IDENTIFICATION OF (NON-) MENDELIAN FACTORS AFFECTING PORK PRODUCTION

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IDENTIFICATION OF (NON-) MENDELIAN FACTORS AFFECTING PORK PRODUCTION

Dirk-Jan de Koning

Proefschrift
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op gezag van de rector magnificus
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Dirk-Jan de Koning. Identification of (non-) Mendelian factors affecting pork production. This thesis describes the analysis of a large experiment in which Chinese Meishan pigs were crossed with pigs from Dutch commercial lines. Three generations of pigs from this experiment were characterized for molecular markers that cover the entire porcine genome. Linkage was studied between these markers and 17 carcass, meat quality, production, and reproduction traits. Among the Quantitative Trait Loci (QTL) that were identified, important effects of genomic imprinting were observed for body composition and growth traits. Genomic imprinting, where only one allele from a specific parent is expressed in the offspring, is generally regarded to be a rare phenomenon, affecting only 1-2 % of all the genes. Following the large imprinting effects that were identified in this study, the characteristics of imprinted genes and their detection were studied in an extensive simulation study. It was concluded that imprinted QTL might remain undetected when only standard Mendelian models are applied. However, extra care must be taken with the design and analysis of experiments to prevent the false detection of imprinting for QTL that are actually Mendelian. It was also demonstrated that the statistical power of a QTL mapping experiment can increase considerably by using identified QTL as cofactors in a multiple QTL analysis. Finally, an application of some of the identified QTL in a commercial pig-breeding program were proposed.

Stellingen

1. Genetische inprenting is een algemener fenomeen dan veelal aangenomen en verdient meer aandacht in de genetica.
   Dit proefschrift

2. Het correct onderscheiden van QTLs met Mendeliaanse en niet Mendeliaanse expressie bij niet-ingeteelde soorten stelt hoge eisen aan het ontwerp en de analyse van een experiment.
   Dit proefschrift

3. Gezien de algemene toepassing van meervoudige QTL analyses bij ingeteelde soorten en de relatieve eenvoud van de implementatie, is het verassend dat meervoudige QTL analyses bij landbouwhuisdieren nauwelijks gebruikt of onderzocht worden.
   R. C. Jansen (1993) Genetics135: 205-211, Dit proefschrift.

4. Bij claims over de rol van genen wordt vaak voorbijgegaan aan het feit dat QTL detectie gebaseerd is op statistische methoden.
   Vrij naar T. F.C Mackay (2001) Nature Reviews Genetics 2: 11-20

5. Als onderzoeker is het gemakkelijker om richtlijnen voor statistische analyse op te stellen, dan om deze consequent toe te passen.

6. Interdisciplinair onderzoek zal niet goed van de grond komen zolang de thematische op- en indeling van wetenschappelijke tijdschriften wordt gehandhaafd.

7. Verkeerd gebruik van de laser pointer bij presentaties leidt tot "presentatie karaoke" en is een grove onderschatting van de intelligentie van het publiek.

8. De mens is het enige dier wat aardig blijft voor zijn prooi, tot dat hij deze op gaat eten (naar Samuel Butler).

Stellingen bij het proefschrift van Dirk-Jan de Koning
"Identification of (non-) Mendelian factors affecting pork production"
Wageningen Universiteit, 7 september 2001

Voorwoord

Dit proefschrift is het resultaat van 4,5 jaar arbeid bij de Leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit. Het beschrijft de resultaten van een experiment waar al meer dan 10 jaar door heel veel mensen aan gewerkt is. Zonder hun inzet was dit proefschrift nooit tot stand gekomen

Johan, ik wil je hartelijk bedanken voor de manier waarop jij mij al die jaren hebt begeleidt. De vrijheid die je mij gaf, gekoppeld aan jouw enthousiasme en waardevolle ideeën, hebben in belangrijke mate bijgedragen aan de kwaliteit van dit proefschrift. Ook Pim, mijn tweede promotor, wil ik hartelijk bedanken. Ondanks je drukke werkzaamheden als hoogleraar-directeur wist je toch altijd de essentie op te pikken en relevante suggesties aan te dragen. Luc, dankzij jouw bijdrage aan het experiment en de eerste begeleiding heb ik echt een vliegende start kunnen maken. Ook je betrokkenheid als lid van de gebruikerscommissie heeft bijgedragen aan de invulling van het onderzoek. Ook Henk Bovenhuis wil ik bedanken voor de prettige samenwerking, met name tijdens de afronding van het project. De enorme hoeveelheid genotyperingen is uitgevoerd onder leiding van Martien Groenen, die ik wil bedanken voor zijn inzet voor de moleculaire kant van het onderzoek. Piet de Groot, Pieter van Oers en Beja de Vries wil ik met naam noemen vanwege hun leeuwenaandeel in de praktische werkzaamheden, zonder daarbij te kort te willen doen aan de andere assistenten die hebben bijgedragen aan het slagen van dit experiment.

Het PIGQTL team werd na een half jaar aangevuld met een oio die de uitdaging werd voorgelegd om de genen achter "mijn" QTLs te vinden. Annemieke, ik heb je leren kennen als een zeer waardevolle collega, die altijd bereid was mij moleculair op de hoogte te houden. Het regelmatige bijkletsen over het onderzoek en andere zaken heb ik erg gewaardeerd. Vervolgens arriveerde Barbara, die in het kader van het Europese FATQTL project bij de leerstoelgroep heeft gewerkt. Jouw onderzoekservaring met varkens en fenomenale literatuurkennis kwamen erg goed van pas. Alle collegae bij de leerstoelgroep wil ik bedanken voor de prettige werksfeer, met een extra pluim voor de secretaresses en het computer ondersteunend personeel.

Mijn onderzoek werd gefinancierd door de Stichting Technische Wetenschappen (STW) van het NWO, die ik daarvoor zeer erkentelijk ben. Ook een woord van dank aan het PVE en de fokkerijinstellingen Hypor BV, Dumeco breeding en Topigs, voor hun financiële bijdrage en deelname aan de gebruikerscommissie in de personen Jan Merks, Gerard Albers en Konrad Broekman. Ook Bernard van Oost wil ik bedanken voor zijn inbreng in de gebruikerscommissie.

A special word of thanks to all the visitors who added some international flavour to our group. I would also like to acknowledge my former colleagues of the MTT Agrifood Research Finland for always providing me with a warm welcome whenever we visit Finland. Also the many colleagues that I have met during international meetings are gratefully acknowledged for fruitful discussions.

Ook een woord van dank aan mijn familie. Pa en moe, het was voor jullie even wennen dat "de jongste" ging studeren en promoveren. Evengoed kon ik wel altijd op jullie steun rekenen, bedankt daarvoor. Marieke, bedankt voor de prachtige omslagillustratie! Voor alle broers, zussen en aanhang: "Het studentje" is nu klaar en kan dus "echt aan het werk"! Jullie nuchterheid heeft mij in ieder geval met beide benen op de grond gehouden.

Lieve Tytti, jij weet als geen ander hoe het werk mijn humeur kan beïnvloeden. Bedankt voor je steun in al die jaren. Ik zal proberen in toekomst breder te kijken dan alleen mijn werk en ik beloof dat ik nu echt de Finse taal ga leren...

CONTENTS

Introduction

In livestock, many traits of commercial interest show a continuous distribution as the results of combined (inter-) action of many genetic and environmental factors. Most quantitative genetic theory is based on the assumption that the genetic component of these traits consists of many genes with an infinitesimal effect. However, with the advancement of molecular and statistical tools during the last decade, it has been demonstrated that also for these quantitative traits, individual gene effects can be detected. The term quantitative trait locus (QTL) was coined to describe chromosomal regions that affect a continuous trait, but where the actual gene is unknown.

Genetic effects can be tested by direct association between a marker or a candidate gene and the trait of interest or by studying linkage between markers and the trait of interest. Early molecular studies in livestock consisted mainly of direct associations between blood group polymorphisms or MHC serotypes and traits (OSTERGAARD *et al.*, 1989). The introduction of the polymerase chain reaction (PCR) and the development of highly informative and abundant microsatellite markers have provided dense marker maps for most livestock species[1]. QTL experiments in livestock can either be carried out with experimental crosses between genetically divergent breeds or by using the pedigree structure of the commercial population. Experimental crosses often have higher power to detect QTL, but QTL that are detected within commercial populations can be implemented directly in the breeding scheme.

For experimental crosses between inbred lines, LANDER AND BOTSTEIN (1989) described maximum likelihood methods to perform interval mapping, after which HALEY AND KNOTT (1992) developed a regression approach for the same purpose. HALEY *et al.* (1994) extended this methodology for the QTL analysis of experimental crosses between outbred lines. Their methods were successfully applied by ANDERSSON *et al.* (1994) on an experimental cross between Wild Boar and Large White pigs, which was not only the first genome scan in pigs, but also in livestock. The line cross analyses are most powerful to detect QTL that explain phenotypic differences between the two lines. However, there may also be an interest to find QTL that explain phenotypic differences within the original lines. For this purpose, half-sib analyses (GEORGES *et al.*, 1995; KNOTT *et al.*,1996) can be very useful.

ANDERSSON (2001) and KIM AND PARK (2001) provide a general overview of gene

[1] For an overview see http://www.thearkdb.org/

detection experiments and methodology in livestock species.

The incentive for QTL detection is not only the possibility for faster genetic progress by using Marker Assisted Selection (MAS), but also to elucidate the genetic background of traits that are in the breeding goal. Therefore the QTL detection procedure should include also a characterization of the QTL. QTL that are detected under a Mendelian model, can be actually Mendelian, but can also show different modes of expression. One example is genomic imprinting, where only the allele originating from the parent of a specific sex, is expressed in the offspring, and contributes to the trait of interest. Also QTL that are on the X chromosome need custom-made models for both their detection as well as their implementation in a breeding program.

This thesis describes the analyses of the Wageningen Meishan experiment for QTL affecting a wide range of production, reproduction, and meat quality traits. The Wageningen Meishan experiment was initially established to investigate the possibilities of introgression of Meishan genes into the Dutch commercial pig lines (JANSS, 1996). The segregation analyses described by JANSS *et al.* (1997a, b) indicated that this experimental population was a promising resource for QTL detection. With close to 1200 F_2 animals, this is the largest QTL experiment in pigs. The large number of F_2 animals, together with > 300 F_1 parents, made the molecular typing for >130 microsatellite markers a formidable task.

Aim and outline of this thesis

The aim of this thesis is to perform QTL analyses on the data from the Meishan experiment, under different genetic models. The analyses are mainly based on standard methodology (HALEY *et al.*, 1994, KNOTT *et al.*, 1996), but extensions to test for genomic imprinting and include unlinked QTL as cofactors are proposed and evaluated. Regression methods are used throughout because of their computational speed and straightforward interpretation of QTL results. Chapter 2 describes the first QTL analyses on 418 animals of the F_2 population for intramuscular fat content and backfat thickness under half-sib and line-cross models. Chapter 3 describes the results of an imprinting analysis for intramuscular fat content, backfat thickness, and muscle depth. A comprehensive QTL analysis of the other meat quality traits is described in Chapter 4. Chapter 5 describes the QTL that were detected for the production and reproduction traits. In Chapter 6, some theoretical aspects of imprinted QTL are described, followed by the results of an extensive simulation study on the detection of imprinted and Mendelian QTL in outbred F_2 designs. Chapter 7 describes a strategy to perform multiple QTL analyses in outbred half-sib designs with an example on dairy cattle. The first of two discussion chapters (Chapter 8) describes an application of the multiple QTL models to a line-cross design with different genetic models for intramuscular fat content and backfat thickness. Subsequently, a permutation approach to test for multiple linked QTL is

introduced. Using all identified QTL, it is evaluated whether the joint QTL effects can account for the major gene effects, described by JANSS *et al.* (1997b).

A possible implementation of the detected QTL in a commercial breeding program is described in Chapter 9, with special emphasis on the unique opportunities offered by imprinted and X-linked QTL.

This Chapter also gives an overview of the imprinted QTL that were detected in the Wageningen Meishan experiment.

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The Pig, if I am not mistaken,
Supplies us sausage, ham, and Bacon.
Let others say his heart is big,
I think it stupid of the Pig.

Ogden Nash, "The Pig"

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

Dirk-Jan de Koning, Luc L.G. Janss, Annemieke P. Rattink, Pieter A.M. van Oers, Beja J. de Vries, Martien A.M. Groenen, Jan J. van der Poel, Piet N. de Groot, E.W. (Pim) Brascamp and Johan A.M. van Arendonk

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. 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 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 X 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).

The present study describes the molecular typing of the crossbred pig population and the subsequent association study to locate QTL that affect intramuscular fat content and backfat thickness. 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. 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.

Material

The Meishan x 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, 1997b). The experiment was designed for the detection of major genes based on phenotypic data. Blood samples were stored in order 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 which 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 five animals from each of the 264 F_2 litters to provide DNA for molecular typing. From these litters about 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 approximately 90 kg of live weight. On 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 had observations 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) intramuscular fat content (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 *M. Longissimus* by petroleum ether extraction (HOVENIER *et al.* 1992), 24 hours after slaughter.

Consumers' demands for lean pork meat have resulted in selection against high backfat thickness (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.

Methods

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 ten markers on SSC1 and two on SSC18. DNA was isolated from blood samples or spleen tissue samples using the PUREGENE® DNA Isolation Kit (Gentra Systems, Inc. USA). 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, USA).

Fragment length of the PCR products was determined with GENESCAN® software (ABI, Perkin Elmer, USA) and marker genotypes were assigned to the animals using GENOTYPER® software (ABI, Perkin Elmer, USA). 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 was used in this step (n=844). The phenotypic data were analyzed assuming a polygenic inheritance model containing non-genetic 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:

$$y = X\beta + Zu + e \qquad (1)$$

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 non-genetic effects estimated under model (1):

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

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 2,000 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. 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, based on 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 cM across the genome the following model is fitted:

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

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 effect 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 are described by Haley *et al.* (1994) and applications to crossbred pig populations are numerous (e.g. ANDERSSON *et al.* 1994, MOSER *et al.* 1998, KNOTT *et al.* 1998).

Half-sib model: The F_2 animals are divided into 19 paternal half-sib groups. Within each group there are six to eight 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 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 centiMorgan (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:

$$y^*_j = a_i + b_i x_{ij} + e_{ij} \qquad (4)$$

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 chromosome-wise 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. Thirdly; the genome-wise significance level is

Table 1. Overall and sex-specific characteristics of the raw measurements for backfat thickness (in mm) and intramuscular fat content (in %)

	Backfat thickness	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

used, which takes account of testing the whole autosomal genome:

$$P_{\text{genome-wise}} = 1-(1-P_{\text{chromosome-wise}})^{1/t} \quad (5)$$

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. 10,000 permutations were sufficient to estimate chromosome-wise 5%, 1% and 0.1% significance thresholds. To estimate smaller risk levels the number of permutations was extended to 50,000.

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 approximately 17 cM.

QTL analysis: An overview of the phenotypic characteristics of the two traits is given in Table 1. The estimated heritabilities

Table 2. Estimated QTL effects under line cross model

Chromosome	Additive effect[a]	SE	Dominance effect[b]	SE
		Backfat thickness (mm)		
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

[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.

were 0.24 and 0.35 for BFT and IMF, respectively

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

The half-sib interval mapping procedure showed genome-wide evidence for a QTL on SSC7 and strong suggestive evidence for a QTL on SSC2 ($P_{\text{genome-wide}}$ ~0.09). Figure 1 shows the development of the test statistic 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 genome-wide 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

Table 3. Overview of estimated QTL effects within families for Backfat Thickness with regard to SSC7

Family	Overall[1a]		Individual families		
	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

[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 mm. The sign of the estimated effect is conditional on the arbitrary assignment of the first parental haplotype and therefore omitted. *, ** and ***denote significance of $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively, based on tabulated values.

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). In Table 4 all QTL that exceeded the level of suggestive linkage in any of the analyses are summarized.

QTL effects for BFT: Under the line cross model the additive and dominance effect of a QTL is 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 of an additive nature. The QTL affecting BFT on SSC1 and SSC6 have a large dominance component (Table 2) which points towards 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 approximately 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. 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 around 6.7 mm (~1.4 S.D.).

