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Detection of Quantitative Trait Loci for Backfat Thickness and Intramuscular Fat Content in Pigs (*Sus scrofa*)

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ABSTRACT

In an experimental cross between Meishan and Dutch Large White and Landrace lines, 619 F_2 animals and their parents were typed for molecular markers covering the entire porcine genome. Associations were studied between these markers and two fatness traits: intramuscular fat content and backfat thickness. Association analyses were performed using interval mapping by regression under two genetic models: (1) an outbred line-cross model where the founder lines were assumed to be fixed for different QTL alleles; and (2) a half-sib model where a unique allele substitution effect was fitted within each of the 19 half-sib families. Both approaches revealed for backfat thickness a highly significant QTL on chromosome 7 and suggestive evidence for a QTL at chromosome 2. Furthermore, suggestive QTL affecting backfat thickness were detected on chromosomes 1 and 6 under the line-cross model. For intramuscular fat content the line-cross approach showed suggestive evidence for QTL on chromosomes 2, 4, and 6, whereas the half-sib analysis showed suggestive linkage for chromosomes 4 and 7. The nature of the QTL effects and assumptions underlying both models could explain discrepancies between the findings under the two models. It is concluded that both approaches can complement each other in the analysis of data from outbred line crosses.

IN pig breeding, experimental populations have been used for detection of quantitative trait loci (QTL), such as the cross between wild boar and Large White pigs described by Andersson *et al.* (1994) and several crosses between Meishan and Western pig breeds (*e.g.*, Rothschild *et al.* 1995; Janss *et al.* 1997a). Meishan pigs have lower lean meat content in their carcasses compared to Western pig breeds, but the lean meat of Meishan pigs is of higher quality (Serra *et al.* 1992). In an experiment with F_2 animals from the Meishan × Dutch pig breed cross, Janss *et al.* (1997a) found evidence for the segregation of major genes that affected a number of meat quality traits. Two of the traits that displayed single-gene activity were related to fatness in pigs: intramuscular fat content (IMF), *i.e.*, the percentage of fat within a loin muscle, and backfat thickness (BFT).

This article describes the molecular typing of the crossbred pig population and the subsequent association study to locate QTL that affect IMF and BFT. The association study was performed under two genetic models: (1) an outbred line-cross model where the purebred lines are assumed to be fixed for different QTL alleles; and (2) a half-sib model, which makes no assumptions about fixation of QTL alleles in the founder lines, because a unique allele substitution effect is fitted within every paternal half-sib family.

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MATERIALS AND METHODS

The Meishan × Dutch population: An F_2 cross between the Chinese Meishan pig breed and commercial Dutch pig lines was available from an experiment involving five Dutch pig breeding companies (Janss *et al.* 1997a,b). The experiment was designed for the detection of major genes on the basis of phenotypic data. Blood samples were stored to facilitate mapping of detected genes. The F_1 was obtained by artificial insemination of purebred females from Large White and Dutch Landrace lines with semen from 19 male pigs from the Meishan breed. From the F_1, males and females were randomly selected to become parents of the F_2 litters. The centrally housed F_1 males provided semen that was used for artificial insemination across companies of the selected F_1 females, which remained at the breeding companies. Blood or tissue samples were taken from the purebred animals, the F_1 parents, and at least 5 animals from each of the 264 F_2 litters to provide DNA for molecular typing. From these litters, ~350 animals were retained as experimental and commercial breeding stock. Performance-tested F_2 animals that were not retained for breeding were slaughtered in a central slaughterhouse at ~90 kg of live weight. From these 844 slaughtered animals, several meat quality traits were measured. For this study, 19

half-sib families were selected for molecular typing from a total of 39 families because they were identified as informative carriers for the single gene affecting intramuscular fat content (Janss *et al.* 1997a). These 19 paternal half-sib families had between 22 and 51 F_2 offspring. From these 619 F_2 offspring, 418 animals were tested for meat quality traits.

The Meishan founders and the selected F_1 fathers were tested for the mutation in the ryanodine receptor (*Ryr*-1), which causes halothane susceptibility and has a large effect on meat quality (Houde *et al.* 1993). None of the tested animals were identified as carriers of the mutation, so the population was "halothane negative."

Fatness traits: In a review by Hovenier *et al.* (1993), IMF was described to affect several organoleptic properties of pig meat, like appearance, tenderness, and juiciness. When IMF is too low the meat tenderness is reduced, which diminishes the eating quality. High levels of IMF are also undesirable because consumers do not appreciate meat with visible amounts of IMF. The optimum level of IMF would be between 2.5 and 3.0%. In this study, IMF was determined on a sample of *Musculus longissimus* by petroleum ether extraction (Hovenier *et al.* 1992) 24 hr after slaughter.

Consumers' demands for lean pork meat have resulted in selection against high BFT. In the Netherlands, backfat and lean thickness are routinely measured with the Hennessy grading probe between the third and fourth rib of a carcass, 6 cm from the spine. Hovenier *et al.* (1993) presented heritabilities of 0.51 for BFT and 0.61 for IMF with a phenotypic correlation of 0.30 and a genetic correlation of 0.37 between the traits. Warris *et al.* (1990) give heritabilities of 0.61 for BFT and 0.52 for IMF with similar phenotypic (0.20) and genetic (0.32) correlations.

DNA isolation, molecular typing, and map construction: The 619 F_2 animals, their 150 F_1 parents, and the F_0 Meishan sires were typed for 127 microsatellite markers. These markers were selected from published linkage maps (Archibald *et al.* 1995; Rohrer *et al.* 1996) and cover all 18 autosomal porcine chromosomes and the X chromosome. The number of markers per chromosome varies between 10 markers on SSC1 and 2 on SSC18. DNA was isolated from blood samples or spleen tissue samples using the Puregene DNA isolation kit (Gentra Systems, Research Triangle Park, NC). Details about the PCR reaction mixtures, PCR conditions, and multiplexes can be found in Groenen *et al.* (1996). PCR products of up to 14 markers were combined and analyzed simultaneously on an automated sequencer (ABI; Perkin Elmer, Norwalk, CT).

Fragment length of the PCR products was determined with Genescan software (ABI; Perkin Elmer), and marker genotypes were assigned to the animals using Genotyper software (ABI; Perkin Elmer). A second examiner evaluated all marker genotypes prior to linkage analyses. Multipoint recombination fractions were calculated with CriMap version 2.4 (Green *et al.* 1990). These recombination fractions were transformed to map distances with the Haldane mapping function. In case there was disagreement with regard to marker order between the two published linkage maps (Archibald *et al.* 1995; Rohrer *et al.* 1996), the marker order was checked using the CriMap-flips option. The marker order with the highest likelihood was chosen.

