotypes used to characterize these traits, there will likely be an increased level of difficulty in the identification of candidate genes within chromosomal regions identified as harbouring QTL. However, the pedigree structure of the Holstein breed is well suited for the application of marker-assisted selection based on closely linked markers, provided that haplotypes, which are diagnostic for the desirable and undesirable QTL alleles, can be characterized within families.

In order to identify new QTL and to confirm previously identified QTL, two sires (which are herein denoted as sire 1 and 2) were chosen for this study based on their milk production and conformational phenotypes. Sire 2 ranks very high within the Holstein breed for milk, fat and protein yield predicted transmitting abilities (PTAs) but has a very poor daughter productive life PTA. Additionally, his daughters seem to have an unusual conformation with deep, wide udders, tall stature and extreme dairy form. Unlike previous mapping studies in the US Holstein population that have used older sires, sire 2 is more contemporary and both he

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and his sons have been heavily used in the breeding programmes of all of the major US AI organizations. Sire 1 was chosen for inclusion in the genome scan because he has high fat yield (FY) and low productive life PTAs and is also the sire of sire 2. Unlike previous genome scans that used several families to identify as many QTL as possible, we were interested in identifying the specific QTL that are segregating in these two sires. Because of the heavy use of their germplasm in today's breeding programmes, the detected QTL may be targeted for future fine-mapping studies.

Materials and methods

Animals and traits

DNA samples were obtained from the Cooperative Dairy DNA Repository (Ashwell & Van Tassell 1999) for bulls comprising two half-sib families. Sire 1 had 96 sons and sire 2 had 212 sons. In order to complete the pedigree and assist in the validation of marker genotypes, members of the paternal sire line, going back to the great-grandsire of sire 1, and six other bulls that appear as maternal grandsires in this pedigree, were also genotyped. DNA from these six sires was obtained from the Dairy Bull DNA Repository (Da *et al.* 1994). In total, six generations were represented in the mapping pedigree. A summary of the traits analysed and the abbreviations used in this manuscript are presented in Table 1. Milk production (MY, FY, FP, PY and PP), productive life (SCS, CE and DPR) phenotypes were obtained from the USDA Animal Improvement Programs Laboratory (May 2004 evaluations). Daughter pregnancy rate is a relatively new evaluation that is defined as the percentage of non-pregnant cows that become pregnant during each 21-day period. A DPR of '1' implies that daughters from this bull are 1% more likely to become pregnant during an oestrus cycle than a bull with an evaluation of zero. Each 1% increase in DPR PTA is equivalent to a decrease of 4 days in days open PTA (VanRaden *et al.* 2004). Conformation phenotypes were obtained from the Holstein Association USA (2002) and male fertility phenotypes were obtained from Dr Kent Weigel of the University of Wisconsin. Daughter yield deviations were used as the dependent variable for MY, FY, FP, PY and PP. Daughter deviations were used for PL and SCS, while PTAs were used for the DPR analyses. All other dependent variables were standardized transmitting abilities. Sample sizes for sire 1 and sire 2 were: milk traits and DPR 61 and 161; type traits 36 and 128; calving ease 62 and 170; productive life 62 and 125; and male fertility 19 and 162, respectively.

Table 1 Traits analysed and the mean and standard deviation for the sons of each sire.

		Sire 1 sons		Sire 2 sons	
Trait	Description	Mean[1]	SD	Mean[1]	SD
MY	Milk yield	43	547	1161	588
FY	Fat yield	24.7	21.6	41.0	19.4
FP	Fat per cent	0.100	0.096	−0.003	0.083
PY	Protein yield	4.5	14.7	37.9	15.2
PP	Protein per cent	0.015	0.047	0.013	0.042
SCS	Somatic cell score	−6.9	23.7	1.4	20.4
DPR	Daughter pregnancy rate	−0.87	0.92	0.21	0.85
PTAT	PTA type traits	0.04	0.58	0.43	0.63
STA	Stature	0.82	0.75	1.07	0.81
STR	Strength	0.60	0.80	0.43	0.78
BD	Body depth	0.79	0.70	0.62	0.71
DF	Dairy form	0.69	0.78	1.35	0.87
RA	Rump angle	0.10	1.12	0.33	0.89
TW	Thurl width	0.11	0.83	−0.10	0.82
RLSV	Rear legs side view	0.39	0.96	−0.14	0.97
RLRV	Rear legs rear view	−0.19	0.94	0.28	1.00
FA	Foot angle	0.58	1.06	0.30	0.94
FLS	Feet leg score	−0.08	0.85	0.05	0.92
FATT	Fore udder attachment	−0.42	0.96	−0.33	0.93
RUH	Rear udder height	−0.57	0.65	0.55	0.94
RUW	Rear udder width	−0.81	0.73	0.75	0.94
UC	Udder cleft	−0.36	0.91	−0.15	1.05
UD	Udder depth	−0.45	0.99	−1.04	1.05
FTP	Front teat placement	−1.10	0.98	−0.36	1.06
TL	Teat length	0.85	0.90	0.43	1.07
CE	Calving ease	9.35	1.17	8.95	1.23
PL	Productive life	−12.31	20.80	−4.49	24.72
MFERT	Male fertility	−0.014	0.022	−0.006	0.027

