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A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition

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Abstract. Molecular genetic markers can be used to identify chromosomal regions that contain quantitative trait loci (QTL) that control meat quality and muscle composition traits in farm animals. To study this in pigs, a resource family was generated from a cross between two Berkshire grand sires and nine Yorkshire grand dams. A total of 525 F_2 progeny from 65 matings of F_1 parents were produced. Phenotypic data on 28 meat quality traits (drip loss, water holding capacity, firmness, color, marbling, percentage cholesterol, ultimate pH, fiber type, and several sensory panel and cooking traits) were collected on the F_2 animals. Animals were genotyped for 125 microsatellite markers covering the entire genome. Least squares regression interval mapping was used for QTL detection. Significance thresholds were determined by permutation tests. A total of 60 QTL were detected at the 5% chromosome level for meat quality traits, on Chrs 1, 2, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 17, 18, and X, of which 9 and 1 QTL were significant at the 5% and 1% genome-wise levels (on Chrs 1, 5, 12, 15, and 17), respectively.

Introduction

Pork quality comprises a set of key fresh meat quality, processing, and sensory characteristics that are important for the future profitability and competitiveness of the swine industry. These include intramuscular fat, cholesterol, ultimate pH, color, water-holding capacity or drip loss, tenderness, cooking loss, and sensory traits involving taste (Sellier 1998). In the past, leanness was considered one of the most important traits. As a result, dramatic improvements in the body composition of pigs have been made. However, it has been shown that lean meat is not always associated with good meat quality (Cameron 1990; Hovenier et al. 1992), and therefore several other traits must be considered to improve pork quality. Improving meat quality genetically is difficult by standard selection methods, but possible if the genes responsible for meat quality are identified and mapped.

A limited number of studies have attempted to map QTL for meat quality traits, but they have generally involved a cross with at least one exotic breed (Andersson-Eklund et al. 1998; Milan et al. 1998; Wang et al. 1998; Moser et al. 1998; Yu et al. 1999; De Koning et al. 2000a, 2000b). These studies have reported the existence of QTL for meat quality traits on almost all chromosomes except 10, 17, and 18. These QTL must be confirmed in other crosses, in particular those involving breeds that are of commercial (economic) interest.

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The Berkshire and Yorkshire are breeds of commercial interest that have demonstrated considerable differences in meat quality, with Berkshire pigs having very positive meat quality traits (Goodwin and Burroughs 1995). In order to identify the chromosomal regions and genes responsible for differences in meat quality traits in these breeds, a three-generation resource family was developed. Malek et al. (2001) reported the identification of several QTL for growth and body composition traits in this population, and Huff-Lonergan et al. (2001) described relationships among meat quality traits. The objectives of this study were to analyze this resource family for QTL for muscle and meat quality traits. This study represents the first genome-wise QTL scan for meat quality traits with both of these commercial breeds.

Materials and methods

Family structure and management. A three-generation resource family was developed by using two purebred Berkshire grand sires (Casino and Count) and nine Yorkshire grand dams. Details on the family structure and management of the pigs are in Malek et al. (2001).

Traits measured. Phenotypic data for a total of 28 meat quality traits were collected on the F_2 animals. Traits measured are listed in Table 1. Measurements were taken primarily at two locations: at the Hormel slaughter plant in Austin, Minnesota at 24 h after slaughter, and at the Iowa State University Meat Laboratory in Ames 48 h after slaughter. All measurements were taken by trained personnel following the guidelines of the National Pork Producers Council (NPPC 1991).

Carcass traits evaluated at the slaughter plant after slaughter and chilling included the subjective quality traits of marbling, firmness, and color in the loin. Subjective traits were scored on a scale from 1 to 5, with higher values indicating greater marbling, greater firmness, and darker color. Objective measurements of color were taken with a Minolta chromometer and a Hunter lab scan. Minolta and Hunter L values measure light reflectance of the muscle. Lower values indicate darker color, which is desirable, and higher values indicate paler, lighter-colored meat. Muscle pH was measured in the longissimus dorsi and the semimembranosus muscles at 24 h after slaughter, using a glass penetration pH electrode. Measurement of Minolta and Hunter L values and pH was repeated at 48 h postmortem in the Ames laboratory.

Two measures of the ability of the muscle to retain moisture, drip loss, and water-holding capacity were taken. Drip loss measures the amount of moisture (purge) lost from the product over a period of time. Water-holding capacity is a complementary measure of the ability of meat to retain water. Drip loss was measured on a size-standardized sample from the longissimus dorsi (3 cm in diameter and 2.5 cm thick; Honikel et al. 1986; Kauffman et al. 1986b) that was collected at 48 h postmortem. The sample was weighed, suspended in a plastic bag, held at 4°C for 72 h, and re-weighed at the end of the holding time. Drip loss was calculated as the percentage of product weight that was lost over the 72-h storage period. This was done with duplicate samples, and the average value was used for analysis. Water-holding capacity was measured by using the filter paper press method (Kauffman et al. 1986a), which evaluates the amount of moisture lost from

Table 1. Means and standard deviations for traits of interest measured on 525 F_2 animals and expected differences between breed means (Berkshire minus Yorkshire)[a].

Trait (Score Range)	Score Interpretation		N	Mean	Std Dev	Berk—York[a]
	Low Value	High Value				
Subjective carcass evaluations						
Color score (1–5)	Pale	Dark	525	3.25	0.48	0.2
Marbling (1–5)	Low	High	525	3.80	0.73	0.6
Firmness (1–5)	Soft	Firm	525	3.42	0.63	0.4
Light reflectance						
Hormel Ham Minolta						
(24-h Semimembranosus Minolta L values)	Dark	Pale	525	17.47	2.90	NA[b]
Hormel Ham Hunter						
(24-h Semimembranosus Hunter L values)	Dark	Pale	525	41.65	3.46	NA
Hormel Loin Minolta						
(24-h loin Minolta L values)	Dark	Pale	525	21.09	5.20	−0.8
Hormel Loin Hunter						
(24-h Hunter L values)	Dark	Pale	525	44.07	6.12	−0.8
Lab Loin Minolta						
(48-h loin Minolta L values)	Dark	Pale	525	22.07	3.24	0.0
Lab Loin Hunter						
(48-h Hunter L values)	Dark	Pale	525	46.87	3.39	−0.6
Muscle pH						
Hormel Ham pH						
(24 h)	Pale	Dark	525	5.89	0.22	NA
Hormel Loin pH						
(24 h)	Pale	Dark	525	5.78	0.17	NA
Lab Loin pH						
(48 h)	Pale	Dark	525	5.83	0.19	0.14
Tissue quality and water-Holding capacity						
Drip loss (%)	Low loss	High loss	525	5.84	1.99	−0.84
Water-holding capacity (g)	Low loss	High loss	525	0.21	0.137	−0.014
Fiber type I %			513	0.08	0.131	NA
Fiber type II ratio			513	1.04	0.77	NA
Glycogen content of the loin						
Average glycogen (μmol/g)			519	8.68	3.34	NA
Average lactate (μmol/g)			519	86.67	13.30	NA
Average glycolytic potential (μmol/g)			518	104.00	16.31	NA
Fat content						
Total lipid (%)			525	3.23	1.32	0.16
Cholesterol (mg/100g)			525	57.72	8.29	0.6
Instrumental tenderness						
Average Instron (Star Probe) force (kg)	Tender	Tough	513	7.84	1.17	0.48
Cooking and sensory panel evaluation						
Percent cooking loss (%)			513	18.23	4.40	−2.0
Tenderness score (1–10)	Tough	Tender	488	4.36	0.86	−0.78
Juiciness score (1–10)	Dry	Juicy	513	6.02	1.49	0.0
Chewiness score (1–10)	Soft	Tough	513	2.42	0.93	−0.32
Flavor score (1–10)	Little flavor	Intense flavor	513	2.85	1.76	0.0
Off-flavor score (1–10)	No off flavor	High off flavor	513	1.59	2.03	0.0