Analysis of phenotypic data: The phenotypes consisted of single measurements on slaughtered F_2 individuals. Prior to the QTL analyses the phenotypic data were adjusted for a number of systematic effects. All data were used in this step ($n = 844$). The phenotypic data were analyzed assuming a polygenic inheritance model containing nongenetic effects of slaughter day, breeding company, sex, and carcass weight. The statistical model to describe the phenotypic observations **y** on the F_2 animals for a given trait was:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e}. \quad (1)$$

β is a vector of fixed effects and the regression coefficient for carcass weight. **X** is a matrix relating observations to their fixed effect levels and the values for covariable carcass weight. Vector **u** contains polygenic effects for all animals in the pedigree. These are linked to observations **y** by the incidence matrix **Z.** Vector **e** contains random errors. The trait score for the interval mapping analyses, y^*, contains the phenotypes corrected for the nongenetic effects estimated under model (1):

$$y^* = Y - X\hat{b}. \quad (2)$$

The estimations were performed using the MaGGiC software package developed by Janss *et al.* (1995). Estimates of effects were obtained from a Gibbs chain of 200,000 iterations with a burn-in of 2000 iterations. For details on matrix descriptions and the construction of the Monte Carlo Markov Chain, see Janss *et al.* (1997a). The file to reconstruct relationships between animals consisted of the purebred animals, all F_1 parents, and the F_2 individuals.

QTL analysis: Two types of interval mapping, both using regression methods, were applied: (1) line-cross analysis following Haley *et al.* (1994), assuming the founder lines to be fixed for different QTL alleles; and (2) analyses nested within half-sib families following Knott *et al.* (1996), making no assumptions about the number of QTL alleles and allele frequencies within the founder lines.

Line-cross model: Under the line-cross model it is assumed that the two founder lines, although they may share alleles at the marker loci, are fixed for different alleles at the QTL affecting the traits of interest. For every F_2 individual it is inferred what the probabilities are that it inherited two Meishan alleles, two Dutch alleles, or one of each line at 1-cM intervals along the genome, on the basis of genotypes of flanking markers. The assumption of fixation of the founder lines at the QTL level allows straightforward calculation of additive and dominance effects of a putative QTL at a given position. The additive QTL effect is defined as half the phenotypic difference between animals that are homozygous for Meishan alleles and animals that are homozygous for alleles from the Dutch lines. A positive value for the additive effect implies that the Meishan allele results in an increase in phenotype. The dominance effect is the deviation of the heterozygous animals from the mean of the two types of homozygous animals. At every centimorgan across the genome the model

$$y_j^* = m + ax_{aj} + dx_{dj} + e_j \quad (3)$$

is fitted, where y_j^* is the adjusted trait score of animal j, m is the population mean, a and d are the estimated additive and dominant effects of a putative QTL at the given location, x_{aj} is the conditional probability of animal j of carrying two Meishan alleles, x_{dj} the conditional probability of animal j of being heterozygous at the given location, and e_j is the residual error. The calculation of these probabilities and QTL effects is described by Haley *et al.* (1994), and applications to crossbred pig populations are numerous (*e.g.*, Andersson *et al.* 1994; Knott *et al.* 1998; Moser *et al.* 1998).

Half-sib model: The F_2 animals are divided into 19 paternal half-sib groups. Within each group there are 6 to 8 full-sib groups, but these groups are too small to perform an analysis using additional relationships from the full-sib families as described by van Kaam *et al.* (1998). For this study, the F_2 animals are treated as 19 unrelated half-sib families, *i.e.*, additional genetic relationships between and within half-sib groups are ignored. In a paternal half-sib design the segregation of possible QTL on chromosome X cannot be evaluated; therefore, only the 18 porcine autosomes were analyzed. The analysis uses the multimarker approach for interval mapping in half-

sib families as described by Knott *et al.* (1996) and as applied to QTL mapping studies in cattle by Spelman *et al.* (1996) and Vilkki *et al.* (1997). The method contains the following steps: In every F_2 offspring the paternal alleles are identified for all markers for which the sire is informative (*i.e.*, heterozygous). Maternal genotypes are used to infer the paternal allele when both sire and offspring are heterozygous for the same marker alleles. The most likely phases of the gametes of the sire of each family are determined by minimizing the number of recombination events in the F_2 offspring. For each offspring the probability of inheriting the sire's first gamete of a chromosome is calculated at 1-cM intervals conditional on the linkage phase of the sire and marker genotypes of the individual and its parents. A QTL with a gene substitution effect is fitted at 1-cM intervals along the chromosome,

$$y_j^* = a_i + b_i x_{ij} + e_{ij}, \quad (4)$$

where y_j^* is the trait score of individual j, originating from sire i; a_i is the average effect for half-sib family i; b_i is the regression coefficient within half-sib family i (*i.e.*, substitution effect for a putative QTL); x_{ij} is the conditional probability for individual j of inheriting the first parental gamete, and e_{ij} is the residual effect. The regression is nested within families because the assignment of the first gamete is random and not all sires are heterozygous for the QTL. Furthermore, the linkage phase between a marker and a QTL can differ between families. The number of QTL alleles is only constrained by the number of families. The test statistic is calculated as an F ratio for every map position within and across families. For details on the calculation of the test statistic see Spelman *et al.* (1996). Once a QTL was detected in the across-family analyses, the tabulated probability of the F ratio for the individual families was used to infer which families were likely to be segregating for the QTL. In the families that were segregating for an identified QTL, it was determined which of the alleles of the F_1 sire gave the higher BFT or IMF. If it could be inferred unequivocally which of the sire's marker alleles originated from the Meishan breed, it could subsequently be determined whether this Meishan allele was associated with an increase or a decrease in phenotype.

Significance thresholds: Following Lander and Kruglyak (1995), three significance levels are defined. The first level is the chromosomewise threshold, which does take account of multiple tests on a specific chromosome but does not correct for testing on the entire genome. The second level is suggestive linkage, where one false positive is expected in a genome scan (Lander and Kruglyak 1995). Expecting one false positive per genome scan, the suggestive significance level for a specific chromosome is proportional to the contribution of that chromosome to the total autosomal genome length. The contribution (r) of a chromosome was obtained by dividing the length of a specific chromosome by the total length of the autosomal genome. Third, the genomewise significance level is used, which takes account of testing the whole autosomal genome:

$$p_{\text{genomewise}} = 1 - (1 - p_{\text{chromosomewise}})^{1/r}. \quad (5)$$

All three significance levels do not take the testing of multiple traits in the present and future studies into account. Comparison between different studies is facilitated by significance levels that take the total genome length into account but that are not affected by the variable number of independent traits in different studies.

Significance thresholds are determined empirically by permutations as described by Churchill and Doerge (1994). Data permutation is used to determine the empirical distribution of the test statistic under the null hypothesis of no QTL associated with the chromosome under study. A total of 10,000 permutations were sufficient to estimate chromosomewise 5, 1, and 0.1% significance thresholds. To estimate smaller risk levels the number of permutations was extended to 50,000.