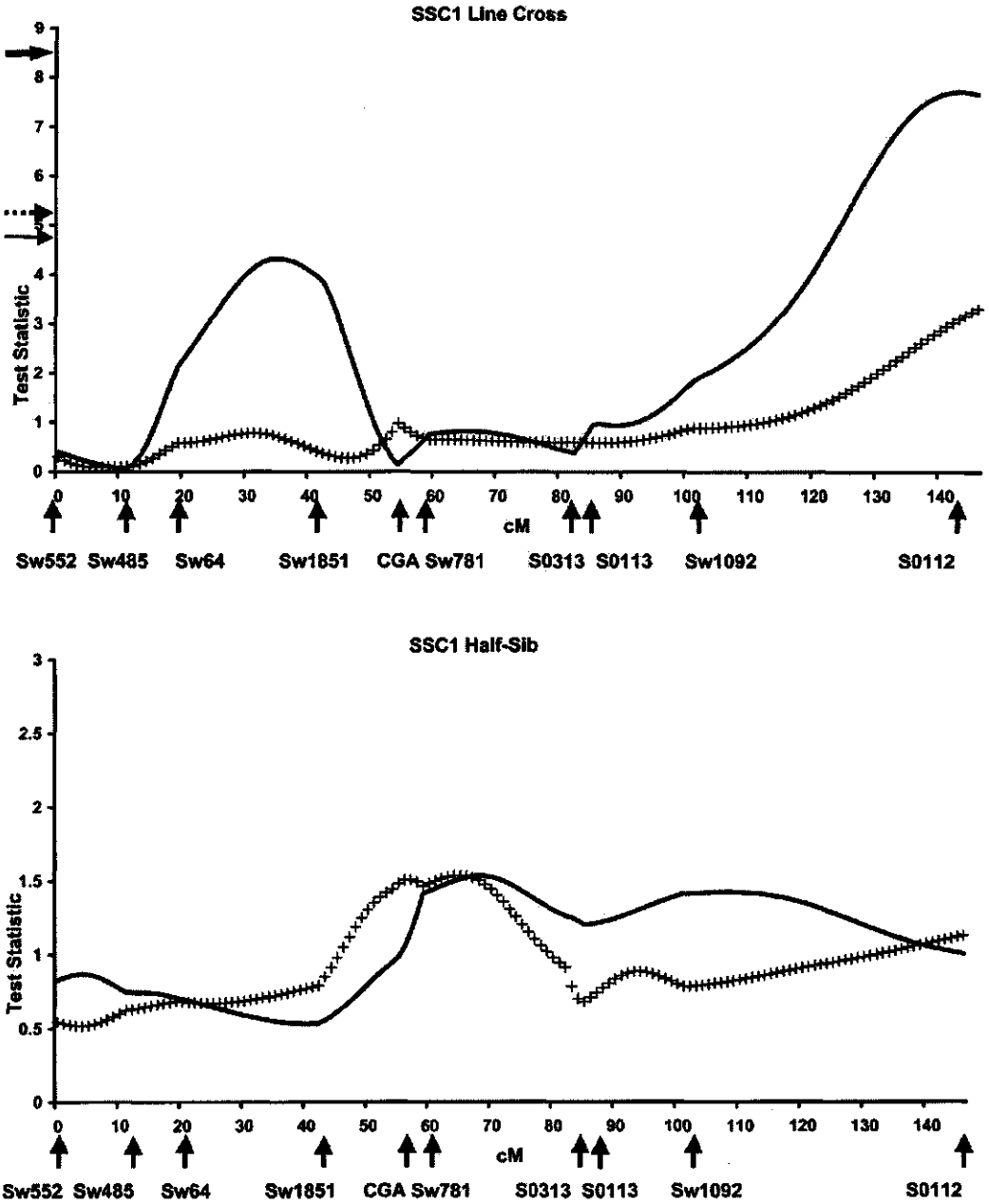
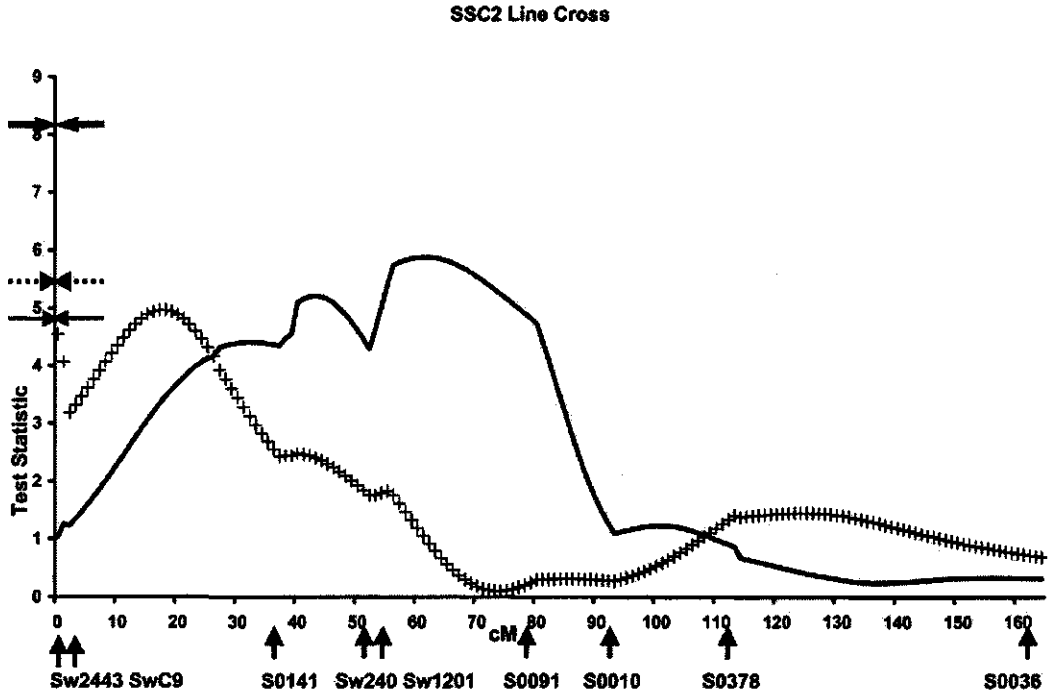


Figure 1 Test Statistic for five chromosomes with regard to BFT and IMF under two models. The solid curve describes the test statistics for BFT and the +++-curve describes the test statistic for IMF. Arrows on the X-axis indicate marker positions and names. Arrows on the Y-axis represent the three thresholds: suggestive (thin arrow), chromosome-wise 5% (dashed arrow) and genome-wise 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.

For some families the best 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



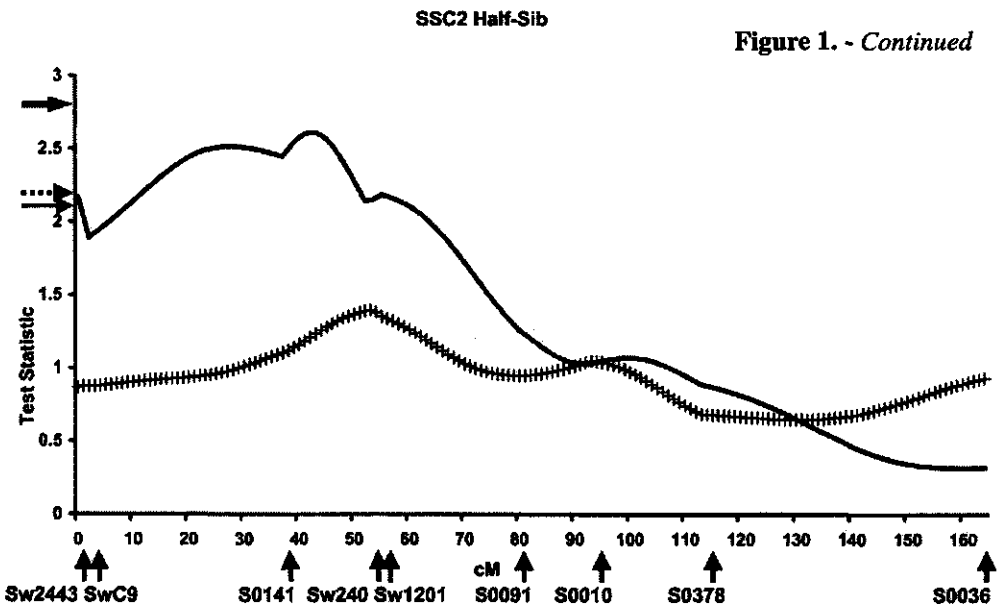
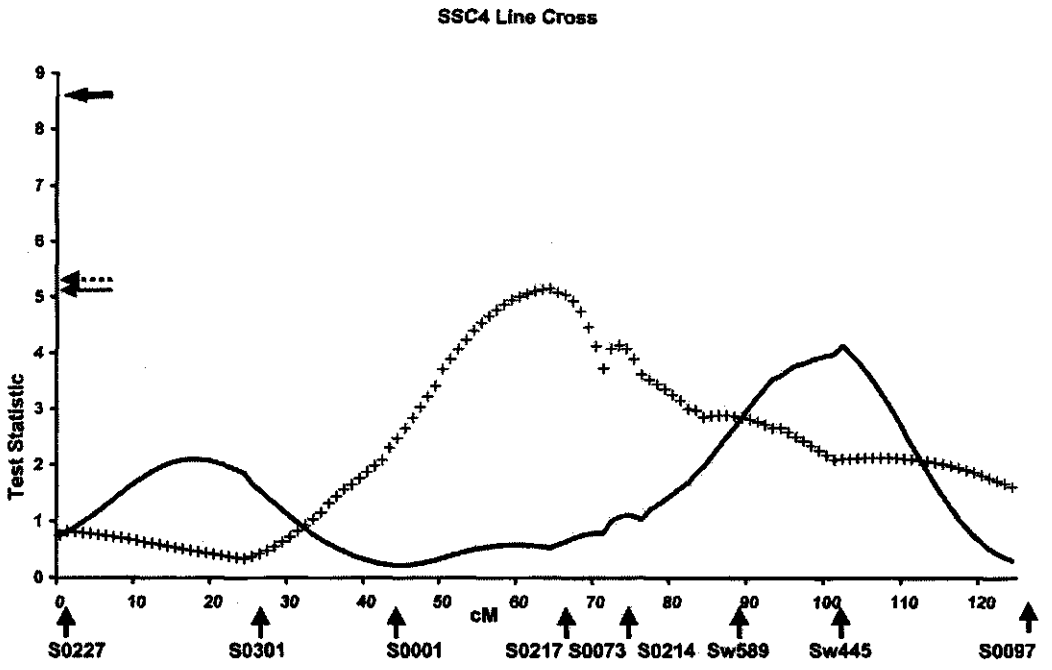


Figure 1. - *Continued*

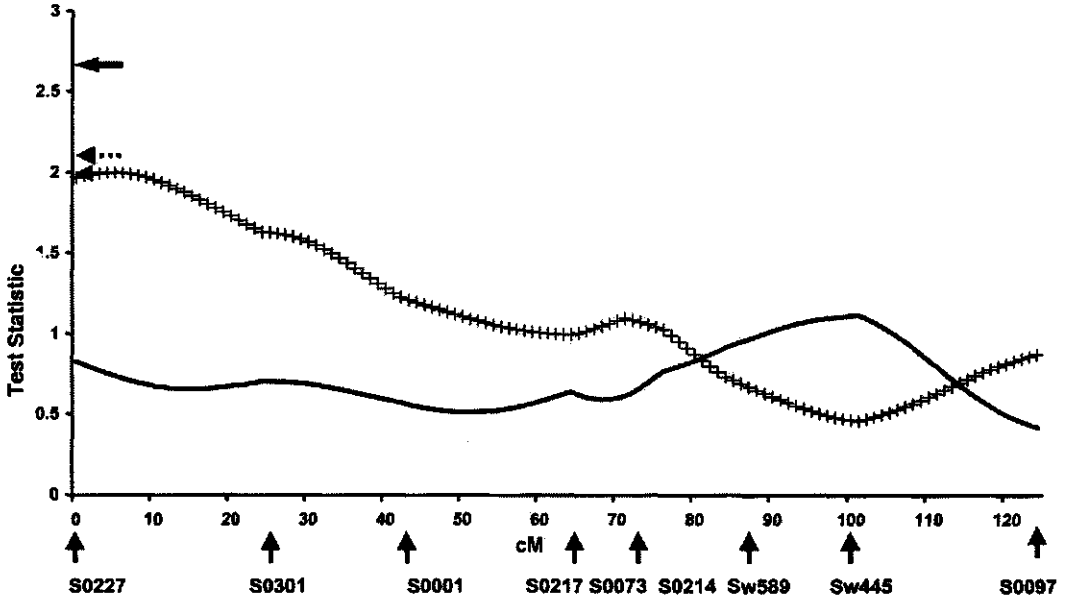
SSC7 in the half-sib analysis (Figure 1).

QTL effects for IMF: The estimated effects of the suggestive QTL that were detected on SSC2, SSC4 and SSC6 in the line cross analysis

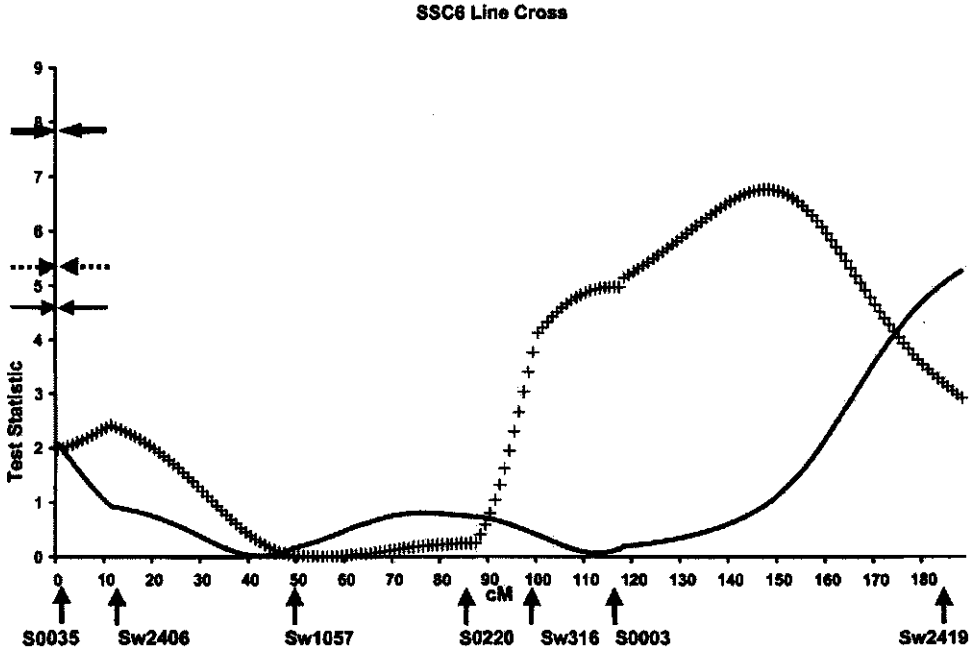


SSC4 Half-Sib

Figure 1. - *Continued*



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.



