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Full Length Research Paper

Detection and mapping of QTL on bovine chromosomes 2 and 5 segregating for live weight, average daily gain and body measurements in Japanese black cattle

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Bovine chromosomes 2 (BTA2) and 5 (BTA5) of purebred, half-sib progeny sired by five Japanese black bulls were genotyped using microsatellite DNA markers. The data were subjected to linkage analysis for the detection and mapping of segregating quantitative trait loci (QTL) influencing live weight, average daily gain and body measurements at weaning. Probability coefficients of inheriting allele 1 or 2 from the sire at specific chromosomal intervals were computed. The phenotypic data on progeny were regressed on these probability coefficients in a within-common-parent regression analysis. Fixed effects of sex, parity and season of birth as well as age as a covariate, were fitted in a linear model to the phenotypic data and subsequently analysed using QTL Express by generating an F-statistic through permutation tests at chromosome-wide significance thresholds over 10, 000 iterations at 1 cM intervals. Highly significant (P<0.01) segregating QTL for body measurements were detected on BTA2 for hip width (1 cM) and chest depth (8 cM) in Sire Family 1 and pin bone width (16 cM) in Sire Family 3. Other significant QTL (P<0.05) detected were withers height (3 cM), hip height (4 cM), body length (4 cM), shoulder width (6 cM), lumbar width (3 cM), thurl width (3 cM) and canon circumference (2 cM) in Sire Family 1, shoulder width (16 cM) in Sire Family 3 and thurl width (24 cM), pin bone width (19 cM), heart girth (26 cM) and abdominal width (69 cM) in Sire Family 4. Significant (P<0.05) QTL for live weight and average daily gain were detected on BTA2 for birth weight (5 cM) and weaning weight (3 cM) in Sire Family 1 and post-weaning average daily gain (68 cM) in Sire Family 4. BTA 5 contained QTL for birth weight, pin bone width and heart girth in Sire Family 3 that were only suggestive and not significant. Such localization of economically important QTL as demonstrated in this study, will expedite genetic improvement via marker-assisted selection, gene introgression and positional cloning in Japanese black cattle.

Key words: QTL mapping, Japanese Black cattle, BTA2, BTA5, body measurements, live weight, daily gain.

INTRODUCTION

Use of DNA markers to account for genetic variation for quantitative traits provides producers a tool to assist in genetic selection of superior animals (Allan et al., 2007). Whereas some marker-assisted selection is currently practiced in the beef cattle industry, a limited number of markers have been developed for use by cattle producers, and these markers explain a relatively small proportion of the genetic variation for a limited number of traits (Dekkers, 2004). Therefore, the need continues for more genetic markers associated with economically important

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traits.

Biotechnological developments in molecular genetics that culminated in the availability of microsatellite DNA markers have been instrumental in the construction of genetic maps across livestock species. In structured pedigree populations, these markers are useful in identifying inheritance patterns of linked segments of the genome. The establishment of significant associations of marker alleles with an animal's phenotype suggests linkage of the markers to quantitative trait loci (Malau-Aduli et al., 2003a). Microsatellites and single nucleotide polymerphisms (SNPs) are among the best genome markers and useful ones can be included in marker-assisted selection programmes to increase the rate of genetic progress (Georges et al., 1993). A plethora of researchers (White et al., 2007; Casas et al., 2007; Lusk, 2007; Rincker et al., 2006; Casas and Stone, 2006; Schenkel et al., 2005; Stone et al., 2005; Casas et al., 2005; White et al., 2005; Nkrumah et al., 2005; Kneeland et al., 2004) have reported quantitative trait loci (QTL) or associations between SNP markers and beef traits in breeds of cattle other than the Japanese black (Wagyu). Continued validation of genetic markers for economically important traits is crucial to establishing marker-assisted selection as a tool in the cattle industry (Allan et al., 2007). Recently, Van Eenennaam et al. (2007) carried out a validation test for the associations between 3 commercially available genetic markers and beef traits in conjunction with the US National Beef Cattle Evaluation Consortium and emphasised the need for unbiased and independent validation studies to help build confidence in DNA marker technology.

Mizoshita et al. (2004) utilised microsatellite markers to detect quantitative trait loci (QTL) for growth and carcass traits in only one family of Japanese black cattle. More recently, we utilised microsatellite markers across five families of purebred Japanese black cattle and reported the detection of QTL for body shape conformation measurements (Malau-Aduli et al., 2005a) and growth (Malau-Aduli et al., 2005b) on bovine chromosome one (BTA1). Preliminary genome-wide scanning in our laboratory using only 30 animals (unpublished data) had suggested BTA1, BTA2 and BTA5 as chromosomes containing segregating QTL significantly influencing growth and body conformation traits in Japanese black cattle. Therefore, in this confirmatory study with a larger data set of genotyped animals, we report the genetic linkage between microsatellite DNA markers and QTL on BTA2 and BTA5 influencing live weight, daily gains and body measurements at weaning of Japanese black cattle.

