Incorporating Mouse Genome
Mammalian Genome
Genes and Phenotypes

Identification of bovine QTL for growth and carcass traits in Japanese Black cattle by replication and identical-by-descent mapping

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Received: 9 August 2006 / Accepted: 4 December 2006

Abstract

To map quantitative trait loci (QTL) for growth and carcass traits in a purebred Japanese Black cattle population, we conducted multiple QTL analyses using 15 paternal half-sib families comprising 7860 offspring. We identified 40 QTL with significant linkages at false discovery rates of less than 0.1, which included 12 for intramuscular fat deposition called marbling and 12 for cold carcass weight or body weight. The QTL each explained 2%–13% of the phenotypic variance. These QTL included many replications and shared hypothetical identical-by-descent (IBD) alleles. The QTL for CW on BTA14 was replicated in five families with significant linkages and in two families with a 1% chromosome-wise significance level. The seven sires shared a 1.1-Mb superior *Q* haplotype as a hypothetical IBD allele that corresponds to the critical region previously refined by linkage disequilibrium mapping. The QTL for marbling on BTA4 was replicated in two families with significant linkages. The QTL for marbling on BTA6, 7, 9, 10, 20, and 21 and the QTL for body weight on BTA6 were replicated with 1% and/or 5% chromosome-wise significance levels. There were shared IBD *Q* or *q* haplotypes in the marbling QTL on BTA4, 6, and 10. The allele substitution effect of these haplotypes ranged from 0.7 to 1.2, and an additive effect between the marbling QTL on BTA6 and 10 was observed in the family examined. The abundant and replicated QTL information will enhance the opportunities for positional cloning of causative genes for the quantitative traits and efficient breeding using marker-assisted selection.

Introduction

Quantitative trait loci (QTL) mapping studies have been performed extensively in many organisms; however, relatively few genes underlying genetically complex traits have been identified (Glazier et al. 2002). There are several problems in detecting QTL. One of the primary problems is the small effect of each contributing locus (Kruglyak 1996). Second, QTL interact with other genes and environmental

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factors. Third, even QTL with significant linkages might be false positives as a result of unsuitable study design and/or the population studied. Finally, a limited number of offspring of the pedigree is an additional problem, particularly for domestic animal species such as cattle.

QTL mapping studies in beef cattle have been performed using experimental crosses between relatively different *Bos indicus* and *Bos taurus* breeds, and several significant QTL have been identified [e.g., Casas et al. 2000; Keele et al. 1999; Stone et al. 1999; the Bovine QTL Viewer (http://www.bovineqtl.tamu.edu/index.html)]. The studies that examined the association between identical-by-descent (IBD) haplotypes and traits were followed up using commercial lines of *Bos taurus*; however, the phases of the associated haplotypes were different between the lines (Li et al. 2002), which resulted in difficulties narrowing down the QTL using this method.

On the other hand, several QTL studies in dairy cattle (Holstein breed) using a granddaughter design have reported multiple QTL for milk yield and composition, such as those on BTA6 (e.g., Georges et al. 1995; Spelman et al. 1996), BTA14 (Coppieters et al. 1998; Heyen et al. 1999), and BTA20 (Arranz et al. 1998; Georges et al. 1995). Furthermore, hypothetical IBD mapping and positional candidate cloning led to the successful identification of causal mutations in *ABCG2* on BTA6 (Cohen-Zinder et al. 2005) and/or *SPP1* on BTA6 (Schnabel et al. 2005), *DGAT1* on BTA14 (Grisart et al. 2002; Winter et al. 2002), and *GHR* on BTA20 (Blott et al. 2003), although relatively large haplotype blocks sometimes impede attempts to narrow down the critical region and identify the causal mutation (de Koning 2006). These identified mutations account for nearly 10% (*GHR F279Y* on protein %) to 44% (*OPN3907* indel on protein % adjusted for *DGAT1*) of the phenotypic variance (Blott et al. 2003; Schnabel et al. 2005). It could be important that a large number of Holstein cattle from a variety of local populations were employed for the analyses and that the mapped QTL were confirmed by replications.

To identify causative genes for beef cattle QTL, we collected more than 10,000 offspring, constructed 15 paternal half-sib families with more than 190 offspring, and searched for QTL replications as the first step. Recently, we confirmed that the progeny design simulated by Weller et al. (1990) and Moody et al. (1997) is useful for mapping QTL in purebred Japanese Black cattle populations (Mizoguchi et al. 2006; Mizoshita et al. 2004). Also, the use of a purebred local population minimizes the genetic background and environmental factors such as feeding, the fattening period, and the need for phenotypic assessment. The objectives of this study were to locate QTL for growth and carcass traits, confirm the QTL by replication, and identify the hypothetical IBD haplotypes harboring the QTL. We found 40 QTL with significant linkages, 15 of which were the replications of the QTL for carcass or body weight on BTA14, marbling on BTA4, and longissimus muscle area on BTA4 and 14. Another eight significant QTL were replicated with less than 5% chromosome-wise significance levels. Shared superior *Q* or inferior *q* haplotypes were observed in the QTL for carcass weight on BTA14 and for marbling on BTA4, 6, and 10. The identified haplotypes will provide useful information for identifying the causal genes as well as for marker-assisted selection in beef cattle.

Materials and methods

Simulation of the power to detect QTL. A simulation was performed to estimate the power to detect a QTL using a progeny design. A chromosome 105 cM long and with a single QTL residing at 52.5 cM was assumed. The eight markers that are all heterozygous in a sire were set to be located at 0, 15, 30, 45, 60, 75, 90, and 105 cM. The marker interval in the simulation (15 cM) was close to the average marker interval in this study (11.9 cM). A set of offspring was generated so that transmission of the QTL and the markers of the offspring occurred probabilistically according to the recombination fractions between the markers and the QTL. The expectation of the informative transmission ratio of paternal alleles was set to 0.6, which was close to the observed ratio in this study (0.625). A normal distribution of the trait values governed by the QTL was assumed. The normal distribution of the trait value variance was generated by an approximation of binomial distribution to normal distribution [$Bi(310, 0.5)$ or $Bi(330, 0.5)$]. The simulation was performed for the QTL explaining 5% and 10% of the trait variance by allele substitution from *q* (inferior) to *Q* (superior), respectively, with increasing the number of offspring by intervals of 50 from 100 to 400. The QTL analyses were performed as described below. The simulation was repeated 1000 times and the number that exceeded either the 1% or 5% chromosome-wise significance level was counted.