[1]DYD for MY, FY and PY reported in pounds; SCS adjusted to log base 2 of the concentration; FP and PP reported as percentage of fat or protein yield/milk yield; PL reported as months of life, limited to 7 years, 10 months of life per lactation; conformation traits as units of genetic standard deviation.

Markers

Microsatellite markers ($n = 221$; Table 2) were chosen primarily from public databases or published marker reports (http://www.marc.usda.gov). The forward PCR primer for each marker was synthesized with one of three fluorescent dye labels. Multiplexed PCR reactions were developed based on the allele size ranges, fluorescent label and the empirically determined ability of each marker to co-amplify. Between four and eight markers were co-amplified in each reaction. Multiplex PCR was performed using 5-μl reactions on an ABI 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) as described in Schnabel *et al.* (2003).

The PCR products were separated on an ABI 3700 Automated Sequencer and sized relative to the GS400HD internal size standard (Applied Biosystems). Fluorescent signals from the dye-labelled microsatellites were detected using GENESCAN 3.1 (Applied Biosystems), and genotypes were assigned using Genotyper 3.7 (Applied Biosystems). Additionally, both sires and their sons were genotyped for the acylCoA:diacylglycerol acyltransferase 1 (*DGAT1*) K232A mutation (Grisart *et al.* 2002).

Data analysis

GENOPROB (Thallman *et al.* 2001a,b) was used to verify genotype scoring using published marker positions (http://www.marc.usda.gov). The complete pedigree information linking each of the genotyped bulls was assembled into a single pedigree to exploit the relationship between the two sires and their sons. Genotype and grand-parental origin probabilities were estimated for each of the genotyped animals using all available information (genotype, map and pedigree). Individual genotypes with low probability as defined by GENOPROB (pGmx < 0.98) were excluded from further analysis.

Half-sib regression based on least squares (LS) using the program QTL Express (Seaton *et al.* 2002) was used to analyse each sire family individually under a granddaughter design model to estimate the segregation status of each sire. Data permutation based on 5000 replicates was used to determine chromosome-wise significance and 1000 replicates for genome-wise significance levels for each sire and each trait (Churchill & Doerge 1994). A strategy analogous to composite interval mapping was used in which an initial scan was performed and the position of the highest test statistic was then incorporated in the model as a cofactor. The genome was then rescanned, incorporating additional cofactors, until no additional tests were significant at the chromosome-wise $P < 0.05$ level. At each step, the cofactors were tested for significance by suspending each cofactor in turn and testing that chromosome for significance. For the milk production traits and the trait DF, the position of *DGAT1* was included in the model as a cofactor to account for the effect of this known QTL.

LOKI v2.4.5 (Heath 1997) was used for a joint multipoint QTL analysis of both sire families. An initial burn in of 1000 iterations was followed by 501 000 iterations where parameter estimates were collected at every iterate for a total of 500 000 data points. A description of the analytical model and the Monte Carlo Markov chain (MCMC) sampling process are presented in Heath (1997). Briefly, the trait is modelled by *k*-biallelic QTL where for the *i*th QTL, genotypes A_1A_1, A_1A_2 and A_2A_2 have genotypic effects a_i, d_i and $-a_i$ respectively. The model for trait y ($n \times 1$; n animals each with a single observation) can be expressed as:

$$y = \mu + \mathrm{X}\beta + \sum_{i=1}^{k} Q_i\alpha_i + \mathrm{Z}u + e,$$

where μ is the overall trait mean, β is an $(m \times 1)$ vector of fixed effects and covariates, α_i is a (2×1) vector of allele substitution effects for the *i*th QTL, u is an $(n \times 1)$ vector of random normally distributed additive residual polygene effects, e is an $(n \times 1)$ vector of normally distributed residuals, k is the number of QTL in the model and X $(n \times m)$, Q_i $(n \times 2)$ and Z $(n \times n)$ are known incidence matrices for the fixed, QTL and polygenic effects, respectively. *DGAT1* genotypes were included in the model as a fixed effect.

Table 2 Summary of genome coverage.

BTA	No. of markers	Average interval (cM)[1]	Centromeric marker (cM)	Telomeric marker (cM)	Genome coverage (cM)
1	10	13.4	8.2	128.7	120.5
2	7	15.2	0.8	92.1	91.3
3	6	18.9	28.3	123.0	94.7
4	5	20.9	3.9	87.4	83.5
5	9	14.2	0.0	113.5	113.5
6	40	2.7	0.0	103.5	103.5
7	8	19.2	0.0	134.1	134.1
8	9	14.5	0.0	116.3	116.3
9	8	12.7	8.1	96.7	88.6
10	9	9.1	25.4	98.4	73.0
11	8	15.2	0.0	106.4	106.4
12	6	18.1	15.1	105.8	90.7
13	3	32.9	19.5	85.3	65.8
14	8	12.0	0.0	84.1	84.1
15	6	16.1	1.0	81.6	80.6
16	11	8.2	11.5	93.2	81.7
17	6	17.3	0.0	86.3	86.3
18	7	12.7	2.8	78.9	76.1
19	7	13.9	15.9	99.5	83.6
20	4	11.3	0.0	33.9	33.9
21	5	17.6	11.7	81.9	70.2
22	4	20.4	0.0	61.1	61.1
23	5	9.2	23.9	60.8	36.9
24	5	14.1	6.0	62.5	56.5
25	4	17.5	12.3	64.9	52.6
26	3	23.4	2.5	49.2	46.7
27	10	7.1	0.0	64.1	64.1
28	3	16.7	2.5	35.8	33.3
29	5	15.4	0.0	61.6	61.6
Average	7.5	15.2			
Total					2291.2

[1]All positions are based on the USDA/MARC map (http://www.marc.usda.gov) and map distances are in Kosambi cM.

LOKI offers the analytical advantage of allowing the number of QTL in the model to vary while simultaneously analysing the entire genome.

Results and discussion

Although numerous genome scans have now been performed in dairy cattle for milk production traits, there are relatively few reports that have examined conformation traits (Schrooten *et al.* 2000; Ashwell *et al.* 2001; Kühn *et al.* 2003). Therefore, in addition to potentially identifying new QTL for milk production traits, these two families were selected to identify novel conformation QTL and to confirm previous results concerning milk production QTL.

Interval analysis using the regression approach of QTL Express produced 157 trait/sire/chromosome combinations that were significant at the chromosome-wise $P < 0.05$ level or better. However, QTL were detected on all 29 chromosomes at this level in order to avoid reporting

false-positives, only results that achieved genome-wise significance are reported (Table 3). At the genome-wise significance level, 10 chromosomes demonstrated no evidence for QTL: BTA4, 12, 13, 17, 19, 22, 24, 27, 28 and 29. The traits for which no QTL were detected were FP, STA, BD, TW, RLSV, PL and MFERT. Somatic cell score and CE had the most QTL detected with 5 each.

Only three chromosomes (BTA2, 9 and 14) showed significant QTL for milk production traits. The QTL affecting FY, PY and DF on BTA2 (Fig. 1) was previously reported by Zhang *et al.* (1998) as affecting FP at an experiment-wise suggestive significance level. Mosig *et al.* (2001) detected a significant association ($P < 0.033$) between PP and *BMS1126*, which is 15 cM telomeric to the most likely QTL position in Fig. 1. Heyen *et al.* (1999) also detected a QTL affecting PP in three families with a significant ($P < 0.05$) association with *TGLA377*, which is approximately 7 cM centomeric from our most likely QTL position. One of the three sires that Heyen *et al.* (1999) found to be segregating for this QTL is the sire/grandsire of the two bulls used in this study. Recently, Ron *et al.* (2004) detected a major QTL towards the telomere of BTA2 affecting protein and milk production and a second QTL located near 70 cM affecting PP. While it is difficult to directly compare the results of this study to previous reports because of differences in the markers and analytical approaches employed, there is clear support for at least one QTL, and possibly as many as three, affecting milk production traits on BTA2.