MATERIALS AND METHODS

Animals and management

One hundred and thirty-two paternal half-sib progeny of five Japanese black sires produced by artificial insemination at the Department of Livestock and Grassland Science, National Agricultural Research Centre for Western Region, Oda, Shimane Prefecture, Japan, were genotyped for this study. Sires 1 and 2 belonged to the average daily gain line while Sires 3, 4 and 5 belonged to the beef marbling score. Routine management of the animals involved recording of weight at birth and monthly thereafter, until 18 months of age. Body shape and conformation measurements on withers height, hip height, hip width, body length, chest width, chest depth, shoulder width, lumbar width, thurl width, pin bone width, rump length, cannon circumference, chest girth, abdominal width and abdominal girth were also taken monthly. Calves were allowed to suckle their dams in addition to being fed 1.5 kg/day/head of concentrate and 1 kg/day/head of corn silage until 5 months of age when they were weaned. After weaning, they were moved to the grower's barn and still raised on concentrates (37% corn grain, 39% rice bran, 17% soybean meal, 7% minerals) and corn silage until 10 months of age. Between 10 and 18 months of age, they were moved to another barn and fed intensively. The proportions of the ration on dry matter basis were: 61% corn grain, 34% soybean and corn glutein meal, 2% bran and 3% mineral. For every 20 kg bag, this ration provided an estimated 21% crude protein, 3.5% crude fat, 5% crude fibre, 7% ash, 0.6% calcium, 0.40% phosphate and a total digestible nutrient of 77%. From 18 to 24 months of age, breeding females were returned to the calving barn while steers were moved to the fattening barn and raised primarily on "Mosa meal" a specially formulated fattening ration containing 77% corn and rye grain, 10.5% wheat and rice bran, 9% soybean oil meal and 3.5% mineral supplement. At all ages, routine veterinary vaccinations and health checks were observed.

Extractions of genomic DNA

Following the method of Sambrook et al. (1989) and described in detail elsewhere (Malau-Aduli et al. 2003b, 2005a), genomic DNA was extracted and prepared from blood leucocytes and sperm.

Polymerase chain reaction (PCR)

PCR pre-mix (13 µl) that comprised of: 10.55 µl of distilled water, 1.04 µl of 2.5 mM dNTP mixture (Takara, Shiga, Japan), 1.3 µl of 10 x buffer containing 15 mM $MgCl_2$ and 0.11 µl of 25 mM of $MgCl_2$ was prepared. A primer set (12.5 pmol/ µl) containing microsatellite DNA markers FAM (blue), HEX (yellow) and TET (Green) supplied by the Shirakawa Institute of Animal Genetics, Fukushima, Japan, based on the bovine genetic map at the U.S. Meat Animal Research Centre (Kappes et al., 1997; http://sol.marc.usda.gov) was added to the PCR pre-mix. Genomic DNA (1 µl) (conc of 20 ng/µl) was added followed by 0.5 µl of Taq polymerase enzyme (conc of 0.75 units/µl) containing 50% glycerol (Takara, Japan). The PCR plates were hotplate-sealed and subjected to PCR in a DNA thermal cycler. The annealing temp settings were: 50, 55 and 60ºC. The PCR products were then mixed with DNA size markers in different loading combinations containing 4 µl of HEX, 1 µl of FAM and 1 µl of TET, properly labelled and stored for genotyping.

Genotyping

Multiplex genotyping was carried out. About 0.8 µl of the mixed PCR products was added to 4.5 µl of DNA size marker, centrifuged for 1 min at 1000 rpm and denatured using the PCR machine at a denaturing temperature of 94ºC for 9 min. The denatured products were subjected to electrophoresis and genotyping in an ABI 377 DNA Sequencer. The number of informative microsatellite DNA markers utilized for the genotyping in each family is portrayed in Tables 1 and 2 for chromosomes 2 and 5 respectively.

Table 1. Microsatellite DNA markers used for genotyping chromosome 2 in the 5 Japanese Black cattle families and their relative positions on the map (cM)