Collection of DNA samples and phenotype data. Sixteen paternal half-sib families of Sire A through Sire O were constructed from carcass data and pedigree records collected by the Japan Wagyu Register Association (Kyoto, Japan). Two families

were constructed for Sire G, each of which consisted of steers and cows, respectively, and were collected from a different local area. The Sire N family and one Sire G family consisted of cows, while other families consisted of steers. Sire DNA was obtained from semen. Offspring DNA samples were collected from adipose tissues around the kidney at the slaughterhouses or from blood at the individual farmer's house. Six traits were analyzed in this study (Supplementary Table 1): body weight at slaughter (BW), cold carcass weight (CW), longissimus muscle area (LMA), rib thickness (RT, thickness of a muscle layer in a rib of beef), subcutaneous fat thickness (SFT), and marbling. Marbling was estimated using a beef marbling score (BMS) that ranks the carcass from 1 to 12 according to the degree of intramuscular fat deposition in which higher scores correspond to more marbling. These traits were systematically measured by certified graders and recorded at the slaughterhouses in Japan. Using farmers' data, the phenotypic values in Sire I and J families were corrected with best linear unbiased estimates of fixed effects estimated by MTDFREML (Boldman et al. 1995). Because offspring of Sire A and Sire C were collected from more than two slaughterhouses, their BMS values were corrected using the deviations.

Genome screen. DNA was extracted from semen, blood, or adipose tissue according to standard protocols. The genome screen was conducted using the microsatellite markers on the Shirakawa-USDA linkage map (Ihara et al. 2004). The markers were selected individually for each sire as heterozygous for the sire and at an approximately 10-cM interval on autosomes. The average marker interval ranged from 8.0 (Sire D) to 16.0 cM (Sire C) in the families. Eleven families of Sire A through Sire J, consisting of 190 to 450 offspring, were individually genotyped, while four families of Sire K through Sire N, consisting of 320 to 760 offspring were selectively genotyped using the 25%–50% extremes for the marbling trait (Supplementary Table 1). The genome screen for Sire O was done using less than 190 offspring and therefore is not presented. Polymerase chain reaction conditions were as described previously (Mizoshita et al. 2004). Polymerase chain reaction products were resolved by electrophoresis in polyacrylamide gels using an ABI 377 sequencer (Applied Biosystems, Foster City, CA) or an ABI 3700 DNA analyzer (Applied Biosystems). Genotype data were captured using GENESCAN and Genotyper software (Applied Biosystems).

Statistical analyses. Marker locations were obtained from the Shirakawa-USDA linkage map (Ihara et al. 2004). QTL analyses were performed with the interval mapping method using a linear regression model for half-sib families (Haley et al. 1994; Seaton et al. 2002), as described previously (Mizoshita et al. 2004). Briefly, phases of the sire's chromosomes were determined at each pair of two consecutive heterozygous markers using allele transmission information to offspring so that recombination between two markers was minimized. Linear regression analysis was performed using the following model:

$$y_i = \mu + \mathrm{Prob(Q)}_i a + e_i,$$

where y_i is the phenotypic value, μ is the fixed effect, a is the allele substitution effect from q to Q, $\mathrm{Prob}(Q)_i$ is the probability of Q phase at a given location, and e_i is the residual error for the ith individual. The analysis was performed at 2-cM intervals along a chromosome. To evaluate whether the QTL effect was well estimated, the information content (IC) was calculated as a variance of $\mathrm{Prob}(Q)_i$ divided by 0.25, the possible maximal variance of $\mathrm{Prob}(Q)_i$ (Knott et al. 1998). The allele substitution effect from q to Q was calculated as an estimator of a. The contribution ratio was calculated as a proportion of the trait variance explained by the paternal allele substitution from q to Q. Thresholds for significance of the F statistic value were obtained by 10,000 random permutations of the phenotypic data (Churchill and Doerge 1994). To control the error rate of multiple trait analysis, we applied the false discovery rate (FDR) proposed by Weller et al. (1998). The FDR was calculated at the marker-existing points in each family. The 95% confidence interval (CI) of the QTL locations was calculated by the bootstrapping method (Visscher et al. 1996).

Secondary screening. Secondary screening was performed for a total of 31 chromosomes of 12 sires [Sires A, C–F, G (cows), H, I, K, M–O]. The families selectively genotyped in the genome scanning (Sires K, M, and N) were individually genotyped and calculated for all traits. Other families were individually genotyped using an increased number of offspring, resulting in 300–872 offspring per family, and calculated for all traits. From 7 to 47 markers were used per chromosome.