[a] Expected difference between breeds means based on twice the difference observed in crossbreds in the NPPC genetic evaluation program (Goodwin and Burroughs 1995).
[b] NA: Not available.

the surface of the loin shortly after cutting. A pre-weighed piece of filter paper, which was exposed to the atmosphere for 10 min, was placed on a fresh cut of the loin muscle 48 h postmortem for 3 s to allow it to absorb surface moisture, and then re-weighed. The difference in weight was used as the measure of water-holding capacity (Kauffman et al. 1986b), with a lower value indicating that less moisture was lost from the tissue, which is more desirable.

At 48 h postmortem, a sub-sample of the loin was frozen and sent to the University of Illinois, where glycogen, free glucose, glucose-6-P, and lactate content were measured in μmol/g (Monin and Sellier 1985). Postmortem metabolism of elevated glycogen stores results in increased production of lactate, which is a pH-lowering by-product of muscle metabolism. Glycolytic potential is a measure of glycogen stores and was calculated as follows: glycolytic potential = 2 × ([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate] (Monin and Sellier 1985; Maribo et al. 1999). Glycolytic potential is expressed in μmol lactate equivalents per gram muscle wet weight. In addition to glycolytic potential and lactate concentration, residual glycogen concentration was used as a trait of interest in this study. Residual glycogen is the glycogen remaining in the muscle that was not converted to lactate and glucose-6-phosphate.

Total lipid in the longissimus dorsi was measured as described by Bligh and Dyer (1959) and expressed as a percentage of tissue weight. Total lipids were then dissolved in isopropanol and assayed for concentration of total cholesterol by using an enzymatic procedure (Sigma Cholesterol Kit No. 352, Sigma Chemical Co., St. Louis, Mo.). Cholesterol was reported in mg per 100 g of tissue.

Muscle fiber type composition was evaluated in 48-h postmortem samples from the longissimus dorsi by separation of myosin isoforms on high porosity SDS-PAGE gels. The procedure used was as described by Talmadge and Roy (1993), but with modifications as described by Huff-Lonergan et al. (2001). Results were expressed as the ratio of the density of the IIa band of myosin to the density of the IIb band within a sample. Porcine diaphragm muscle (extracted as described in Huff-Lonergan et al. 2001) was used as a standard on each gel to aid in identifying the myosin isoforms. Diaphragm muscle contains primarily type IIa, IIx, and type I associated myosin isoforms (Talmadge and Roy 1993).

To evaluate the sensory characteristics of the meat, vacuum-packaged boneless chops from the longissimus dorsi of each animal were taken 48 h after slaughter and stored for 10 days at 4°C. Following the storage period, chops were broiled to 71°C in an electric oven broiler (Amana Model ARE 60) that had been preheated to 210°C. The temperature of each chop was monitored in the center of the chop with thermocouples (Chromega/Alomega) attached to an Omega digital thermometer (Model DSS-650, Omega Engineering). Cooking loss was calculated from weights taken before and after broiling and was expressed as a percentage. Instrumental measurement of tenderness of the broiled chops was evaluated by using a circular five-pointed star-probe (9 mm in diameter with 6 mm between points) attached to an Instron Universal Testing Machine (Model 1122). A

100-kg load cell was used with a crosshead speed of 200 mm/min. The star-probe attachment was used to determine the amount of force needed to puncture and compress the chop to 80% of the sample height. Each chop was punctured three times, and the average was recorded.

Sensory evaluation of the broiled chops was done using three highly trained professional sensory panelists. Panelists were seated in individual booths with red lighting overhead to mask any differences in product color. Cubes, 1.3 cm in size, were removed from the center of the broiled loin chops, placed in preheated, individually coded glass petri dishes, and served to each panelist. Room temperature deionized, distilled water and unsalted crackers were used to cleanse the palates of the panelists between samples. Samples were evaluated for degree of juiciness, tenderness, chewiness, pork flavor, and off-flavor by using a 10-point category scale. The scale was anchored on the left end with a term representing a low degree of juiciness, tenderness, chewiness, flavor, and off-flavor intensity. On the right end of the scale was a term representing a high degree of each characteristic. Any flavor that was not associated with normal pork flavor was considered as an off-flavor. The values for each pork chop were averaged across the three panelists.

DNA isolation, marker selection, and genotyping. Usual methods were used to collect samples and isolate DNA. All animals were genotyped for 125 markers as described by Malek et al. (2001). Of the original 525 F_2 animals, likely parentage (or collection) problems existed for 13 F_2 animals, and these were removed, leaving 512 animals for analysis.

QTL analyses. Marker linkage maps and the QTL analyses used are explained in Malek et al. (2001). Significance levels at the 5% chromosome-wise and at the 5% and 1% genome-wise levels were determined by permutation as described by Malek et al. (2001).

Results and Discussion

Arithmetic means and standard deviations measured for each trait on the F_2 animals are listed in Table 1. Measurements were not available for all traits on all animals owing to occasional sampling problems. Results conformed to the usual range of measurement scores. Relationships among traits are described in Huff-Lonergan et al. (2001). Expected differences between the Berkshire and Yorkshire breeds for the traits evaluated are also in Table 1. Breed differences are based on crossbred results from the National Pork Producer Council's Genetic Evaluation Program (Goodwin and Burroughs 1995).

QTL results. Results for QTL that were detected at the 5% chromosome-wise level are in Table 2 and summarized by trait in Table 3. For the pig genome, the 5% chromosome-wise significance level roughly corresponds to the genome-wise suggestive level (Lander and Kruglyak 1995; De Koning et al. 1999). The QTL graphs for chromosomes with evidence for QTL at the 5% and 1% genome-wise levels are presented in Fig. 1. To avoid double counting, cases where evidence for QTL extended over multiple adjacent marker intervals were reported as a single QTL in Tables 2 and 3. Further dissection of these QTL will require additional statistical analyses.