Family	Marker	Position	Family	Marker	Position	Family	Marker	Position	Family	Marker	Position	Family	Marker	Position
1	TGLA44	2.6	2	TGLA44	2.6	3	BM3627	7.6	4	BMS1928	6.9	5	BM8139	8.2
1	TGLA431	11.1	2	DIK621	4.6	3	BMS803	44.0	4	BMS711	21.3	5	BMS2321	14.0
1	TEXAN2	24.2	2	ILSTS026	9.6	3	RM356	55.1	4	TGLA57	46.2	5	BMS711	21.3
1	ETH121	36.9	2	DIK1172	16.8	3	BM4440	58.3	4	BMS4035	55.0	5	BMS2725	41.8
1	BMS803	44.0	2	DIK1081	25.6	3	BMS1264	63.3	4	BMS4029	61.3	5	BMS4002	47.9
1	ILSTS082	62.0	2	ETH121	36.9	3	RM041	72.3	4	BM9019	67.5	5	BMS4012	51.0
1	BMS1866	86.1	2	MNB-187	57.3	3	BMS1866	86.1	4	BMS4008	71.7	5	RM326	55.6
1	BM6444	91.8	2	BMS1126	59.7	3	BM6444	91.8				5	BMS4030	59.2
1	BM1223	95.9	2	BM2808	63.3	3	INRA135	102.6				5	BMS4029	61.3
1	INRA135	102.6	2	BMS2	65.0	3	BM4117	104.9				5	INRA119	68.7
1	BM4117	104.9	2	TEXAN1	72.0	3	BL1028	109.7				5	BMS4008	71.7
1	BL1028	109.7	2	BM6444	91.8		IDVGA37	112.0				5	BM8246	76.2
1	IDVGA-37	112.0	2	INRA135	102.6		IDVGA-2	121.8				5	BMS4006	79.4
1	IDVGA-2	121.8	2	BL1028	109.7									
1	OARFCB11	124.4	2	DIK1155	113.1									
Total	15			15			13			7			13	

Traits analyzed

Offspring of the five sires born between 1997 and 2002 were evaluated (SAS, 2002) for live weight, average daily gain and the following 15 body shape and conformation measurements at weaning (5 months of age): withers height, hip height, hip width, body length, chest width, chest depth, shoulder width, lumbar width, thurl width, pin bone width, rump length, cannon circumference, chest girth, abdominal width and abdominal girth.

QTL analysis

We adopted the methods of Knott et al. (1996), Haley and Knott (1992) and de Koning et al. (1998, 2001) for the detection and mapping of QTL in half-sib populations using least squares simple regression. We used the QTL Express (http://qtl.cap.ed.ac.uk/) developed by Seaton et al. (2002) and based on the methods of the researchers mentioned above for the QTL analysis. The half-sib model of QTL computer program with a web-based user interface Express run within and across sires, implemented the analysis in a two-step procedure: Firstly, microsatellite DNA marker data on progeny and their common parent (sire) were combined in a multi-point approach to calculate the probabilities of inheriting allele 1 or 2 from the sire at specific chromosomal intervals. These probabilities were combined into coefficients with values between 0.0 and 1.0. Secondly, the phenotypic data on progeny were regressed on these coefficients in a within-common-parent regression analysis. A linear model containing the fixed effects of sire, sex, parity and season of birth as well as age as a covariate, was fitted to the probability coefficients and phenotypic data. Appropriate F-statistic thresholds for a $P<0.05$ chromosome-wise type 1 error rate were generated by permutation analysis as described by Churchill and Doerge (1994), Doerge and Churchill (1996) (and applied to other half-sib studies by Spelman et al. (1996) and Vilkki et al. (1997). In determin-ing significant and suggestive thresholds, the QTL Express software (Seaton et al. 2002) computed both the F-statistics and the F-threshold at $P<0.05$ chromosome-wise level. QTL was classified as significant when the F-statistic exceeded the F-threshold indicating a marker-trait association. For the determination of a suggestive association, which is the expected number of type I errors in the experiment when the null hypothesis of no QTL segregating is true, we followed the procedure of de Koning et al. (1998). They stated that the overall significance used for the chromosomal test when undertaking a scan of n independent linkage groups (or n [linkage groups x independent traits]) can be calculated from the nominal significance level applied to a single linkage group following Bonferoni:

$$P_{overall} = 1 - (1 - P_{nominal})^{n}.$$

A very good and simple approximation for the solution to this equation is,

$$P_{nominal} \approx P_{overall}/n.$$

The suggestive significance level can then be obtained from the binomial distribution as:

$$P_{suggestive} = 1/n.$$

RESULTS

Informative microsatellite DNA markers and positions on the linkage map

Portrayed in Tables 1 and 2 are the microsatellite

Table 2. Microsatellite DNA markers used for genotyping chromosome 5 in the 5 Japanese Black cattle families and their relative positions on the map (cM)