Results and discussion

Simulation and experimental framework. **Number of offspring.** The progeny design can detect only QTL with moderate to large effects (Moody et al. 1997). To determine how many offspring should be used for the analysis, we simulated the power of detecting a QTL (Fig. 1). The probability of detecting QTL accounting for 5% and 10% of the trait variance

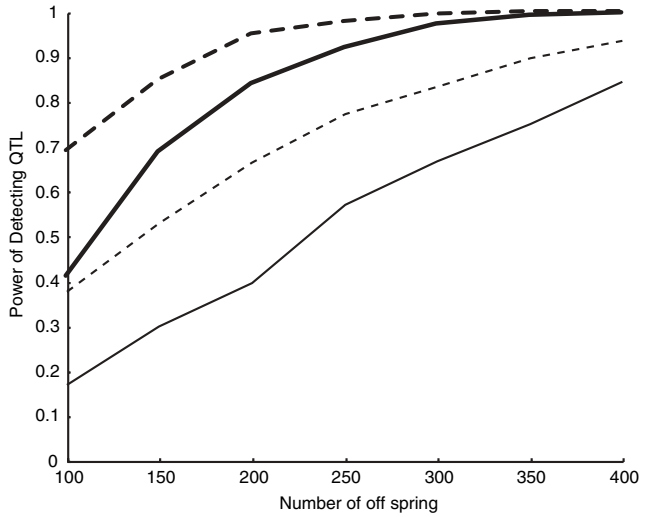


Fig. 1. Simulation of the power of detecting a QTL. The power of detecting a QTL explaining 5% (thin lines) or 10% (bold lines) of the trait variance was estimated. The broken and the solid lines indicate the power of detecting a QTL at 5% and 1% chromosome-wise significance levels, respectively.

was 0.66 and 0.95, respectively, at the 5% chromosome-wise significance level using 200 offspring, although this threshold might produce one or two false positives as type I errors in a whole-genome scan. The power of detecting a QTL accounting for 5% of the trait variance reached 0.94 by increasing the number of offspring to 400 under the same conditions. In this study, we used 15 families whose sires were produced by line breeding or line cross (Fig. 2). Eleven families, consisting of 190–450 offspring, were individually genotyped and analyzed for growth and carcass traits, and in the remaining four families, consisting of 320–760 offspring, the 25%–50% extremes were selectively genotyped for the marbling trait only (Supplementary Table 1). According to Lander and Botstein (1989), the power obtained from the genotyping of the 33% extremes (greater than 1 SD from the mean) in a 25% larger population is almost equivalent to the power obtained from individual genotyping of the original population size. The simulation suggested that a QTL accounting for 5% of the trait variance could be detected at more than 66% probability in our study.

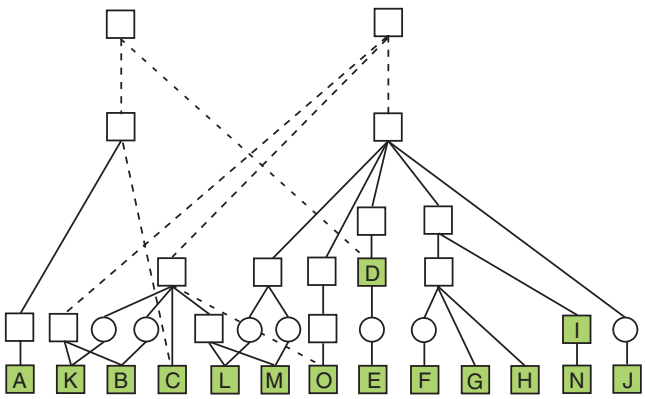


Fig. 2. Pedigrees of the sires used in this study. A dotted line indicates a distant relationship with more than two generations.

Selection of markers. In general, the average heterozygosity of microsatellite markers in the Japanese Black cattle population was almost the same as that in Holstein and slightly lower than that in the MARC reference population, which includes *Bos indicus* (Bishop et al. 1994) [0.71 in Japanese Black (N = 96); 0.73 in Holstein (N = 96); 0.79 in the MARC reference population; 78 microsatellites examined (data not shown)]. To QTL-analyze the 15 half-sib families, we selected heterozygous microsatellites for each sire at approximately 10-cM intervals. The average marker interval in the family ranged from 8.0 (Sire D) to 16.0 cM (Sire C). The overall marker interval averaged 12 cM, ranging from 3.3 to 72 cM. Ninety-five percent of the intervals were less than 27 cM and the remaining intervals lacked any available heterozygous markers. The average heterozygosity of the markers in the family ranged from 0.61 (Sire I) to 0.71 (Sire A). The heterozygosity of the markers in detected QTL regions was comparable to that in other regions (data not shown). The ratio of identified paternal alleles was 0.62 (589,066 among 945,561 total genotypes), ranging from 0.45 (Sire B) to 0.70 (Sire D) in the families, which assured correct determination of the phase of the sire's chromosomes.

Correlation between traits. Six traits were analyzed in this study: BW, CW, LMA, RT, SFT, and marbling. Phenotypic data for carcass traits of each half-sib family are available in Supplementary Table 1. Pairwise correlation coefficients between the traits were calculated per family (Supplementary Table 2). CW was highly correlated with BW (r = 0.96) and moderately correlated with RT (r = 0.59). Interestingly, there was a weak to moderate correlation between LMA and both CW and beef marbling score (BMS; r = 0.43), whereas the correlation between CW and BMS varied among families (r = 0.06-0.39). BMS and SFT were not correlated (r = 0.03).

Overall QTL analyses. Table 1 summarizes the QTL detected in genome scanning. Eleven families of Sire A through Sire J were analyzed for five or six traits and the remaining four families of Sire K through Sire N were selectively genotyped and ana-

Table 1. Summary of the QTL with significant linkages detected in genome scanning

