Detection and Analysis of Quantitative Trait Loci Affecting Production and Secondary Traits on Chromosome 7 in Israeli Holsteins

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ABSTRACT

A total of 5,459 Israeli Holstein cows, daughters of 11 sires, were genotyped for 29 microsatellites spanning chromosome 7 and analyzed by the daughter design for 9 economic traits: milk, fat, and protein yield, fat and protein percentage, somatic cell score, female fertility, herd life, and milk persistency. Quantitative trait loci at chromosome-wise 0.05 significance were obtained for fat and protein yield, fat percentage, somatic cell score, and female fertility. Peak *F*-values were obtained at 29 cM for fat and protein yield and fat percentage, at 60 cM for somatic cell score, at 74 cM for herd life, and at 11 cM for female fertility. The 0.95 confidence intervals for quantitative trait loci locations were 20 cM for kilograms of fat, 27 cM for fertility, and 51 cM for somatic cell score. Two loci affecting fertility at opposite ends of the chromosome are apparently segregating in the population. A quantitative trait locus for fertility near the centromere was confirmed by application of the modified grand-daughter design to a single family. Estimated frequency of the economically favorable allele in the Israeli Holstein cattle was less than 0.5. Significant genetic gain for fertility seems possible by marker-assisted selection.

Key words: quantitative trait loci, genetic marker, female fertility, dairy cattle

INTRODUCTION

Although traditional selection has been very efficient for milk production traits, which have moderate heritability, this is not the case for secondary traits, which generally have lower heritability and are more difficult to measure. Genetic trend for fertility was negligible throughout the last 20 yr in the Israeli Holstein population (Weller and Ezra, 2004), and fertility has declined in the US Holstein population (Washburn

et al., 2002). Many studies have shown that genetic progress can be enhanced by selection on markers linked to QTL (Weller, 2001). Although QTL for traits such as fertility have great potential for use in genetic improvement, the lower the heritability, the more difficult it should be to identify QTL affecting these traits (Weller et al., 1990).

Ron et al. (2004) completed a genome scan for QTL affecting economic traits in the Israeli dairy cattle population, based on a daughter design analysis of 11 sire families. Eight traits were analyzed, including female fertility, but no significant effects were found for this trait. Although only 3 markers on *Bos taurus* autosome (BTA) 7 were included in the genome scan, significant effects were detected in 2 families for the 5 milk production traits, SCS, and herd life. Thus, we decided to fine-map the QTL on this chromosome for all 11 families.

Significant associations of genetic markers with ovulation rate (Kappes et al., 2000; Kirkpatrick et al., 2000) and twinning rate (Lien et al., 2000) have been identified in dairy cattle. Arias and Kirkpatrick (2004) and Cruickshank et al. (2004) described a QTL affecting multiple ovulations and twinning in cattle in the centromeric region of BTA7. In both cases the QTL was identified in families with Holstein-Friesian ancestry. Arias and Kirkpatrick (2004) analyzed specific families from a genetically diverse USDA research population selected for ovulation and twinning rate. The ancestor of the family in which the BTA7 QTL was identified was a Swedish Friesian sire, son of a Canadian Holstein bull.

In this study, we report on significant effects for female fertility on BTA7 in the same regions in which Arias and Kirkpatrick (2004), Cruickshank et al. (2004), and Lien et al. (2000) found QTL affecting multiple ovulations and twinning rate. To estimate QTL allelic frequencies, we applied the modified grand-daughter design to a single family (Weller et al., 2002). In addition, QTL in different regions on this chromosome affecting milk production traits, SCS, and herd life are also reported.

Received May 15, 2007.

Accepted October 10, 2007.

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Table 1. Number of daughter genotypes per sire by marker