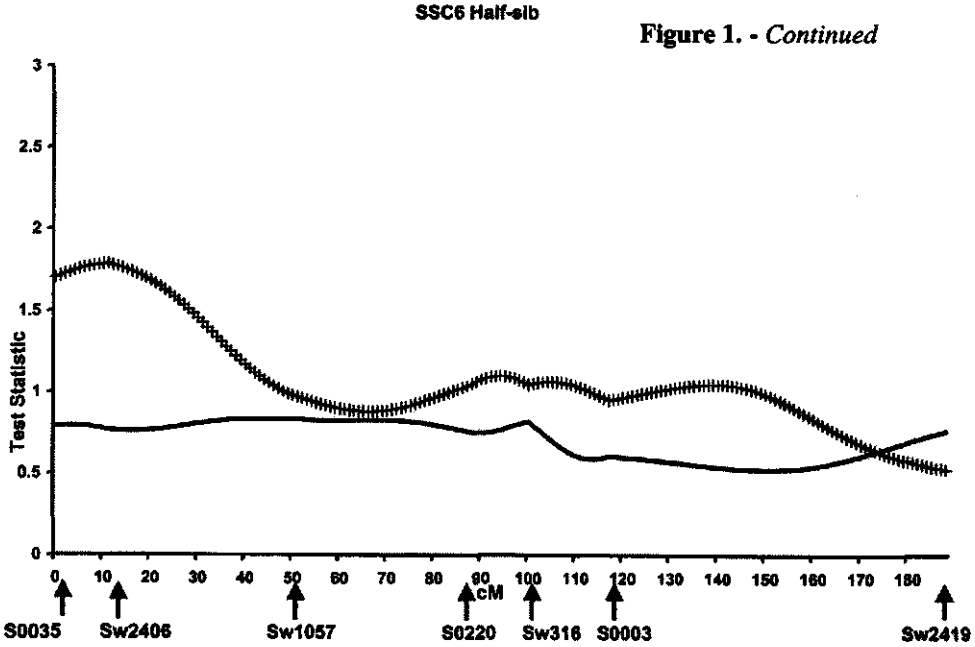
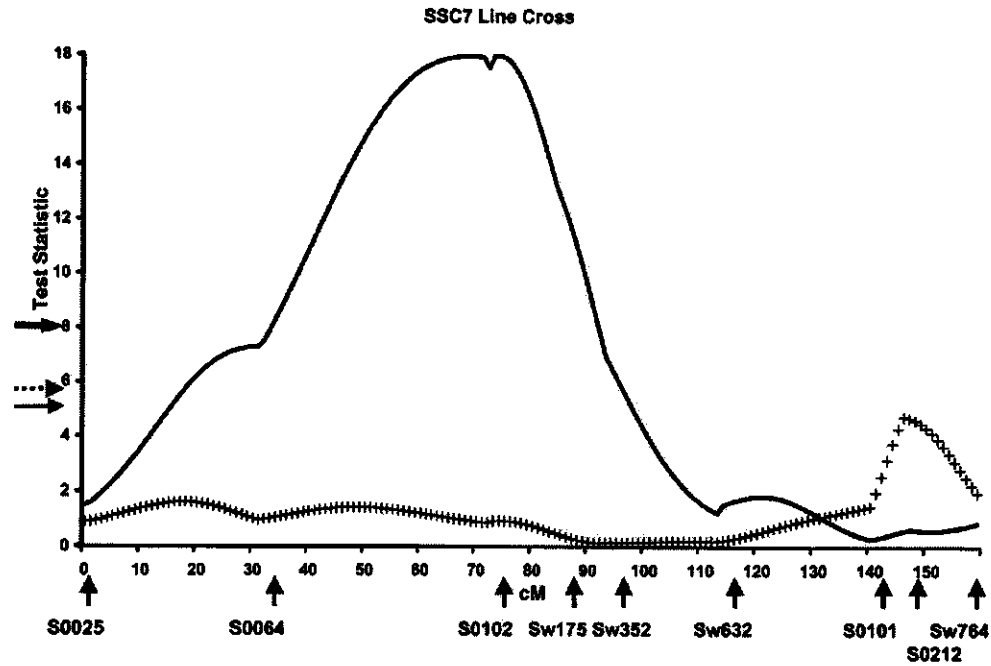


Figure 1. - *Continued*

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 effects within these families



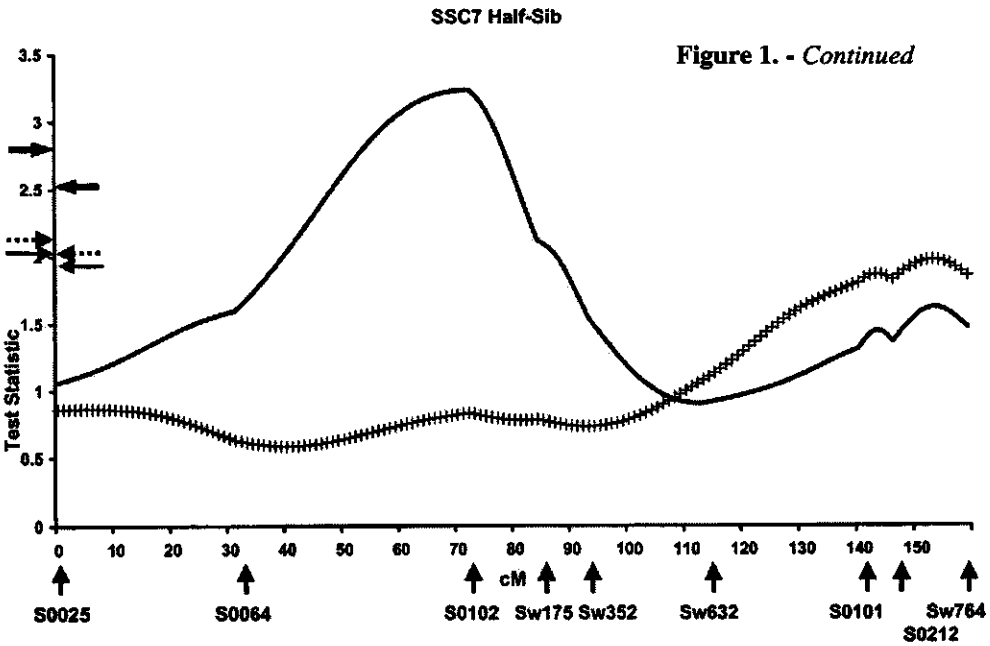


Figure 1. - *Continued*

at their individual best position 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 backfat thickness could be traced back to the Meishan grandparents in all but one of the families that were segregating for this QTL. This would suggest that this higher allele might be absent or very rare in the purebred Dutch lines. In all 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 intramuscular fat content. 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 would represent the single gene for that trait the test statistic for that QTL would diminish if the data was 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 pre-adjusted 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. It was tested with a standard F test whether the best two QTL on a chromosome explained significantly more variance that 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 be overdominance (Table 2). The finding of completely dominant or overdominant QTL alleles gives rise to the question whether these are true effects of single genes or whether they

Table 4 Most likely positions for QTL affecting Backfat thickness or Intramuscular fat content under two genetic models

SSC	Founder lines fixed for different QTL alleles			No assumptions about QTL alleles and frequency		
	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]	S0102 (73)	3.23	0.006
			Intramuscular fat content			
2	Swc9-S0141 (19)	4.97	0.61^{s}	Sw240-Sw1201 (54)	1.39	NS
4	S0217 (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 chromosome-wise and suggestive significance, respectively; NS, not significant (not exceeding suggestive or chromosome-wise significance).

[a] The genome-wise *P* value.

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

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, 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 since only part of the F_1 families are inferred as heterozygous 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 bi-allelic 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 upwards since 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 to 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 since thresholds were determined empirically. Although programs for simultaneous estimation of non-genetic, 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 genome-wide 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 towards 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 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). Since DE KONING *et al.* (1998) performed only single marker comparisons this difference might well be explained by difference in marker information.

Comparison to other studies: This is the first study that describes a genome-wide scan for QTL affecting intramuscular fat content.

This study did not confirm the existence of a QTL affecting backfat thickness on SSC4 that was identified by ANDERSSON *et al.* (1994) and confirmed by WALLING *et al.* (1998). Recently, KNOTT *et al.* (1998) describe the detection of a suggestive QTL affecting BFT in the same region on SSC2 as the QTL in this study. GELDERMANN *et al.* (1996) report 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. Since 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) report the detection of QTL affecting fatness traits in a Meishan x 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 as reported here.

Backfat and SSC7: SSC7 harbors the Swine Lymphocyte antigen (SLA) complex, the major histocompatibility complex (MHC) 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) present a review of many studies concerning possible associations between SLA polymorphism and immunology, production and reproduction traits. With regard to backfat thickness they report 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 and MOSER *et al.* 1998).

MOSER *et al.* (1998) and ROHRER and KEELE (1998) also report that for the QTL on SSC7 the allele with the higher backfat thickness

originates from the western breed and not from the Meishan pigs. This suggests that although there has been strong selection against high backfat thickness 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 backfat thickness. 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. Firstly, the 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 are 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 model animal for human genetic research compared to mice due to higher genetic conservation between human and pigs (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 bi-directional chromosomal painting. So far, 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.* (1995) 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 to 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 (GAUGUIER *et al.* 1996, WEST *et al.* 1994) 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.

Further 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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Genome-wide Scan for Body Composition in Pigs Reveals Important Role of Imprinting

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Abstract – The role of imprinting in body composition was investigated in an experimental cross between Chinese Meishan pigs and commercial Dutch pigs. A whole genome scan revealed significant evidence for five quantitative trait loci (QTL) affecting body composition, of which four were imprinted. Imprinting was tested with a statistical model that separated the expression of paternally and maternally inherited alleles. For back fat thickness, a paternally expressed QTL was found on *Sus scrofa* chromosome 2 (SSC2), and a Mendelian expressed QTL was found on SSC7. In the same region of SSC7 a maternally expressed QTL affecting muscle depth was found. Chromosome 6 harbored a maternally expressed QTL on the short arm and a paternally expressed QTL on the long arm, both affecting intramuscular fat content. The individual QTL explained from 2% up to 10% of the phenotypic variance. The known homologies to human and mouse did not reveal positional candidate genes. This study demonstrates that testing for imprinting should become a standard procedure to unravel the genetic control of multifactorial traits.

It is well established that quantitative trait loci (QTL) underlying the genetic variance of multifactorial traits can be mapped in experimental as well as outbred populations (1, 2). Whole-genome scans have revealed a number of genomic regions contributing to genetic variation and have provided insight into the form of gene action. The genome scans can also be used to search for non-Mendelian forms of expression (3) but these opportunities have not been exploited systematically. Knowledge of mode of inheritance of identified QTL is important for medical and agricultural applications.

Parental genomes undergo modifications during gametogenesis, resulting, for some genes, in parent-of-origin-specific expression in the offspring. This phenomenon of genomic imprinting, as a form of epigenetic gene regulation, has been shown to influence several sub-chromosomal areas in mammals (4). In human and mouse, most imprinted genes are arranged in chromosomal clusters[1] and their linked organization suggests coordinated mechanisms controlling imprinting and gene expression (5, 6). It is generally viewed that imprinting is involved in fetal growth and brain development (7).

Different approaches have been used over time to identify imprinted areas in the genome. Both Robertsonian and reciprocal

[1] A World Wide Web Site is provided by C.V. Beechey, B. M. Cattanach, R. L. Selley, MRC Mammalian Genetics Unit, Harwell, Oxfordshire. http://www.mgu.har.mrc.ac.uk/imprinting/imptables.html

translocations resulting in mice with uniparental disomy for portions of the genome have been used to identify imprinted regions on six chromosomes (8). Furthermore chromosomal anomalies associated with imprinted diseases in humans helped to identify imprinted genes and to narrow regions of interest (9, 10). More recently, molecular genetic approaches taking advantage of, for example, methylation patterns observed for imprinted genes, have been used to isolate imprinted genes (11-14). The number of known genes is increasing rapidly, but imprinting has been reported only for about 30 (8). In livestock, evidence for imprinting was found for one specific chromosomal region in sheep and one in pigs (15-17). Imprinting effects, however, have not been studied systematically for multifactorial traits. We present results of a genome-wide approach to detect imprinted regions for multifactorial traits in an experimental cross of pigs.

Material and Methods

Experimental population. Boars from the Chinese Meishan pig breed were crossed with sows from commercial Dutch pig lines. From the resulting F_1, randomly selected boars and sows were mated to create the F_2 population (18). This experimental population facilitates the dissection of the genetics underlying phenotypic differences between these breeds for body composition traits. Meishan pigs are characterized by high fatness compared to Dutch pigs, which have been selected for lean growth for many generations. On 785 F_2 pigs we recorded three body composition traits after slaughter: back fat thickness and muscle depth measured between the third and fourth rib, and percentage of intramuscular fat inside the *Musculus longissimus* (18). The phenotypic mean (± SD) of the F_2 population was 22.0 (± 5.7) mm for back fat thickness, 40.6 (± 6.7) mm for muscle depth and 1.84 (± 0.87) % for intramuscular fat content (18). Assuming Mendelian expression, analyses for back fat thickness and intramuscular fat content on part of this population revealed significant evidence for QTL on chromosome 2 and on chromosome 7 affecting back fat thickness (19).

Genotyping and statistical analyses. A whole-genome scan including a test for imprinting was used to map autosomal QTL on the F_2 population. Genotypes were obtained for 132 microsatellite markers, covering more than 90% of the porcine genome, which were selected after testing many markers on the individual Meishan grandfathers and DNA pools of the grandmother lines (19). Genotypes were obtained for the F_2 animals, their F_1 parents, and the purebred Meishan grandparents.

The statistical analyses were based on the line cross concept (20), where original breeds are assumed homozygous for different QTL alleles but can have marker alleles in common. Extension of this model to test for imprinting has been suggested (3) and used in the analysis of the *IGF2* region in pigs (17). Analysis with this model, however, provided evidence for imprinting but a separate test was

needed to infer paternal or maternal expression. The model for imprinting (3), therefore was re-parameterized to enable a direct test for the contribution of the paternally and maternally inherited effect. For every F_2 individual we inferred the probabilities of inheriting two Meishan alleles (P_{11}), two Dutch alleles (P_{22}), or one from each line (P_{12} or P_{21}, different subscripts according to parental origin; first subscript is paternally inherited allele) at 1-centimorgan (cM) intervals across the genome. Using multiple marker information for a given location in the genome, we calculated the probability of the two alleles in an offspring corresponding to any of the four possible combinations (3, 20). The probabilities are functions of the recombination rates between the location under consideration and the flanking informative markers, which may vary from progeny to progeny depending on the genotype of the F_1 parents and the Meishan grandparents. Under the traditional line cross approach, an additive effect (a) and a dominance effect (d) are estimated using the regression of the phenotypes on $P_a = P_{11} - P_{22}$ and $P_d = P_{12} + P_{21}$. To separate the contribution of the parents, we introduced the probability that the individual inherited a Meishan allele from its father ($P_{pat} = [P_{11} + P_{12}] - [P_{22} + P_{21}]$) or from its mother ($P_{mat} = [P_{11} + P_{21}] - [P_{22} + P_{12}]$). A saturated model, which included a paternal (P_{pat}), a maternal (P_{mat}) and a dominance component (P_d), was fitted at 1-cM intervals across the genome. For each position of a QTL, the mode of inheritance of the QTL was inferred based on the contribution of each of the three components. The contribution of a component was measured by the reduction in total sum of squares caused by incorporating that component in the model after fitting the other components. The F statistic was used to evaluate the significance of each component. This evaluation facilitated discrimination between QTL showing exclusive paternal expression, exclusive maternal expression or Mendelian expression.

Significance thresholds and confidence intervals. For the inferred genetic models the significance thresholds and the confidence intervals of the QTL position were determined empirically. The significance threshold was set at the 5% genome-wise risk level (21). This threshold accounted for testing the entire genome but not for testing multiple traits. These thresholds were determined by permutation with at least 10,000 replicates (19).

Empirical confidence intervals for the QTL position were obtained by bootstrapping the data followed by analysis of the replicates under the inferred genetic model. From each of 10,000 bootstrap replicates, the best test statistic was stored. The 95% cut-off point of the sorted (in descending order) test statistics provided an empirical threshold to define the boundaries of the confidence interval. This method is an alternative to other bootstrapping strategies in which QTL positions of the replicates are sorted to determine an empirical confidence interval (3). The method used here allows for non-continuous confidence

Table 1. Genetic model for QTL affecting three body composition raits.

Location	F ratio* Paternal effect	Maternal effect	Dominance	Inferred Genetic model	QTL effect§
Backfat thickness (mm)					
SSC2, 36 cM	24.07†	2.85	0.51	Paternal expression	0.95 (0.20)
SSC7, 57 cM	30.27†	49.35†	0.04	Mendelian expression	–2.30 (0.25)
Muscle depth (mm)					
SSC7, 56 cM	4.74	50.33†	2.20	Maternal expression	-1.69 (0.24)
Intramuscular fat content (%)					
SSC6, 23 cM	0.07	14.53†	0.00	Maternal expression	0.14 (0.04)
SSC6, 117 cM	14.71†	1.34	0.31	Paternal expression	-0.13 (0.03)

* Partial *F* ratios for the individual components of a model including a paternal, maternal and dominance component at the most likely position of the QTL. † $p < 0.0001$ ‡ Empirical confidence intervals obtained by bootstrapping for the relevant model. § Estimates of QTL effects for the inferred genetic model. The additive effect (Mendelian expression) and the paternal or maternal effect (imprinting) are expressed as the deviation of the Meishan allele. Standard errors of the estimates are in parentheses.

intervals and is closer to the traditional logarithm of odds drop-off methods.