Family	Marker	Position	Family	Marker	Position	Family	Marker	Position	Family	Marker	Position	Family	Marker	Position
1	BMS1928	6.9	2	BM8139	8.2	3	BMS2321	14.0	4	BMS1928	6.9	5	BM8139	8.2
1	BMS711	21.3	2	TGLA57	46.2	3	ILSTS104	28.2	4	BMS711	21.3	5	BMS2321	14.0
1	ILSTS104	28.2	2	BMS4012	51.0	3	BMS4002	47.9	4	TGLA57	46.2	5	BMS711	21.3
1	MB055	32.0	2	BMS4013	61.3	3	BMS4012	51.0	4	BMS4035	55.0	5	BMS2725	41.8
1	TGLA57	46.2	2	BMS4001	64.7	3	BMS4035	55.0	4	BMS4029	61.3	5	BMS4002	47.9
1	BMS4012	51.0	2	BM9019	67.5	3	RME36	63.0	4	BM9019	67.5	5	BMS4012	51.0
1	BMS4035	55.0	2	BL26_1	77.7	3	BM8246	76.2	4	BMS4008	71.7	5	RM326	55.6
1	RM326	55.6	2	BMS4006	79.4	3	BMS119	88.6	4	BMS4048	76.2	5	BMS4030	59.2
1	RME36	63.0	2	URB038	80.6	3	BMS4019	98.8	4	URB038	80.6	5	BMS4029	61.3
1	INRA049	67.5	2	MCM130	83.3	3	UWCA46	113.8	4	BMS4010	87.1	5	INRA119	68.7
1	BM65O6	69.2	2	BMS4010	87.1	3	BMS599	125.8	4	BM864	88.2	5	BMS4008	71.7
1	URB038	80.6	2	BM864	88.2				4	BMS1170	92.8	5	BM8246	76.2
1	BMS4052	94.6	2	BMS1170	92.8				4	BMS4019	98.8	5	BMS4006	79.4
1	BMS4028	95.6	2	BMS4028	95.6				4	BMS4011	102.1	5	BMS4010	87.1
1	BMS4040	98.8	2	BMS4019	98.8				4	BMS4049	114.3	5	BMS4019	98.8
1	BMS1789	100.9	2	BMS1789	100.9				4	BMS918	118.1	5	BMS1757	108.3
1	BMS4044	128.7	2	BMS1939	104.1				4	BMS599	125.8	5	BMS4044	128.7
1	BMS2263	135.1	2	BMS4039	108.3				4	BMS4044	128.7			
			2	BM3205	113.8				4	BMS922	135.5			
			2	BMS599	125.8									
			2	BMS4043	128.7									
			2	BMS2263	135.1									
			2	BMS4014	135.5									
Total	18			23			11			19			17	

DNA markers and their relative positions on the BTA2 and BTA5 linkage maps respectively, that were utilized in genotyping the sires and half-sib progeny. The tables show that on BTA2, 15, 15, 13, 7 and 13 markers were informative for families 1, 2, 3, 4 and 5 respectively (Table 1), while on BTA5, 18, 23, 11, 19 and 17 markers were informative for the families 1, 2, 3, 4 and 5, respectively (Table 2).

Live weight, daily gains and body measurements at weaning

The means and standard deviations of live weight (kg), daily gains (kg/day) and body conformation measurements (cm) at weaning in the 5 Japanese black families are shown in Table 3. It was evident that in all families, almost all of the body conformation measurements within traits were similar. The only clearly visible sign of significant differences between families was in chest girth (CHESTGTH) measurements in which Families 1 and 2 (125.9 and 127.2 cm respectively) were higher than in Families 3, 4 and 5 (121.7, 123.4

Table 3. Means ±S.D. of live weight (kg), daily gains (kg/day) and body measurements at weaning (cm) in the progeny of 5 Japanese Black sire families.