In total, 60 QTL were detected at the 5% chromosome-wise level for the 28 traits evaluated (Table 2), of which 9 were significant at the 5% genome-wise level. One QTL, for Hormel Loin pH on Chr 15 (Fig. 1G), was significant at the 1% genome-wise level. Thus, substantially more QTL were detected for the 28 traits evaluated than the 28, 1.4, and 0.3 QTL that would be expected at the suggestive, 5% and 1% genome-wise levels by chance alone. Significant QTL were detected for nearly all traits and on all chromosomes, except on Chr 3, 9, and 16. Most QTL accounted for 2%–5% of the F_2 variance, but one reached 10% (color score on SSC 12). As noted by Malek et al. (2001), variance accounted by individual QTL (Table 2) may be overestimated. However, total variances explained by QTL by trait, as reported in Table 3, may be underestimated because the existence of multiple QTL in adjacent marker intervals was ignored. Both breeds had favorable QTL alleles on separate chromosomes, despite Berkshires having more desirable meat quality breed characteristics for most traits (Table 1). In the following presentation, QTL detected will be discussed on a trait basis and related to literature findings.

Color and light reflectance. Color is one of the most important visual parameters for meat quality. Color determines initial acceptance or rejection in the marketplace. Lighter colored pork is often associated with more drip loss, poorer water holding capacity, and lower pH. Huff-Lonergan et al. (2001) also found significant negative correlations of ultimate pH with Hunter L values and drip loss in our F_2 population.

Three QTL were found for subjective color in this study (Table 3), of which two were significant at the 5% genome-wise level (Fig. 1E and I). In total, 19 QTL were detected for the subjective and objective measurements related to color, of which 4 were significant at the 5% genome-wise level (Tables 2 and 3). Because of the relationships between these traits, several QTL likely represent a single QTL with pleiotropic effects.

There was suggestive evidence of QTL for four color traits, including subjective color, within a 60 cM region of SSC2 (Table 2). Two and three objective reflectance traits, respectively, showed QTL at the same positions on SSC4 and SSC5. A QTL for subjective color was detected on SSC12 at the 5% genome-wise level, but this QTL was not supported by QTL for reflectance traits. The phenotypic correlation of subjective color with Lab Loin Hunter was −0.7 in this population (Huff-Lonergan et al. 2001); thus, this could represent a QTL for subjective color that is not pleiotropic for reflectance traits. Chr 15 showed QTL for three reflectance traits within a 30-cM region. Chr 17 had the greatest evidence for QTL, with significance at the 5% genome-wise level at the same position for three traits, including subjective color.

For two of the three QTL for subjective color, Berkshire alleles were associated with lighter colored meat than were Yorkshire alleles (Table 2). Berkshire alleles were associated with better (lower) reflectance scores on SSC 14, 15, and 17, but with higher reflectance on SSC 2, 4, 5, 7, and 18.

Andersson-Eklund et al. (1998) found some evidence that the proportion of Wild Boar alleles on Chr 2, 10, 12, and 15 was associated with QTL affecting meat color in a cross between the Wild Boar and Large White breeds, although no QTL reached genome-wise significance. Wang et al. (1998) reported suggestive QTL on SSC 4 and SSC 7 affecting color. These QTL were significant in individual Chinese by Western breed crosses, but not pooled over all crosses evaluated in their study. Jeon et al. (1999) reported a paternally inherited QTL for reflectance on SSC 2, but at the beginning of the chromosome, near IGF-2, not at the distal end as in our study. We did, however, not test for imprinted QTL. De Koning et al. (2000a) found a total of nine suggestive and three significant QTL at the genome-wise level for various measures of reflectance: five QTL, on Chr 1, 3, 4, 13, and 14 affecting Color-L (lightness, which is the same as Hunter or Minolta of our study). In addition, they found four QTL affecting Color-A (green to redness) on Chr 3, 13, 14, and 15, and three QTL affecting Color-B (blue to yellowness), on Chr 4, 13, and 14. They reported that the QTL for color found by Wang et al. (1998) on SSC 4 was at approximately the same position as a significant QTL affecting Color-B in their study. For Color-L, which was the only trait in common with our study, our QTL on SSC 14 for Hormel Ham Hunter was on a different region of the chromosome than the QTL found by De Koning et al (2000a).

Tissue quality and water-holding capacity. Water-holding capacity and drip loss measure the ability of the muscle to retain moisture. Less moisture loss prior to cooking is also often associated with better color, greater firmness and higher pH, which was sub-

Table 2. Evidence for QTL significant at the 5% chromosome-wise level for various meat quality traits by chromosome. Estimated significance levels (F value), location, gene effects, and % of F_2 variance explained by each QTL.