Marker	Sire										
	2278	2283	2357	3070	3089	3099	3208	3212	3241	3258	3274
BM7160	—	—	424	—	543	—	455	—	—	353	—
MNS91	—	—	—	666	548	228	541	—	—	348	—
SRC040	—	—	406	—	546	—	—	—	—	—	—
RM12	—	—	—	—	—	—	549	—	—	—	309
BM9289	656	409	—	540	452	—	—	—	425	—	—
IDVGA90	—	—	—	—	—	227	570	—	—	—	310
BP41	43	—	—	—	—	222	506	—	—	375	—
DIK2211	647	—	—	682	535	231	—	501	410	393	223
BL5	641	411	—	561	475	—	—	—	418	—	—
TGLA176	—	—	—	—	—	232	—	—	—	391	—
BMS713	—	—	406	—	480	—	—	—	—	364	—
DIK4378	43	—	—	—	—	—	—	—	—	—	298
ILSTS001	—	—	426	—	—	—	—	—	—	—	—
DIK5321	—	410	—	682	—	—	—	504	431	392	—
DIK4421	654	387	—	680	—	229	568	—	425	391	296
RM006	333	235	452	464	303	—	144	273	—	—	—
BMS1116	—	—	—	531	—	—	—	492	—	369	262
IL4	—	—	—	608	—	—	—	—	—	—	—
BM6105	—	—	407	507	—	204	—	—	510	382	305
TGLA303	—	—	420	445	—	217	—	263	—	385	308
BM7247	—	—	398	456	—	217	—	—	506	—	306
UWCA20	375	188	509	472	469	—	229	510	—	—	—
BMS2840	—	—	318	307	—	—	—	—	—	—	—
INRA112	—	—	320	275	—	—	—	—	—	—	—
BMS792	—	—	339	320	—	227	—	—	494	389	286
INRA192	—	—	—	—	—	—	—	—	498	371	—
RME30	—	—	—	—	—	—	—	—	498	393	—
BMS1331	—	—	331	—	—	—	—	—	—	—	—
ILSTS006	255	296	307	522	328	137	385	—	320	—	245
Total daughters	679	420	512	692	593	233	579	513	521	407	311

MATERIALS AND METHODS

Population

Semen samples of the 11 Israeli-Holstein sires and blood samples of 6,040 of their putative daughters were analyzed. All cows were genotyped for at least 5 microsatellites to confirm paternity as described by Weller et al. (2004). Cows that did not have either of the alleles of their putative sire for at least 2 loci were assumed to have incorrect paternity identification and were deleted from further analysis, leaving 5,639 cows with confirmed paternity. There were 5,459 cows with at least 1 marker genotyped on BTA7 and genetic evaluations for production traits. The number of daughters per sire is given in the last row of Table 1.

Genotyping Methods

The DNA from frozen blood or semen was extracted by the salting-out procedure (Ma et al., 1996). The DNA was diluted to 7 ng/ μ L, and 5 μ L was aliquoted to 96-well and 384-well plates using the Hydra robotic system (Robbins Scientific, Sunnyvale, CA). The DNA in plates was dried and stored at -20°C . The PCR protocols for DNA isolated from semen and blood cells

were as described by Ron et al. (1995a) using a DNA engine thermocycler (MJ Research Inc., Waltham, MA). Annealing temperatures of PCR ranged from 55 to 64 degrees, with 30 cycles of amplification. The PCR reactions were run on the ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA). Automated fragment analysis, size calling, and binning were then used by GeneScan (Version 3.1) and Genotyper (Version 2.0) genetic software (Applied Biosystems) to identify the alleles of each of the microsatellite loci.

Sires were genotyped for a total of 29 microsatellites spanning nearly the length of BTA7. In a daughter design, markers are genotyped only for those microsatellites for which their sires were heterozygous. The number of cows genotyped per marker and the number of informative daughters per marker are given in Table 2. For a daughter design analysis, markers are informative only if the daughter genotype is different from the sire (Ron et al., 1995b). The numbers of daughters genotyped per sire per marker are given in Table 1. Each family was genotyped for at least 6 markers. The total number of valid genotypes was 47,083. Of these, 33,007 (0.70) were informative, which is similar to previous results for this population (Ron et al., 2004). Only recombination events of the sires are of interest