TABLE 1

Overall and sex-specific characteristics of the raw measurements for backfat thickness and intramuscular fat content

	Backfat thickness (mm)	SE	Intramuscular fat content (%)	SE
Overall mean	22.01	±5.69	1.84	±0.87
Minimum	7.60		0.20	
Maximum	44.00		6.10	
Male mean	21.33	±5.60	1.77	±0.81
Female mean	23.14	±5.66	1.95	±0.94

RESULTS

Genotyping and map construction: The heterozygosity of the microsatellite markers, which was measured on the 19 F_1 sires, ranged from 0.2 to 1.0 with a mean of 0.87 (±0.15). With regard to SSC7, there was disagreement between the two published maps (Archibald *et al.* 1995; Rohrer *et al.* 1996) for markers employed in this study. Archibald *et al.* (1995) report the order SW352–SW632–SW175, while Rohrer *et al.* (1996) proposed the order SW175–SW352–SW632. Applying the CriMap-flips option to marker data from this study gave evidence for the order proposed by Rohrer *et al.* (1996). Unexplained jumps in the test statistic for SSC4 gave reason to evaluate the marker order for that chromosome as well. Applying the CriMap-flips option showed that the order S0073–S0214–Sw589 was more likely than the published order S0073–SW589–S0214 (Archibald *et al.* 1995; Rohrer *et al.* 1996), but the difference in LOD was only 2.7, which implies that the original order cannot be excluded. The total autosomal map length was 2115 cM (Haldane), and the average marker interval was ~17 cM.

QTL analysis: An overview of the phenotypic characteristics of the two traits is given in Table 1. The estimated heritabilities were 0.24 and 0.35 for BFT and IMF, respectively.

QTL analyses for BFT: The QTL analyses following the line-cross model showed genomewide evidence for a QTL affecting BFT on SSC7, strong suggestive linkage for SSC1, and suggestive evidence for a QTL on SSC2 and SSC6. The genomewide risk level of the QTL on SSC7 is very small but could not be estimated because the test statistic was not exceeded by chance during 50,000 permutations. The suggestive QTL at SSC1 had a genomewide risk level of 0.08.

The half-sib interval mapping procedure showed genomewide evidence for a QTL on SSC7 and strong

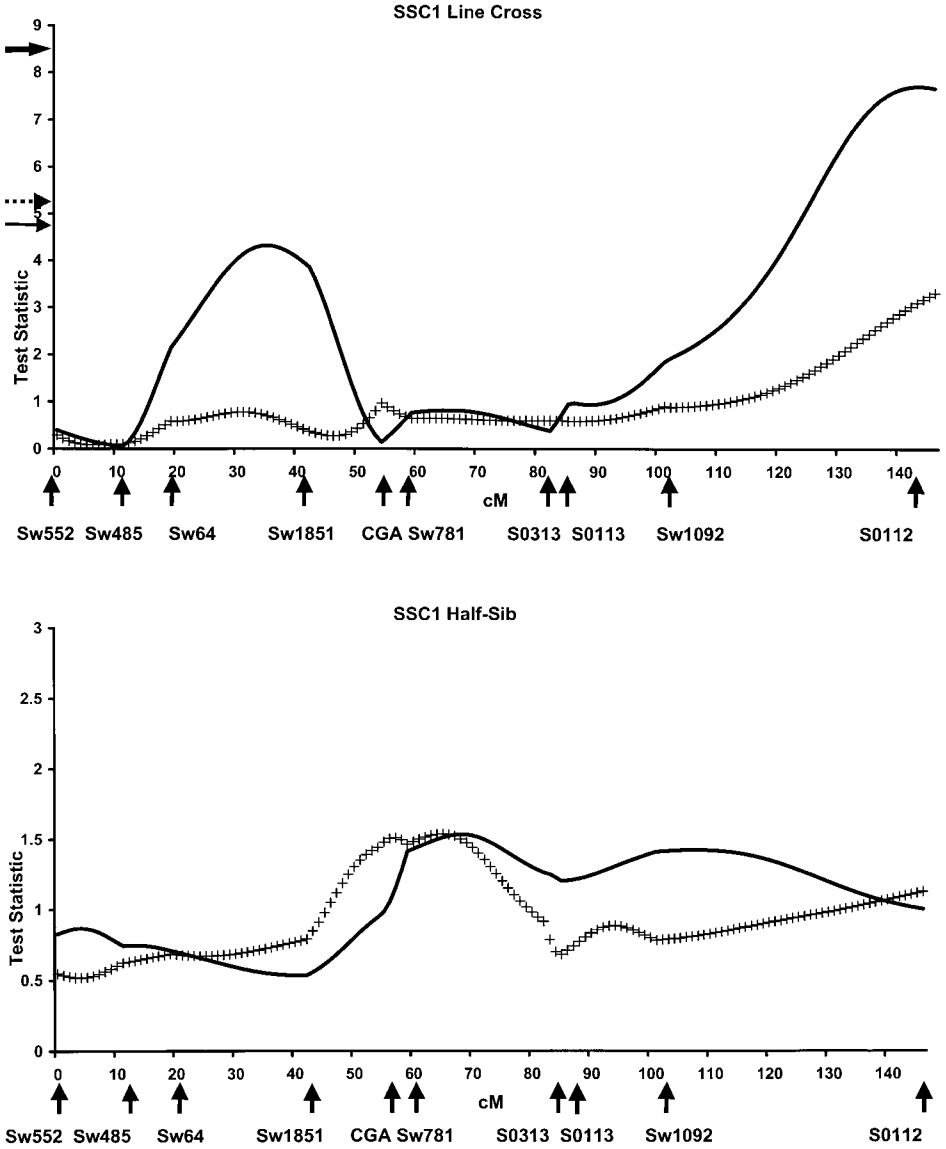


Figure 1.—Test statistics for four chromosomes with regard to BFT and IMF under two models.(—) The test statistics for BFT; (+++) the test statistics for IMF. Arrows on the *x*-axis indicate marker positions and names. Arrows on the *y*-axis represent the three thresholds: suggestive (thin arrow), chromosomewise 5% (dashed arrow), and genomewise 5% (thick arrow). Arrows on the left of the *y*-axis indicate thresholds for BFT, and arrows on the right side indicate thresholds for IMF.