Results

Our genome scan resulted in five significant QTL affecting body composition traits, of which four were imprinted. For back fat thickness, there was strong evidence for a paternally expressed QTL on *Sus scrofa* chromosome 2 (SSC2, Table 1). For the QTL affecting back fat thickness on SSC7, both the paternal and maternal component were highly significant implying Mendelian expression for this QTL. For muscle depth, a highly significant QTL mapped to the same area as the QTL for back fat thickness on SSC7. In contrast to the QTL for back fat thickness, the QTL for muscle depth was maternally expressed (Table 1). From these results, it cannot be determined whether there are two linked loci or one locus with pleiotropic effects that shows imprinting during one stage of development and Mendelian expression during another.

With a model ignoring imprinting, suggestive evidence for a Mendelian QTL for intramuscular fat content was reported on the long arm of SSC6 (19). The present analysis, however, revealed that this effect was caused by a significant paternally expressed QTL (Table 1). In addition, a maternally expressed QTL affecting the same trait was found on the short arm of the same chromosome. The phenotypic variance explained by the individual QTL varied from 2% for the QTL affecting intramuscular fat content on SSC6, to 10% for the QTL affecting back fat thickness on SSC7.

A graphical comparison of results obtained under the imprinting and Mendelian models is in Fig 1. The imprinted QTL for back fat thickness on SSC2 maps 35 cM from the *IGF2* region, for which an imprinted QTL for

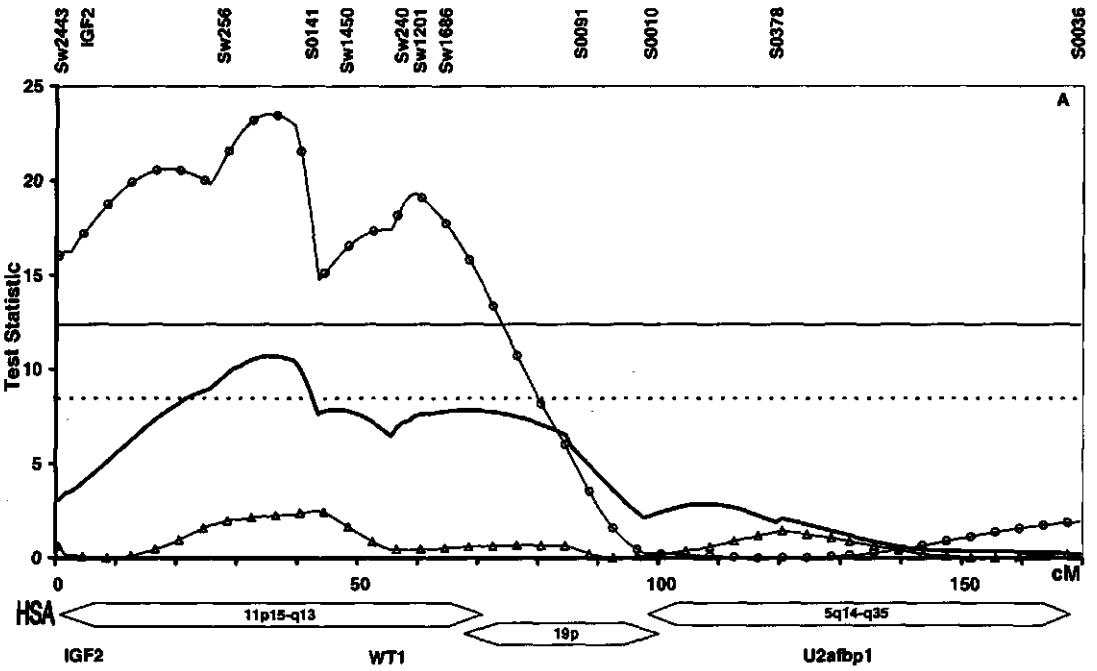

Fig. 1. Test statistic profiles for three porcine chromosomes that exhibit imprinting effects for one of the body composition traits: SSC2 and back fat thickness (**A**), SSC6 and intramuscular fat content (**B**), SSC7 and muscle depth (**C**), and SSC7 and back fat thickness (**D**). The black solid line represents the test statistic for a Mendelian QTL vs. no QTL. The circled line (-o-o-) represents the test statistic for a paternally expressed QTL vs. no QTL. The triangles line (-Δ-Δ-) represents the test statistic for a maternally expressed QTL vs. no QTL. The solid horizontal line denotes the 5% genome-wise threshold for the Mendelian model, and the dotted horizontal line indicates the same threshold for the imprinting models (thresholds for maternal and paternal expression were very similar and well within the sampling variance associated with permutation testing). Homologous regions in humans are indicated as bars (22-24, 26)[2]. Imprinted genes located within these human chromosomal areas are listed at the bottom (5,25).

muscularity and fat deposition has been reported (16, 17). Although the confidence interval does not exclude *IGF2* as a candidate gene, our results indicate that an additional imprinted QTL is present more proximal on this chromosome. The reported QTL in the *IGF2* region primarily controlled muscularity (16, 17) whereas in the present study we found no evidence for a QTL affecting muscle depth on SSC2. All three studies provided convincing evidence for a QTL, which rules out chance as a cause for the observed differences in affected traits between studies. The discrepancies, however, might very well be due to the differences in founder populations, in particular between the Piétrain, wild boar, and Meishan breeds. Also,

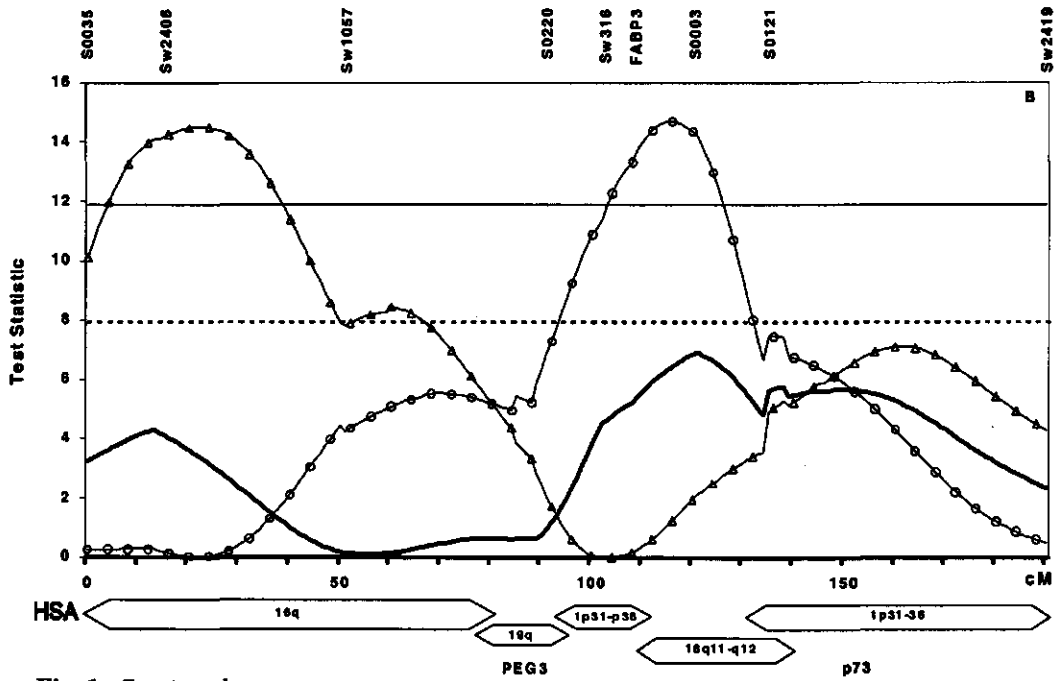

Fig. 1 -*Continued*

differences in age and weight at which carcass composition was measured may play a role.

The general outline of the comparative map between pig and human for the regions of interest has been established[2] by using bidirectional chromosome painting, a somatic cell hybrid panel and fluorescent in situ hybridization (refs. 22-24, Fig. 1). Genes that have been mapped more precisely in pigs, by linkage analysis or on the radiation hybrid panel (26), facilitated further refinement of the comparative map. We realize that the comparative map presented herein is not comprehensive and that some genes originating from other chromosomes are reported but not represented in Fig. 1.

QTL affecting body composition traits in pigs can have implications for obesity research in humans (20). Although several obesity-related disorders that are reported in humans and mice map to homologous regions of the imprinted QTL found in this study (27), imprinting has been reported only for the Prader-Willi Syndrome (HSA15q11.2-q12, refs. 9 and 10).

The QTL on SSC7 can be narrowed to a region homologous with HSA6p21.3-p22. This region contains the major histocompatibility complex, including *LTA*, and shows extensive conservation in gene order (28). Imprinted genes have not been reported for this region in humans or mice (5).

[2] The comparative map of the pig can be viewed at http://www.toulouse.inra.fr/lgc/pig/cyto/cyto.htm. Alignment of the porcine cytogenetic and linkage map is adapted from http://sol.marc.usda.gov/genome/swine/

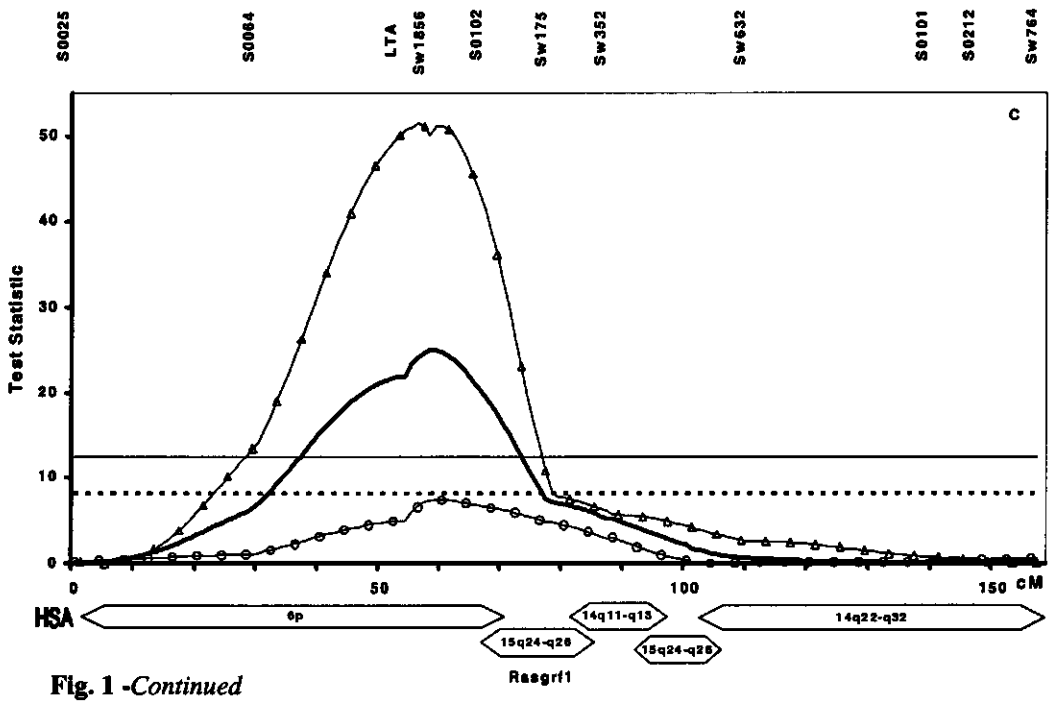

Fig. 1 -*Continued*

For the maternally expressed QTL affecting intramuscular fat content on SSC6p, several genes that map to the area are located on HSA16q22-ter. No imprinted genes have been reported for this region in humans. For the paternally expressed QTL affecting intramuscular fat content on SSC6q, candidate genes *MC5R* (29), *FABP3* (30) and *UOX* (26) map between markers SW316 and S0003. These genes are located on human chromosomes 18p11.2, 1p33-p32, and 1p22, respectively, and in humans, imprinting has not been reported for these regions. However, the confidence interval of this QTL extends on both sides to homologous regions in humans, where imprinted genes have been reported: *p73* on HSA1p36 and *PEG3* on HSA19q13.4 (imprinted only in mice).

For SSC2, imprinting is reported for the *IGF2* area, but until now homology to other imprinting clusters could not be established clearly. Data on imprinting of the Wilms Tumor gene 1 (*WT1*) on HSA11p13 are contradictory (5).

Discussion

The progress of the genome projects, in particular the large number of polymorphisms that have been characterized in many species, has boosted the search for genes involved in multifactorial traits such as obesity, diabetes, and schizophrenia. Genomic imprinting, however, is regarded to be a rare phenomenon and consequently is ignored in most studies. Our results indicate that genomic imprinting might be a more common phenomenon than

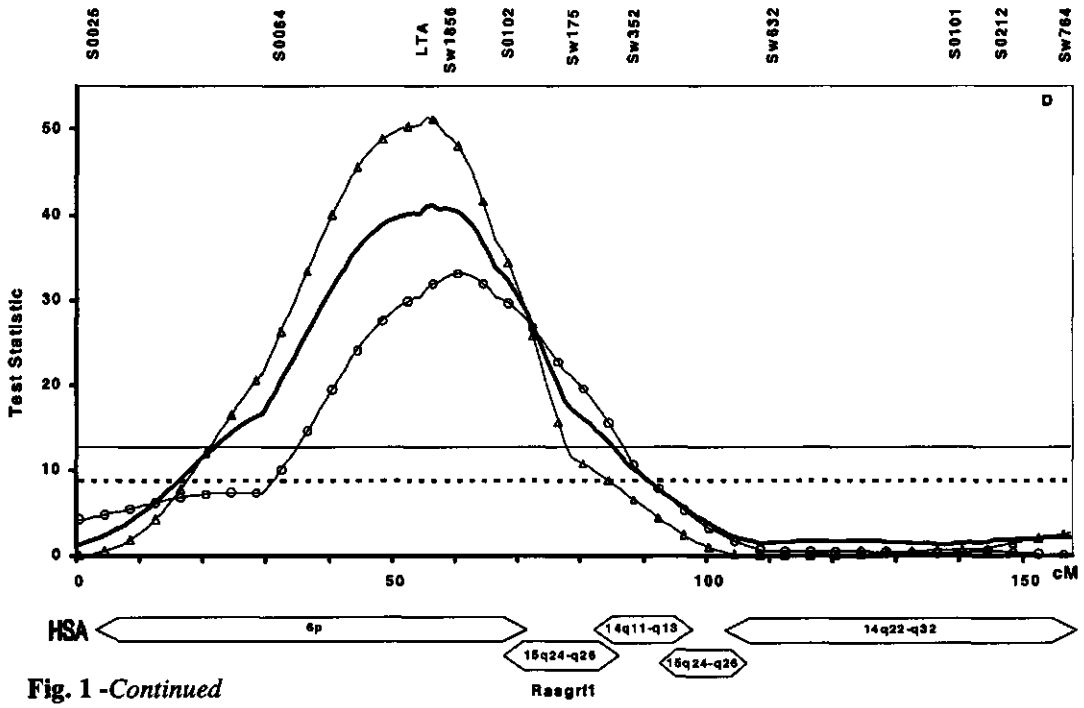

Fig. 1 -*Continued*

previously thought. We detected five QTL, of which four were subject to imprinting. For at least two of these regions, imprinting has not been reported in pigs, and the known homologies to humans and mice did not reveal obvious positional imprinted candidates. To our knowledge, only one study has considered imprinting in a genome-wide analysis, and these results indicated that uni-parental expression, both paternal and maternal, might indeed be involved in diabetes (31).

The statistical analysis presented herein provides information on the mode of expression of genes. In addition, analysis under different modes of expression increases the power of finding genes. This increase is exemplified by the results for intramuscular fat content on SSC6, where significant evidence for QTL was found only under the imprinting model. The approach is implemented in this study for a cross between outbred lines but can be extended to other designs and methods of analysis, including mapping methods used in human genetic studies. For implementation of the method proposed herein, it is essential that parental origin of marker alleles can be derived for the offspring. This requirement excludes studies based on F_2 crosses or a single backcross between inbred lines that are commonly used in mice and rats (3). These model species have contributed enormously to the current understanding of genetic variation. The inability to detect imprinting in the most commonly used mapping designs has certainly contributed to the current feeling that

imprinting is a rare phenomenon. The problem can be overcome by producing one backcross population from F_1 fathers as well as one from F_1 mothers as applied by Clapcott *et al.* (32) to demonstrate genomic imprinting for a major QTL controlling susceptibility to trypanosomiasis in mice. Outbred crosses, such as the cross between two pig breeds in our study, are the ideal resource for detection of imprinted regions.