We have identified a QTL near 50 cM on BTA9 affecting both protein and fat yield. Several studies have identified QTL affecting the milk yield traits on BTA9; however, most of these reports position the QTL telomeric of the position we identified (Georges *et al.* 1995; Vilkki *et al.* 1997; Wiener *et al.* 2000). Our data indicate that while the QTL near position 45–50 cM affects both fat and protein yield, there is also evidence for a second QTL near position 98 cM affecting only FY, which is consistent with the previously reported positions for a FY QTL (data not shown).

Coppieters *et al.* (1998) first identified a major QTL on BTA14 affecting milk FP that was subsequently positionally cloned and identified as *DGAT1* by Grisart *et al.* (2002). All of the animals used in this study were genotyped for the *DGAT1* K232A mutation in order to account for the variance attributed to the *DGAT1* polymorphism. A significant QTL affecting MY and PP was unexpectedly identified in the interstitial region of BTA14 at position 53 cM (Table 3 and Fig. 2) when the *DGAT1* genotypes were incorporated into the model. The precipitous decline in the test statistic values between 40 and 50 cM in Fig. 2 is because of the inclusion of *DGAT1* in the model at 0.0 cM and because sire 2 was not informative for *BMS1941* (33.5 cM). Thus, after conditioning on *DGAT1*, there was no additional chromosomal architecture information available until *BMC1207* (43.9 cM). Removal of *DGAT1* from the model had no effect on the evidence for this QTL (data not shown), indicating that the detection of this QTL is not an artifact of the known milk production QTL on this chromosome.

While *DGAT1* has been the most frequently identified QTL on BTA14, three studies have reported evidence of an additional QTL in the interstitial region of the chromosome. Mosig *et al.* (2001) found a significant ($P < 0.033$) association between *BL1036* (79.7 cM) and PP. Heyen *et al.* (1999) found a significant ($P < 0.01$) association between *BM4305* (66.4 cM) and MY in two families. Ashwell *et al.* (2001) found a significant ($P = 0.016$ trait-wise) association between *BM6425* (85.7 cM) and PP in one sire family. This sire is the maternal grandsire of the bull found to be segregating for the QTL in this chromosomal region in this study. Thus, our results confirm and refine previous marker association studies supporting the existence of a second milk production QTL on BTA14.

The DPR evaluations are relatively new, being first published in February 2003; therefore, there has only been one report of QTL for this trait. Ashwell *et al.* (2004) identified six chromosomes with chromosome-wise $P < 0.01$ effects for DPR. The only chromosome with QTL in common with the current study was BTA14 where Ashwell *et al.* (2004) placed the DPR QTL at 11 cM, between *ILSTS11* and *CSSM66*. Our results place a DPR QTL at 60 cM, between *BMC1207* (44 cM) and *BMS1899* (62 cM), at a genome-wise significance level of $P < 0.05$. However, the estimated allele substitution effects were virtually identical between the two studies (0.84 vs. 0.85). They suggest that the DPR QTL may be explained by the effects of the nearby *DGAT1* gene. In order for that to be true, there must be a second DPR QTL on this chromosome because the sire segregating for DPR in this study was homozygous for the *DGAT1* K232A polymorphism. Given the relatively sparse maps used in both studies, a more likely explanation would be a single QTL located between the positions reported by Ashwell *et al.* (2004) and this study. Recently, Gonda *et al.* (2004) identified a QTL on BTA14 affecting ovulation rate in the USDA MARC twinning herd. Their interval analysis placed the ovulation rate QTL at 59 cM (Kosambi), approximately 6 cM telomeric of *BMS947*. The DPR QTL detected in this study is about 8 cM centromeric of this ovulation rate QTL and potentially represents the same gene.