Table 2. The marker locations and the number of cows genotyped per marker

Markers ¹	Location		Daughters	
	MARC ²	CRI-MAP	Genotyped	Informative ³
BM7160	0	0	1,775	1,444
MNS91	0	7.6	2,331	1,801
SRC040	2.2	11.5	952	779
RM12	8.4	20.2	858	522
BM9289*	14.7	23.2	2,482	1,803
IDVGA90	14.9	23.2	1,107	650
BP41	15.6	26.1	1,146	603
DIK2211*	15.6	26.6	3,622	2,279
BL5*	16.1	27.7	2,506	1,987
TGLA176	16.1	27.9	623	483
BMS713	16.8	29.2	1,250	846
DIK4378	16.8	31.0	341	204
ILSTS001	17.6	34.8	426	232
DIK5321*	22.3	34.9	2,419	1,998
DIK4421*	22.7	37.6	3,630	2,790
RM006	25.4	42.4	2,204	757
BMS1116*	30.5	43.9	1,654	1,024
IL4	32.0	51.1	608	261
BM6105	36.9	57.7	2,315	1,849
TGLA303	39.3	64.3	2,038	1,656
BM7247	57.2	85.6	1,883	1,031
UWCA20	58.6	88.0	2,752	2,088
BMS2840	65.3	102.5	625	578
INRA112	72.9	108.8	595	515
BMS792	79.6	113.4	2,055	1,335
INRA192	82.5	115.3	869	582
RME30	83.5	119.4	891	640
BMS1331	90.7	123.7	331	211
ILSTS006	116.6	144.2	2,795	2,059
Total			47,083	33,007

¹Markers denoted with an asterisk were also genotyped for the maternal granddaughter design analysis.

²The US Meat Animal Research Center (MARC) linkage map (<http://www.marc.usda.gov/genome/cattle/cattle.html>).

³Daughter genotype differs from its sire.

for this analysis. The male genetic map was computed by the fixed option of CRI-MAP (<http://linkage.rockefeller.edu/soft/crimap/>), using the marker order given by the US Meat Animal Research Center (MARC) linkage map (<http://www.marc.usda.gov/genome/cattle/cattle.html>). Marker map locations by CRI-MAP and the MARC map are also given in Table 2. The map derived in our analysis was 24% longer than the corresponding MARC map. This is somewhat surprising considering that the male recombination map is generally shorter than the female recombination map, and the MARC map is sex-averaged. However, our map is based on the meioses of only 11 bulls, and the number of bulls contributing to the map at the extreme ends is even less. Major discrepancies between the 2 maps were obtained at the beginning of the chromosome and in the region of marker *IL4*. Deleting these markers from the analysis did not appreciably affect the map length. Furthermore, an alternative map was constructed using the build option of CRI-MAP, but this

map was 8 cM longer than that derived from the MARC order. The accuracy of MARC marker order was also tested by the flip option of CRI-MAP. This option compares the likelihood obtained by the primary map, in this case the MARC order, to the likelihood obtained by reversing the order of each pair of markers sequentially. Two flips, between BM9289 and IDVGA90 and between BMS713 and DIK4378, resulted in increased likelihood. The flip option was again applied on the revised order, but no significant new flips were found. Length of the revised map was then determined by the fixed CRI-MAP option. Although the base-10 logarithm likelihood was increased by 15 relative to the MARC order, the map length was only reduced by 1 cM. Therefore, the MARC order was used for all further analyses.

Marker information content was computed over all families and is plotted in Figure 1. Information content was highest near position 30 cM, and there was a secondary peak near position 90. Information content was greater than 0.5 from the beginning of the chromosome to position 100 cM.

Genetic Evaluations

The official Israeli Holstein genetic evaluations are computed twice yearly at the Agricultural Research Organization. All analyses were based on the June 2006 evaluations, and the genetic base was the mean EBV of cows born in 2000. The EBV for 305-d milk, fat, and protein production, SCS, female fertility, and milk persistency were computed by multitrait animal models, preadjusted for calving age and month (Weller and Ezra, 2004). Milk persistency was defined as the predicted milk production 180 d after peak divided by peak production in percent (Weller et al., 2006). Female fertility was computed as the inverse of the number of inseminations to conception (Weller and Ezra, 1997). Evaluations for herd life were computed by a single trait animal model as described by Settar and Weller (1999). The EBV for fat percentage were derived as follows:

$$BV_{FP} = (BV_F + M_F)/(BV_M + M_M) - M_{FP},$$

where BV_{FP} , BV_F , and BV_M = the EBV of the cow for fat percentage, fat yield, and milk, and M_F , M_M , and M_{FP} = mean adjusted first-parity fat yield, milk, and fat percentage of cows born in 2000. The EBV for protein percentage were computed similarly with protein yield and percentage, instead of fat yield and percentage. Numbers of records and the means and standard deviations of the EBV of the cows genotyped for the 5 production traits, SCS, herd life, female fertility, and

Figure 1. Marker information content for all 5,459 cows from the 11 families genotyped. Positions of markers are indicated by arrows.

milk persistency are given in Table 3, and the correlations among the evaluations are given in Table 4.