The model of analysis assumes that alleles at the QTL are fixed in the parental lines. The QTL will be detected when the parental lines carry different alleles, which is likely given the marked morphological divergence between European and Chinese Meishan pigs. If the fixation assumption is violated and the alleles at the QTL are still segregating in either or both of the lines, the power of its detection will be greatly reduced, and its effect will be underestimated (33). Extreme QTL allele frequency differences between male and female parents could lead to the false identification of imprinting for a Mendelian QTL. In our study, this risk is small because male and female parents were selected randomly from the same F_1 population. Furthermore, a large number of parents reduces the chance of allele frequency differences caused by sampling.

Genome-wide screens for QTL often result in estimates of QTL position that lack precision, which complicates the identification of the responsible gene. Knowledge of the fact that the QTL is subject to imprinting will help in identifying the genes. Expression studies aimed at the identification of mono-allelic expression of positional candidates will further aid the identification of the gene(s) responsible for the observed QTL effect. Genotypes of the parents can be used to discriminate between random inactivation and parent-of-origin effects.

For the practice of animal breeding, identification of major imprinted loci affecting body composition has several implications. Our results call for a revision of methods for genetic evaluation that currently ignore non-Mendelian expression. The net result of gametic imprinting is a reduction of the expected phenotypic covariance between parents and offspring relative to that between siblings. Identification of imprinted loci opens new perspectives for crossbreeding, which is common practice in pig breeding. Imprinted genes could further accommodate differentiation between sow lines, which are required to have optimal body composition to support their reproductive performance, and between boar lines, to ensure high-quality pork.

Although the mechanisms underlying imprinting are not totally unraveled (5), this study clearly demonstrates the important role of imprinting for body composition traits. We strongly urge, therefore, the inclusion of statistical testing for imprinting in human and animal genetic research, both in genome scans and in evaluating candidate genes.

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Detection and characterization of quantitative trait loci for meat quality traits in pigs

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Abstract – In an experimental cross between Meishan and Dutch Large White and Landrace lines, 785 F_2 animals with carcass information and their parents were typed for molecular markers covering the entire porcine genome. Linkage was studied between these markers and eight meat quality traits. Quantitative trait locus analyses were performed using interval mapping by regression under two genetic models: 1) the line-cross approach, 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 38 half-sib families. The line-cross approach included tests for genomic imprinting and sex-specific QTL effects. In total, three genome-wide significant and 26 suggestive QTL were detected. The significant QTL on chromosomes 3, 4, and 13, affecting meat color, were only detected under the half-sib model. Failure of the line-cross approach to detect the meat color QTL suggests that the founder lines have similar allele frequencies for these QTL. This study provides information on new QTL affecting meat quality traits. It also shows the benefit of analyzing experimental data under different genetic and statistical models.

In livestock, selection for meat quality traits is limited because phenotypic information for estimating breeding values is only available for relatives of candidates for selection and not for the candidates themselves. Therefore, marker-assisted selection (MAS) may be very profitable for these traits. MEUWISSEN and GODDARD (1996) showed benefits up to 64% for carcass traits by using MAS compared to traditional selection.

Several studies have reported QTL affecting growth and production traits in pigs (ANDERSSON *et al.*, 1994; ROHRER and KEELE, 1998). However, only ANDERSSON-EKLUND *et al.* (1998) presented a whole genome scan that included meat quality traits in pigs.

Analyses of F_2 designs are often based on the line-cross approach suggested by HALEY *et al.* (1994). KNOTT *et al.* (1998) and DE KONING *et al.* (2000) recently introduced extensions of the Mendelian model including methods to test for imprinting effects. ALFONSO and HALEY (1998) showed that the power of the line cross model is reduced when the assumption of fixation in the founder lines is violated. The half-sib QTL analysis (KNOTT *et al.*, 1996) does not make the assumption of fixation, but it has lower power than the line-cross approach when alleles are fixed. Comparing results from different analyses may provide additional insight in the actual genetic composition of the founder lines.

The objective of the present study was to locate QTL that affect eight meat quality traits

Table 1. Measured traits[a], number of F_2 animals with observation (N_{fen}), number of F_2 animals included in QTL analyses (N_{QTL}), phenotypic means, and standard deviations. Adapted from JANSS *et al.* (1997)

Trait	Full name, measurement unit	N_{fen}	N_{QTL}	Mean	SD
Drip	Drip loss, %	844	788	2.70	1.54
Cook	Cooking loss, %	845	789	26.4	3.46
Shear	Shear force, N	845	789	39.6	10.5
PH	pH	845	789	5.66	0.26
pH-s	pH in *M. Semimembranosus*	846	790	5.82	0.30
Color-L	CIELAB L* color coordinate (lightness of the meat)	844	788	53.9	4.83
Color-A	CIELAB a* color coordinate (green-redness of the meat)	846	790	17.3	1.90
Color-B	CIELAB b* color coordinate (blue-yellowness of the meat)	845	789	9.59	1.92

[a]Measurements are on a sample of *M. longissimus*, except pH-s.

using an experimental cross between Meishan and Dutch commercial lines (DE KONING *et al.*, 1999). Earlier analyses of this population (DE KONING *et al.*, 2000; HARLIZIUS *et al.*, 2000; RATTINK *et al.*, 2000) revealed a number of QTL affecting body composition. This is the first study using a comprehensive set of analyses in a genome scan to detect QTL affecting meat quality traits.

Material and Methods

The Meishan x 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, which has been described in detail by JANSS *et al.* (1997). Performance tested F_2 animals that were not retained for breeding were slaughtered in a central slaughterhouse at approximately 90 kg of live weight. On these slaughtered animals, eight meat quality traits were measured (JANSS *et al.*, 1997). The Meishan founders all tested negatively for the mutation in the ryanodine receptor (*Ryr*-1), so they were halothane negative. The commercial lines have been selected against this mutation for several generations so the frequency of the mutation was expected to be very low. All 39 F_1 sires tested negative for the *Ryr*-1 mutation, ensuring that the F_2 population was halothane negative. It cannot be excluded that some F_2 animals were heterozygous, but their number is expected to be very low.

Molecular Typing. The F_2 animals, their F_1 parents, and the F_0 Meishan sires were typed for 132 microsatellite markers. The number of markers per chromosome varies between 15 markers on SSC2 and two on SSC18. Marker maps were re-estimated on the complete data using CRI-MAP. The marker order, and discrepancies between this and published maps has been described by DE KONING *et al.* (1999)

Phenotypic Data. Eight meat quality traits were measured on carcasses 24 h. after slaughter. On samples of the loin muscle (*M. Longissimus*), pH, drip loss, cooking loss, shearforce, and three color scores were measured. Another pH measurement was taken on a sample of a ham muscle (*M. Semimembranosus*). An overview of the meat quality traits and their acronyms that will be used throughout the manuscript is given in Table 1. Details on the measurements can be found in JANSS *et al.* (1997) and HOVENIER *et al.* (1992). Prior to the QTL analyses, the phenotypic data were adjusted for a number of systematic effects. The phenotypic data were analyzed under a polygenic inheritance model containing slaughter day, breeding company, and sex as fixed effects and carcass weight as a covariate. These estimations were performed using the MAGGIC software package developed by JANSS *et al.* (1995).

QTL Analysis. Interval mapping, using regression methods, was applied for distinct genetic models: 1) line-cross analyses following HALEY *et al.* (1994), assuming the founder lines to be fixed for different QTL alleles. These analyses included the extensions suggested by KNOTT *et al.* (1998) and DE KONING *et al.* (2000). 2) Paternal half-sib analyses following KNOTT *et al.* (1996) making no assumption on 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 segregate at the marker loci, are fixed for alternative 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 (P_{11}), two Dutch alleles (P_{22}), or one from each line (P_{12} or P_{21}, different subscripts according to parental origin; first subscript is paternally inherited allele) at 1 cM intervals across the genome. Under the traditional line-cross approach (Mendelian inheritance), an additive effect (a) and a dominance effect (d) are estimated using the regression of the phenotypes on $P_a = P_{11} - P_{22}$ and $P_d = P_{12} + P_{21}$. At every cM across the genome the following model is fitted:

$$y_j = m + ap_{aj} + dp_{dj} + e_j \qquad [1]$$

where y_j is the trait score of animal j (adjusted for systematic effects), m is the population mean, a and d are the estimated additive and dominant effect of a putative QTL at the given location, P_{aj} is the conditional probability of animal j to carry two Meishan alleles, P_{dj} the conditional probability of animal j to be heterozygous, and e_j is the residual error. The calculation of these probabilities and QTL effects are described in more detail by HALEY *et al.* (1994) and applications to crossbred pig populations are numerous (e.g., ANDERSSON *et al.*, 1994; KNOTT *et al.*, 1998).

A test for sex-specific QTL effects was performed following KNOTT *et al.* (1998). The model with sex specific QTL effects was accepted if the F test against the model with equal effects for both sexes was significant ($P < 0.05$).

Extension of the line-cross model to test for imprinting has been suggested (KNOTT *et al.*,

1998) and used in the analysis of the *IGF2* region in pigs (JEON *et al.*, 1999). This model, however, gives no indication whether there is paternal or maternal expression. In addition, differential expression of the paternally and maternally inherited effect might also result in a significant interaction component. The model for imprinting (KNOTT *et al.*, 1998) was re-parameterized to enable a direct test for the contribution of the paternally and maternally inherited effect (DE KONING *et al.*, 2000). To separate the contribution of the parents, we introduced the conditional probability that the individual inherited a Meishan allele from its father ($P_{pat} = [P_{11} + P_{12}] - [P_{22} + P_{21}]$) or from its mother ($P_{mat} = [P_{11} + P_{21}] - [P_{22} + P_{12}]$). This re-parameterization allowed additional models to be fitted with exclusive paternal or maternal expression. All putative QTL locations from the three models were subsequently evaluated with a saturated model that contained a paternal, a maternal and a dominance component:

$$y_j = m + a_{pat} p_{patj} + a_{mat} p_{matj} + dp_{dj} + e_j \quad [2]$$

The genetic model for a putative QTL was evaluated based on the contributions of the individual components of the saturated model and the residual variance. Imprinting was inferred if only one of the parental contributions was significant and no dominance was present (i.e., by comparing the full model with a model containing only one component). After derivation of the genetic model, the significance level and the QTL effects were calculated under the inferred genetic model.

Half-sib Model. For the half-sib analysis, the F_2 animals are treated as 38 unrelated half-sib families, i.e., additional genetic relationships between and within half-sib groups are ignored. The analysis uses the multi-marker approach for interval mapping in half-sib families as described by KNOTT *et al.* (1996) and applied to QTL mapping studies in cattle (VILKKI *et al.*, 1997) and pigs (DE KONING *et al.*, 1999). In these analyses contrasts are made between the two haplotypes of every F_1 boar. Within every half-sib family a QTL with a gene substitution effect is fitted at 1 cM intervals along the chromosome:

$$y_{ij} = m_i + b_i P_{ij} + e_{ij} \quad [3]$$

where y_{ij} is the trait score of individual j (adjusted for systematic effects), originating from boar i; m_i is the average effect for half-sib family i; b_i is the substitution effect for a putative QTL; P_{ij} is the conditional probability for individual j of inheriting the first paternal haplotype; and e_{ij} is the residual effect. The regression is nested within families because the first haplotype is randomly assigned, not all boars are heterozygous for the QTL, and the linkage phase between a marker and a QTL can differ between families. The test statistic is calculated as an F ratio for every map position within and across families. For details on half-sib analyses see DE KONING *et al.* (1999). Once a QTL was detected in the across-family analyses, tabulated values ($P < 0.05$) of the F ratios for the individual families were used to infer which families were likely to be segregating for the QTL. In the families that

were inferred to be segregating for an identified QTL, it was determined whether the Meishan allele was associated with an increase or a decrease in phenotype.

X Chromosome. Quantitative trait loci on the X chromosome cannot be detected under a paternal half-sib model. Therefore, the X chromosome was only analyzed under the line-cross model following KNOTT *et al.* (1998). Since the founder sows were all from the Dutch breeds, all F_2 females will have at least one X chromosome originating from the Dutch lines (inherited through their F_1 sire). Consequently, QTL effects were estimated separately for F_2 males and females because they are only equal if a QTL is additive. The probabilities for recombination between a marker and a putative QTL are different compared to the autosomes because the majority of the X chromosome cannot recombine within the male parent.

Significance Thresholds. Although calculated as an F ratio, the distribution of the test statistic under the H_0 of no QTL is unknown for both the line-cross and half-sib analyses. Therefore, chromosome-wide significance thresholds were determined empirically by permutation for individual chromosomes (CHURCHILL and DOERGE, 1994). Two significance thresholds were applied. The first level was suggestive linkage where one false positive is expected in a genome scan (LANDER and KRUGLYAK, 1995). The chromosome-wide *P* value for suggestive linkage for a specific chromosome equals the contribution (r) of that chromosome to the total genome length, which was obtained by dividing the length of a chromosome by the total length of the genome. For claiming significant linkage we applied the 5% genome-wide significance level (LANDER and KRUGLYAK, 1995). To derive genome-wide significance levels from the chromosome-wide significance levels, the following Bonferoni correction was applied.

$$p_{\text{genome-wide}} = 1 - (1 - p_{\text{chromosome-wide}})^{1/r} \quad [4]$$

This is computationally more efficient than performing genome-wide permutations, because in the current approach permutations only have to be performed for chromosomes that show evidence for QTL. Both significance levels do not take account of the testing of multiple traits and multiple models in the present and future studies. It should be noted that these significance levels are based on the realized marker density and not on an infinitely dense map as proposed by LANDER and KRUGLYAK (1995).

Results

All the QTL that exceeded the threshold for suggestive linkage in the analyses are reported. For the line-cross analyses, only the results of the genetic model that best fit the data are presented.