Trait/Acronym	Family 1	Family 2	Family 3	Family 4	Family 5
BWT Birth weight (kg)	34.7 ± 4.7[a]	34.0 ± 4.8[a]	28.7 ± 5.1[b]	28.7 ± 3.3[b]	26.9 ± 5.4[b]
WT6 Weaning weight (kg)	163.9 ± 21.7[b]	172.6 ± 19.4[a]	177.6 ± 20.5[a]	176.7 ± 22.9[a]	174.3 ± 31.7[a]
WT12 Yearling weight (kg)	300.9 ± 31.8[a]	284.7 ± 34.1[b]	299.9 ± 33.5[a]	283.6 ± 29.1[b]	281.7 ± 35.2[b]
PREWADG (kg/day)	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.2
POSTWADG (kg/day)	0.8 ± 0.2[a]	0.7 ± 0.2[a]	0.6 ± 0.1[b]	0.6 ± 0.2[b]	0.6 ± 0.2[b]
WHT Withers height	99.5 ± 3.9	100.9 ± 3.5	98.5 ± 3.5	97.5 ± 2.7	97.6 ± 4.0
HIPHT Hip height	103.1 ± 3.9	103.0 ± 3.0	101.0 ± 3.8	101.1 ± 3.8	98.7 ± 4.1
BL Body length	106.5 ± 5.9	108.1 ± 5.0	103.2 ± 7.5	103.2 ± 4.5	101.9 ± 5.3
CHESTWD Chest width	28.1 ± 2.2	29.5 ± 2.3	27.7 ± 2.6	26.9 ± 2.2	27.2 ± 1.6
SHOUWD Shoulder width	31.2 ± 2.6	31.4 ± 2.1	28.4 ± 2.2	28.6 ± 2.0	27.4 ± 2.3
CHESTDP Chest depth	46.3 ± 1.8	46.6 ± 1.5	44.7 ± 1.8	45.5 ± 1.4	43.8 ± 2.2
HIPWDT Hip width	28.3 ± 1.8	29.0 ± 1.3	26.3 ± 2.1	28.1 ± 1.4	27.4 ± 1.5
LUMBARWD Lumbar width	22.7 ± 1.5	23.1 ± 1.0	21.1 ± 2.1	22.6 ± 1.3	22.0 ± 1.3
THURLWD Thurl width	33.0 ± 2.2	33.6 ± 1.7	31.0 ± 1.6	31.3 ± 1.9	31.0 ± 2.0
PINBWD Pin bone width	20.5 ± 2.1	20.6 ± 1.3	18.6 ± 1.9	18.9 ± 1.0	18.1 ± 1.4
RUMPL Rump length	35.2 ± 2.1	35.8 ± 1.8	34.6 ± 1.7	35.3 ± 1.4	34.4 ± 1.7
CANNONCIR Cannon circumference	14.4 ± 0.9	14.7 ± 0.9	13.8 ± 1.0	13.5 ± 0.8	13.4 ± 0.9
CHESTGTH Chest girth	125.9 ± 5.2[a]	127.2 ± 4.3[a]	121.7 ± 4.9[b]	123.4 ± 3.8[b]	120.2 ± 5.9[b]
ABDWD Abdominal width	37.0 ± 2.5	37.7 ± 2.5	35.4 ± 2.9	36.5 ± 2.2	35.5 ± 1.9
ABDGTH Abdominal girth	144.0 ± 7.0	143.5 ± 6.1	138.5 ± 7.4	140.6 ± 6.0	138.0 ± 7.2
No. of progeny	40	36	19	17	20

Means in rows bearing different superscripts significantly differ between families.

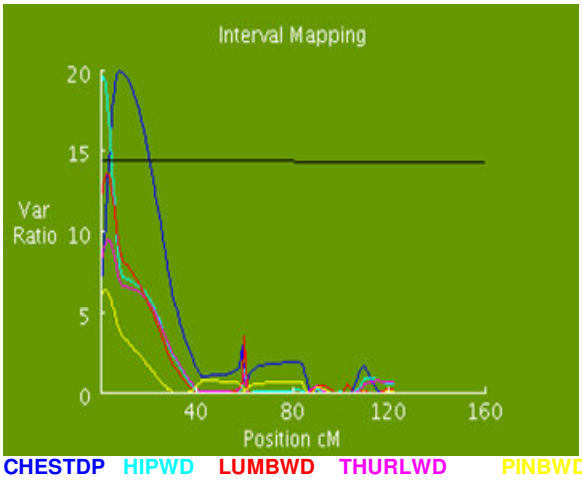

Figure 1. BTA2 map of F-statistics in Sire Family 1 depicting highly significant (P<0.01) QTL for chest depth (CHESTDP) at 8cM and hip width (HIPWD) at 1cM. Bold line represents the chromosome-wide F-threshold.

and 120.2 cm respectively).

Allele substitution effects, estimated QTL locations and chromosome-wide thresholds

Allele substitution or sire QTL effects, estimated QTL locations and chromosome-wide threshold statistics for live weight and body conformation traits in the 5 Japanese black families are shown in Table 4. Highly significant (chromosome-wide P<0.01) segregating QTL for body measurements were detected on BTA2 for hip width (1 cM), chest depth (8 cM) in Sire Family 1 (Figure 1) and pin bone width (16 cM) in Sire Family 3 (Figure 2). BTA 2 harboured significant QTL (Figure 3) at the chromosome-wide P<0.05 level for 10 other body measurement traits: withers height (3 cM), hip height (4 cM), body length (4 cM), shoulder width (6 cM), lumbar width (3 cM), thurl width (3 cM) and canon circumference (2 cM) in Sire Family 1, shoulder width (16 cM) in Sire Family 3 and thurl width (24 cM), pin bone width (19 cM), heart girth (26 cM) and abdominal width (69cM) in Sire Family 4. Significant (P<0.05) QTL for live weight and average daily gain were detected on BTA2 for birth weight (5 cM) and weaning weight (3 cM) in Sire Family 1 (Figure 4) and post-weaning average daily gain (68 cM) in Sire Family 4. BTA 5 contained QTL for birth weight, pin bone width and heart girth in Sire Family 3 that were only suggestive and not significant

DISCUSSION

Approximately 43% of domestic beef production in Japan is supplied by the Japanese Black breed of cattle and its genetic superiority for meat quality and carcass value is well known (Mukai et al. 2004). A study on consumer re-

Table 4. Allele substitution/Sire QTL effects ± standard errors (ß ± S.E.) and estimated QTL locations (cM) for liveweight (kg), average daily gains (kg/day) and body measurement (cm) traits in Japanese Black cattle families.