Half-sib family	*Number of offspring*	*BTA*	*Number of markers*	*Average IC*	*Trait*	*Position (cM)*	*F statistic*	*IC on the position*	*Q to q allele substitution effect*[a]	*Contribution ratio (%)*	*Chromosome-wise significance level*[b]	*Experiment-wise significance level*[c]	*FDR*
Sire A	372	9	37	0.84	BMS	36	29.3	0.90	1.1	7.1	***	@@@	0.000
		28	6	0.83	SFT	56	14.6	0.80	0.3	3.5	***	@	0.026
Sire B	328	20	11	0.76	BMS	52	16.6	0.90	1.0	4.6	***	@	0.000
Sire D[d]	236	1	17	0.83	BW	76	16.3	0.83	29.4	6.1	**	@	0.000
		4	17	0.84	LMA	64	15.4	0.89	4.0	5.8	**	@	0.000
		4			BMS	74	17.0	0.96	1.1	6.4	***	@	0.008
		14	18	0.86	BW	46	28.8	0.92	36.3	10.6	***	@@@	0.000
		14			CW	46	28.1	0.92	25.4	10.3	***	@@@	0.000
Sire E	192	14	31	0.86	LMA	68	15.9	0.94	3.8	7.2	**	@	0.090
Sire F	246	2	9	0.79	SFT	50	16.1	0.74	0.5	5.8	**	@	0.000
		7	10	0.79	BW	80	14.8	0.98	21.4	5.3	**	@	0.056
Sire G	379	12	9	0.52	SFT	108	14.1	0.60	0.4	4.2	**	@	0.043
(steers)		14	15	0.83	LMA	34	15.4	0.91	3.8	4.6	***	@	0.000
		14			CW	38	15.1	0.85	22.3	4.5	**	@	0.032
Sire H	353	3	5	0.52	RT	64	15.4	0.81	0.4	3.9	***	@	0.036
		10	6	0.56	BW	50	16.0	0.61	34.4	4.1	***	@@	0.000
		10			CW	48	13.8	0.66	20.8	3.5	**	@	0.000
Sire I[e]	282	13	6	0.67	SFT	28	13.5	0.85	0.3	4.6	***	@	0.062
		14	11	0.67	CW	26	14.0	0.76	16.0	4.8	**	@	0.068
		21	11	0.76	BMS	78	20.9	0.88	1.1	7.2	***	@@	0.000
Sire J	258	2	10	0.77	LMA	52	15.1	0.78	3.6	6.0	**	@	0.030
		14	15	0.72	CW	48	33.6	0.82	26.1	12.9	***	@@@	0.000
		14			LMA	56	26.9	0.79	4.7	10.5	***	@@@	0.000
Sire K	158[g]/524[h]	14	11	0.73	BMS	22	19.9	0.61	NC[f]	NC[f]	***	@@	NC[f]
Sire N	190[g]/760[h]	6	10	0.76	BMS	84	15.5	0.82	NC[f]	NC[f]	***	@	NC[f]
		10	9	0.75	BMS	72	25.9	0.88	NC[f]	NC[f]	***	@@@	NC[f]

The QTL that did not exceed 5% experiment-wise significance level are shown in Supplementary Table 4.
[a]The unit is kg for BW and CW, cm^2 for LMA, and cm for RT and SFT, respectively.
[b]*, **, and *** mean <5%, <1%, and <0.1% chromosome-wise significance levels, respectively.
[c]@, @@, and @@@ mean <5%, <1%, and <0.1% experiment-wise significance levels, respectively.
[d]Mizoshita et al. 2004.
[e]Mizoguchi et al. 2006.
[f]NC, = not calculated for the selectively genotyped families.
[g]The number of the offspring selectively genotyped.
[h]Total number of the offspring.

lyzed for marbling (Supplementary Table 1). In the 11 families, a total of 200 regions reached the 5% chromosome-wise significance level. To control for type I errors due to multiple tests for multiple traits, we calculated the FDR for each region (Weller et al. 1998). One hundred forty-three regions failed to pass the FDR threshold (< 0.1) (Weller et al. 1998), which comprised one third of the regions detected at a less than 1% chromosome-wise significance level (23/74) and most of the regions detected at the 5% chromosome-wise significance level (120/126) (Supplementary Table 3). These regions were eliminated from the study, although some of them might be real because FDRs were calculated irrespective of the phenotype correlation. For the four selectively genotyped families, 24 regions were detected at a less than 5% chromosome-wise significance level. In total, 81 regions were detected as provisional QTL (Supplementary Table 4). Each QTL explained 2.2%–12.9% of the phenotypic variance in the family, as expected from the simulated data (Fig. 1). Among them, six QTL were highly significant (<0.1% experiment-wise significance level) and 20 were significant (<5% experiment-wise significance level) according to the criteria formulated by Lander and Kruglyak (1995) (Table 1).

Based on the genome scanning results, secondary screening was performed for a total of 31 chromosomes of 12 sires with increased numbers of offspring and markers. We detected 65 QTL at a less than 5% chromosome-wise significance level, including 40 that were not detected as provisional QTL in genome scanning (Table 2; Supplementary Table 5). On the other hand, one QTL that was detected at the 1% chromosome-wise significance level by selective genotyping did not reach the 5% chromosome-wise significance level after individual genotyping (Supplementary Table 4). Fourteen QTL newly exceeded 0.1% chromosome-wise significance level and therefore we regarded them as significant QTL (Table 2). Finally, we obtained 40 QTL with highly significant or significant linkages composed of 12 for marbling, 7 for CW, 5 for BW, 11 for LMA, 4 for SFT, and 1 for RT, and 80 other provisional QTL.

QTL locations with a 95% CI are shown in Fig. 3. Highly significant or significant QTL were distributed on 15 chromosomes (BTA1–4, 6–10, 12–14, 20, 21, and 28), and other provisional QTL were distributed on 25 chromosomes. As expected from the correlation between the traits, the QTL for CW were also detected for BW, and vice versa for the QTL for BW. Also, some of the QTL detected for LMA overlapped with the QTL detected for CW or marbling.

Several QTL were replicated. Significant linkages for marbling and LMA on BTA4 were detected in Sire D and Sire I families, and for CW and LMA on BTA14 were five families, respectively (Sires D, G, I, J, and N for CW and Sires E, G, J, K and O for LMA). In addition, the significant QTL for marbling on BTA6, 7, 9, 10, 20, and 21 and for BW on BTA6 were replicated at a 1% or 5% chromosome-wise significance level among families. The replicate results supported the presence of the QTL as well as the reliability of the analyses. These QTL are expected to be effective independent of the genetic background because they were detected in various local populations. Next, we further examined the replicated QTL for CW, BW, and marbling.

QTL for CW and BW. Twelve highly significant or significant QTL were detected for BW or CW, which were distributed on BTA1, 6, 7, 10, and 14 (Tables 1 and 2; Fig. 3). Thirteen other regions were detected at a less than 5% chromosome-wise significance level (Supplementary Tables 4 and 5). The QTL on BTA6 and 14 were replicated in different pedigrees as described above.