SSC	Trait	F-value[a]	Location (cM)	Additive Effect[b]	S.E.	Dominance Effect	S.E.	% Variance[c]
1	Marbling	8.42*	48	−0.16	0.04	0.16	0.07	4.34
1	Total lipid (%)	6.06	51	−0.28	0.08	0.13	0.13	2.90
1	Drip loss (%)	7.15	90	−0.53	0.14	−0.13	0.27	4.66
2	Color score	5.33	141	−0.10	0.03	0.04	0.05	2.41
2	Hormel Loin Minolta	5.90	77	0.91	0.31	−0.94	0.48	3.83
2	Lab Loin Minolta	7.24	127	0.83	0.22	0.19	0.38	3.94
2	Lab Loin Hunter	6.33	128	0.80	0.23	0.18	0.39	3.39
2	Drip loss (%)	5.68	122	0.43	0.13	−0.26	0.23	3.53
2	Drip loss (%)	5.07	40	0.44	0.14	0.12	0.27	3.22
2	Water-holding capacity (g)	5.85	139	0.03	0.01	−0.01	0.01	2.67
2	Water-holding capacity (g)	5.90	71	0.03	0.01	0.01	0.01	2.94
2	Chewiness score	6.76	143	0.20	0.06	0.07	0.08	2.62
2	Tenderness score	7.99	143	−0.26	0.07	−0.15	0.11	3.08
2	Firmness	5.31	86	−0.11	0.04	−0.09	0.06	2.39
2	Flavor score	5.93	143	−0.35	0.10	0.03	0.15	2.45
2	Off-flavor score	5.84	45	0.50	0.15	0.03	0.30	4.18
2	Off-flavor score	5.17	143	0.35	0.11	0.07	0.16	2.08
4	Lab Loin Hunter	6.15	130	0.54	0.20	0.67	0.29	2.66
4	Lab Loin Minolta	6.01	130	0.55	0.19	0.56	0.28	2.58
5	Hormel Loin Minolta	6.88	112	0.64	0.30	−1.38	0.46	4.11
5	Hormel Loin pH	8.56*	113	−0.03	0.01	0.05	0.02	4.85
5	Lab Loin Hunter	7.41	113	0.48	0.22	−1.09	0.34	4.30
5	Lab Loin Minolta	7.95	113	0.49	0.21	−1.07	0.33	4.59
5	Lab Loin pH	6.20	81	−0.04	0.01	0.01	0.17	3.38
6	Hormel Ham pH	6.82	53	−0.03	0.01	0.05	0.02	2.90
7	Lab Loin Hunter	5.83	80	0.52	0.21	−0.79	0.33	3.00
8	Fiber type I	5.97	52	−0.03	0.01	−0.01	0.01	2.88
8	Marbling	5.92	40	−0.14	0.05	0.16	0.08	3.61
10	Star Probe Force (kg)	5.83	71	−0.20	0.06	−0.06	0.10	3.82
10	Marbling	5.11	3	−0.14	0.05	−0.13	0.10	3.24
11	Drip loss (%)	5.95	7	0.44	0.13	0.59	0.23	6.01
11	Glycogen (μmol/g)	4.73	0	0.65	0.21	−0.12	0.34	2.33
11	Glycolytic potential (μmol/g)	5.91	0	3.36	1.03	−1.54	1.66	2.68
12	Chewiness score	5.13	73	0.10	0.08	0.43	0.14	6.63
12	Color score	8.33*	73	−0.14	0.04	−0.22	0.07	10.13
13	Water-holding capacity (g)	6.14	43	0.03	0.01	0.09	0.01	2.75
14	Hormel Ham Hunter	5.16	0	−0.21	0.22	−1.06	0.35	3.19
14	Hormel Ham pH	5.79	110	−0.05	0.02	0.01	0.03	3.59
14	Percent cooking loss (%)	7.14	31	−1.03	0.28	−0.40	0.47	3.29
14	Tenderness score	5.77	70	0.28	0.08	0.23	0.11	2.83
15	Hormel Loin Hunter	6.31	96	−1.07	0.32	0.62	0.50	3.16
15	Lab Loin Hunter	5.04	66	−0.68	0.22	0.17	0.33	2.46
15	Lab Loin Minolta	6.30	66	−0.73	0.21	0.17	0.31	3.05
15	Hormel Ham pH	8.42*	72	0.05	0.01	−0.02	0.02	4.00
15	Hormel Loin pH	12.15**	76	0.05	0.01	−0.01	0.02	5.61
15	Lab Loin pH	9.05*	45	0.04	0.01	−0.04	0.02	5.14
15	Glycogen (μmol/g)	8.25*	65	−0.77	0.22	0.71	0.34	4.27
15	Glycolytic potential (μmol/g)	6.21	67	−3.67	1.05	0.77	1.59	2.95
15	Tenderness score	5.22	44	0.24	0.08	−0.20	0.14	3.00
15	Star Probe Force (kg)	5.25	42	−0.17	0.05	0.09	0.09	2.88
15	Flavor score	6.41	91	0.36	0.11	−0.37	0.18	3.73
17	Color score	8.75*	82	0.11	0.03	−0.09	0.04	3.63
17	Lab Loin Hunter	9.11*	82	−0.83	0.20	0.22	0.29	3.73
17	Lab Loin Minolta	9.91*	82	−0.83	0.19	0.25	0.28	4.04
17	Lactate (μmol/g)	6.40	82	−1.48	0.77	3.37	1.11	2.80
17	Glycolytic potential (μmol/g)	5.01	82	−1.47	0.10	4.05	1.44	2.22
17	Juiciness score	6.36	30	0.23	0.12	−0.70	0.24	8.03
18	Hormel Loin Minolta	6.40	26	0.12	0.29	−1.58	0.45	3.82
18	Cholesterol (mg/100g)	4.67	26	−0.15	0.56	2.60	0.86	2.62
X	Off-flavor score	4.90	69	−0.58	0.19	−0.12	0.20	5.78

[a] Chromosome-wise F-statistic thresholds at the 5% level, as determined by permutation test were as follows: (1) 5.08, (2) 5.12, (3) 5.14, (4) 5.14, (5) 4.99, (6) 5.32, (7) 5.25, (8) 5.03, (9) 5.09, (10) 5.11, (11) 4.59, (12) 4.78, (13) 5.03, (14) 5.02, (15) 5.02, (16) 4.34, (17) 4.86, (18) 4.45, (X) 4.80.

[b] Additive (a) and dominance (d) QTL effects correspond to genotype values of +a, d, and −a for, respectively, individuals having inherited two Berkshire alleles, heterozygotes, and individuals with two Yorkshire alleles. Positive additive effects indicate that Berkshire alleles increased the trait, negative that the Berkshire alleles decreased it. Dominance effects are relative to the mean of the two homozygotes.

[c] % variance = genetic variance at the QTL based on estimated additive and dominance effects and allele frequencies of ½, as a percentage of the residual variance in the F_2.

* Significant at the 5% genome-wise level (F > 8.22).

** Significant at the 1% genome-wise level (F > 9.96).

stantiated by moderately high (0.2 to 0.3) correlations observed in our data (Huff-Lonergan et al. 2001).

Our results found seven significant QTL at the 5% chromosome-wise significance level for water-holding capacity and drip loss, on Chr 1, 2, 11, and 13 (Table 2). Multiple peaks were found for drip loss and water-holding capacity on SSC 2 (Fig. 1B) and for drip loss on SSC 11 (not shown). These peaks may be due to multiple QTL on those chromosomes. Yorkshire alleles were associated with more desirable quality for all QTL related to moisture loss, except for the QTL for drip loss on SSC 1. Based on breed means (Table 1), Yorkshires are expected to have more drip loss.

Andersson-Eklund et al. (1998) reported QTL on Chr 1, 2, and 12 affecting drip loss and on Chr 12, 13, and 18 for water-holding

Table 3. Summary of QTL significant at the 5% chromosome-wise level (%5 chr), the 5% genome-wise level (%5 gen) (F > 8.22) and the 1% genome-wise level (%1 gen) (F > 9.96) by trait.

Trait	# of Significant QTL			% of F_2 Variance Explained
	%5 chr	%5 gen	%1 gen	
Marbling	2	1		11.2
Total lipid %	1			2.9
Cholesterol concentration	1			2.6
Hormel Ham pH	2	1		10.5
Hormel Loin pH		1	1	10.5
Lab Loin pH	1	1		8.5
Hormel Loin Hunter score	1			3.2
Hormel Ham Hunter score	1			3.2
Lab Loin Hunter score	5	1		19.5
Hormel Loin Minolta	3			11.8
Lab Loin Minolta	4	1		18.2
Color score	1	2		16.2
Firmness	1			2.4
Water-holding capacity	3			8.4
Drip loss	4			17.4
Percent cooking loss	1			3.3
Juiciness score	1			8.0
Star Probe Force	2			6.7
Tenderness score	3			8.9
Chewiness score	2			9.3
Flavor score	2			6.2
Off-flavor score	3			12.0
Lactate	1			2.8
Glycogen	1	1		6.6
Glycolytic potential	3			7.9
Fiber type I	1			2.9

capacity, but none reached genome-wise significance. However, they found that the average proportion of wild boar alleles across the genome had highly significant effects on drip loss. De Koning et al. (2000a, 2000b) detected four QTL for drip loss, some with imprinted effects, on Chr 4, 6 (maternal), 14 (Mendelian), and 18 (paternal). We were not able to confirm the QTL found by Andersson-Eklund et al. (1998) and De Koning et al. (2000a, 2000b).