Results of Line-cross Analyses. The line-cross analyses for the eight meat quality traits revealed 24 QTL exceeding the threshold for suggestive linkage (Table 2). The number of QTL per trait varied from two QTL for Cook, Shear, pH, and Color-B up to five suggestive QTL for pH-s. Although none of these exceeded the 5% genome-wide threshold, seven QTL exceeded the 20% genome-wide significance level. The strongest suggestive QTL was found for a paternally expressed QTL

Table 2. Location and characterization of QTL affecting meat quality that exceed suggestive linkage under the line-cross models

SSC	Marker [bracket] (position, cM)	Genetic model[a]	Test statistic (*P*)[b]	a (SE)[c]	d (SE)[c]
			Drip, %		
SSC4	S0301-S0001 (32)	Paternal	7.15 (0.54)	-0.17 (0.06)	.
SSC6	SW1057-S0220 (74)	Maternal	9.57 (0.18)	-0.23 (0.07)	.
SSC14	SW857 (1)	Mendelian	6.08 (0.24)	0.23 (0.08)	-0.32 (0.14)
SSC18	SW1023-SW787 (24)	Paternal	6.22 (0.80)	-0.15 (0.06)	.
			Cook, %		
SSC7	S0064- LTA (40)	Mendelian	6.69 (0.20)	0.68 (0.19)	-0.33 (0.34)
SSC18	SW787 (31)	Maternal	8.92 (0.38)	-0.35 (0.12)	.
			Shear N		
SSC9	S0295-SW174 (101)	Maternal	9.06 (0.27)	-0.99 (0.33)	.
SSC13	SW398-S0287 (115)	Mendelian	5.03 (0.53)	0.91 (0.46)	2.06 (0.86)
			PH		
SSC4	S0301-S0001 (33)	Paternal	7.27 (0.54)	0.027 (0.01)	.
SSC9	S0295 (96)	Maternal	10.03 (0.17)	0.029 (0.01)	.
			pH-s		
SSC11	SW1377 (54)	Paternal	8.15 (0.39)	0.033 (0.01)	.
SSC14	S0007-SW1557 (96)	Paternal	10.85 (0.10)	0.037 (0.01)	.
SSC14	SW857 (1)	Sex specific	3.59 (0.32)	♂ -0.065 (0.02) ♀ 0.027 (0.02)	♂ 0.012(0.03 ♀ 0.007(0.03)
SSC18	SW787 (31)	Mendelian	7.06 (0.16)	0.049 (0.01)	-0.013 (0.02)
SSCX	SW2534 (1)	X-linked	4.64 (0.59)	♂ 0.031 (0.01) ♀ 0.025 (0.02)	.
			Color-L		
SSC1	CGA (64)	Paternal	10.28 (0.13)	0.53 (0.16)	.
SSC3	S0216 (72)	Mendelian	5.13 (0.57)	0.64 (0.28)	1.15 (0.52)
SSC4	S0073-S0214 (78)	Sex specific	3.75 (0.38)	♂ 1.04 (0.32) ♀ -0.81 (0.40)	♂ 0.12 (0.42 ♀ 0.12 (0.48)
SSC14	SW295-SW210 (51)	Sex specific	4.24 (0.15)	♂ -1.08 (0.32) ♀ 0.64 (0.40)	♂ -0.82 (0.32 ♀ -0.26 (0.51)
			Color-A		
SSC13	SW225-SW398 (95)	Paternal	8.01 (0.35)	0.19 (0.08)	.
SSC14	SW857-SW295 (35)	Maternal	7.95 (0.37)	0.25 (0.09)	.
SSC15	S0088-SW906 (59)	Mendelian	5.83 (0.37)	0.24 (0.11)	-0.59 (0.21)
			Color-B		
SSC13	S0219-S0076 (13)	Maternal	6.50 (0.59)	-0.21 (0.08)	.
SSC14	S0007-SW1557 (90)	Mendelian	5.85 (0.28)	-0.25 (0.11)	-0.53 (0.22)

[a]Genetic model which is most appropriate for the QTL. [b]Test statistics for the inferred genetic model vs the H_0 of no QTL, *P* values are genome-wide significance levels. [c]Estimated QTL effects for the inferred genetic model. The additive effect a is expressed as the deviation of the Meishan allele and the dominance effect d is expressed (where appropriate) as the deviation of the heterozygous animals from the mean of the homozygotes. For QTL with sex-specific expression, ♂ and ♀ indicate the estimated effect for males and females, respectively.

Table 3. Location of QTL affecting meat quality that exceed suggestive linkage under the half-sib model

Trait	Chromosome	Marker [bracket] (position, cM)[a]	Test statistic (P)[b]
pH	SSC9	SW21-SW911 (32)	2.01 (0.10)
Color-L	SSC13	S0076-S0068 (55)	2.55 (< 0.0001)
Color-A	SSC2	SW256 (26)	1.71 (0.47)
Color-A	SSC3	S0164-S0216 (41)	2.03 (0.028)
Color-B	SSC4	S0217-S0073 (71)	2.18 (0.007)

[a]Best position across families. [b]Test statistic across families for the H_0 of no QTL, P values are genome-wide significance levels.

significance level of 10%. For this QTL, a paternally inherited Meishan allele gives rise to a higher pH (Table 2). From the 24 suggestive QTL, seven show paternal expression, six show maternal expression, and one is located on the X chromosome. From the 10 QTL with Mendelian expression, two QTL affecting Color-L (SSC4 and SSC14) and another QTL affecting pH-s (SSC14) show significant differences in estimated QTL effects between sexes (Table 2). In all three cases, the direction of the effect is opposite in the two sexes. There is no obvious biological explanation why genes would act antagonistically in different sexes. It cannot be ruled out that these QTL with opposite effects between sexes are statistical artifacts.

Results of Half-sib Analyses. In the half-sib analysis, QTL effects are fitted within half-sib families and the test statistics are calculated from the pooled sums of squares of the 38 families. Consequently, the values of the test statistic as well as the significance thresholds are lower in the half-sib analyses compared to the line-cross analyses. The half-sib-analyses revealed three significant QTL and two suggestive QTL (Table 3). A highly significant QTL affecting Color-L mapped to SSC13 (Table 3, Figure 1). Six half-sib families that were inferred to be heterozygous for this QTL showed allele substitution effects between 4.3 and 23.5 color-L coordinate units (Table 4). A significant QTL affecting Color-A mapped to SSC3. The allele substitution effect for this QTL varied between 1.23 and 2.9 in color coordinate units across nine heterozygous families (Table 4). The third significant QTL affecting meat color mapped to SSC4 and affected Color-B. The allele substitution effect of the QTL varied between 1.8 and 2.5 units across seven heterozygous families (Table 4). Additional suggestive QTL were detected for pH and Color-A on SSC9 and SSC2, respectively (Table 3).

Discussion

Under the various genetic models of the line-cross approach, 24 suggestive QTL were detected, affecting meat quality traits (Table 2). In addition, the half-sib analyses revealed three significant and two suggestive QTL (Table 3). In the following paragraphs we will compare results for the different traits and models used in this study, compare results with findings for different traits in the same population, and

Table 4. Estimated allele substitution effects for half-sib families that are informative for the QTL affecting meat color

Test statistic[a]	QTL position[b], cM	QTL effect[c] (SE)
	Color-L SSC13, 55 cM	
49.02	56	-23.45 (3.35)
19.36	4	7.93 (1.80)
12.84	100	-6.44 (1.80)
9.90	59	8.76 (2.78)
8.61	142	-4.32 (1.47)
7.78	58	-13.48 (4.83)
	Color-A SSC3, 41 cM	
18.56	38	-2.96 (0.69)
18.53	38	-2.88 (0.67)
14.38	84	2.71 (0.71)
12.96	28	2.00 (0.56)
12.90	122	-2.36 (0.66)
9.54	88	2.55 (0.83)
9.52	37	1.23 (0.40)
8.56	93	-2.41 (0.82)
7.90	104	-1.87 (0.66)
	Color-B SSC4, 71 cM	
29.47	37	2.48 (0.46)
12.92	70	1.79 (0.50)
11.18	82	-1.80 (0.54)
10.15	12	-1.47 (0.46)
8.65	17	1.90 (0.65)
8.40	55	-2.50 (0.86)
8.38	38	-2.02 (0.70)

[a]Test statistic for individual family for the most likely position of a QTL within that family. [b]The most likely position of a QTL within a family. [c]The estimated allele substitution effect of the Meishan haplotype (CIELAB color coordinate) for the individual family at the most likely position of a QTL within that family.

relate our findings for meat quality to those from other studies.

Comparison of Results for Different Traits. Two paternally expressed QTL, affecting respectively Drip and pH, map to the same region on SSC4 (Table 2). The opposite direction of the QTL effect for these two traits is in agreement with the negative genetic correlation between these traits (-0.60, DE VRIES *et al.*, 1994). The concordance of position, type of expression, and direction of effect suggest a single gene on SSC4 affecting both traits. Similarly, two maternally expressed QTL affecting Shear and pH map to the same marker interval on SSC9. The opposite signs of the QTL effects is in agreement with the negative genetic correlation (-0.27) reported by DE VRIES *et al.* (1994). Three suggestive QTL affecting Drip, Cook, and pH-s map to the same

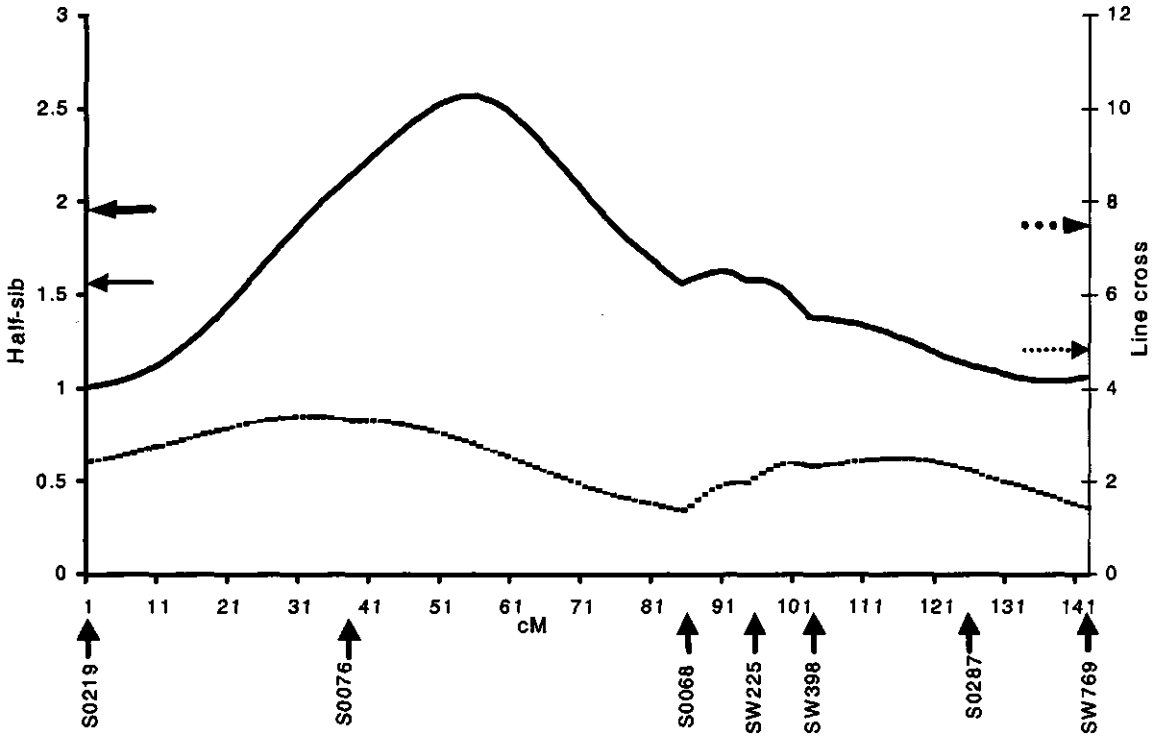

Figure 1. Results for porcine chromosome 13 and Minolta color coordinate L (lightness of the meat). The curves show the behavior of the test statistic under two alternative statistical models. The solid curve shows the test statistic for the half-sib analysis (primary Y-axis) whereas the dashed line shows the test statistic for the line-cross analysis (secondary Y-axis). The vertical arrows denote genome-wide suggestive (thin arrow) and significant (thick arrow) threshold levels. Marker names are given on the X-axis with arrows indicating their respective location.

region on SSC18 but show paternal, maternal and Mendelian expression, respectively.

Under the line-cross models, five suggestive QTL map to SSC14, showing the full range of Mendelian, paternal, maternal, and sex-specific expression across the chromosome (Table 2). It must be noted that these QTL are only significant at the suggestive level, although four of them have genome-wide significance levels below 25%. The traits that are affected are Color-A, Color-B, pH-s, and Drip. Given the variety in estimated QTL position and genetic model, plus the lack of obvious relations between the affected traits, it is unlikely that the observed QTL effects result from one single gene with pleiotropic effects.

Recently, KNOTT and HALEY (2000) have presented a method for multivariate QTL analysis in a least squares framework. The present analysis could be extended to handle multiple traits following their procedure. This could provide additional power for the

chromosomes where we find suggestive QTL for several traits and also provide a test whether there is pleiotropy or close linkage. The multivariate methods are developed for a Mendelian model but need extensions for fitting other genetic models.

Comparison of Results with Other Studies. So far, only ANDERSSON-EKLUND *et al.* (1998) have investigated meat quality traits in a genome-wide scan in pigs. They did not report any suggestive or significant QTL affecting meat quality traits. However, some linkage groups for which they infer a 'chromosome substitution effect' for pH (SSC4) or meat color (SSC2, SSC15) are shown in the present study to contain suggestive QTL affecting these traits. WANG *et al.* (1998) reported a suggestive QTL affecting meat color on SSC4 in one breed cross. Two markers (~ 20 cM) separate the best position of their QTL from the QTL affecting Color-B in the present study. Different types of color measurements used in the two studies further compromise any conclusions whether both studies detected the same QTL.

Comparison of Results for Different Models and Traits. Earlier studies on this experimental population revealed several highly significant QTL affecting body composition of which several where imprinted (DE KONING *et al.*, 2000; RATTINK *et al.*, 2000) or on the X chromosome (HARLIZIUS *et al.*, 2000). Compared to those results, the number of QTL detected in the present study might seem rather low. Likewise, in an F_2 cross between Large White and Wild Boar, several significant QTL were detected for growth and fatness (ANDERSSON *et al.*, 1994; KNOTT *et al.*, 1998), whereas little evidence was found for QTL affecting meat quality (ANDERSSON-EKLUND *et al.*, 1998). The lower power of these designs to detect meat quality QTL could have several reasons. The QTL effects may be smaller compared to those of the QTL affecting body composition traits. Furthermore, the founder lines might not be genetically divergent with regard to meat quality genes because there has been relatively little selection on meat quality within the commercial breeds. The latter is supported by the fact that the three significant QTL affecting meat quality were only detected under the half-sib analyses. This is illustrated for Color-L and SSC13 in Figure 1 where the test statistic under the half-sib analysis is compared with that of the line-cross model. The estimated allele substitution effects for the individual half-sib families (Table 4) are expressed as the effect of the Meishan haplotype within a family. The estimates in Table 4 show clearly that for all three QTL, about half of the heterozygous families show a higher value of color coordinate for the Meishan allele, whereas the other half show a negative effect. This suggests that the assumption of fixation of founder lines for alternative alleles is not valid for the meat color QTL. It is more likely that the founder lines were segregating for similar alleles of these QTL.

All line-cross analyses depend heavily on the assumption of fixation although it has been demonstrated that the line-cross model is robust to some deviations of this assumption (ALFONSO and HALEY, 1998). To our knowledge, this is the first study in which it is

shown that the violations of the fixation assumption are so severe that the half-sib approach is superior to the line-cross model for the detection of QTL.

Statistical models for F_2 designs that do not depend on the assumption of fixation of the founder lines have been suggested but are only feasible when the F_2 consists of a single full sib family (KNOTT *et al.*, 1997; SILLANPÄÄ and ARJAS, 1999). The half-sib approach does not put a restriction on the number of alleles which complicates a direct comparison of methods. Currently, (half-sib) family sizes in QTL detection experiments in pigs are often too small to perform half-sib or full-sib analyses and these crosses are only analyzed under a standard line-cross model (ANDERSSON *et al.*, 1994; ROHRER and KEELE, 1998).

In general, the results show little correspondence between the findings under the line-cross models (Table 2) and the half-sib model (Table 3). For instance, it seems surprising that the paternally expressed QTL on SSC14 (Table 2) was not detected under the half-sib model. In case a Mendelian QTL is analyzed under a line-cross model, the variance explained by the model is at maximum (Henk Bovenhuis, personal communication):

$$2pq[a + d(q - p)]^2 + [2pqd]^2 \qquad [5]$$

When the same QTL is analyzed under a paternal half-sib model, the variance explained by incorporating a QTL is at maximum:

$$\frac{\theta}{4}[a + (q - p)d]^2 \qquad [6]$$

where θ is the proportion of F_1 sires that is heterozygous for the QTL. For an additive Mendelian QTL that is fixed for alternative alleles in the founder lines, the variance explained by the QTL to the model under the line-cross approach ($\frac{1}{2}a^2$) is twice as large compared to the variance explained by this QTL under the half sib-model ($\frac{1}{4}a^2$). For a completely dominant QTL, the QTL variance from the line-cross model ($\frac{1}{2}a^2 + \frac{1}{4}d^2$) can be three times higher than that of the half-sib design. This difference in variance explained by the QTL becomes smaller with increased levels of allele sharing between founder lines. When founder lines are segregating for the same alleles at equal frequencies, the variance explained by the line cross model reduces to zero, and only the half-sib model has some power to detect QTL. With more moderate levels of allele sharing in the founder lines, the line-cross model is expected to have considerable more power than the half-sib analyses. As a result, the line-cross analyses are best suited to detect QTL that explain phenotypic differences between the Meishan and the commercial lines, whereas the half-sib analyses are useful to detect QTL that explain phenotypic differences within the founder lines.