The *CW-1* QTL was originally mapped to BTA14 using the Sire D family and its location was narrowed down to the 1.1-Mb region by linkage disequilibrium mapping (Mizoshita et al. 2004, 2005). Highly significant or significant linkages for CW on BTA14 were detected in four additional families (families from Sires G, I, J, and N) and suggestive linkages were detected in two families (families of Sires K and O; Tables 1 and 2; Supplementary Tables 4 and 5). Because the QTL analyses were individually performed, in which different markers were used among the analyses (Fig. 4A), the haplotypes of the sires were examined more precisely around the *CW-1* region (41.7–49.8 cM) by adding more microsatellite markers. The superior *Q* and inferior *q* haplotypes of the seven sires were classified into two (*Q1* and *Q2*) and three haplotypes (*q1*, *q2*, and *q3*), respectively, and *Q1* and *Q2* shared a common haplotype between DIK7013 and NRKM-040, which corresponds to the critical region refined by the linkage disequilibrium mapping (Mizoshita et al. 2005) (Fig. 4B). The fact that many sires harbored *CW-1 Q* suggested that this QTL was already successfully selected. The *Q* frequency in Japanese Black cattle was estimated to be nearly 50% (Mizoshita et al. 2005), which means that this QTL is still important for increasing CW. Also, the relatively high frequency suggests that the QTL has no apparent disadvantage for animal health or for the economically important carcass traits analyzed in this study. Interestingly, marbling and/or LMA QTL, with significant or suggestive linkages, were concomitantly mapped around the *CW-1* locus (Fig. 3), at which each superior (*Q*) allele resided in

Table 2. Summary of the QTL with significant linkages detected in secondary screening

Half-sib family	*Number of offspring*	*BTA*	*Number of markers*	*Average IC*	*Trait*	*Position (cM)*	*F statistic*	*IC on the position*	*Q to q allele substitution effect*[a]	*Contribution ratio (%)*	*Chromosome-wise significance level*[b]
Sire A	785[c]	9	47	0.87	BMS	40	38.1	0.98	0.8	4.5	***
		9			LMA	38	24.3	0.99	2.5	2.9	***
Sire C	563[d]	21	18	0.68	BMS	40	16.8	0.89	1.1	2.7	***[h]
Sire D[e]	384	4	32	0.89	BMS	56	33.5	0.98	1.2	7.8	***
		4			LMA	60	19.0	0.94	3.4	4.5	***
	348	14	17	0.82	BW	50	45.9	0.92	39.6	11.5	***
		14			CW	50	42.0	0.92	26.7	10.6	***
Sire E	393	6	32	0.83	BW	42	29.9	0.88	41.7	6.9	***
		6			CW	38	24.7	0.90	24.1	5.7	***[h]
Sire F	496	7	15	0.84	BMS	28	28.0	0.93	1.0	5.2	***
					BW	82	14.9	0.92	15.0	2.7	**
Sire H	410	10	23	0.80	BW	52	13.0	0.70	26.5	2.9	**
		10			CW	44	11.5	0.96	14.3	2.5	**
Sire I[f]	872	4	14	0.61	BMS	66	55.7	0.90	0.9	5.9	***[h]
		4			LMA	42	15.1	0.79	1.9	1.6	***[h]
		14	12	0.73	CW	36	55.0	0.70	18.2	5.8	***
		14			LMA	24	29.6	0.75	2.7	3.2	***[h]
		21	35	0.78	BMS	80	18.0	0.96	0.5	1.9	***
Sire K	524	9	28	0.88	BMS	80	20.6	0.92	0.8	3.6	***
		14	20	0.88	BMS	38	17.1	0.89	0.7	3.0	***
		14			LMA	48	14.9	0.89	2.6	2.6	***[i]
Sire N	760	6	45	0.71	BMS	94	21.2	0.95	0.7	2.6	***
		10	25	0.73	BMS	78	27.6	0.98	0.8	3.4	***
		14	25	0.80	CW	34	26.0	0.95	15.4	3.2	***[i]
		14			LMA	30	16.5	0.89	2.1	2.0	***[i]
Sire O[g]	300	8	28	0.86	BMS	4	22.7	0.83	1.0	6.8	***
		14	28	0.86	LMA	72	23.7	0.88	3.9	7.1	***

Provisinal QTL detected in chromosome scanning are shown in Supplementary Table 5.
[a]The unit is kg for BW and CW, cm^2 for LMA, and cm for RT and SFT, respectively.
[b]*, **, and *** mean <5%, <1%, and <0.1% chromosome-wise significance levels, respectively.
[c]Three hundred forty-eight steers from Market 1 and 437 from Market 4.
[d]Three hundred twenty-five steers from Market 1, 143 from Market 2, and 95 from Market 3.
[e]Mizoshita et al. 2004.
[f]Mizoguchi et al. 2006.
[g]Genome scanning was done using less than 190 offspring and therefore was not included in Table 1.
[h]The QTL was FDR>0.1 or not detected at 5% chromosome-wise significance level in genome scanning.
[i]The trait was not examined in genome scanning because of selective genotyping for BMS.