Sire Family	Trait	Chromosome	QTL location	Sire QTL/ Allele substitution effect (ß ± S.E)	F-statistics	F-threshold	Significance (chromosome-wide)
1	BWT	2	5cM	-6.2 ± 1.87 kg	10.78	8.78	P<0.05
1	WT6	2	3cM	-29.7 ± 9.60 kg	9.57	8.97	P<0.05
1	WHT	2	3cM	-4.8 ± 1.52 cm	10.04	9.15	P<0.05
1	HIPHT	2	4cM	-5.0 ± 1.58 cm	10.03	9.18	P<0.05
1	BL	2	4cM	-8.2 ± 2.30 cm	12.82	9.29	P<0.05
1	SHOUWD	2	6cM	-3.2 ± 0.97 cm	10.69	8.91	P<0.05
1	**CHESTDP**	**2**	**8cM**	**-2.7 ± 0.61 cm**	**20.00**	**14.07**	**P<0.01**
1	**HIPWD**	**2**	**1cM**	**-3.4 ± 0.75 cm**	**19.59**	**14.37**	**P<0.01**
1	LUMBWD	2	3cM	-2.0 ± 0.65 cm	9.55	9.13	P<0.05
1	THURLWD	2	3cM	-3.3 ± 0.89 cm	13.64	9.31	P<0.05
1	CANCIR	2	2cM	-1.0 ± 0.31 cm	11.66	8.80	P<0.05
2	POSTWADG	2	60cM	0.24 ± 0.08 kg/day	8.62	8.68	Suggestive
2	WT12	2	60cM	40.6 ± 0.08 kg	7.57	8.58	Suggestive
3	SHOUWD	2	16cM	4.55 ± 1.25 cm	13.20	11.78	P<0.05
3	HIPWD	2	21cM	3.45 ± 0.98 cm	12.29	12.45	Suggestive
3	THURLWD	2	14cM	3.17 ± 0.94 cm	11.32	11.44	Suggestive
3	**PINBWD**	**2**	**16cM**	**6.07 ± 1.01 cm**	**35.87**	**23.87**	**P<0.01**
4	POSTWADG	2	68cM	-0.26 ± 0.04 kg/day	37.44	31.57	P<0.05
4	POSTWADG	2	24cM	17.40 ± 2.74 cm	40.36	37.81	P<0.05
4	PINBWD	2	19cM	9.08 ± 1.17 cm	60.34	30.67	P<0.05
4	HEARTGT	2	26cM	53.39 ± 6.53 cm	66.71	44.60	P<0.05
4	ABDWD	2	69cM	-5.26 ± 0.79 cm	44.07	40.06	P<0.05
3	BWT	5	69cM	4.74 ± 1.59 kg	8.83	11.11	Suggestive
3	PINBWD	5	108cM	2.34 ± 0.81 cm	8.24	11.77	Suggestive
3	HEARTGT	5	117cM	4.27 ± 1.46 cm	8.49	11.66	Suggestive

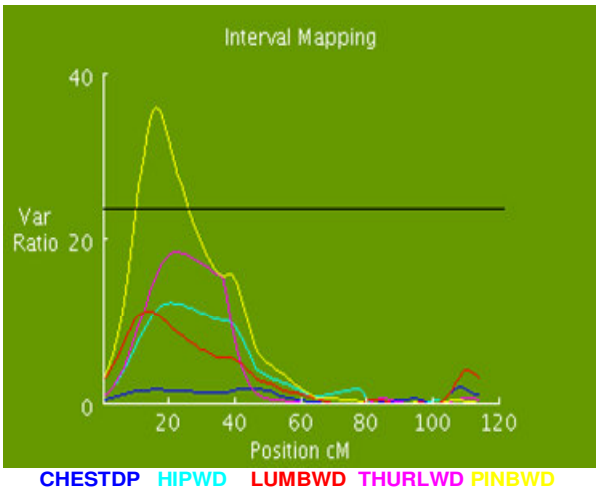

Figure 2. BTA2 map of F-statistics in Sire Family 3 depicting highly significant (P<0.01) QTL for pin bone width (PINBWD) at 16cM. Bold line represents the chromosome-wide F-threshold.