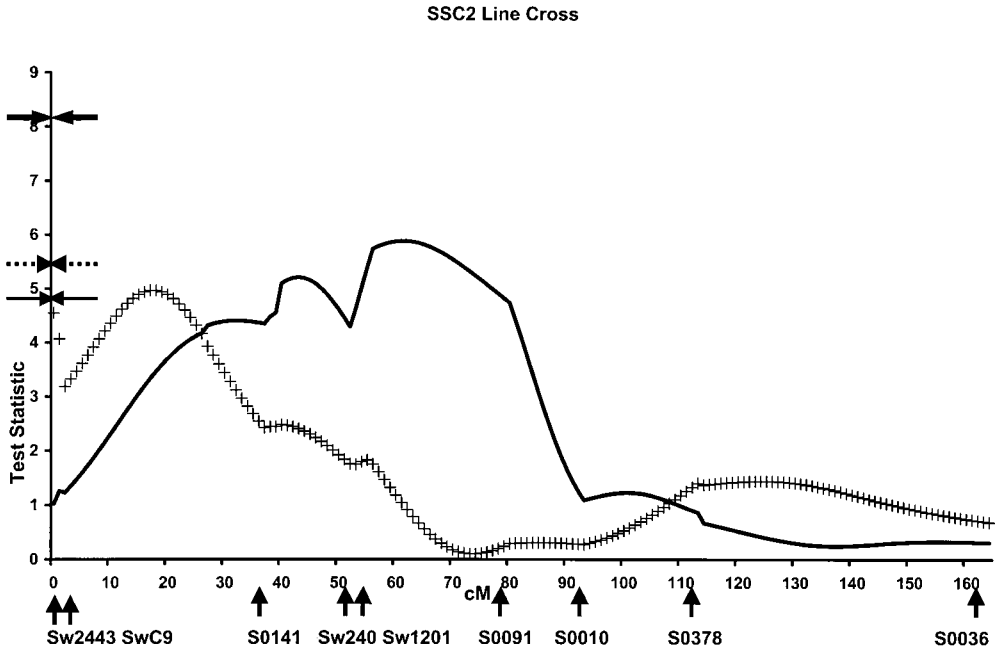
suggestive evidence for a QTL on SSC2 ($p_{\text{genomewide}} \sim 0.09$). Figure 1 shows the development of the test statistics and the threshold levels along SSC1, SSC2, SSC4, SSC6, and SSC7 for both BFT and IMF. The estimated position of the QTL on SSC7 is very similar under both models. The estimate of the QTL position on SSC2 is 62 cM under the line-cross model and 43 cM in the half-sib analysis. However, Figure 1 shows a rather flat curve for SSC2 under both analyses, and therefore it is likely that the same QTL is detected under both models. The suggestive QTL on SSC1 and SSC6 both map to the end of the chromosome.

QTL analyses for IMF: The line-cross analysis showed the strongest linkage for SSC6 with a genomewide risk level of 0.13. Other suggestive QTL affecting IMF were detected on SSC2 and SSC4 under the line-cross model. Like the suggestive QTL for BFT, the suggestive QTL for IMF on SSC6 maps to the last marker bracket of that chromosome. The suggestive QTL on SSC2 maps to the second marker bracket on that chromosome, and the putative QTL on SSC4 has its most likely position in the middle of the linkage group.

The half-sib analysis showed suggestive linkage for SSC4 and SSC7. The most likely position of a QTL affecting IMF on SSC7 is at the end of the linkage group, where also the test statistic for BFT showed a small peak (Figure 1). The line-cross analysis of SSC7 also gave a peak for IMF at the end of the linkage group, but it was not significant (Figure 1). The suggestive QTL for IMF on SSC4 maps to the first marker bracket of that chromosome (Figure 1). All QTL that exceeded the level of suggestive linkage in any of the analyses are summarized in Table 4.

QTL effects for BFT: Under the line-cross model the additive and dominance effects of a QTL are calculated across the whole population, whereas in a half-sib analysis a unique allele substitution effect (Falconer 1989) is fitted within every half-sib family. The estimated effects under the line-cross model are given in Table 2.

The QTL affecting BFT on SSC2 and SSC7 are mainly



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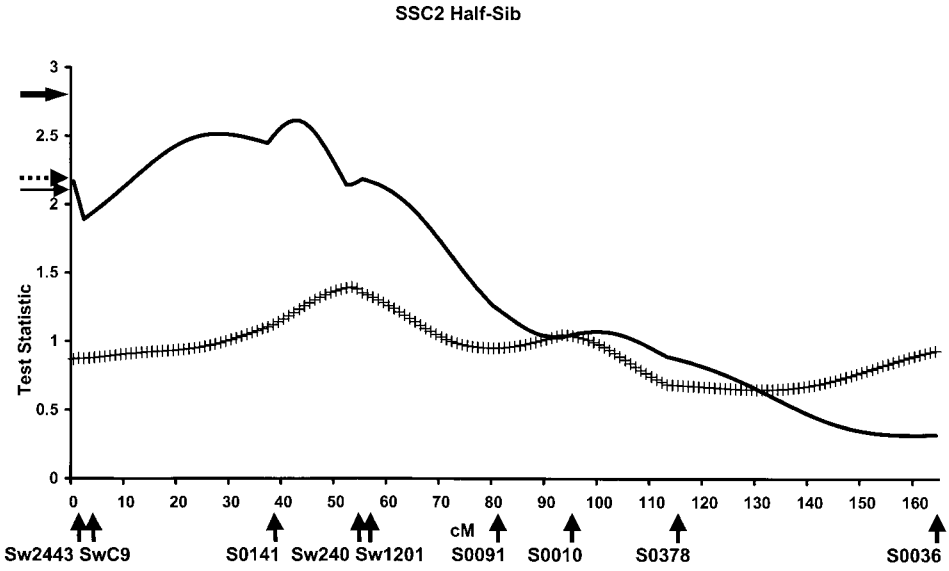
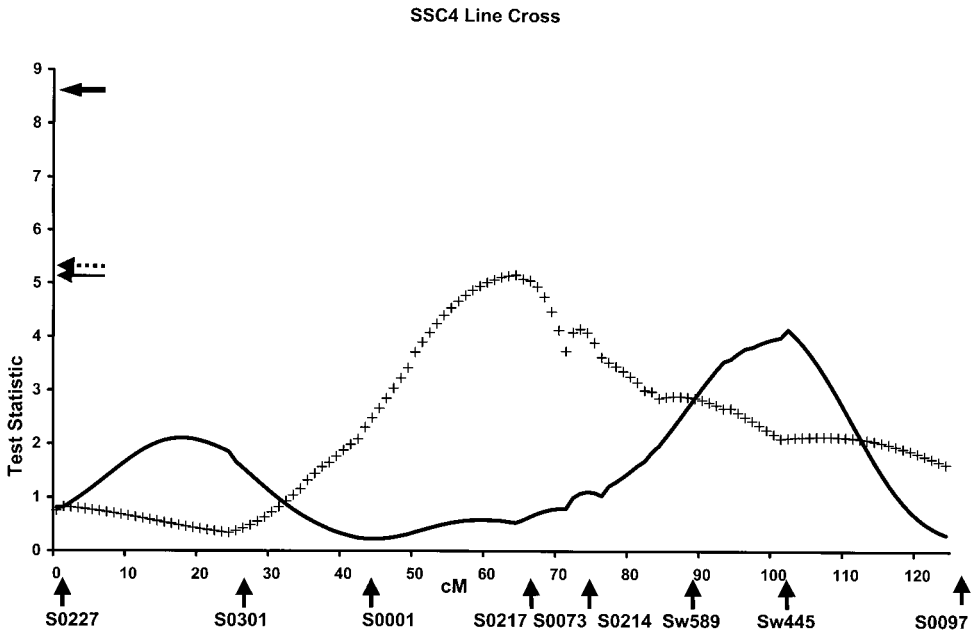


of an additive nature. The QTL affecting BFT on SSC1 and SSC6 have a large dominance component (Table 2), which points toward overdominance.