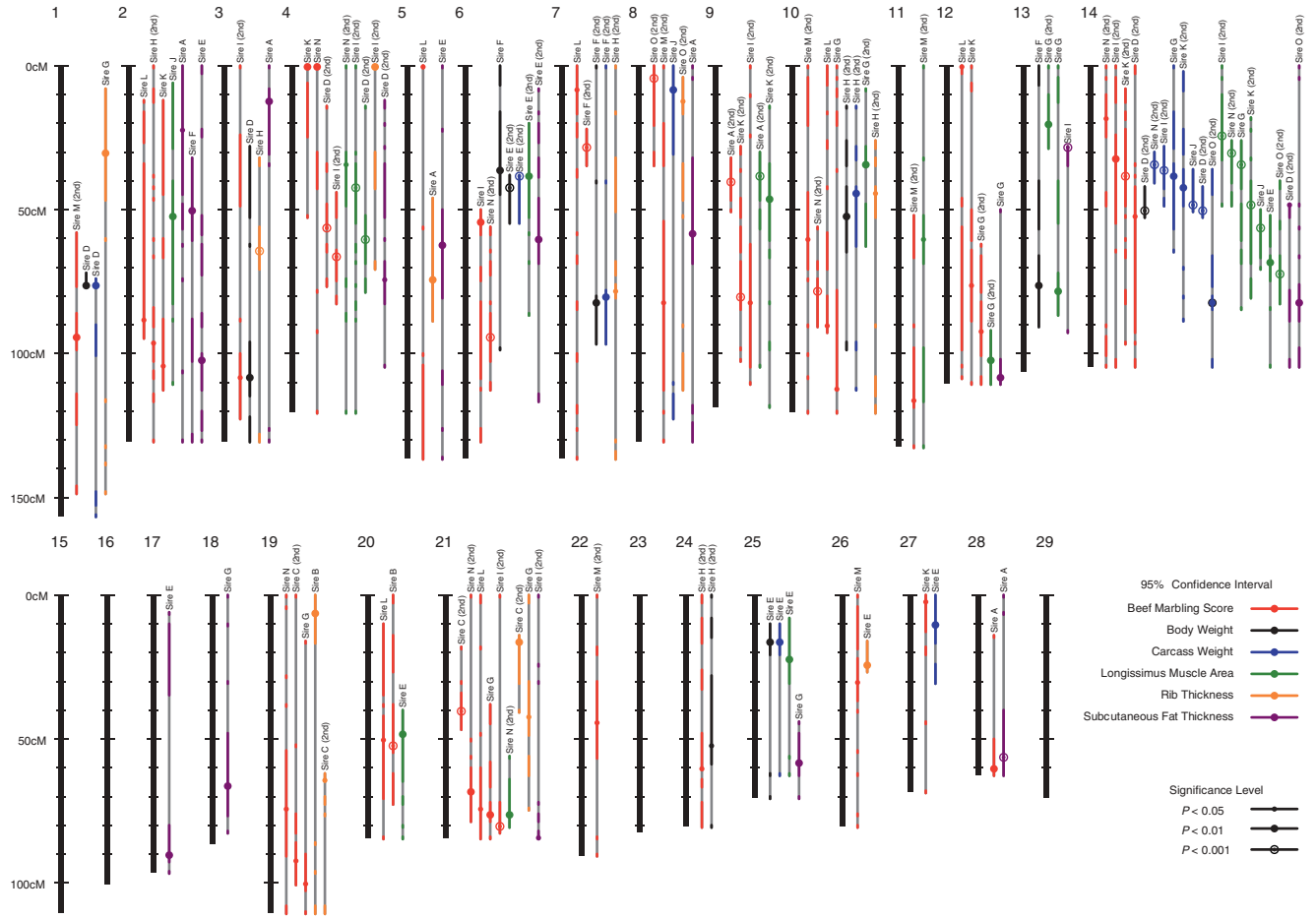


Fig. 3. Bovine QTL map. The QTL detected at less than 5% chromosome-wise significance level with less than 0.1 false discovery rate (FDR) are shown with 95% CI. For the QTL submitted to chromosome scanning, 95% CI of the fine-mapped regions are shown.

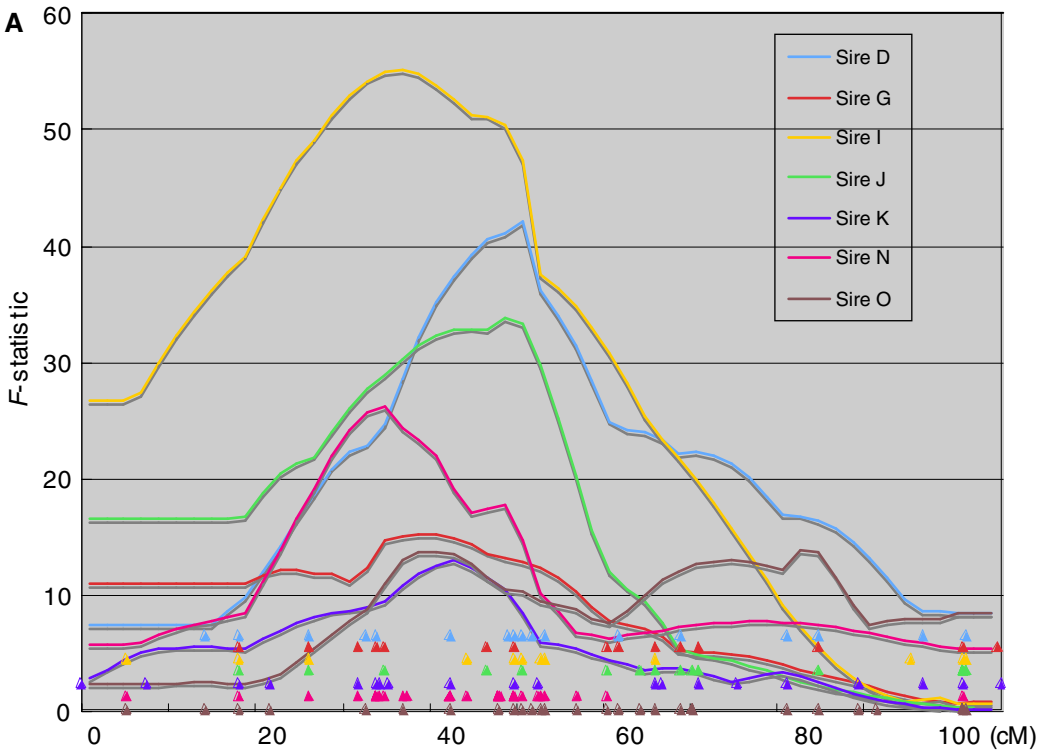
the same haplotype as the *CW-1 Q* allele, except for the marbling QTL detected at the 5% chromosome-wise significance level in the Sire D family (data not shown). It is likely that the selection for CW acts mainly at the *CW-1* locus because of a possible hitchhiking effect on the marbling and/or LMA QTL.