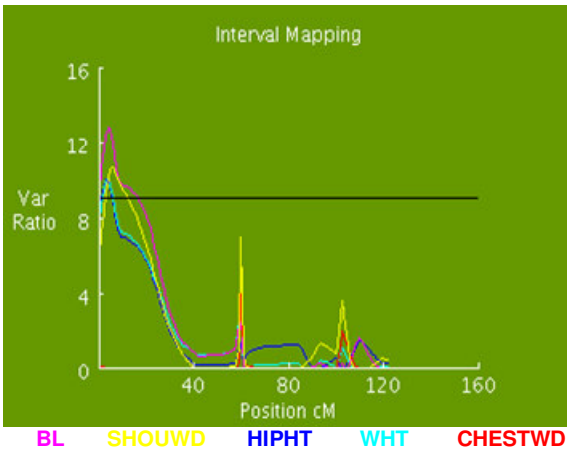

Figure 3. BTA2 map of F-statistics in Sire Family 1 depicting significant (P<0.05) QTL for withers height (WHT) at 3cM, hip height (HIPHT) at 4cM, body length (BL) at 4cM and shoulder width (SHOUWD) at 6cM. Bold line represents the chromosome-wide F-threshold.

quirements for beef quality in Japan (Sasaki and Mitsumoto, 2004) indicated the need for beef cattle breeders to meet the specifications and increasing demands of Japa-

nese consumers. In order to meet some of these challenges, we investigated the relationship between body measurements and body weight (Malau-Aduli et al., 2004a) and correlations between mitochondrial DNA polymerphism, maternal lineage and postnatal growth to yearling age (Malau-Aduli et al., 2004b) of Japanese black cattle. Animal improvement has been achieved by selection based on either phenotype or predicted additive genetic merit of superior animals for production traits. Molecular biology techniques allow the identification of genetic variation at specific loci and the association between QTL and production traits. The final goal is to use marker assisted selection to improve the genetic gain achieved by selection as a result of higher accuracy on the estimation of an animal's genetic value (Tambasco et al., 2003). The phenomenon of genetic linkage means that each marker can be used to follow the inheritance of a section of the linked chromosome. However, markers have to be very closely linked to the causative mutation in the trait gene if they are to remain associated with specific QTL alleles through several generations of selection and therefore be useful in practical breeding programmes. If a genetic marker and a trait are significantly linked, there is a tendency for such associations to be maintained at a population level. This phenomenon of linkage disequilibrium could be exploited to locate the trait genes using single nucleotide polymorphism (SNPs) that is where two DNA sequences differ by a single base. Napolitano et al. (1996) reported the lo-calization of three microsatellites IDVGA-2, IDVGA-3 and IDVGA-46 on bovine chromosomes 2, 11 and 19 respectively, and their association with beef performance traits in F_1 Piemontese x Chianina

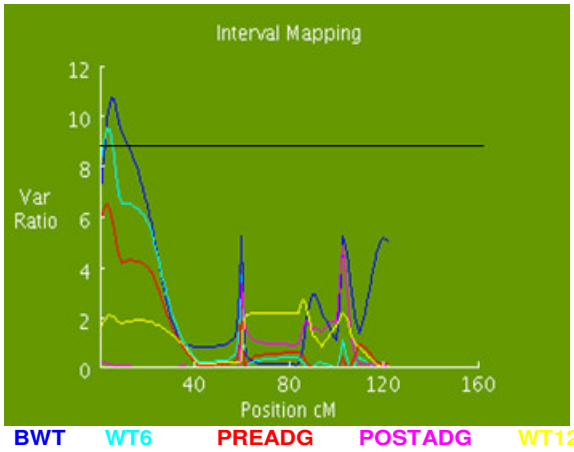

Figure 4. BTA2 map of F-statistics in Sire Family 1 depicting significant (P<0.05) QTL for live weight at birth (BWT) at 5cM and liveweight at 6 months of age (WT6) at 3cM. Bold line represents the chromosome-wide F-threshold.

crossbred cows. Of the three microsatellites, IDVGA-46 was reported to be the best marker for most beef conformation traits in this crossbred population and that animals homozygous for allele 205 gave the best results in terms of linkage with segregating QTL for beef conformation (Napolitano et al., 2001). Their study examined only 7 body conformation measurements–Withers height, body length, chest width, chest depth, chest girth, rump length and pelvis width. In our present study, we examined 15 body conformation measurements and detected highly significant QTL on chromosome 2 for hip width and chest depth located at 1 cM and 8 cM respectively (Sire Family 1) and for pin bone width at 16cM in Sire Family 3. The implication is that the bracketing microsatellite markers TGLA44 and TGLA431 flanking this interval in Sire Family 1 and BM3627 and BMS803 in Sire Family 3 can be used in marker-assisted selection to introduce or retain the beneficial QTL allele. Our findings in this study clearly demonstrated that the chromosomal interval 1 - 6cM on BTA2 harboured significant QTL (P<0.05) for birth weight, weaning weight, withers height, hip height, body length, shoulder width, lumbar width, thurl width and canon circumference. In other breeds of cattle, Casas et al. (1998) reported that a locus near the centromere of bovine chromosome 2 was responsible for muscle hypertrophy in two half-sib families of Belgian Blue x MARC III and Piedmontese x Angus and confirmed the location to be 4 cM with a 95% confidence interval between 2 – 6 cM. This interval has been recognised as one harbouring the myostatin gene (Casas et al., 2000). Bovine chromosome 2 has also been shown to harbour QTL significantly linked to carcass quality, for instance, MacNeil and Grosz (2002) detected a significant QTL for marbling in Hereford x Composite Double backcross cattle located at 122 cM with a 95% confidence interval from 112–132 cM between the microsatellite markers IDVGA-2 and FCB11. Grosz and Macneil (2001) using this same population, had earlier reported a significant QTL for birth weight on chromosome 2 located at 114 cM in the interval between BM2113 and OarFCB11 microsatellite markers. In a half-sib family of Brahman x Hereford cattle, Casas et al. (2003) reported the detection of putative QTL for birth weight on bovine chromosomes 1, 2 and 3 Kim et al. (2003) also detected a signify-cant QTL for birth weight on bovine chromosome 2 in a cross-bred population of Angus x Brahman. More recently, Li et al. (2004a) identified and fine-mapped QTL for backfat on bovine chromosomes 2, 5, 6, 19, 21 and 23 in a commercial line of Beefbooster cattle. Our detection of significant QTL for birth weight on bovine chromosome 2 in this study is in agreement with these reports.