QTL Linkage Analysis

Preliminary QTL analysis for all markers was by the following model:

$$BV_{ijkl} = S_{ij} + M_{ijk} + e_{ijkl},$$

where BV_{ijkl} = the EBV for trait i of cow l , daughter of sire j , that received paternal allele k ; S_{ij} = the effect of sire j on trait i ; M_{ijk} = the effect of paternal allele k of sire j on trait i ; and e_{ijkl} = the random residual associated with each record. A significant paternal al-

lele effect is indicative of a segregating QTL linked to the genetic marker.

For all 9 traits, interval mapping was performed by the algorithm of Spelman et al. (1996) considering all 11 families jointly. Chromosome-wise significance values were determined by a permutation analysis (Churchill and Doerge, 1994) separately for each trait. Two thousand permutation samples were generated, and the empirical 0.005, 0.01, and 0.05 significance levels were compared with the nominal, or comparison-wise, significance levels of the highest F -value for each sample.

Traits with significant within-family effects (absolute value of the contrast > twice the standard error) for at least 3 families were reanalyzed. The 0.95 and 0.90 confidence intervals (CI) for the QTL location were determined by the bootstrap method (Visscher et al., 1996; Ron et al., 2001) including only families with significant contrasts at the overall F -value peak. Chromosome-wise significance values for each number of families with significant contrasts were also determined by the permutation analysis of a single trait, SCS. In each permutation sample, interval mapping was performed on the families with significant contrasts. Critical F -values for the 0.01 and 0.05 significance levels were determined separately for each number of families with significant contrasts. Interval mapping was not performed for permutations in which there were no families with significant contrasts.

Table 3. Number of cows, means, and standard deviations of the genetic evaluations of the traits analyzed

Trait	Cows (n)	Mean	SD
Milk (kg)	5,459	91	494
Fat (kg)	5,459	-5.3	18.1
Protein (kg)	5,459	-9.4	11.4
Fat percentage (%)	5,459	-0.076	0.193
Protein percentage (%)	5,459	-0.110	0.097
SCS ¹	5,448	0.103	0.328
Herd life (d)	5,409	-10	109
Female fertility (%)	5,275	-0.48	2.73
Milk persistency (%)	5,314	-0.19	3.53

¹Lower values are economically favorable.

Table 4. Correlations among the genetic evaluations of the traits analyzed

Trait	Fat	Protein	Fat percentage	Protein percentage	SCS	Herd life	Fertility	Milk persistency
Milk (kg)	0.297	0.708	-0.566	-0.662	0.165	0.117	-0.203	0.319
Fat (kg)		0.355	0.619	-0.042	-0.111	0.176	0.142	0.068
Protein (kg)			-0.277	0.060	0.093	0.192	-0.160	0.313
Fat percentage (%)				0.507	-0.232	0.054	0.291	-0.207
Protein percentage (%)					-0.131	0.038	0.115	-0.119
SCS						-0.298	-0.262	-0.028
Herd life (d)							0.562	0.372
Fertility (%)								0.152

In addition, all 11 families were analyzed separately for the traits with significant contrasts in the joint analyses. The program of Spelman et al. (1996) was modified so that haplotype effects at each centimorgan were printed. If in the individual family QTL analysis 2 test statistic peaks were obtained for a single trait, and the sign of the effect changes in the valley between the 2 peaks, then the 2 effects are in repulsion for this sire. Otherwise, the 2 QTL are in coupling phase.

Modified Granddaughter Design

Significant effects were verified by application of the modified granddaughter design to a single sire family, 3070 (Weller et al., 2002). This family was chosen, because it was segregating for QTL effects on most of the traits analyzed, and a large number of maternal granddaughters were available for analysis. Six markers heterozygous in this grandsire spanning positions 14.7 to 30.5 cM of BTA7 were genotyped for 605 maternal granddaughters and 15 of their sires. These markers are denoted by an asterisk in Table 2. As described by Weller et al. (2002), granddaughter records were deleted if there were less than 2 microsatellites with ascertainable genotypes or if there was a conflict between genotypes of granddaughters and of their re-

spective putative sires. After these edits, there were 540 valid granddaughter records. For each trait, putative position of the QTL was varied across the chromosomal segment to obtain the location with the greatest contrast between the grandpaternal alleles. Allelic frequencies were estimated for significant QTL effects as described by Weller et al. (2002).