Firmness and fiber type could also be considered part of precooked tissue quality. Huff-Lonergan et al. (2001) found that fiber type II ratio was negatively correlated with Hunter L values and drip loss in our F_2 population, although the magnitude of the relationships was not high (−0.10). Our analyses found QTL for the subjective measure of firmness on SSC 2 and for fiber type on SSC 8. Andersson-Eklund et al. (1998) reported that the proportion of Wild Boar alleles on SSC 2 in their Wild Boar by Large White cross was associated with sarcoplasmic protein extractability, which may be associated with firmness. Milan et al. (1998) reported QTL for muscle fiber type differences on SSC 3, but this QTL was not confirmed in our study.

Fat content. Visual (subjective) marbling scores correspond to intramuscular lipid content, with a correlation of 0.57 in this F_2 population (Huff-Lonergan et al. 2001). Higher lipid content is generally considered more desirable as it adds to flavor and cooking properties and improves tenderness, although correlations between these traits were not very high in our data (<0.25; Huff-Lonergan et al. 2001).

Strongest evidence for a QTL for intramuscular fat was on SSC 1, which showed a QTL for marbling score at the 5% genome-wise level and a suggestive QTL for lipid percentage in the same region (Table 2, Fig. 1A). Berkshire alleles were associated with less intramuscular fat (unfavorable) for both QTL, which is opposite to expectations based on breed means (Table 1). Two additional QTL were found for marbling on Chr 8 and 10, both at the suggestive level (Table 2). Again, Yorkshire alleles were superior to Berkshire alleles. Only one QTL was found for cholesterol concentration (on SSC 18). This QTL was not related to QTL for lipid % or marbling.

De Koning et al. (2000a, 2000b) found six QTL for intramuscular fat, with different types of gene expression. These were on Chr 4, 6 (maternally inherited), 6 (paternal), 8 (sex specific), 13 (maternal), and the X chromosome. The *H-FABP* gene, a candidate gene associated with fat levels, is known to map to SSC 6 (Gerbens et al. 1997). Yu et al. (1999) also reported a suggestive QTL for marbling on SSC 13. We were not able to confirm any of these QTL in our cross, and our evidence for a QTL on SSC 1 was not corroborated by other studies.

Measures of pH. Ultimate pH is the most commonly used trait to assess pork quality and usually is measured at 24 and 48 h postmortem. Ultimate pH of pork is not a direct measure of quality, but it is correlated with the quality traits of color, drip loss, and water-holding capacity. Muscle pH postmortem is also correlated with sensory panel traits such as tenderness and juiciness. A higher level of acidity within the muscle (lower pH) causes muscle proteins to denature and lose their ability to hold water. Therefore, meat with higher pH will tend to have more desirable characteristics such as darker color, less drip loss, more firmness, and higher tenderness. In our data, correlations of ultimate pH with measures of water-holding capacity, color, and sensory quality were moderately high (0.15 to 0.35; Huff-Lonergan et al. 2001).

Seven QTL were detected for pH-related traits at the 5% chromosome-wise significance level (Table 3), of which three were significant at the 5% genome-wise level (on Chr 5 and 15) and one at the 1% genome-wise level (on SSC 15). Yorkshire alleles had higher (better) pH for the QTL on SSC 5, 6, and 14, but Berkshire alleles were better for the QTL on SSC 15.

Two QTL for pH were detected in SSC 5, both for pH in the loin but measured at different times (Table 2, Fig. 1D). The QTL for pH at 24 h was significant at the 5% genome-wise level and at the distal part of the chromosome, where also QTL were found for three reflectance traits. These likely represent the same QTL, for which Yorkshire alleles were desirable (higher pH and lower reflectance). Other studies have not detected QTL for pH or reflectance on SSC 5.

A suggestive QTL for 24-h pH in the ham was found on SSC 6 (Table 2). This QTL was near the *HAL* gene (Fujii et al. 1991), although the positive (detrimental) *HAL* allele was not present in our population. Geldermann et al. (1996) also demonstrated a QTL for pH on SSC 6 near the *HAL* gene but using *HAL*-positive pigs. Our results, however, suggest that some mutation other than the well-known detrimental allele might be present in *HAL* or another closely linked gene.

The suggestive QTL for 24-h pH in the ham on Chr 14 (Table 2) was in the same location as a paternally imprinted QTL that was found by De Koning et al (2000a, 2000b). Additional analyses are needed to determine whether our QTL is also subject to imprinting effects. De Koning et al. (2000a, 2000b) also found another QTL on SSC 14 affecting pH, which showed significant differences in estimated QTL effects between sexes. We were not able to confirm this QTL.

Strongest evidence for QTL for pH was on SSC 15 (Table 2, Fig. 1G), which showed QTL in the central and distal regions of the chromosome for three pH measures. These QTL were significant at the 5% genome-wise level for two traits and significant at the 1% genome-wise level for Hormel Loin pH. These QTL were in the same region as QTL for reflectance, glycolytic potential, and sensory traits, and will be discussed in greater detail in the following.

De Koning et al. (2000a, 2000b) also found QTL affecting pH on SSC 4, 9, 11, 18, and X, with a variety of modes of gene expression. We were not able to confirm these results.

Glycolytic potential. During the first 6–24 h postmortem, glycogen reserves in the muscle are reduced, lactic acid builds up, and