Four QTL were detected for SCS: BTA8, 10, 11 and 21. Heyen *et al.* (1999) reported a QTL on BTA21 at 32 cM and Schulman *et al.* (2004) identified a QTL affecting mastitis at 23 cM, both of which are near the QTL reported here. Sire 2 appears to be segregating for two QTL affecting SCS on BTA11 at positions 33 and 93 cM. A two-QTL model placed both QTL at the same positions as in the single QTL model, with substitution effects of 16.08 for position 33 cM and −13.39 for position 93 cM. Zhang *et al.* (1998) reported a QTL at 46 cM while Schulman *et al.* (2004) reported QTL affecting SCS and mastitis but at different chromosomal positions. Of the four families Schulman *et al.* (2004) found

Table 3 Test statistics and locations for quantitative trait loci (QTL) using QTL Express and LOKI.

			QTL Express			LOKI		
BTA	Sire	Trait	Position[1]	F^2	Effect	Bayes factor	Position[1]	Marker interval (position)
1	2	DPR	9	15.10	−0.76	1.25	151	*BM8139* (9)–*BMS711* (24)
		UC	39	16.17*	0.72	1.55	37	*BMS711* (24)–*BM4307* (40)
2	2	FY	39	19.86*	14.27	16.36	37	*TGLA44* (0)–*ETH121* (44)
		DF	45	17.97*	0.58	36.79	45	*ETH121* (44)–*BMS803* (52)
		PY	45	19.91*	9.96	26.89	46	*ETH121* (44)–*BMS803* (52)
		CE	77	15.94	0.72	1.44	85	*BY32* (76)–*RM041* (85)
3	2	FTP	45	19.52*	−0.76	14.66	41	*BMS2904* (36)–*BMS482* (45)
5	2	FTP	22	16.47*	0.82	0.74	132	*BMS1095* (0)–*BP1* (22)
6	1	CE	113	16.70	1.15	2.12	88	*BM8124* (96)–*BMC4203* (114)
7	1	STR	110	15.49	−1.02	1.40	109	*BB719* (105)–*BM9065* (122)
8	1	SCS	85	26.20*	−23.94	1.23	86	*BMS2072* (69)–*IDVGA52* (88)
		FLS	86	40.69*	−1.50	4.03	62	*BMS2072* (69)–*IDVGA52* (88)
		RLRV	109	17.29	−1.45	0.65	53	*BM711* (98)–*CSSM47* (131)
9	1	RA	106	43.72*	1.61	39.41	107	*BMS2295* (106)–*BMS1943* (112)
	2	FY	45	15.60	11.41	4.59	39	*BMS817* (43)–*UWCA9* (51)