In a half-sib model the most likely position of a QTL across families is not necessarily the most likely position of a QTL within families. Table 3 shows the estimates of the QTL effects at the overall best position on SSC7 and the individual best position for the families that exceed a tabulated risk level of 0.05. Five families have their maximum in an interval of ∼30 cM around the overall best position of a QTL. The difference in most likely positions between these families can be partly explained by marker information. The estimates of the QTL effects at the overall best position were quite different between families, whereas the estimates at the individual best position would suggest that the same QTL allele was segregating in families 1, 8, 12, 17, and 19 with an effect of around 6.7 mm (∼1.4 phenotypic standard deviation). For some other families the most likely position of a QTL affecting BFT on SSC7 is at the last marker of the chromosome. This explains the additional peak in the test statistic profile at the end of SSC7 in the half-sib analysis (Figure 1).

QTL effects for IMF: The estimated effects of the suggestive QTL that were detected on SSC2, SSC4, and SSC6 in the line-cross analysis are also summarized in Table 2. The effect on SSC2 seems completely dominant, whereas the suggestive QTL on SSC4 and SSC6 seem to act in an additive way.

In the half-sib analysis for SSC4 there were four families that showed a significant QTL ($P < 0.01$) in the first 35 cM of that chromosome. The estimated QTL



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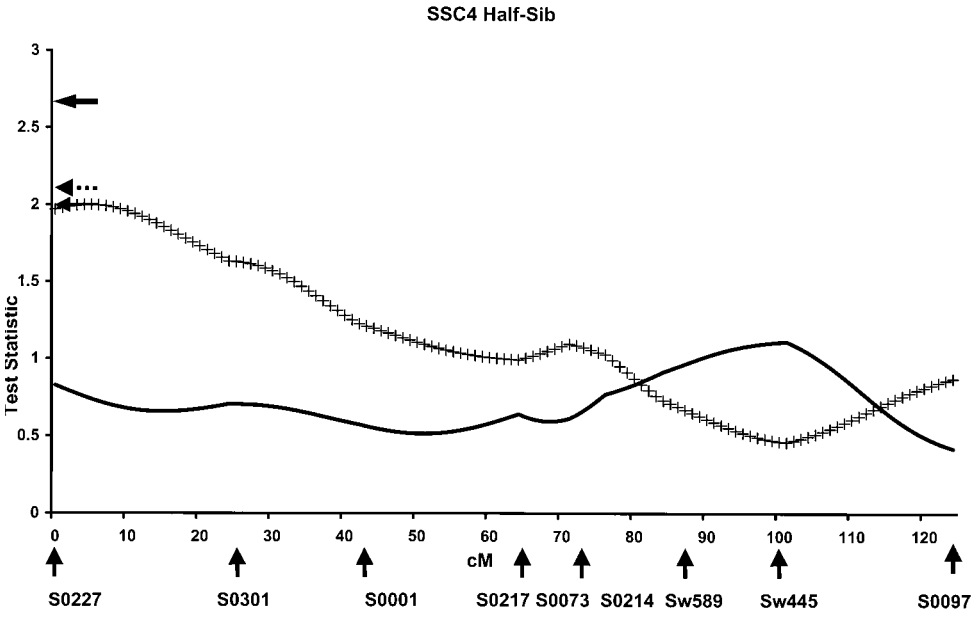
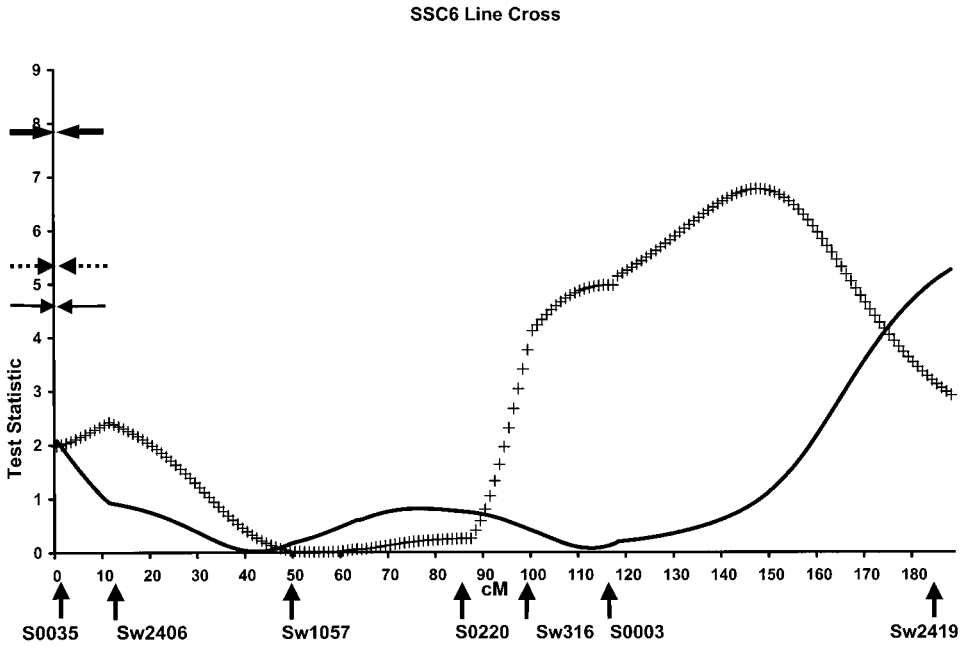


effects within these families at their individual best positions varied between 0.74 and 1.56% of IMF.

For SSC7 the most likely position of a QTL affecting IMF across families was at the end of the chromosome, where the test statistic of six individual families exceeded the tabulated level of $P < 0.05$ in the initial analyses. Estimated effects at their individual best positions varied between 0.8 and 1.5% of IMF.

Origin of QTL alleles from the half-sib analysis: For the identified QTL affecting BFT on SSC2, the marker alleles associated with a higher BFT could be traced back to the Meishan grandparents in all but one of the families that were segregating for this QTL. This suggests that this higher allele might be absent or very rare in the purebred Dutch lines. In all of these families it was possible to determine which Meishan allele the F_1 sire inherited for at least one of the flanking markers of the QTL. For the QTL affecting BFT on SSC7, the alleles associated with higher BFT were all traced back to the purebred Dutch lines. For the families that were segregating for the QTL affecting IMF on SSC4 and/or SSC7, the Meishan alleles were associated with both higher and lower levels of IMF. This indicates that both the Meishan and the purebred Dutch lines are segregating for the same QTL alleles at the same loci affecting IMF.