The *Q* to *q* allele substitution effect of *CW-1* ranged from 13.7 to 26.7 kg in the seven families (Tables 1 and 2), which is comparable with the effect examined using 1700 steers: 23.6 kg for the first *Q* and an additional 15.2 kg for the second *Q* (Mizoshita et al. 2005). Interestingly, the average CW of the family differed by 60.5 kg (95%CI: 54.4–66.6 kg) between the Sire K and Sire J families (Supplementary Table 1). The difference was bigger than the effect of the *CW-1* locus, which should be due to the result of other CW QTL rather than a difference in the maternal *CW-1 Q* frequencies in the local populations.

The BW QTL on BTA6 were detected at the 0.1% and 1% chromosome-wise significance levels in the Sire E and Sire F families, respectively (Table 2, Supplementary Table 4). This QTL, designated *CW-2*, was fine-mapped between 38 and 54 cM in the Sire E family (Fig. 3). There were no apparent shared haplotypes between Sire E and Sire F in the region, although they harbored common *Q* alleles for some markers (data not shown). The allele substitution effect on BW was 41.7 kg (Sire E) and 26.5 kg (Sire F), respectively, which corresponded to 24.1 kg and 13.4 kg on CW, respectively (Tables 1 and 2, Supplementary Table 4). Therefore, the effect of *CW-2* was nearly equal to that of *CW-1*. Because Sire E harbored a homozygous *Q* in the *CW-1* region (Fig. 4B), *CW-2* is expected to act additively on *CW-1*.

The QTL for carcass yield and/or growth rate on BTA1, 6, 10, and 14 were previously mapped using experimental crosses (Bovine QTL Viewer, http://www.bovineqtl.tamu.edu/index.html). Searching the *Q* haplotypes in other breeds might be useful if they have common founders. In humans and mice, many genes affecting body weight have been identified (Mouse Genome Informatics, http://www.informatics.jax.org/). In the human, the region

A



B

	Marker	BMS1941	DIK7008	DIK7012	DIK7013	DIK7015	DIK7018	NRKM-040	DIK7020	BM4630	DIK519	DIK062
	cM	41.707						47.116		45.799	48.925	49.828
Sire D	*Q1*	2	1	1	3	3	2	3	5	1	1	2
	q1	1	1	2	2	4	3	3	2	1	2	1
Sire O	*Q1*	2	1	1	3	3	2	3	5	1	1	2
	q1	1	1	2	2	4	3	3	2	1	2	1
Sire K	*Q1*	2	1	1	3	3	2	3	5	1	1	2
	q1	1	1	2	2	4	3	3	2	1	2	1
Sire I	*Q2*	1	2	3	3	3	2	3	3	2	2	1
	q2	3	1	2	3	2	1	1	4	2	3	2
Sire N	*Q2*	1	2	3	3	3	2	3	3	2	2	1
	q2	3	1	2	3	2	1	1	4	2	3	2
Sire J	*Q2*	1	2	3	3	3	2	3	3	2	2	1
	q3	1	1	2	1	1	2	2	1	1	3	2
Sire G	*Q2*	1	2	3	3	3	2	3	3	2	2	1
	q3	1	1	2	1	1	2	2	1	1	3	2
Sire E	*Q1*	2	1	1	3	3	2	3	5	1	1	2
	Q1	2	1	1	3	3	2	3	5	1	1	2

CW-1, 1.1-Mb region

Fig. 4. (**A**) *F*-statistic profiles for CW on BTA14. Marker locations are indicated by triangles colored for each family. (**B**) Haplotype analysis around the *CW-1* region. The microsatellite markers are ordered according to the physical map described in Mizoshita et al. (2005). The superior *Q* and inferior *q* haplotypes of the seven sires are grouped into two (*Q1* and *Q2*) and three (*q1*, *q2*, and *q3*) haplotypes, respectively.

corresponding to *CW-1* (HSA 8q12.1–12.2) contains no apparent candidate genes (Itoh et al. 2005; Mizoshita et al. 2005). Identification of the *CW-1* gene and other CW QTL genes might elucidate a novel molecular mechanism underlying growth and meat quantity in mammals.

QTL for marbling. Marbling is ranked according to the degree of intramuscular fat deposition and is highly related to beef quality. We mapped 12 significant and 33 provisional QTL for marbling (Tables 1 and 2, Supplementary Tables 4 and 5). The significant QTL were distributed on BTA4, 6,

A. BTA 4

	Marker	DIK4920	BMS1237	DIK2956	DIK4159	BMS2646	DIK2875	BMS1840	MAF50	BMS2172	DIK1139	INRA072	DIK4293	BL21	DIK2472	DIK4682	DIK2293	DIK2353	DIK026
	cM	30.8	34.4	35.5	41.1	43.2	45.5	46.5	51.2	53.3	58.5	63.0	64.7	67.2	70.2	73.0	77.6	80.8	86.2
Sire I	*Q1*	2	3	1	2	2	1	3	1	3	1	1	2	2	3	1	1	2	2
	q1	1	1	3	2	2	3	2	2	1	3	2	1	3	2	2	2	1	1
Sire D	*Q2*	3	2	2	1	1	2	1	2	2	2	1	1	1	1	2	3	2	1
	q1	1	1	3	2	2	3	2	2	1	3	2	1	3	2	2	2	1	1