RESULTS

The F -values, comparison-wise, and corresponding chromosome-wise probabilities from the permutation analysis of all 11 families are presented in Table 5 for the 0.005, 0.01, and 0.05 chromosome-wise probabilities. Results are not presented for percentage of protein, milk persistency, and herd life, because F -values for the actual data did not approach nominal significance for these traits (Table 6). The F -values at each significance level were similar for all 6 traits, but minor differences in the F -values result in major differences in the nominal probabilities at extreme values. The average F -value and comparison-wise probability for all 6 traits are given in the last row. Using these values, the chromosome-wise probabilities are approximately 7-fold the comparison-wise probabilities at all 3 significance levels. The results of the analyses of the

Table 5. The F -values, comparison-wise, and corresponding chromosome-wise probabilities for interval mapping of BTA7 as derived by permutation analysis of SCS

Trait	Chromosome-wise probability						Denominator ² (df)
	0.005		0.01		0.05		
	<i>F</i> -value	CWP ¹	<i>F</i> -value	CWP	<i>F</i> -value	CWP	
Milk	2.85	0.00099	2.74	0.00154	2.31	0.00804	5,437
Fat	2.91	0.00078	2.74	0.00154	2.35	0.00693	5,437
Protein	2.92	0.00075	2.77	0.00137	2.36	0.00667	5,437
Fat percentage	2.84	0.00103	2.72	0.00167	2.34	0.00719	5,437
SCS	3.10	0.00036	2.96	0.00064	2.41	0.00553	5,426
Fertility	2.98	0.00059	2.80	0.00121	2.31	0.00804	5,253
Overall average	2.93	0.00075	2.79	0.00133	2.35	0.00707	

¹Comparison-wise F -probability.

²There were 11 numerator degrees of freedom for all traits.

Table 6. Maximum *F*-values, their chromosomal positions, and families with significant contrasts with all 11 families analyzed jointly

Trait	Denominator (df)	Maximum <i>F</i>		Families with significant contrasts ¹
		Value	Position	
Milk (kg)	5,437	2.21	91	3070
Fat (kg)	5,437	4.58***	29	2283, 2357, 3212, 3241, 3274
Protein (kg)	5,437	2.70*	29	2357, 3070, 3212, 3241
Fat percentage (%)	5,437	3.20***	29	2283, 2357, 3274
Protein percentage (%)	5,437	1.65	44	3274
SCS	5,426	2.43*	60	2357, 3070, 3258
Herd life (d)	5,382	1.95	20	2357, ² 3070, ² 3274
Fertility (%)	5,253	2.40*	11	2278, 3070, 3089, 3208, 3258, 3274 ²
Milk persistency (%)	5,387	1.37	27	

¹*t*-values > 2.²Contrast was not significant at the peak likelihood of the joint analysis of all families but was significant in the individual family analyses.**P* < 0.05; ****P* < 0.005.

families with significant contrasts from the permutation analysis of SCS are presented in Table 7. There were 391 permutations with no significant within-family contrasts but only 19 permutations with 4 significant within-family contrasts. For permutations with 3 significant family contrasts, the 0.05 and 0.01 critical *F*-values were 8.4 and 9.2.

The maximum *F*-values, the comparison-wise *F*-probabilities, and the denominator degrees of freedom for the interval mapping analyses of all 11 families are presented in Table 6. The *F*-values for fat yield and percentage were significant at the 0.005 chromosome-wise level, whereas protein yield, SCS, and fertility were significant at the 0.05 chromosome-wise level. The families with significant contrasts (*P* < 0.05) are also listed. There were 6 families with significant contrasts for female fertility, 5 families for fat yield and female fertility, and 3 families for each of the other traits with chromosome-wise significance. Of the 6 families with significant contrasts for fertility, 5 were significant at the position with the overall peak *F*-value. There were also 3 families with significant contrasts for herd life, even though the *F*-value was below

the critical value, but only 1 was significant at the position of the peak *F*-value.