Additional analyses: To test whether any of the identified QTL would represent the single genes identified by Janss *et al.* (1997a), additional analyses were carried out in which the phenotypes were also corrected for the effects of these single genes. If one of the identified QTL represented the single gene for that trait, the test



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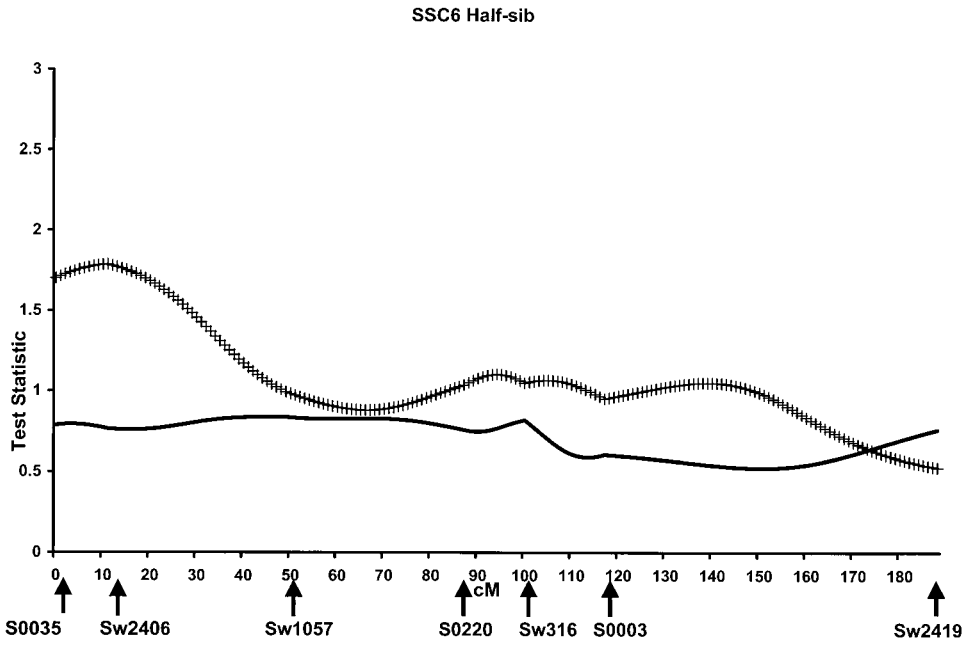

statistic for that QTL would diminish if the data were corrected for the single gene effect. This phenomenon was only observed for the putative QTL affecting BFT at the distal end of SSC1. The test statistic under the line-cross model dropped dramatically when the phenotypes were preadjusted for the putative single gene. For BFT the maximum test statistic on SSC1 dropped from 7.7 to 3.9. This was not observed for any of the other QTL locations.

To test whether there could be more than a single QTL on a chromosome affecting the trait of interest, a grid search fitting two QTL was performed on all linkage groups that exceeded suggestive linkage for any of the traits. This analysis was only carried out under the half-sib model. A standard *F*-test was used to test whether the best two QTL on a chromosome explained significantly more variance than the best single QTL. From a 5-cM grid search, it was for BFT on SSC7 that two QTL at 71 and 151 cM explained significantly ($P < 0.05$) more variance than a single QTL at 73 cM.

DISCUSSION

All putative QTL affecting BFT or IMF that exceeded the thresholds for suggestive linkage are summarized in Table 4. The strongest evidence for QTL was found for BFT on SSC7, SSC1, and SSC2. For the suggestive QTL on SSC1 and SSC6 affecting BFT, there seems to