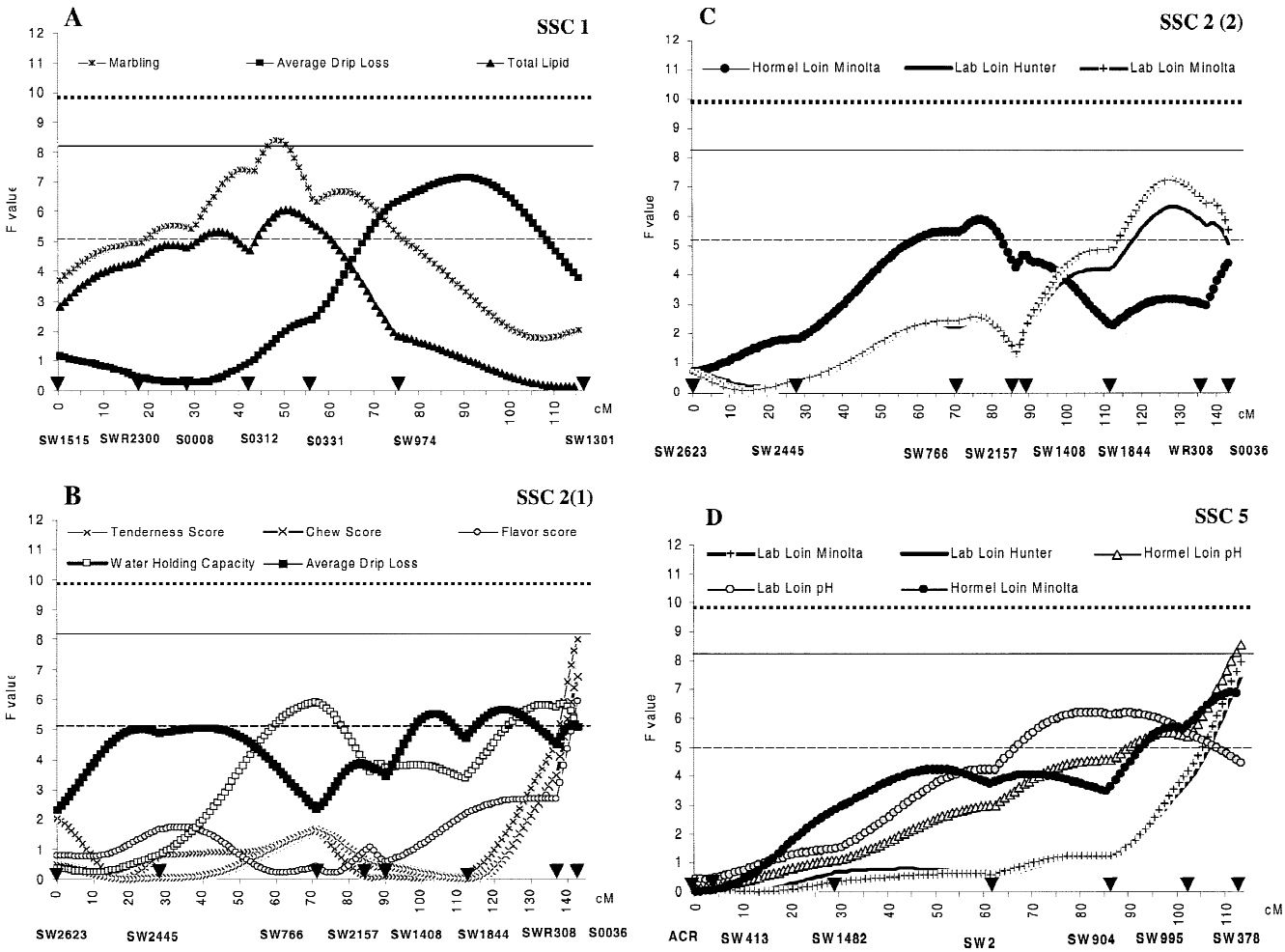

Fig. 1. F-ratio curves for evidence of QTL. The x-axis indicates the relative position on the linkage map. The y-axis represents the F-ratio. Arrows on the x-axis indicate the position where a marker was present. Three lines are provided for 5% chromosome-wise (-----), 5% genome-wise (——) and the 1% genome-wise (▪▪▪▪) significance. (*Continued on next page.*)

muscle metabolism stops (Lundberg and Vogel 1986). Lactate is a pH-lowering by-product of muscle metabolism. Greater amounts of glycogen in the tissue at harvest provide the potential for sustained glycolysis in the muscle after slaughter, which could result in lower ultimate pH. Glycolytic potential is a measure of the amount of energy stored in the muscle at harvest. Huff-Lonergan et al. (2001) showed that glycolytic potential had a significant positive correlation with Hunter L values (0.30) and drip loss (0.36) in our F_2 population, and was significantly negatively correlated with color (−0.30) and pH (−0.38). Lower glycolytic potential was associated with a more tender product, with a correlation of −0.31 (Huff-Lonergan et al. 2001).

Very high glycolytic potential values and significantly lower ultimate pH values have been observed in meat from pigs with the Rendement Napole (RN) or acid meat gene (Monin and Sellier 1985; Milan et al. 2000), which has major effects on meat quality. Based on segregation analysis of phenotypic data, LeRoy et al. (1990) first described the RN allele as being dominant. The unfavorable allele reduced water-holding capacity, yield of cured cooked ham, and pH, and resulted in lighter colored meat. The RN-defect has been identified only in Hampshire pigs and is associated with a 70% increase in muscle glycogen content (LeRoy et al. 1990). Recently, Milan et al. (2000) discovered the causative mutation of the RN-effect in the PRKAG3 gene on SSC15 (Protein kinase AMP activated-γ 3 subunit).

In our study, in total six QTL were found for glycogen and lactate content and for glycolytic potential, on Chr 11, 15, and 17 (Tables 2 and 3), of which one on Chr 15 for glycogen content, was significant at the 5% genome-wise level. On SSC 11, QTL were found for both glycogen content and glycolytic potential at the proximal end of the chromosome. These QTL were in the same region as a QTL for drip loss and likely represent the same locus. Yorkshire alleles were favored for these QTL, with lower glycogen and glycolytic potential and less drip loss. These QTL were not found in other studies and could be specific to these breeds.

Two QTL were detected at the same position on SSC 15, one for glycogen content, which was significant at the 5% genome-wise level, and one for glycolytic potential (Table 2, Fig. 1F). Several additional QTL for other meat quality traits were found to be located in the same region of the chromosome (Table 2, Figs. 1G and H). Further analysis is needed to determine whether these represent the same QTL. Berkshire alleles were superior to Yorkshire alleles for all QTL on SSC 15 (Table 3). Berkshire alleles had lower glycogen content, lower glycolytic potential, lower reflectance, higher pH, better tenderness, and better flavor. These results are consistent with trait correlations (Huff-Lonergan et al. 2001).

Milan et al. (2000) mapped the PRKAG3 (RN) gene between SW1683 and SW1983 (Figs. 1F, G and H), which is central to the QTL regions detected in our study. Further testing revealed, however, that the RN^- mutation found by Milan et al. (2000) is not present in our population. Additional mutations in this or closely linked genes may be present in our population.