		UD	46	15.96	−0.69	8.33	39	*BMS817* (43)–*UWCA9* (51)
		PY	50	15.23	8.41	2.00	37	*BMS817* (43)–*UWCA9* (51)
10	1	**CE**	74	21.78*	−1.19	4.57	70	*INRA071* (68)–*INRA037* (81)
	2	**CE**	68	14.33	−0.67	–	–	
		TL	68	14.03	−0.64	1.43	70	*INRA071* (68)–*INRA037* (81)
		SCS	75	13.18	−12.03	5.58	85	*INRA071* (68)–*INRA037* (81)
11	2	SCS	33	19.55*	16.08	3.57	27	*BM827* (0)–*INRA177* (32)
		SCS	93	13.92	−13.38	–	–	*BMS2047* (86)–*BL1103* (106)
14	1	DPR	60	15.73	0.85	11.48	55	*BMC1207* (44)–*BMS1899* (62)
		RUW	79	28.17*	1.23	0.57	29	*BM4305* (78)–*BL1036* (92)
	2	PP	53	23.39*	−0.037	18.44	55	*BMC1207* (44)–*BMS1899* (62)
		MY	61	16.32*	415	22.04	57	*BMC1207* (44)–*BMS1899* (62)
15	1	**RA**	36	21.85*	1.78	6.54	50	*BMS1004* (7)–*MB076* (49)
	2	**RA**	44	16.75	−0.71	–	–	
16	1	CE	63	26.73*	−1.33	1.55	80	*IDVGA49* (62)–*INRA048* (81)
18	1	FATT	3	17.60	1.25	0.78	12	*BMS1355* (3)–*ILSTS021* (12)
	2	FA	90	13.73	0.61	0.77	86	*BMS929* (73)–*BM6507* (91)
		TL	90	14.33	0.66	0.88	90	*BMS929* (73)–*BM6507* (91)
20	2	FATT	38	15.31	−0.65	2.38	38	*RM310* (27)–*BMS1128* (39)
		PTAT	38	18.91*	−0.50	3.35	38	*RM310* (27)–*BMS1128* (39)
		RUH	38	14.32	−0.66	4.05	38	*RM310* (27)–*BMS1128* (39)
		RUW	38	21.44*	−0.78	5.22	38	*RM310* (27)–*BMS1128* (39)
21	2	SCS	16	13.32	12.88	8.65	6	*RM151* (13)–*BM103* (35)
		PTAT	36	15.06	−0.40	1.59	34	*BM103* (35)–*BMS868* (56)
		RUW	36	17.93*	−0.65	1.15	37	*BM103* (35)–*BMS868* (56)
		FTP	40	26.55*	−0.86	1.31	37	*BM103* (35)–*BMS868* (56)
		FATT	42	15.43	−0.62	3.25	28	*BM103* (35)–*BMS868* (56)
		TL	56	23.48*	0.85	2.89	55	*BM103* (35)–*BMS868* (56)
23	2	UD	57	16.29	−0.71	3.62	56	*RM185* (53)–*BM1818* (59)
25	1	RA	75	19.15	−1.24	1.05	74	*BMS1353* (52)–*BM1864* (75)
26	2	CE	2	15.35	−0.79	4.20	4	*BMS651* (0)–*BM4505* (52)
29	2	FTP	64	15.64	0.74	0.69	52	*BMS1600* (33)–*BMS1948* (71)