The value of applying a comprehensive set of analyses is best illustrated by comparing the current results with those that would have been obtained if only the standard line-cross approach was used to analyze the data. The imprinting analyses revealed 13 additional QTL that were not detected under the standard model. The analysis of sex specific alleles for QTL with Mendelian expression revealed three additional suggestive QTL. The half-sib analysis revealed three genome-wide significant

QTL and two suggestive QTL that were not detected under any of the models that assume fixation of QTL alleles in the founder lines. The benefit of applying the comprehensive set of models is three genome-wide significant and 18 suggestive QTL. Therefore, experimental crosses should be designed in a way that allows several genetical and statistical models to be evaluated.

In the present study, we did not adjust the thresholds for testing multiple models on the data. The line-cross model and the half-sib model can be considered as two separate analyses, which differ in their genetic model. Under the line-cross model, Mendelian, maternal, and paternal models are fitted independently to the data to detect all putative QTL, after which a single test is used to distinguish between Mendelian, paternal, and maternal expression. The test for sex specific QTL effects is another addition to the line-cross model that may reveal additional QTL. Adjustment of the significance thresholds could be done by an additional Bonferoni correction for the number of models that are applied. However, the different models are not independent so taking the total number of tests would be too conservative and deriving the number of independent tests is not straightforward. We prefer to give genome-wide thresholds, without additional corrections for number of traits and (or) number of models to facilitate comparisons across studies.

A comprehensive QTL analysis in an experimental pig cross, including testing for imprinting and sex specific QTL effects has only been performed by KNOTT *et al.* (1998) for growth and fatness data. Extending the analyses of published experiments with testing for sex-specific QTL effects and specific testing for paternal and maternal expression is strongly recommended. Furthermore, analyses that are free of the assumption of fixation in the founder lines, like half-sib or full-sib analyses, should be considered. Altogether, these will provide additional QTL or at least a better characterization of QTL that were already detected. Furthermore, it will allow better comparisons for the QTL presented in this study and give insight in how many of the suggestive QTL reported here are likely to be true QTL.

Implications

Several QTL have been identified for eight meat quality traits. The strongest effects were found for QTL affecting meat color. Improvement of meat quality by traditional selection is often complicated because most of the traits can only be measured on slaughtered animals. Detection of genes affecting these traits allows characterization of the genetic potential of living and even unborn animals. For some of the QTL described here only one of the parental alleles is expressed in the animals. This process of genomic imprinting, which is not covered in traditional selection programs, offers new opportunities for breeding programs where crossing selection lines is common practice. This also applies to the QTL that are located on the X chromosome. This study shows the benefits of analyzing experimental data under several genetic models. The statistical evidence for the different QTL varies and verification is

necessary before implementation in commercial breeding programs.

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Detection and characterization of quantitative trait loci for growth and reproduction traits in pigs

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Abstract – A genome scan was performed on F_2 animals of a cross between Meishan and Dutch commercial pigs. Phenotypic data were available for growth traits and ultrasonic backfat thickness on 942-1151 animals, and for litter size on 249 and 206 animals at first and second parity, respectively. QTL analyses were performed using interval mapping by regression under the line-cross approach complemented by tests for genomic imprinting and sex-specific QTL effects. For backfat thickness, the analyses revealed significant QTL on chromosomes 2, 7, 14, and X, with significant imprinting for chromosomes 2 and 14. Significant QTL were detected for the different growth traits on chromosomes 1, 4, 7, and 8. Both the QTL on chromosome 4 and chromosome 8 showed maternal expression for a specific growth stage. The QTL analyses for litter size revealed one suggestive QTL for first parity and three suggestive QTL for the second parity. Analyses under a half-sib model did not reveal additional significant QTL, but confirmed several of the QTL detected under the line-cross models. This study provides confirmation of several QTL affecting growth and fat deposition in pigs and adds interesting new insight into their mode of expression. Furthermore, additional significant and suggestive QTL were identified.

In pig breeding, strong selection pressure is applied to growth and reproduction traits.

Using modern molecular technology it has been possible to identify QTL affecting growth, fatness, and litter size by scanning the entire genome (e.g. ANDERSSON *et al.*, 1994; ROHRER and KEELE, 1998; ROHRER *et al.*, 1999) or using a candidate gene approach (ROTHSCHILD *et al.*, 1996; KIM *et al.*, 2000). Genome scans are often performed on crosses between genetically distant pig breeds and analyzed under the line-cross model proposed by HALEY *et al.* (1994). KNOTT *et al.* (1998) introduced extensions to this model to test for sex specific QTL effects, QTL on the X chromosome, and genomic imprinting effects.

Using only phenotypic data, DE VRIES *et al.* (1994) were the first to show that genomic imprinting may influence growth rate and body composition in pigs. This was corroborated by a report of an imprinted QTL affecting fatness on SSC4 (KNOTT *et al.*, 1998) and an imprinted QTL affecting muscularity and fatness in pigs in the *IGF2* region on SSC2 (JEON *et al.*, 1999; NEZER *et al.*, 1999). Subsequently, DE KONING *et al.* (2000) showed significant imprinting effects on chromosomes 2, 6, and 7 for backfat thickness, intramuscular fat content, and muscle depth, respectively.

ROHRER and KEELE (1998) described a QTL affecting backfat thickness on the X

Table 1. Measured traits with number of F2 animals with observation (N_{obs}), number of F2 animals included in QTL analyses (N_{QTL}), phenotypic means and standard deviations (SD). Adapted from Janss et al. (1997).

Trait	Full name, measurement unit	N_{obs}	N_{QTL}	Mean[a]	SD[a]
EGR	Early growth, g/day	1020	942	448.3	79.1
TGR	Test growth, g/day	1020	942	657.0	126.5
LGR	Life growth, g/day	1246	1151	522.6	76.2
BFT	Ultrasonic backfat thickness, mm	1218	1131	15.6	3.8
LS1	Litter size (total number born) at first farrowing	269	249	11.0	3.2
LS2	Litter size (total number born) at second farrowing	222	206	11.7	3.2

[a] For all the animals that have phenotypic information

chromosome. This QTL was confirmed by HARLIZIUS *et al.* (2000), who showed that this QTL also affected intramuscular fat content.

The present study describes an effort to locate QTL that affect growth rate, backfat thickness, and litter size using animals of an experimental cross between Meishan and Dutch commercial lines (DE KONING *et al.*, 1999, 2000). The traditional line-cross analyses were complemented with systematic tests for imprinting and sex specific QTL effects. The production traits were also analyzed under a half-sib model in order to investigate the segregation of QTL alleles and effects within the F_1.

Material and Methods

Population, phenotypes, and markers. 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, which has been described in detail by JANSS *et al.* (1997). An F_1 was obtained by artificial insemination of sows from the commercial lines of the breeding companies with semen from 19 boars from the Meishan breed. The commercial lines consisted mainly of the Large White breed but other breeds were also present (e.g. Dutch Landrace). From the F_1, 264 sows and 38 boars were randomly selected to become parents of the F_2 litters. Using F_1 boars across all five companies prevented the confounding of genetic effects with company effects. F_2 animals were performance tested and three growth traits were defined: 1) early growth (**EGR**), daily gain from weaning to approximately 25 kg; 2) test growth (**TGR**), daily gain from approximately 25 to 90 kg; and 3) life growth (**LGR**), daily gain during entire life, not adjusting for birth weight. At the end of the performance test, backfat thickness (**BFT**) was measured ultrasonically and averaged over 4-8 measurements along the spine. A selection of F_2 sows was inseminated with boars from the breeding company where they were kept and litter size, including stillborn piglets, was recorded at two parities (**LS1** and **LS2**). An overview of the traits is given in Table 1.

Prior to the QTL analyses, the phenotypic data were adjusted for a number of systematic

effects following JANSS *et al.* (1997). The phenotypic data were analyzed under a polygenic inheritance model containing a fixed effect of period by company for all traits, an additional sex by company effect for the performance traits, and body weight at end of test as a covariable for BFT. These estimations were performed using the MAGGIC software package developed by Janss *et al.* (1995). JANSS *et al.* (1997) included phenotypic information on the F_1 animals in the analysis whereas the current analyses were only based on phenotypes from the F_2.

The F_2 animals, their F_1 parents and the F_0 Meishan boars were typed for 132 microsatellite markers. The average marker spacing was 16 cM. The number of markers per chromosome varied between 15 markers on SSC2 and two on SSC18. Details on laboratory protocols and map construction can be found in DE KONING *et al.* (1999).

QTL analysis. For all traits, interval mapping using regression methods was applied, following the line-cross approach proposed by HALEY *et al.* (1994). Under the line-cross approach it is assumed that the two founder lines are fixed for alternative alleles at the QTL affecting the traits of interest, although they may share alleles at the marker loci. Using multi-marker information, four probabilities are calculated at 1 cM intervals along the genome. P_{11} is the probability that an F_2 animal inherited two Meishan alleles, P_{22} that it inherited two Dutch alleles, and P_{12} or P_{21} that it inherited one from each line (different subscripts according to parental origin, first subscript is paternally inherited allele). At every cM across the genome, an additive effect (a) and a dominance effect (d) are estimated using the regression of the phenotypes on a linear combination of the probabilities of line origin:

$$y_j = m + aP_{aj} + dP_{dj} + e_j \qquad (1)$$

Where y_j is the trait score of animal j (adjusted for systematic effects), m is the population mean, a and d are the estimated additive and dominant effect of a putative QTL at the given location, P_{aj} is the conditional probability of animal j to carry two Meishan alleles (P_{11} - P_{22}), P_{dj} the conditional probability of animal j to be heterozygous ($P_{12} + P_{21}$), and e_j is the residual error. A detailed description of these methods is given by HALEY *et al.* (1994) and applications to crossbred pig populations are numerous (e.g. ANDERSSON *et al.*, 1994; KNOTT *et al.*, 1998).

The line-cross analyses were extended with a test for sex-specific QTL effects following KNOTT *et al.* (1998). The model with sex-specific QTL effects was accepted if the *F*-test against the model with equal effects for both sexes was significant ($P < 0.05$).

Imprinting was tested following the procedures presented by DE KONING *et al.* (2000). The contribution of the parents was separated using the probability that the individual inherited a Meishan allele from its father ($P_p = [P_{11} + P_{12}] - [P_{22} + P_{21}]$) or from its mother ($P_m = [P_{11} + P_{21}] - [P_{22} + P_{12}]$). This

re-parameterization allowed additional models to be fitted with exclusive paternal or maternal expression. All putative QTL locations from the three models were subsequently evaluated with a saturated model that contained a paternal, maternal and dominance component:

$$y_j = m + a_p P_{pj} + a_m P_{mj} + dP_{dj} + e_j \quad (2)$$

Using *F* ratios for the individual components of the model, imprinting was inferred if only one of the parental contributions was significant and no dominance was present.

The X chromosome was analyzed under the line-cross model as described by KNOTT *et al.* (1998) and implemented for this experimental population by HARLIZIUS *et al.* (2000). The analyses accounted for the non-reciprocal nature of the F_2 cross and differences in probabilities for recombination between a marker and a putative QTL compared to the autosomes. QTL effects on the X chromosome were estimated separately within F_2 males and females for the performance traits.

QTL analyses were also performed under a paternal half-sib model (KNOTT *et al.*, 1996). However, half-sib family sizes were too small for litter size so these analyses were only carried out for the performance traits. The half-sib model makes no assumption on the number of QTL alleles and allele frequencies within the founder lines. For the half-sib analysis, the F_2 animals are treated as 38 unrelated half-sib families and contrasts are made between the two haplotypes of every F_1 boar. Within every half-sib family a QTL with a gene substitution effect is fitted at 1 cM intervals along the chromosome:

$$y_{ij} = m_i + b_i P_{ij} + e_{ij} \quad (3)$$

Where y_{ij} is the adjusted trait score of individual j, sired by boar i; m_i is the average effect for half-sib family i; b_i is the substitution effect for a putative QTL; P_{ij} is the conditional probability for individual j of inheriting the first paternal haplotype; and e_{ij} is the residual effect. The test statistic is calculated as an *F* ratio for every map position within and across families. For details on half-sib analyses applied to this experimental population see DE KONING *et al.* (1999). In the families that were inferred to be segregating for an identified QTL it was determined whether the Meishan allele was associated with an increase or a decrease in phenotype.

Significance of QTL was evaluated using two thresholds. The first level was suggestive linkage where one false positive is expected in a genome scan (LANDER and KRUGLYAK, 1995). The suggestive significance level is proportional to the contribution (r) of a specific chromosome to the total genome length, which was obtained by dividing the length of a chromosome by the total length of the genome. For claiming significant linkage, the more stringent 5% genome-wide significance level was used. Both significance levels do not take the testing of multiple traits and models in the present and future studies into account.

Although calculated as an F ratio, the distribution of the test statistic under the H_0 of no QTL is unknown for both the line-cross and half-sib analyses. Therefore, significance thresholds were determined empirically by permutation for individual chromosomes (CHURCHILL and DOERGE, 1994). To derive genome-wide significance levels from these chromosome-wide significance levels, the following Bonferroni correction was applied.

$$P_{\text{genome-wide}} = 1-(1-P_{\text{chromosome-wide}})^{1/r} \quad (4)$$

The test statistics for the different models have different degrees of freedom. Furthermore, there are differences in number of animals per trait, which further complicates comparisons between different analyses. As a result the empirical genome-wide significance threshold for the test statistic varied between ~2.0 for the half-sib analyses and ~12.5 for an imprinted QTL under the line-cross analyses. To facilitate graphical comparisons of different models, a transformation was applied to the test statistics. The tabulated P value was obtained for every test statistic, using an F distribution with the appropriate degrees of freedom. In the graphs, the negative logarithm of these P values is presented $[-\log_{10}(P)]$. Applying this transformation, the empirical threshold levels varied between 2.0 and 2.1 for suggestive linkage and between 3.4 and 3.7 for significant linkage, across all chromosomes, models and traits. Within the same trait or chromosome the range was even smaller. The thresholds in the graphs are averaged over all traits and models that are represented in a graph.

Results

Results for performance traits. For the performance traits, the line-cross analyses revealed 12 genome-wide significant QTL and 25 suggestive QTL (Table 2). The half-sib analyses revealed five genome-wide significant, and three suggestive QTL (Table 3). For each of the performance traits, the highly suggestive and significant QTL will be discussed in the following paragraphs.

Results for EGR. For EGR, genome-wide significant QTL were detected on SSC4 and SSC8. The QTL on SSC4 showed significant differences in QTL effects between the sexes, both for the sign of the additive effect and for the size of the additive and the dominance effect (Table 2). A graph of the test statistics of all QTL on SSC4 that were detected in the present study is given in Figure 1. The QTL on SSC8 was maternally expressed with an estimated difference of 23 g/day lower growth for an animal with a maternally inherited Meishan allele compared to that with a maternal allele from the Dutch lines. An overview of the test statistic for the maternally expressed QTL affecting growth on SSC8 is given in Figure 2.

Four highly suggestive QTL, with genome-wide P values between 0.06 and 0.07, were detected on SSC1, SSC6, SSC10, and on an additional region of SSC4 (Table 2, Figure 1). The suggestive QTL on SSC1 shows sex specific QTL effects with a larger effect in the F_2 males (Table 2, Figure 3). The suggestive QTL on SSC6 and SSC10 are imprinted, with exclusive maternal and paternal expression,

Table 2. Location and characterization of QTL affecting performance traits that exceed suggestive linkage under the line-cross models

Chromosome	Marker (position, cM)	Genetic model[a]	Test statistic (p)[b]	a (s.e.)[c]	d (s.e.)[c]
EGR (g/day)					
SSC1	SW552-SW485 (2)	Sex specific	4.79 (0.06)	♂ -19.0 (5.5) ♀ -12.1 (6.0)	♂ -9.5 (7.3) ♀ 4.4 (7.9)
SSC1	S0112 (148)	Paternal	9.45 (0.21)	16.7 (5.4)	.
SSC4	S0227-S0301 (21)	Sex specific	7.32 (< 0.001)	♂ 11.6 (6.0) ♀ -28.2 (6.9)	♂ 16.2 (9.0) ♀ 27.3 (9.4)
SSC4	S0214 (82)	Mendelian	8.00 (0.07)	-12.6 (3.8)	14.3 (5.8)
SSC5	SWR453-SW332 (30)	Mendelian	5.38 (0.46)	0.8 (4.6)	26.0 (7.9)
SSC6	SW2419 (191)	Maternal	11.81 (0.07)	-9.6 (2.8)	.
SSC8	SW2410-SW905 (10)	Maternal	13.54 (0.03)	-11.5 (3.1)	.
SSC8	SW905-SW268 (23)	Sex specific	3.74 (0.47)	♂ -12.7 (5.5) ♀ -12.4 (5.9)	♂ -6.1 (7.5) ♀ 11.5 (8.0)
SSC10	SW1041 (85)	Paternal	10.93 (0.07)	-9.0 (2.7)	.
SSC13	S0076-S0068 (43)	Maternal	6.38 (0.61)	-10.3 (4.1)	.
TGR (g/day)					
SSC1	CGA-S0313 (73)	Mendelian	8.5 (0.03)	-26.2 (6.4)	-1.6 (10.6)
SSC2	SW256 (25)	Paternal	7.14 (0.57)	-13.7 (6.2)	.
SSC4	S0073-S0214 (81)	Maternal	14.26 (0.02)	-15.5 (4.1)	.
SSC6	SW2406-SW1057 (31)	Mendelian	5.83 (0.31)	-20.7 (7.3)	9.5 (9.1)
SSC7	SW1856 (59)	Mendelian	29.70 (< 0.001)	40.8 (5.5)	15.8 (8.4)
SSC8	SW268-S0017 (53)	Maternal	6.98 (0.56)	-15.3 (5.8)	.
SSC12	S0090-S0106 (79)	Paternal	7.39 (0.43)	-11.6 (4.3)	.
SSC13	S0076-S0068 (67)	Mendelian	6.54 (0.17)	-1.9 (7.8)	57.1 (15.8)
SSC14	SW210-S0007 (65)	Paternal	7.36 (0.43)	-13.3 (4.9)	.