The peak *F*-values for the 3 milk production traits were all at 29 cM. Sire 2357 had significant contrasts for all 3 traits. Furthermore, the directions of the effects were the same for all 3 traits; the haplotype associated with increased fat was also associated with increased protein and fat percentage. This was also the case for sires 2283, 3241, and 3274. For sire 3212, the significant effects on fat and protein were in the same direction, but the nonsignificant effect on fat percentage was not. Thus, it is likely that the same QTL is responsible for the effects observed on these 3 traits. The QTL affecting female fertility is clearly different from the other effects detected.

The interval mapping results for the significant families are plotted in Figures 2 to 7 for fat yield, fat percentage, protein yield, SCS, female fertility, and herd life. The overall *F*-value for all families with significant effects and the individual family *F*-values are plotted. In addition to the 5 sires with significant contrasts for fertility near the centromere, 2 sires had significant contrasts for fertility near the acrocentric end of the chromosome, 3258 and 3274. Sire 3258 had significant contrasts at both locations. The effects of the 2 QTL are in repulsion, because the signs of 2 effects were different. Thus, the haplotype associated with increased fertility near the beginning of the chromosome is associated with reduced fertility near the distal end. In this case, the positions of both peaks should be biased outward (Ron et al., 2001).

The maximum *F*-values, their positions, and the CI for QTL location for families with significant contrasts are given in Table 8 for traits with 0.05 chromosome-wise significance and herd life. Although the probabil-

Table 7. Frequencies of families with *t*-values >2 in the permutation analysis of SCS and *F*-values for 0.05 and 0.01 chromosome-wise significance

Number of families with <i>t</i> -values >2	Frequency	Critical <i>F</i> -value	
		0.05	0.01
0	391	—	—
1	936	11.5	15.2
2	508	8.9	10.7
3	142	8.4	9.2
4	19	—	—
5	4	—	—

Figure 2. Interval mapping for fat yield. —, all 5 bulls; ···, sire 2283; — —, sire 2357; — — —, sire 3212; — · —, sire 3241; — · · —, sire 3274. Positions of markers are indicated by arrows.

ity values listed are for the comparison-wise F -values, SCS was nearly significant at the 0.05 chromosome-wise level, and fat percentage was significant at the 0.01 level, as determined by the permutation analysis

results in Table 7. Both fat yield and fertility can be considered significant at the 0.005 level, because there were only 4 permutation samples out of 2,000 (0.002) with 5 within-family significant contrasts.

Figure 3. Interval mapping for fat percentage. —, all 3 bulls; ···, sire 2283; — —, sire 2357; — · —, sire 3274. Positions of markers are indicated by arrows.

Figure 4. Interval mapping for protein yield. —, all 4 bulls; ···, sire 2357; ---, sire 3070; — · —, sire 3212, — - —, sire 3241. Positions of markers are indicated by arrows.

Considering only the families with significant contrasts, the peak F -value for herd life shifted from position 20 to 74 cM. Two of the 3 sires with significant contrasts, 2357 and 3070, had peak F -values near this

position. It is possible that the same QTL affecting SCS is also responsible for the marginal effect observed on herd life near the middle of the chromosome. The locations of the peak F -values are similar, and both

Figure 5. Interval mapping for SCS. —, all 3 bulls; ···, sire 2357; ---, sire 3070; — · —, sire 3258. Positions of markers are indicated by arrows.

Figure 6. Interval mapping for female fertility. —, all 6 bulls; ···, sire 2278; ---, sire 3070; — · —, sire 3089; — — —, sire 3208; — · · —, sire 3258; - - -, sire 3274. Positions of markers are indicated by arrows.

families with significant contrasts near the middle of the chromosome for herd life also had significant contrasts for SCS. For all 4 sires with significant effects for either SCS or herd life, the haplotype associated with decreased SCS was associated with increased

herd life. This result corresponds with the -0.3 correlation between the genetic evaluations for these traits (Table 4).

The estimated substitution effects for the individual families are also given (Table 8). The substitution ef-

Figure 7. Interval mapping for herd life. —, all 3 bulls; ···, sire 2357; ---, sire 3070; — · —, sire 3274. Positions of markers are indicated by arrows.