Even though there was suggestive evidence in our study that chromosome 5 harboured QTL for birth weight (69 cM), pin bone width (108 cM) and heart girth (117 cM), these were not significant as the F-statistics were below the chromosome-wide threshold. However, published literature has demonstrated that bovine chromosome 5 harboured significant QTL for preweaning average daily gain at 55 – 70 cM, average daily gain at 70 – 80 cM and birth

weight at 0 – 30 cM (Li et al., 2002), fat depth (40 – 80 cM), yield grade and retail product yield at 62 – 72 cM (Casas et al., 2000), birth weight at 49 cM (Kim et al., 2003), carcass yield at 45 -54 cM (Mizoshita et al. 2004) and backfat at 65.4 – 70 cM (Li et al., 2004a, 2004b). Other earlier studies in cattle that had detected QTL on chromosome 5 include those of Davis et al. 1998 (birth weight located at 90 cM) and Stone et al. (1999) who reported that the interval 50 – 80 cM harboured QTL for rib bone and dressing percentage. Some of the above cattle herds were purebreds, others were crossbreds and different population sizes, sire families and estimation procedures were utilised, hence an expected variation in results.

There were significant differences between the sire families in birth weight (BWT), postweaning average daily gain (POSTWADG) and chest girth (CHESTGTH) in which Families 1 and 2 were higher than in Families 3, 4 and 5. This was not entirely surprising because Sires 1 and 2 had been selected for average daily gain (daily gain line) while Sires 3, 4 and 5 belonged to the beef marbling score (BMS) line. Chest girth is an important body conformation measurement that has been reported in Japanese black cattle. For instance, Mukai et al. (1995) studied the genetic relationships between body measurements, growth and field carcass performance traits and reported highly significant and positive genetic correlations between chest girth and carcass weight at the beginning, middle and end of performance testing of 0.64, 0.77 and 0.79 respectively. They concluded that it was possible to improve total merit of the carcass by introducing chest girth into performance testing of Japanese black cattle. Other studies (Oyama et al., 1996; Kitamura et al., 1999) on genetic relationships among recorded body measurement traits, reproductive traits of breeding females and carcass traits in Japanese black cattle buttress the finding of Mukai et al. (1995) that there is an unfavourable or low correlation between chest girth and beef marbling score (-0.07, 0.28 and 0.21 at the beginning, middle and end of performance testing respectively). It is this low correlation that has been ob-served in this present study with the BMS line families having lower chest girth measurements than the daily gain line families. Other body conformation measurements like chest depth, thurl width and withers height were also found to be genetically correlated with field carcass weight ranging from 0.64 to 0.90 (Mukai et al. 1995, 2000), indicating that body conformation measurements can be valuable in selection for meat quality as well. Data from our group (Malau-Aduli et al., 2004a, 2004b) portray a significant and positive relationship between body conformation measurements and average daily gain to weaning and yearling age. Thus, the identification of significant QTL in the present study holds hope for the utilization of markers closely linked to these traits for the implementtation of marker-assisted selection for growth and carcass traits. We hypothesise that single nucleotide polymerphism (SNP) of the myostatin gene, which acts as a negative regulator of muscle growth, could possibly account for the segregating QTL influencing these growth and body measurement traits detected in our study. Sequencing to find SNPs in four regions (promoter, exon 1, exon 2 and exon 3) of the myostatin gene in these sires is now in progress in our laboratory. We conclude that the detection of these QTL boosts the prospects of implementing marker-assisted selection for live weight, average daily gain and body measurement traits in Japa-nese black cattle. Furthermore, this finding could pave the way for positional cloning using candidate genes in Japa-nese black cattle.

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