For description of traits see Table 1.

*Genome-wise $P < 0.01$

[1]Haldane cM.

[2]Significance of F-statistic is genome-wise $P < 0.05$ unless otherwise noted. Traits in bold denote those for which both sires were segregating for the QTL.

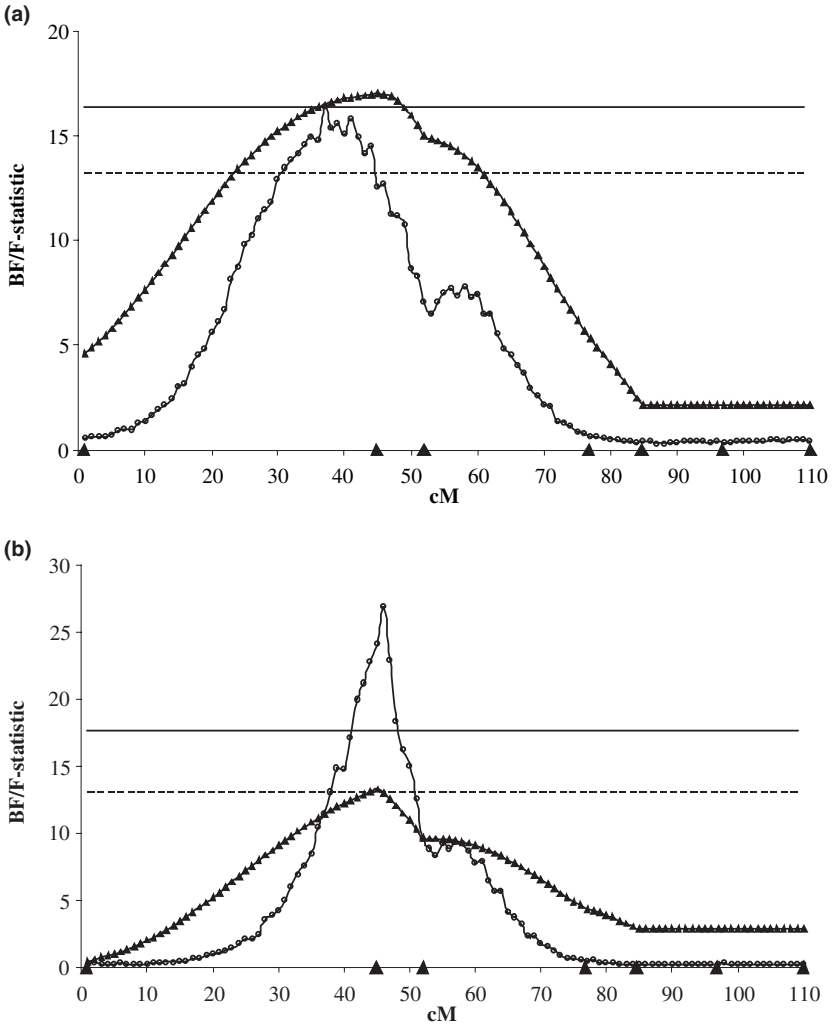

Figure 1 Interval analysis of BTA2 for fat yield (panel a) and protein yield (panel b). Least squares (LS) results are for sire 2 while Monte Carlo Markov chain (MCMC) results are for both sires jointly. LS results (▲) are an *F*-statistic and MCMC (●) results are a Bayes factor (BF). Horizontal lines represent $P < 0.05$ (dashed) and $P < 0.01$ (solid) genome-wise significance levels for LS analysis. Marker locations (Haldane cM) are indicated by triangles.

to be segregating, family 12 was segregating for two QTL. The QTL for mastitis in their family 12 is very near the QTL we identified at 33 cM, while the second QTL in their family 12 at 65 cM is about 30 cM distal from our second QTL. These results strongly suggest that there are at least two QTL affecting SCS on BTA11.

Five chromosomes showed evidence for QTL affecting CE; BTA2, 6, 10, 16 and 26. Of these, only the QTL on BTA10 at 68–74 cM is concordant with previous reports. Both sire 1 and sire 2 were significant for this QTL indicating that it is likely the same QTL identical by descent and the estimates of position differ simply because of marker informativeness and family size. Kühn *et al.* (2003) detected a QTL affecting stillbirth and dystocia at 79 and 83 cM, respectively, which independently supports the existence of this QTL. On BTA2, Schrooten *et al.* (2000) reported a QTL affecting CE near *BM2113* at 139 cM which is in agreement with reported QTL from beef cattle affecting birth weight (Grosz & MacNeil 2001; Kim *et al.* 2003). As sire 2 was not informative for the last two markers at positions 96 and 110 cM on BTA2, we cannot exclude the possibility that the CE QTL identified at position 77 cM is the same QTL previously detected for birth weight and CE near the telomeric end of the chromosome. Nevertheless, these results provide evidence for at least one QTL on BTA2 that affects CE. Schrooten *et al.* (2000) and Kühn *et al.* (2003) detected QTL for CE and stillbirth on BTA6 near *BMS690* (44 cm) and *DIK82* (58 cM) respectively. Considering the high density of markers ($n = 40$) that we used on BTA6, it is likely that the CE QTL detected at 113 cM represents a novel QTL.

Many of the conformation trait QTL detected in this study appear to be novel. The only previously reported QTL in common with the present study are for RA on BTA9 and FATT on BTA20 (Ashwell *et al.* 2001). The four conformation trait QTL on BTA20 for which sire 2 was segregating were all related to the udder, including PTAT, which is a measure of overall conformation. All three analyses were in agreement as to the most likely location of this QTL, placing it at *BMS1128* (38 cM). Although *BMS1128* was the most distal marker genotyped on this chromosome, it lies approximately 41 cM from the most telomeric marker known. Blott *et al.* (2003) identified a mutation in the