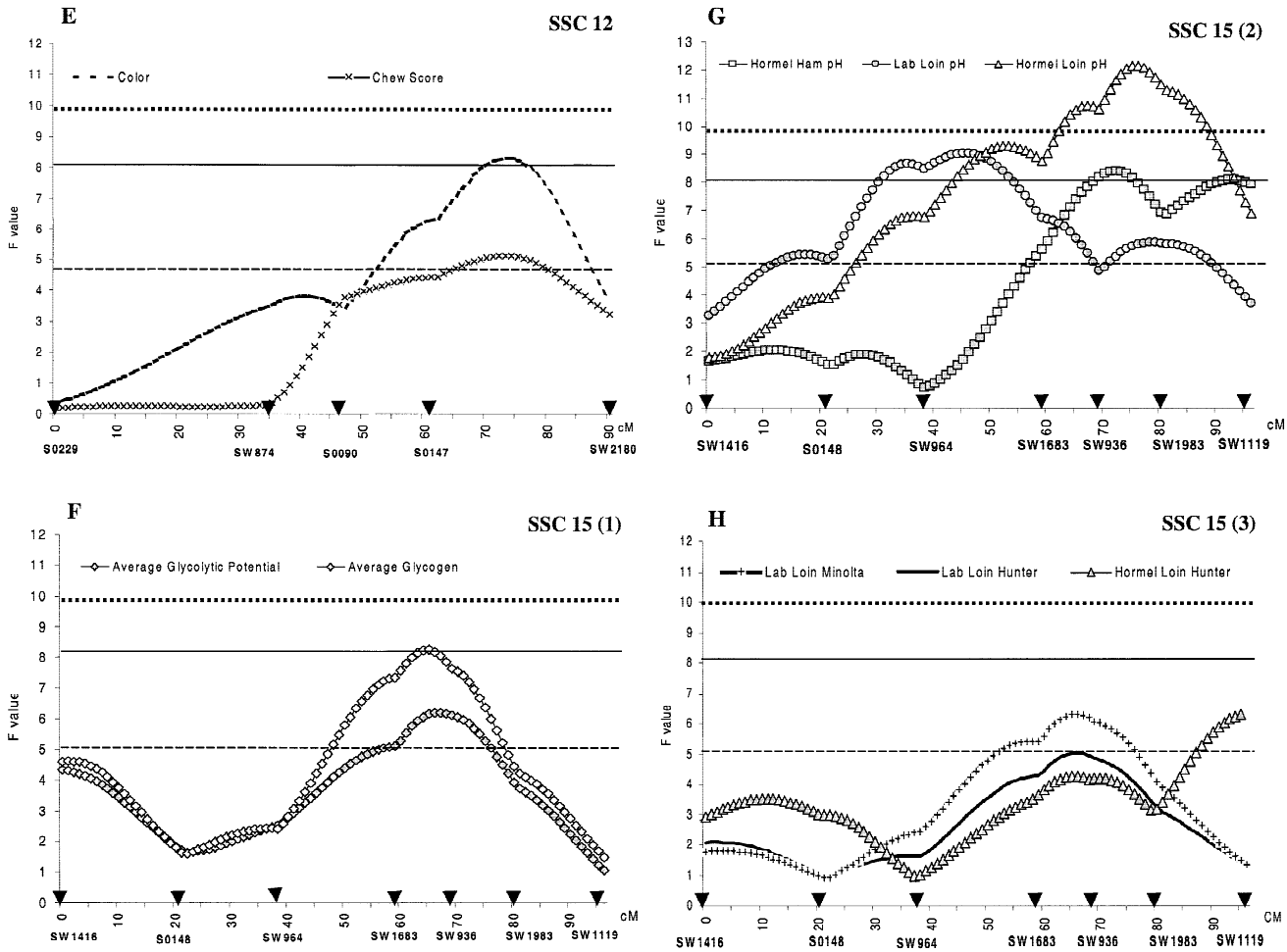

Fig. 1. *Continued.*

The final QTL for traits associated with glycogen metabolism were detected on SSC 17, with suggestive QTL for lactate content and glycolytic potential (Table 2, Fig. 1I). Both QTL were in the same region as the 5% genome-wise significant QTL for color and reflectance and likely represent the same QTL. Berkshire alleles were favored for the QTL in this region, with lower lactate content and glycolytic potential, and better color and lower reflectance. These QTL were not confirmed by other studies.

Cooking and sensory evaluation traits. Traits associated with sensory evaluation were measured both objectively, using star probe force as a measure of tenderness, and subjectively, using trained panelists. Huff-Lonergan et al. (2001) showed that lower average star probe force values were associated with better subjective tenderness scores (correlation −0.54), and tenderness of the product, whether measured objectively or subjectively, was moderately correlated with light reflectance in our F_2 population. Product that was darker in color (lower Hunter L value) was evaluated as being more tender (lower Star Probe values and higher sensory tenderness scores). Measures of tenderness were also moderately but favorably correlated with drip loss, cooking loss, and subjective evaluations of firmness and juiciness.

A total of nine suggestive QTL were detected for objective and subjective traits associated with tenderness, juiciness, and cooking loss. Chr 2 showed QTL for two subjective traits related to tenderness (Table 2, Fig. 1B), of which the QTL for tenderness approached 5% genome-wise significance at the distal part of the chromosome. A QTL for chewiness was detected in the same region on SSC2 as for firmness. Yorkshire alleles were associated with greater tenderness and less chewiness for these QTL.

Suggestive QTL for individual traits associated with tenderness were identified on Chrs 10 (star probe force), 12 (chewiness), 14 (cooking loss and tenderness), and 17 (juiciness) (Table 2). The QTL for chewiness on SSC 12 was in the same region as the 5% genome-wise significant QTL that was detected for color score.

Suggestive QTL for star probe force and subjective tenderness were detected at the same position on SSC 15 (Table 2). These QTL were in the central region of the chromosome, where QTL associated with pH and glycogen metabolism were also found. Berkshire alleles were associated with greater tenderness.

Except for the QTL on SSC 2, none of our QTL associated with tenderness could be confirmed based on literature results. Andersson-Eklund et al. (1998) found that the proportion of Wild Boar alleles on SSC 3 was associated with shear force, but we were not able to confirm this result.

Flavor is an important parameter for meat quality from a consumer perspective. Any flavor that can not be associated with normal pork flavor is considered off-flavor. Flavor and off-flavor scores had a substantial negative correlation in our data (−0.62; Huff-Lonergan et al. 2001). Better flavor scores tended to be associated with higher pH (correlations of 0.25 to 0.32), less glycolytic potential (−0.24), and greater lipid concentration (0.23, Huff-Lonergan et al. 2001). Opposite relationships held for off-flavor score.

We found three suggestive QTL for off-flavor score and two suggestive QTL for flavor score (Tables 2 and 3). Past studies did

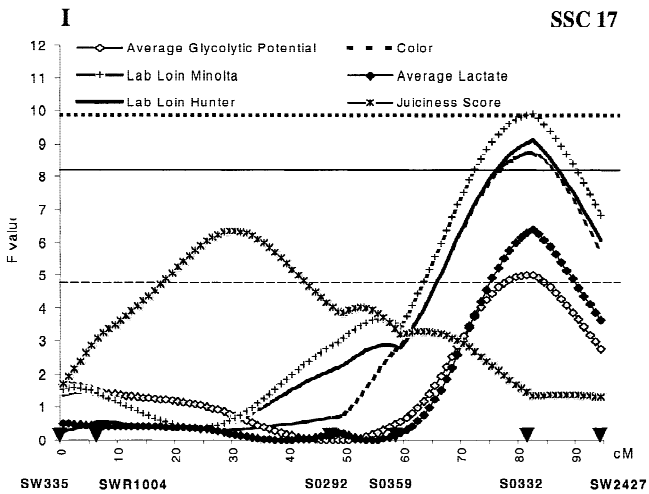

Fig. 1. *Continued.*

not report QTL for these two traits. Chr 2 showed QTL for both flavor and off-flavor score, but at different positions. The QTL for flavor score was in the same region as the QTL associated with tenderness, color, and reflectance. The QTL for off-flavor score did not appear to be associated with QTL for other traits.

An additional QTL for flavor was detected on Chr 15 (Table 2). This QTL was in the same region as the QTL for reflectance, pH, and glycogen metabolism that were detected on this chromosome and near the RN gene. Berkshire alleles were associated with more flavor, consistent with the favorable Berkshire effects observed for other traits on this chromosome.