B. BTA 6

	Marker	BM1329	BM143	BMS690	BM4322	BMS483	ILSTS097	BMS360	DIK2163	BM415	MNB-210	EL03	ILSTS087	BMS739	BM9257	BM2320
	cM	35.4	53.7	56.4	63.9	67.8	72.4	72.9	77.6	82.0	83.4	88.2	89.7	120.3	120.3	127.3
Sire I	*Q1*	2	2	2	2	2	2	1	2	2	1	2	2	1	1	ND
	q1	2	3	3	1	1	1	2	2	1	3	2	2	2	2	ND
Sire N	*Q2*	2	2	2	2	2	2	1	2	2	1	2	2	1	2	1
	q2	1	1	1	3	1	1	2	1	1	2	1	1	2	1	2

C. BTA 10

	Marker	DIK2014	BMS419	DIK2866	BR1603	BR6027	DIK4288	TGLA433	CSRM60	INRA037	BMS1620	DIK4078	DIK020	BMS614
	cM	54.4	59.5	65.8	68.1	69.5	71.1	74.0	77.8	79.0	80.4	84.8	90.1	100.0
Sire N	*Q1*	2	3	1	3	3	2	2	2	1	1	2	3	3
	q1	4	2	2	2	1	3	1	1	2	2	1	2	4
Sire M	*Q2*	1	1	3	1	2	1	1	2	2	3	2	1	4
	q2	3	4	2	2	4	3	1	1	2	2	1	3	2
Sire L	*Q2*	1	1	3	1	2	1	1	2	2	3	2	1	4
	q3	3	4	2	2	4	3	3	2	1	3	2	2	1

Fig. 5. Haplotype analyses for the replicated marbling QTL regions. (**A**) BTA4, (**B**) BTA6, (**C**) BTA10. The arrows indicate 95% CI of the QTL with significant linkages. Shared *Q* and *q* haplotypes were shown by grayed and bold-lined boxes, respectively.

7, 8, 9, 10, 14, 20, and 21, which were replicated at a less than 5% chromosome-wise significance level except for the QTL on BTA8 and 14 (Fig. 3). These haplotypes in the replicated regions were compared between sires using the microsatellites on the Shirakawa-USDA linkage map (Ihara et al. 2004). In BTA6, an IBD superior *Q* haplotype was inherited from Sire I to Sire N (Figs. 2 and 5B). In BTA10, Sire L and Sire M shared an IBD *Q* haplotype inherited from a common sire, whereas no apparent shared *Q* haplotype was observed between the two sires and Sire N (Figs. 2 and 5C). Alternatively, Sire N shared an inferior *q* haplotype with Sire M in the QTL region. Also in BTA4, an IBD inferior *q* haplotype was shared between Sire D and Sire I (Fig. 5A). On the other hand, there was no significant shared haplotype for either *Q* or *q* in other QTL regions, although the QTL peaks in BTA9 and 20 were too broad to compare the haplotypes in detail (data not shown). Because the haplotype blocks of the QTL regions might be smaller than those examined, more detailed comparisons might reveal a shared *Q* haplotype.

The allele substitution effect of the significant marbling QTL ranged from 0.5 to 1.2 (Tables 1 and 2). Those of shared alleles were 0.9–1.2 (BTA4), 0.7–0.9 (BTA6), and 0.7–0.8 (BTA10), respectively (Supplementary Tables 4 and 5). The regression analyses suggested that the QTL on BTA4 and 21, BTA9 and 14, and BTA6 and 10 were additive in Sire I, Sire K, and Sire N families, respectively (Mizoguchi et al. 2006; Supplementary Table 6). The size of the effect and the interaction among the loci should be further examined in a general population.

It has been shown that linkage disequilibrium mapping is a useful method to narrow down the QTL regions (Farnir et al. 2002; Mizoshita et al. 2005). We expect it is also workable for replicated QTL using a general population collected throughout Japan. On the other hand, the *Q* haplotypes of some marbling QTL were inherited in maternal lineages, and their frequencies were too low in the population to examine the association between the alleles and marbling (data not shown). The QTL analyses of descendant sires might be useful to confirm and narrow down the QTL.

Our investigation outlined marbling QTL in Japanese Black cattle. The QTL for marbling score or meat tenderness have been mapped on several chromosomes using crossbred populations (Bovine QTL Viewer, http://www.bovineqtl.tamu.edu/index.html), while QTL on BTA4, 7, 8, and 21 were not detected previously. The ability to deposit intramuscular fat that accounts for high marbling might be a characteristic of Japanese Black cattle, and the *Q* allele frequencies of these QTL might be low in other breeds.

The genes involved in adipogenesis have been studied extensively. More than 1000 genes are transcriptionally regulated in adipogenesis (Soukas et al. 2001; Tseng et al. 2005). In cattle, approximately 80 genes have been isolated whose expression levels are different between high-marbled Japanese Black and low-marbled Holstein biopsied

muscle (Sasaki et al. 2006). Physical assignment of these genes might help us find a candidate gene for mapped QTL (Yamada et al. 2006). So far, the transgenic mouse overexpressing ADAM 12 under the muscle creatine kinase promoter is the only animal model in which intramuscular fat is deposited in skeletal muscle (Kawaguchi et al. 2002) Identification of the marbling QTL genes will provide new insight into adipogenesis.

Perspectives. We detected 40 QTL for growth and carcass traits with moderate to large effects using 15 paternal half-sib families of purebred Japanese Black cattle, and we further confirmed their presence by replication and IBD mapping. This QTL information will be applied to breed descendant sires, followed by confirmation of the effects using the resulting half-sib family. Some QTL regions detected in this study were overlapped with the QTL detected using crossbred populations in previous reports. Our QTL results using a purebred Japanese Black population might be applicable to other bovine breeds. Recently, the bovine SNP consortium produced more than 120,000 SNP, and the 10k SNP array is commercially available (Affymetrix, Santa Clara, CA). Genome-wide linkage disequilibrium mapping is now possible using the array; it might be useful to examine the size of the effect and narrow down the region of each QTL.

Acknowledgments

The authors thank the technical staff at the Shirakawa Institute of Animal Genetics and the collaborating institutes for technical assistance. The work was partly supported by the Ministry of Agriculture, Forestry, and Fishery, Japan, and by the Japan Racing and Livestock Promotion Foundation.

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