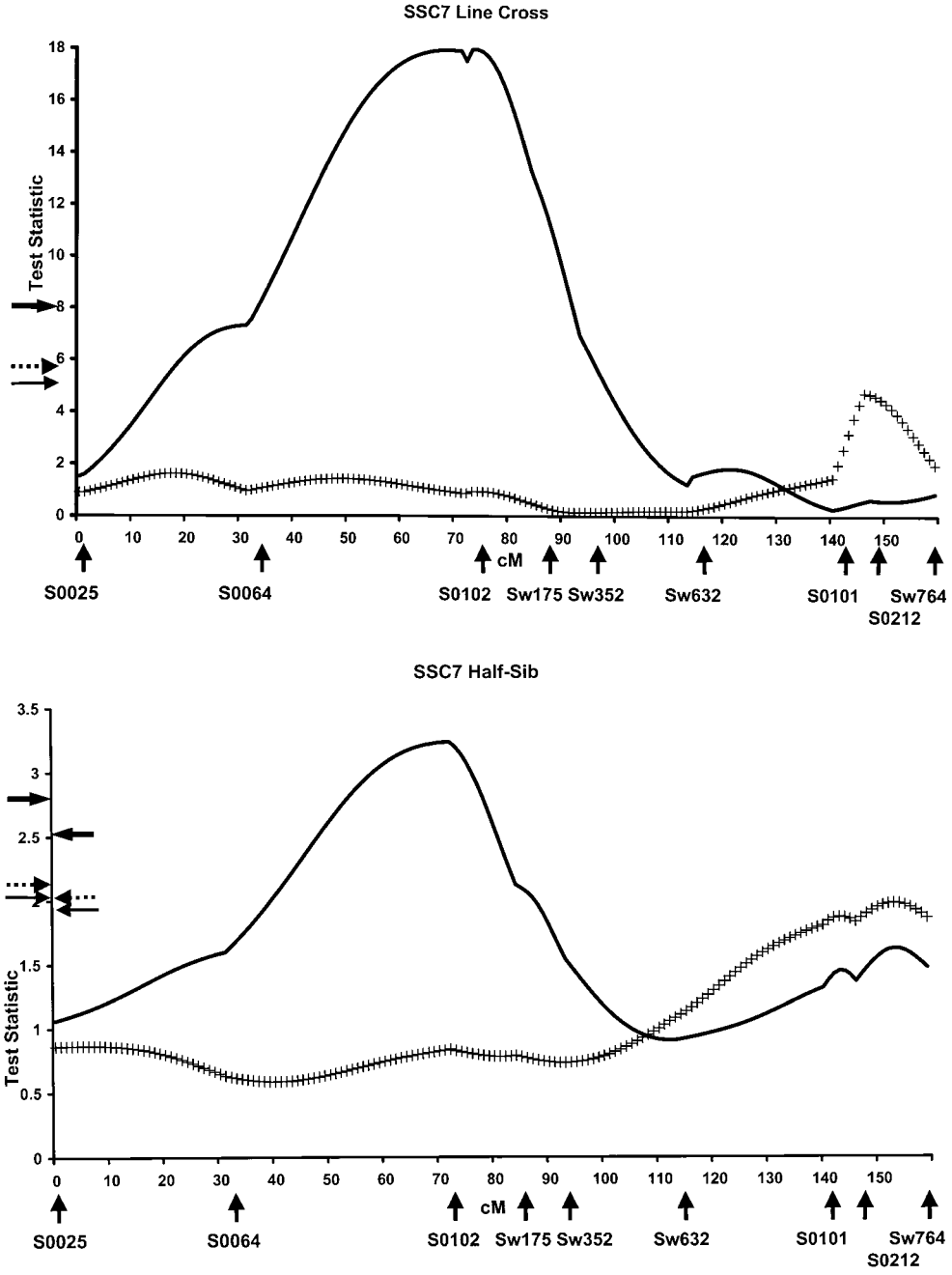

Figure 1.—*Continued.*

be overdominance (Table 2). The finding of completely dominant or overdominant QTL alleles gives rise to the question of whether these are true effects of single genes or whether they arise from a cluster of closely linked genes. It should be noted that for both linkage groups the last marker interval is rather large, which gives lower information content in these regions. This could have resulted in inflated estimates if the QTL effects.

Statistical analysis: The application of both the line-cross and the half-sib model provides a useful tool to explore different *a priori* assumptions about the QTL genotypes in the founder lines. The findings for QTL affecting BFT on SSC2 and SSC7 are consistent under both models. For IMF and the other putative locations for QTL affecting BFT, the two models point toward different chromosomes and/or locations (Table 4). The validity of the underlying assumptions and/or the nature of the detected QTL can explain these apparent discrepancies.

In the half-sib analysis it was inferred for both the QTL on SSC2 and SSC7 that the "high" or "low" QTL alleles could consistently be traced back to one of the founder lines. It is therefore not surprising that these QTL were also detected under the line cross model, which assumes unique QTL alleles for the founder lines. However, the assumption of fixation of the founder lines for these unique alleles is not supported, because only part of the F_1 families are inferred as heterozygous

TABLE 2

Estimated QTL effects under the line-cross model

Chromosome	Additive effect[a]	SE	Dominance effect[b]	SE
	Backfat thickness			
1	1.46	±0.68	−5.04	±1.37
2	1.37	±0.40	−0.31	±0.65
6	−0.61	±0.40	−1.77	±0.63
7	−2.08	±0.35	0.29	±0.54
	Intramuscular fat content			
2	−0.24	±0.09	−0.31	±0.16
4	0.22	±0.07	−0.07	±0.10
6	−0.45	±0.12	−0.09	±0.33

The estimates are in millimeters backfat and percentage intramuscular fat content.

[a] The effect of the Meishan allele estimated as half the difference between the two homozygous genotypes.

[b] The estimated deviation from the mean of the two homozygous genotypes.

for these QTL. This can also be seen from the much larger estimates of the allele substitution effect within families compared to the estimated additive effect in the line-cross analysis.

For the suggestive QTL affecting IMF on SSC4 and SSC7, it was inferred under the half-sib model that the high alleles originated from both the Meishan and the Western pigs. In this case, an analysis, which assumes the lines to be fixed for different alleles, has little power to detect these QTL. It is, therefore, not surprising that these two QTL were not detected under the line-cross model.

The suggestive QTL affecting BFT at SSC1 and SSC6 are not detected under the half-sib analysis. These putative QTL are both of an (over)dominant nature, and dominance effects contribute little to the allele substitution effect that is estimated in the half-sib analysis.

The line-cross analysis is very powerful when the QTL alleles are unique for the founder lines and when QTL effects are of a dominant nature. Even when the founder lines are not completely fixed for these unique alleles, the method still proves very useful (Alfonso and Haley 1998). When a founder line is not completely fixed for a line-specific allele of a biallelic QTL, the estimated effects under the line-cross analysis are a function of the true allelic effects and the allele frequency in the founder lines (Alfonso and Haley 1998). The estimated allele substitution effect and the test statistic for the individual families from the half-sib analysis provide more insight into the real effect and frequency of a line-specific allele. The estimated allele substitution effects from the half-sib analysis might be biased upward because a test on the individual families is used to determine which families are segregating for the QTL. When there are more than two QTL alleles, a half-sib analysis would use a more realistic genetic model, but the inference of the number of QTL alleles and their respective effects from the individual family tests and estimates is not straightforward.

The half-sib approach has similar power as the line-cross approach when QTL effects are mainly additive. The half-sib approach is particularly useful to detect QTL for which the founder lines carry similar or identical alleles. The combined application of both types of analyses provides more insight into the number of QTL affecting the traits of interest and their mode of action than only using a single method of analysis.

Both methods did not take litter effects and additional genetic relationships within the population into account. Although this might lead to correlated residuals, this does not pose a serious problem because thresholds

TABLE 3

Overview of estimated QTL effects within families for backfat thickness with regard to SSC7

	Overall[a]		Individual families		
Family	QTL effect[b]	SE	Position (cM)	QTL effect[b]	SE
1	4.15*	1.85	50	7.37**	2.27
4	1.11	1.62	151	3.39	1.7
6	1.11	1.42	139	3.26*	1.37
7	1.42	2.00	124	4.11	2.05
8	5.46*	1.96	73	5.46*	1.96
11	3.24*	1.53	85	3.55*	1.51
12	4.15**	1.27	58	5.88**	1.58
13	6.82*	2.64	145	7.64*	2.78
16	2.68	1.33	154	3.01*	1.31
17	5.60**	1.72	55	7.38**	1.99
18	0.29	2.50	151	4.82	2.80
19	6.97**	1.69	79	7.20**	1.72

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, based on tabulated values.

[a] Estimates at 73 cM, the most likely position of a QTL from the analysis across families.

[b] Absolute values of the allele substitution effect in millimeters. The sign of the estimated effect is conditional on the arbitrary assignment of the first parental haplotype and therefore omitted.

TABLE 4

Most likely positions for QTL affecting backfat thickness or intramuscular fat content under two genetic models

	Founder lines fixed for different QTL alleles			No assumptions about QTL alleles and frequency		
Chromosome	Marker bracket (position)	Test statistic	Risk level[a]	Marker bracket (position)	Test statistic	Risk level[a]
			Backfat thickness			
1	Sw1092-S0112 (144)	7.70	$0.08^{c/s}$	Sw781-S0313 (70)	1.56	NS
2	Sw1201-S0091 (62)	5.88	$0.33^{c/s}$	S0141-Sw240 (43)	2.61	$0.09^{c/s}$
6	S0003-Sw2419 (189)	5.24	$0.42^{c/s}$	S0220-Sw316 (101)	0.82	NS
7	S0102-Sw175 (75)	17.95	0.0[b]	S0064-S0102-Sw175 (73)	3.23	0.006
			Intramuscular fat content			
2	Swc9-S0141 (19)	4.97	0.61^{s}	Sw240-Sw1201 (54)	1.39	NS
4	S0001-S0217-S0073 (65)	5.15	0.61^{s}	S0227-S0301 (6)	2.00	0.64^{s}
6	S0003-Sw2419 (148)	6.76	0.13^{cs}	S0035-Sw2406 (12)	1.74	NS
7	S0212-Sw764 (147)	4.73	0.69	S0212-Sw764 (154)	1.97	0.66^{s}

Superscripts c and s denote chromosomewise and suggestive significance, respectively; NS, not significant (not exceeding suggestive or chromosomewise significance).