A QTL for off-flavor score was also observed on the X Chr, which was the only QTL observed across all traits on this chromosome (Table 2). Berkshire alleles were associated with less off-flavor.

Conclusions

Despite the limited differences between the two breeds used in this cross compared with exotic crosses, in total, 60 QTL were detected at the 5% chromosome-wise significance level for the 28 traits evaluated in this study. Of the 60 suggestive QTL, 9 and 1 QTL were significant at the 5%, and 1% genome-wise levels (Table 3), respectively. If no QTL were present for any trait, 28, 1.4, and 0.3 QTL would be detected at these levels by chance alone. We chose to report all QTL significant at the 5% chromosome-wise level. This will aid other researchers as additional experiments are reported for these meat, muscle, and sensory traits. Our study reports many QTL that had not been previously reported, and we were able to confirm only a limited number of QTL that were described previously. These differences with literature results may be due to the fact that two commercial breeds were used in our study, compared with literature results, which generally involved one exotic breed.

Significant QTL existed for nearly all traits. They varied in size, but most accounted for 3–5% of the total F_2 variance. Some QTL exceeded this considerably, and one QTL reached 10%. Both breeds had favorable QTL on separate chromosomes for meat quality. Overall, Chrs 15 and 17 contributed highly to the Berkshire superiority in meat quality, but Yorkshires were superior for Chrs 2, 5, and 11. There was some evidence on several chromosomes that cryptic alleles existed which favored the breed least expected to have them.

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References

Andersson-Eklund L, Marklund L, Lundstrom K, Haley CS, Andersson K et al. (1998) Mapping quantitative trait loci for carcass and meat traits in a Wild boar x Large White intercross. J Anim Sci 76, 694–700

Bligh EG, Dyer WJ (1959) A rapid method for total lipid extraction and purification. Can J Biochem Physiol 3, 911–917

Cameron ND (1990) Genetic and phenotypic parameters for carcass traits, meat and eating quality traits in pigs. Livest Prod Sci 26, 119–135

De Koning D, Janss LLG, Rattink AP, van Oers PAM, de Vries BJ et al. (1999) Detection of quantitative trait loci for back fat thickness and intramuscular fat content in pigs (*Sus scrofa*) Genetics 152, 1679–1690

De Koning D, Harlizius B, Rattink AP, Bovenhuis H, Groenen MAM et al. (2000a) Detection and characterization of quantitative trait loci for carcass and meat quality traits in pigs. Proc Eur Assoc Anim Prod 6, 3

De Koning D, Rattink AP, Harlizius B, van Arendonk AMJ, Brascamp WE et al. (2000b) Genome- wide scan for body composition in pigs reveals important role of imprinting. Proc Natl Acad Sci 97, 7947–7950

Fujii J, Otsu K, Zorzato F, De Leon S, Khanna VK et al. (1991) Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253, 448–451

Gerbens F, Rettenberger G, Lenstra JA, Veerkamp JH et al. (1997) Characterization, chromosomal localization, and genetic variation of the porcine heart fatty acid-binding protein gene. Mamm Genome 8, 328–332

Geldermann H, Mueller E, Beeckman P, Knoor C et al. (1996) Mapping quantitative trait loci by means of marker genes in F2 generations of Wild Boar, Pietrain and Meishan pigs. J Anim Breed Genet 113, 381–387

Goodwin R, Burroughs S (1995) Genetic evaluation terminal line program results. National Pork Producers Council, Des Moines, Iowa

Honikel KO, Kim CJ, Hamm R, Roncales P (1986) Sarcomere shortening of prerigor muscles and its influence on drip loss. Meat Sci 16, 267–282

Hovenier R, Kanis E, Van Asseldonk Th and Westerink NG (1992) Genetic parameters of pig meat quality traits in a halothane negative population. Livest Prod Sci 32, 309–321

Huff-Lonergan E, Baas TJ, Malek M, Dekkers JCM, Pursa K et al. (2001) Correlations among selected pork quality traits. J Anim Sci submitted

Jeon J, Carlborg O, Tornsten A, Giuffra E, Amarger V et al. (1999) A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. Nat Genet 21, 157–158

Kauffman RG, Eikelenboom G, van der Wal PG, Merkus G, Zaar M (1986a) The use of filter paper to estimate drip loss of porcine musculature. Meat Sci 18, 191–200

Kauffman RG, Eikelenboom G, van der Wal PG, Engel B, Zaar M (1986b) A comparison of methods to estimate water-holding capacity in postrigor porcine muscle. Meat Sci 18, 307–322

Lander ES, Kruglyak L (1995) Genetic dissection of complex traits: guide lines for interpreting and reporting linkage results. Nat Genet 11, 315–324

LeRoy P, Naveau J, Elsen JM, Sellier P (1990) Evidence for a new major gene influencing meat quality in pigs. Genet Res 55, 33–40

Lundberg P, Vogel HJ (1986) Carbon-13 and Proton NMR studies of post-mortem metabolism in bovine muscle. Meat Sci 18, 133–160

Malek M, Dekkers JCM, Lee HK, Baas T, Rothschild MF (2001) A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. I. Growth and body composition. Mamm Genome, submitted

Maribo H, Stoier S, Jorgensen PF (1999) Research note: procedure for determination of glycolytic potential in porcine m. longissimus dorsi. Meat Sci 52, 191–193

Milan D, Bidanel JP, Le Roy P, Chevalet C, Woloszy N (1998) Current status of QTL detection in Large White X Meishan crosses in France. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production 26, 414–417

Milan D, Jeon J, Looft C, Amarger V, Robic A et al. (2000) A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science 288, 1248–1251

Monin G, Sellier (1985) Pork of low technological quality with a normal rate of muscle pH falls in the immediate post-mortem period: the case of the Hampshire breed. Meat Sci 13, 49:63

Moser G, Mueller E, Beeckmann P, Yue G, Geldermann H. (1998) Mapping of QTLs in F_2 generations of Wild Boar, Pietrain and Meishan pig. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production 26, 478–481

NPPC (1991) Procedures to Evaluate Market Hogs. National Pork Producers Council, Des Moines Iowa.

Sellier P (1998) Genetics of meat and carcass traits. In: Rothschild MF, Ruvinsky A, eds. *The Genetics of the Pig,* (Wallinford, UK: CAB International), pp463–510

Talmadge RJ, Roy RR (1993) Electrophoretic separation of rat skeletal muscle heavy-chain isoforms. J Appl Physiol 75, 2337–2340

Wang L, Yu TP, Tuggle CK, Liu HC, Rothschild MF (1998) A directed search for quantitative trait loci on chromosomes 4 and 7 in the pig. J Anim Sci 76, 2560–2567

Yu TP, Wang L, Tuggle CK, Rothschild MF (1999) Mapping fatness and growth on pig chromosome 13: a search in the region close to the pig PIT1 gene. J Anim Breed Genet 16, 269–280