Table 8. Maximum *F*-values, their positions, and 0.95 confidence intervals for QTL location for families with significant contrasts

Trait	Degrees of freedom		Maximum <i>F</i>			0.95 Confidence interval (cM)	Within-family substitution effects ¹
	Numerator	Denominator	Value	Position (cM)	Probability		
Fat (kg)	5	2,267	9.15	30	1.2×10^{-8}	23 to 43	5.4, 7.9, 4.3, 3.9, 5.1
Protein (kg)	4	2,230	5.25	29	0.0003	0 to 92	2.8, 2.9, 2.3, 2.4
Fat percentage (%)	3	1,237	9.42	29	3.7×10^{-6}	26 to 64	0.033, 0.060, 0.051
SCS	3	1,604	8.02	60	2.6×10^{-5}	34 to 85	0.095, 0.060, 0.097
Fertility ² (%)	5	2,860	4.63	11	0.0003	0 to 27	0.27, 0.36, 0.37, 0.29, 0.44, 0.66
Herd life (d)	3	1,485	5.08	74	0.0017		17.4, 20.0, 49.0

¹Substitution effects were computed from interval mapping analyses of the individual bulls.

²The joint interval mapping analysis for fertility did not include sire 3274, and estimation of the 0.95 confidence interval did not include sires 3258 and 3274.

fects ranged in magnitude from 0.1 to 0.5 units relative to the phenotypic standard deviations in Table 3. Because EBV of cows were analyzed, these values underestimate the actual substitution effects (Israel and Weller, 1998). On the other hand, the effects deemed significant are a selected sample and therefore overestimate the actual substitution effects, unless the estimated effect is much greater than the critical value for selection (Weller et al., 2005). The substitution effects were estimated from the peak with the highest *F*-value regardless of chromosomal location. Sire 2357 had the greatest contrast for fat yield and percentage but not protein yield.

In general, effects for bulls were similar for each trait. The exceptions were the effects of sire 3274 on fertility and herd life. For herd life, the effect associated with this bull was more than double the effects associated with the other bulls. For both traits, the contrasts estimated were in 2 different chromosomal locations, as shown in Figures 6 and 7. For female fertility, the peak *F*-values for bulls 3258 and 3274 were near positions 120 cM, whereas for the other bulls, the peak values were near the centromere. Sire 3258 was apparently heterozygous for both QTL, but the peak near position 120 cM was higher. For herd life, the maximum *F*-value for sire 3274 was near the centromere, whereas the peak values for the other 2 bulls were near the middle of the chromosome. Sire 3274 was not heterozygous for any of the markers from positions 0 to 24 cM. The fact that the *F*-value for this bull was relatively high from positions 0 through 120 cM indicates that probably more than a single QTL affecting herd life is segregating for this sire.

A CI was not computed for herd life, because this trait was not significant at the 0.05 chromosome-wise level, and the curve for sire 3274 was clearly different from the other 2 bulls. The 0.95 CI are presented for all other traits. As expected, the CI generally decreased with increase in significance. The 0.95 CI for fat yield spanned only 20 cM. The CI for fertility and

SCS did not overlap, clearly indicating that different QTL are responsible for these effects. Because 2 QTL are apparently segregating for fertility in sire 3258, the CI for female fertility was estimated without this sire. There was minimal overlap between the CI for the QTL affecting female fertility and the QTL affecting fat yield.

Results of the modified granddaughter design analysis for sire 3070 are presented in Table 9. Significant contrasts were found for female fertility and herd life, confirming the daughter design results for these traits but not for SCS. However, the maximum *F*-value for SCS for sire 3070 was at position 57, well outside the chromosomal segment analyzed by the modified granddaughter design. For both traits, the maximum contrast was obtained for the interval between DIK2211 and BL5, at 27 cM, which was 1 cM from the location of the peak value for fertility for sire 3070. The substitution effects obtained by the modified granddaughter design were very similar to the effects obtained by the daughter design analysis. For both traits, the estimated frequencies for the positive QTL alleles were <0.5, but the standard errors of these estimates were so large that the entire range of 0 to 1 is within the CI.