[a] The genomewise *P* value.

[b] Test statistic not exceeded during 50,000 permutations.

were determined empirically. Although programs for simultaneous estimation of nongenetic, polygenic, and QTL effects are currently available (Bink and van Arendonk 1999), their application in a whole-genome scan is limited because they are very computer-intensive.

Previous studies on this experimental population: There is some evidence from this study that the strongly suggestive QTL at the end of SSC1 affecting BFT might represent the major gene identified by Janss *et al.* (1997a). This QTL at SSC1 is detected at a 0.08 genomewide risk level under the line-cross model only. For IMF there was no indication that any of the identified loci represented the major gene from the segregation analysis. Failure to detect a single major locus affecting IMF in the present study suggests that the results of one of the studies are misleading. Possible explanations for lack of conclusive evidence could be the recessive nature of the single genes that were identified by Janss *et al.* (1997a) or insufficient marker coverage.

A preliminary study with these data by de Koning *et al.* (1998) pointed toward SSC1 to harbor the major genes affecting BFT and possibly IMF described by Janss *et al.* (1997a). In their study, inferences from the segregation analysis were used to assign major gene genotypes to the F_2 animals and were followed by a standard linkage analysis with the molecular markers. Under the half-sib analysis the test statistic profiles for both traits for SSC1 showed a maximum near the region indicated by de Koning *et al.* (1998), but they were not significant. The suggestive QTL at SSC1 detected under the line-cross model maps to the end of the chromosome, which is 40 cM from the area indicated by de Koning *et al.* (1998). Because de Koning *et al.* (1998) performed only single-marker comparisons, this difference might well be explained by a difference in marker information.

Comparison to other studies: This is the first study that describes a genomewide scan for QTL affecting IMF.

This study did not confirm the existence of a QTL affecting BFT on SSC4 that was identified by Andersson *et al.* (1994) and confirmed by Walling *et al.* (1998). Recently, Knott *et al.* (1998) described the detection of a suggestive QTL affecting BFT in the same region on SSC2 as the QTL in this study. Geldermann *et al.* (1996) reported highly significant effects on carcass traits for a region on SSC6, which contains the mutation that causes halothane susceptibility (Houde *et al.* 1993). The suggestive QTL detected on SSC6 both map to the last marker interval, which is ~70 cM away from the halothane susceptibility locus. In the present study this *Ryr* locus is located in the interval between Sw1057 and S0220. Because the experimental population was screened against that mutation and found to be negative, it was not expected to find effects of the halothane locus in this study (Janss *et al.* 1997a).

Rohrer and Keele (1998) reported the detection of QTL affecting fatness traits in a Meishan × White backcross. They detected a significant QTL affecting BFT on SSC1 in the same area where the present study detected a strongly suggestive QTL affecting BFT. They also detected a significant QTL affecting BFT on SSC7 in a similar region to that reported here.

Backfat and SSC7: SSC7 harbors the swine lymphocyte antigen (SLA) complex, the major histocompatibility complex of the *Sus scrofa* species. According to Rohrer *et al.* (1996), its position is between marker S0064 and S102 in the present study. Vaiman *et al.* (1988) presented a review of many studies concerning possible associations between SLA polymorphism and immunology, production, and reproduction traits. With regard

to BFT they reported effects between −2.23 and +3.7 mm backfat for specific SLA haplotypes. The QTL affecting BFT around the SLA region has been confirmed in several crosses between Meishan and commercial breeds (Rothschild *et al.* 1995; Milan *et al.* 1998; Moser *et al.* 1998).

Moser *et al.* (1998) and Rohrer and Keele (1998) also reported that for the QTL on SSC7, the allele with the higher BFT originates from the Western breed and not from the Meishan pigs. This suggests that although there has been strong selection against high BFT, there are still cryptic alleles segregating in the Dutch lines that increase BFT. An explanation for this could be that the alleles are recessive and can therefore remain at a reasonable frequency in the breeding stock. This does not agree with the mainly additive nature of the QTL effect (Table 2). Another explanation could be that the allele, although it is undesirable for BFT, might have a favorable effect on other production traits like growth and/or reproduction. Furthermore, the close linkage with, or possible direct effect of, the SLA complex might give rise to favorable fitness effects linked to or caused by the same alleles that cause higher BFT. The fact that the SLA region is associated with many production and health parameters in pigs would complicate the implementation of the QTL for selection against thick backfat within commercial lines.

Comparative mapping: The conservation of genomic regions between mammalian species can be exploited in two directions. First, molecular research in livestock species can benefit from the massive resources being allocated to human genome research. Establishment of direct links with regard to gene mapping, sequencing, and functional information via comparative mapping is very valuable, especially in the candidate gene approach (Carver and Stubbs 1997). On the other hand, livestock populations, as well as laboratory animals, offer the possibility to design specific experiments with large families that are unseen in human populations. In this context pigs might be a more promising animal model for human genetic research compared to mice due to higher genetic conservation between human and pig (Johansson *et al.* 1995), with much less genomic rearrangements than the rodent chromosomes (Graves 1996).

Goureau *et al.* (1996) determined this correspondence between the human and the porcine genome by bidirectional chromosomal painting. At present, 97% of the total length of the porcine genome matches with the humane genome. Using the comparative map of Goureau *et al.* (1996), the region on SSC7, which harbors the QTL affecting BFT, has its human homologues on HSA 6 or HSA 15. An important chromosomal region on HSA 6 is the TNFα locus, for which Norman *et al.* (1997) found linkage with obesity in Pima Indians. On the porcine genome, TNFα maps to the SLA region on SSC7, near the location of the QTL for BFT. The area on SSC2, where another QTL affecting BFT was detected, corresponds to HSA 11.

The regions identified for IMF in the porcine genome on SSC7 and SSC4 match HSA 14 and HSA 8, respectively. Three rodent studies report QTL for body mass and/or adiposity, which correspond to these regions on the human genome: two on HSA 8 (West *et al.* 1994; Gauguier *et al.* 1996) and one on HSA 14 (Warden *et al.* 1995). However, it is difficult to infer synteny between rodents and pigs on the basis of rodent-human and pig-human comparative maps.

Future research will be aimed at fine mapping of the regions of interest found in this experiment and positional comparative candidate gene analysis. Hopefully, this will eventually lead to the characterization and isolation of the genes of interest.

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