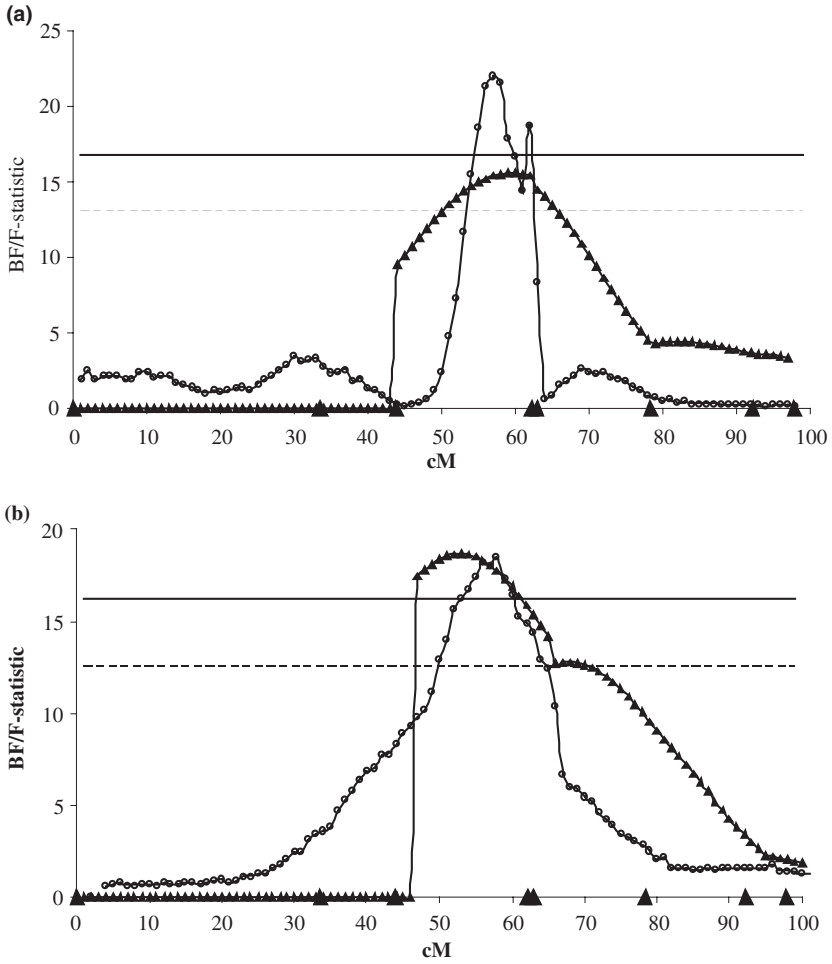

Figure 2 Interval analysis of BTA14 for milk yield (panel a) and protein per cent (panel b). Least squares (LS) results are for sire 2 while Monte Carlo Markov chain (MCMC) results are for both sires jointly. LS results (▲) are an *F*-statistic and MCMC (●) results are a Bayes factor (BF). Horizontal lines represent $P < 0.05$ (dashed) and $P < 0.01$ (solid) genome-wise significance levels for LS analysis. Marker locations (Haldane cM) are indicated by triangles.

growth hormone receptor (*GHR*) gene, which is located approximately 4 cM telomeric of *BMS1128*, that has a large effect on milk production traits. Given the proximity of these udder-related QTL to *GHR*, additional markers in the distal region of BTA20 must be genotyped to refine the position of this QTL and to evaluate the effect of *GHR* polymorphisms on these traits.

Chromosome 21 possessed by far the highest density of QTL influencing conformation (five QTL; Table 3). As found for BTA20, these results suggest the presence of at least one QTL influencing udder conformation on BTA21. However, in contrast, the estimates of the most likely QTL positions were not consistent among the traits. Either there is more than one QTL on BTA21 influencing conformation traits, or the phenotypes for conformation traits are not well defined. Subjectivity in assigning individual trait scores and the combination of scores for several underlying traits that define conformation may be responsible for the variation in QTL position on BTA21.

We used LOKI to complement the traditional regression approaches used in dairy QTL mapping and to evaluate this MCMC approach for QTL detection in a whole-genome scan. Clearly, there are discrepancies between the magnitude of the Bayes factors obtained using LOKI and the significance levels from LS regression (Table 3). These differences are because of the differences in the underlying models and their adequacy to represent the true QTL architecture in a mapping population, which is usually not known *a priori*. De Koning *et al.* (2003) discuss differences between the model assumptions of the two approaches and how violations can affect QTL detection in livestock populations. It appears clear that half-sib regression should be the method of choice for preliminary QTL detection. Using available half-sib families as the approach allows the dissection of the population into families in which different QTL may be segregating for a trait on any one chromosome. However, full-pedigree analysis, such as implemented in LOKI, appears to be valuable for QTL fine-mapping after the chromosomal QTL architecture is understood and a full analysis of a multigeneration pedigree is attempted (Schnabel *et al.* 2005).

In addition to confirming the existence of several milk production QTL, our results indicate a highly significant QTL on BTA2 affecting protein and FY, a new QTL affecting PP and MY on BTA14 and a QTL affecting DPR on BTA14. Sire 2 was chosen for inclusion in this study because of the

seemingly unusual udder conformation among his daughters. Our data indicate that this sire is likely segregating for a gene on BTA20 and a second gene on BTA21, which are responsible for variation in udder conformation and several udder-related conformation traits. These chromosomes are now candidates for fine mapping to more precisely estimate the locations and magnitudes of these QTL.

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