DISCUSSION

Even though a highly significant effect on milk persistency was found in the individual marker analyses for DIK2211, the *F*-value from the interval mapping analysis was not significant for this trait. Previous studies in other populations did not find significant effects for milk persistency on BTA7 (Boichard et al., 2003; Harder et al., 2006). The large effect on fat yield found in this study was not found in other populations, although an effect on protein yield was found in French Holsteins in the same chromosomal region (Boichard et al., 2003) and also near position 115 cM (Heyen et al., 1999). Effects on SCS near the middle of the chromosome were found in US and German Holsteins

Table 9. Results of the modified granddaughter design (MGD) analysis for sire 3070¹

Trait	Daughter design (substitution effect)	MGD contrasts		Estimated QTL allele frequency ³
		Substitution effect	X - q ²	
Herd life (d)	20.0 ± 7.6	18.3 ± 8.7	2.7 ± 7.9	0.15 ± 1.04
Fertility (%)	0.355 ± 0.128	0.336 ± 0.148	0.128 ± 0.136	0.38 ± 0.55

¹All effects are given ± their standard error.

²Difference between granddaughters that inherited neither grandpaternal allele and granddaughters that received the negative QTL allele.

³Positive allele.

(Ashwell et al., 2001, 2004; Kuhn et al., 2003) and near the distal end of BTA7 (Heyen et al., 1999). The effect on herd life was not significant at the 0.05 chromosome-wise level but was significant in the modified granddaughter design analysis of a single family. Effects on female fertility were detected in the same chromosomal regions that Arias and Kirkpatrick (2004), Cruickshank et al. (2004), and Lien et al. (2000) found effects on twinning and ovulation rate. Of course, the number of QTL claims in dairy cattle is now so extensive, especially for production traits, that QTL for all traits analyzed have been detected on nearly all chromosomes. Therefore, the question of verification is of paramount importance.

The verification of female fertility by the modified granddaughter design is the second case in which a QTL detected by a daughter or granddaughter design was verified by this method (Weller et al., 2002) and the first case for a nonproduction trait. Unlike the QTL for protein percentage on BTA6, it was not possible in the current analysis to obtain accurate estimates for the QTL allelic frequencies for either fertility or herd life, because the QTL effects in the current study were much smaller relative to their standard errors. The relationship between the standard error for allelic frequency and the QTL effect was demonstrated by Weller et al. (2002) for simulated data. Nevertheless, assuming the estimated frequency of 0.38 for the allele that increases fertility, then the expected frequency of heterozygous sires, assuming Hardy-Weinberg equilibrium, is 0.47. In fact, their frequency for the QTL near the centromere was $5/11 = 0.45$, which is nearly perfect correspondence. Likewise, assuming the estimated frequency of 0.15 for the favorable allele for herd life, then the Hardy-Weinberg frequency of heterozygous sires is 0.27, is in agreement with the realized number of heterozygote sires $(3/11) = 0.25$. The modified granddaughter design analysis indicated that the frequencies of the favorable alleles in the Israeli Holstein population are <0.5 for both fertility and herd life, implying that there is significant scope to apply marker-assisted selection. This was not the case

for the *ABCG2* polymorphism on BTA6, in which the favorable allele for milk protein percentage is already at high frequencies in nearly all dairy cattle populations (Cohen-Zinder et al., 2005; Ron et al., 2006). Application of the modified granddaughter design to identify QTL with favorable alleles at low frequencies may be used to determine priority for quantitative trait nucleotide identification.

Sartori et al. (2006) found that multiple ovulating cows have a significantly higher circulating progesterone concentration on d 7 and a tendency for a greater conception rate on d 25 to 32 than single ovulators. Such hormonal change could have positive effects on fertilization and early embryonic development (Kerber et al., 1997). Thus, it is possible that the QTL affecting ovulation rate and twinning detected in the centromeric region of BTA7 (Lien et al., 2000; Arias and Kirkpatrick, 2004; Cruickshank et al., 2004) is the same QTL that was found to affect conception rate in the current study.

Recently, Ron and Weller (2007) reviewed methods to determine the polymorphism responsible for QTL detected in animal populations. The methods described, including determination of likely candidates and linkage disequilibrium mapping, will be used to ascertain the causative polymorphisms for the QTL described in this study.

ACKNOWLEDGMENTS

This research was supported by grants from the Israel milk marketing board and the European Sixth Research and Technological Development Framework Programme, Proposal No. 016250-2 SABRE. We thank E. Ezra (Israeli Cattle Breeders' Association) for providing phenotypic data for analysis, R. Spelman (Livestock Improvement Corp. Ltd.) for use of his interval mapping program, and N. Silinakov (ARO, The Volcani Center) for useful discussions.

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