[a] Genetic model which is most appropriate for the QTL. [b] Test statistics for the inferred genetic model vs. the H0 of no QTL, p values are genome-wide significance levels. [c] Estimated QTL effects for the inferred genetic model. The additive effect a is expressed as the deviation of the Meishan allele and the dominance effect d is expressed (where appropriate) as the deviation of the heterozygous animals from the mean of the homozygotes.

respectively. Further suggestive evidence was obtained for a paternally expressed QTL at the end of SSC1, an over-dominant QTL on SSC5, a QTL with opposite dominance effects between the sexes on SSC8, and a maternally expressed QTL on SSC13 (Table 2). For all putative QTL affecting EGR the Meishan allele gives rise to lower growth except for the suggestive imprinted QTL on SSC1 where a paternally inherited Meishan allele gives rise to higher growth. The half-sib analyses showed no evidence for QTL affecting EGR.

Results for TGR. The line-cross analyses revealed genome-wide significant QTL affecting TGR on SSC1, SSC4, and SSC7 (Table 2). The QTL affecting TGR on SSC1 maps to a different region than both putative QTL affecting EGR on SSC1 (Figure 3). This QTL affects TGR mainly additively with an estimated additive effect of –26.2 g/day. The significant QTL on SSC4 maps to the same position as the highly suggestive QTL affecting EGR, but for TGR this QTL is imprinted with exclusive maternal expression

Table 2. *-continued*

Chromosome	Marker (position, cM)	Genetic model[a]	Test statistic (p)[b]	a (s.e.)[c]	d (s.e.)[c]
LGR (g/day)					
SSC1	SW64-SW1851 (38)	Mendelian	9.9 (0.007)	-16.1 (3.7)	-5.9 (6.3)
SSC3	SW72 (1)	Paternal	9.11 (0.26)	-6.9 (2.3)	.
SSC3	S0206-SW902 (20)	Mendelian	6.07 (0.28)	-11.2 (3.4)	-8.3 (5.4)
SSC4	S0073-S0214 (79)	Mendelian	9.42 (0.02)	-12.8 (3.3)	11.1 (5.1)
SSC5	S0005-IGF1(80)	Maternal	6.74 (0.64)	-7.4 (2.8)	.
SSC6	SW2406-SW1057 (33)	Mendelian	6.53 (0.17)	-12.5 (4.2)	17.7 (8.1)
SSC6	S0035-SW2406 (7)	Maternal	6.72 (0.57)	-6.8 (2.6)	.
SSC7	SW1856 (59)	Mendelian	35.01 (< 0.001)	22.2 (3.1)	18.7 (4.7)
SSC8	SW905-SW268 (29)	Maternal	12.63 (0.07)	-9.6 (2.7)	.
SSC13	S0076-S0068 (72)	Mendelian	5.03 (0.53)	0.6 (4.3)	27.1 (8.5)
SSC17	SWR1004 (1)	Maternal	8.56 (0.40)	-6.5 (2.2)	.
BFT (mm)					
SSC2	IGF2-SW256 (5)	Paternal	31.2 (< 0.001)	0.61 (0.11)	
SSC4	Afabp-S0217 (63)	Mendelian	7.25 (0.13)	-0.35 (0.15)	-0.69 (0.23)
SSC5	S0005-IGF1 (83)	Paternal	9.67 (0.18)	0.37 (0.12)	.
SSC6	S0220-SW316 (91)	Maternal	8.43 (0.30)	0.33 (0.12)	.
SSC7	SW1856-S0102 (63)	Mendelian	57.86 (< 0.001)	-1.5 (0.14)	0.17 (0.22)
SSC14	SW857-SW295 (30)	Maternal	13.65 (0.02)	0.55 (0.15)	.
SSCX	SW2456-SW2467 (58)	X-linked	43.66 (< 0.001)	♂ 1.28 (0.16) ♀ 0.77 (0.15)	.

[a] Genetic model which is most appropriate for the QTL. [b] Test statistics for the inferred genetic model vs. the H0 of no QTL, p values are genome-wide significance levels. [c] Estimated QTL effects for the inferred genetic model. The additive effect a is expressed as the deviation of the Meishan allele and the dominance effect d is expressed (where appropriate) as the deviation of the heterozygous animals from the mean of the homozygotes.

(Figure 1). The most significant QTL was detected near the SLA region on SSC7. The estimated additive effect of 40.8 g/day indicates higher growth for a Meishan allele compared to an allele of the Dutch lines. This QTL was genome-wide significant under the half-sib model (Table 3), where nine families exceeded the nominal 5% threshold. These families show allele substitution effects between 76 and 155 g/day with higher growth for the Meishan allele in all these families.

The line-cross analyses showed six suggestive QTL affecting TGR. Of these, paternal expression was inferred for the suggestive QTL on SSC2, SSC6, SSC12, and SSC14. A suggestive QTL on SSC8 was maternally expressed but mapped to a different region than the maternally expressed QTL affecting EGR on SSC8 (Figure 2). The strongest suggestive QTL mapped to SSC13 and showed strong over-dominance (Table 2). For all putative QTL affecting TGR the Meishan allele is associated with lower growth, apart from the QTL on SSC7 (Table 2).

Results for LGR. Under the line-cross analyses, three significant QTL affecting LGR were found on SSC1, SSC4, and SSC 7. Furthermore, eight suggestive QTL were detected on SSC3 (2), SSC5, SSC6 (2), SSC8,

Table 3. Location of QTL affecting performance traits that exceed suggestive linkage under the half-sib model

Trait	Chromosome	Marker (position, cM)[a]	Test statistic (p)[b]
TGR	SSC7	LTA (55)	2.02 (0.04)
LGR	SSC1	CGA-S0313 (81)	2.01 (0.03)
LGR	SSC3	SW72 (1)	1.74 (0.38)
LGR	SSC6	SW2419 (191)	1.67 (0.46)
LGR	SSC7	SW1856-S0102 (61)	2.85 (< 0.001)
BFT	SSC2	IGF2-SW256 (10)	2.15 (0.02)
BFT	SSC7	SW1856-S0102 (61)	2.89 (< 0.001)
BFT	SSC14	SW857 (1)	1.67 (0.63)

[a] Best position across families.
[b] Test statistic across families for the H0 of no QTL, p values are genome-wide significance levels.

SSC13, and SSC17 (Table 2). The half-sib analyses showed significant QTL affecting LGR on SSC1 and SSC7, and suggestive QTL on SSC3 and SSC6 (Table 3).

Under the line-cross model, the significant QTL affecting LGR on SSC1 maps at 38 cM, exactly between the sex specific QTL affecting EGR and the QTL affecting TGR (Figure 3). A 5 cM grid search, fitting two QTL simultaneously (KNOTT *et al.* 1998), resulted in two QTL affecting LGR at 16 and 71 cM, but this was no significant improvement compared to fitting the single best QTL at 38 cM. Under the half-sib approach, a significant QTL on SSC1 mapped to the same interval as the QTL for TGR under the line-cross model (Table 2, Table 3, and Figure 3). Estimated allele substitution effects at this position for seven informative families varied between 50 and 100 g/day. For five families it was deduced that the Meishan gave lower growth whereas for one family the Meishan allele gave higher growth.

The QTL affecting LGR on SSC4 mapped to the same region as those detected for EGR and TGR (Figure 1). The estimated additive and dominance effects are comparable to those of the Mendelian QTL affecting EGR.

The QTL on SSC7 is similar to that detected for TGR, with a positive effect on growth for the Meishan allele, albeit smaller than the effect for TGR (Table 2). Under the half-sib model, the estimated effects for 13 informative families are also smaller compared to TGR: between 52 and 102 g/day. Within these families the Meishan allele was consistently associated with lower growth.

A highly suggestive, maternally expressed QTL (P = 0.07) mapped to the neighboring interval of the maternally expressed QTL affecting EGR on SSC8 (Table 2, Figure 2). On SSC3, a suggestive QTL is detected at the beginning of the linkage group under both the line-cross (paternally expressed) and the half-sib approach, with an additional suggestive QTL at 20 cM under the line-cross approach. For SSC6, the line-cross model showed a maternally expressed QTL at 7 cM and a Mendelian QTL at 33 cM, the latter coinciding with a suggestive QTL affecting

Table 4. Location and characterization of QTL affecting litter size that exceed suggestive linkage under the line-cross models

Chromosome	Marker (position, cM)	Genetic model[a]	Test statistic (p)[b]	a (s.e.)[c]	d (s.e.)[c]
LS1 total number born					
SSC7	S0025-S0064 (10)	Maternal	8.73 (0.38)	0.75 (0.25)	.
LS2 total number born					
SSC12	S0090 (71)	Mendelian	5.92 (0.26)	0.29 (0.32)	1.70 (0.51)
SSC14	SW210-S0007 (62)	Maternal	7.20 (0.51)	-0.75 (0.28)	.
SSC17	SW840-SW1031 (43)	Mendelian	5.35 (0.61)	-0.91 (0.39)	1.39 (0.66)

[a] Genetic model which is most appropriate for the QTL. [b] Test statistics for the inferred genetic model vs. the H_0 of no QTL, p values are genome-wide significance levels. [c] Estimated QTL effects for the inferred genetic model. The additive effect a is expressed as the deviation of the Meishan allele and the dominance effect d is expressed (where appropriate) as the deviation of the heterozygous animals from the mean of the homozygotes.

TGR (Table 2). In contrast, the half-sib analysis showed a suggestive QTL at 191 cM of SSC6 (Table 3). The suggestive QTL affecting LGR on SSC13 mapped to the same area as the suggestive QTL affecting TGR and was also over-dominant.

Results for BFT. The line-cross analyses showed significant QTL affecting BFT on SSC2, SSC7 , SSC14, and the X chromosome. Additional suggestive QTL were detected on SSC4, SSC5, and SSC6 (Table 2). Under the half-sib analyses significant QTL were detected on SSC2 and SSC7 as well as a suggestive QTL on SSC14.

The highly significant QTL on SSC2 was paternally expressed with an estimated effect 0.6 mm BFT for the paternally inherited Meishan allele. Under the half-sib model, 12

Table 5. Contributions of the paternal, maternal, and dominance components for the QTL on SSC4 and SSC7. The QTL are ordered with increasing degree of imprinting within a linkage group.

Trait	Position	F ratios for individual components of the model		
		Paternal	Maternal	Dominance
EGR	SSC4 82 cM	5.29	5.81	6.00
LGR	SSC4 79 cM	5.08	10.65	4.75
BFT	SSC4 63 cM	0.24	7.67	8.30
TGR	SSC4 81 cM	2.23	15.16	2.27
LGR	SSC7 59 cM	22.13	28.60	15.92
BF-HGP[†]	SSC7 57 cM	30.27	49.35	0.04
TGR	SSC7 59 cM	18.14	36.37	3.66
BFT	SSC7 63 cM	36.19	81.02	0.49
MD-HGP[‡]	SSC7 56 cM	4.74	50.33	2.20

[†] Backfat thickness, measured with Hennessy grading probe, results from DE KONING *et al.* (2000)
[‡] Muscle depth, measured with Hennessy grading probe, results from DE KONING *et al.* (2000)

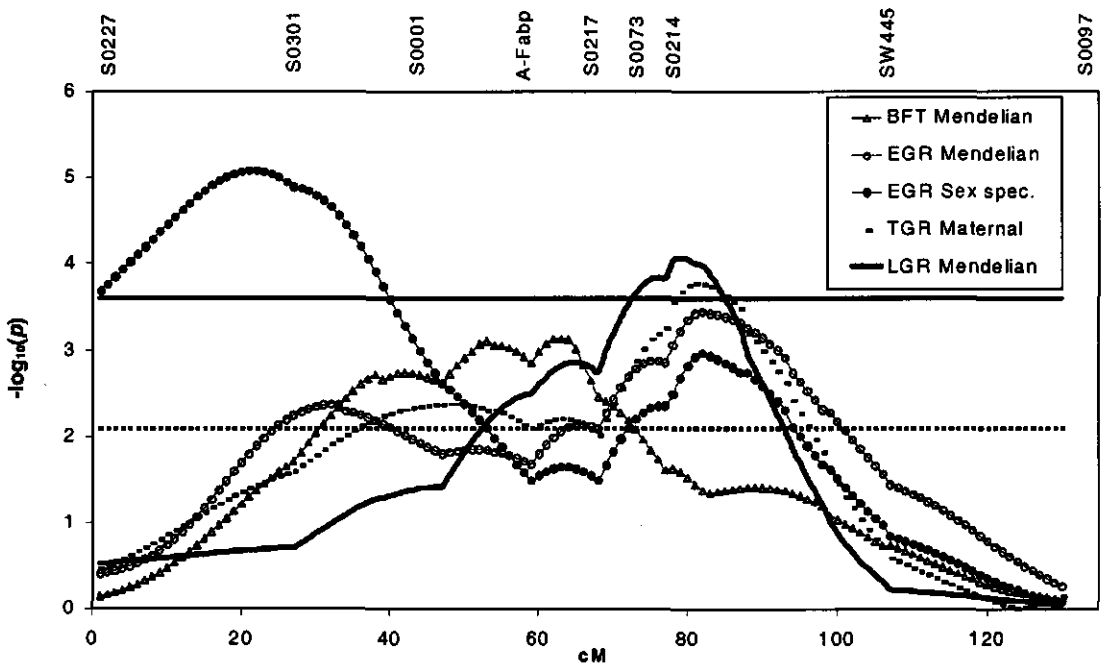

Figure 1. Test-statistic along SSC4 for QTL affecting early growth (EGR), test growth (TGR), life growth (LGR), or ultrasonic backfat thickness (BFT) for the inferred genetic models. The dashed and solid horizontal lines denote the thresholds for suggestive and genome-wide significant linkage, respectively. Marker names are given above the graph.

informative families showed allele substitution effects between 2.1 and 3.9 mm of BFT at the best position across families. Within all these families, the Meishan haplotype was associated with higher BFT. To illustrate the exclusive paternal expression at the distal tip of SSC2p, the test statistics of four different models are compared in Figure 4.

The QTL on SSC7 mapped to the same region as the QTL affecting TGR and LGR. Like the growth QTL, the effect of the Meishan allele is against expectation because it gives rise to lower BFT (Table 2). For the half-sib analysis, 14 families exceeded the nominal 5% threshold at the overall best position of the QTL, with estimated allele substitution effects between 2.0 and 4.4 mm of BFT. For all these families the Meishan haplotype was associated with lower BFT.

The QTL on SSC14 was maternally expressed with an estimated effect of 0.55 mm of BFT for a maternally inherited Meishan allele. Given the maternally expressed QTL at 30 cM (Table 2), it was surprising that a suggestive QTL was detected in the same marker interval under the (paternal) half-sib analyses.

The QTL on the X chromosome was highly significant with a much larger effect in the F_2

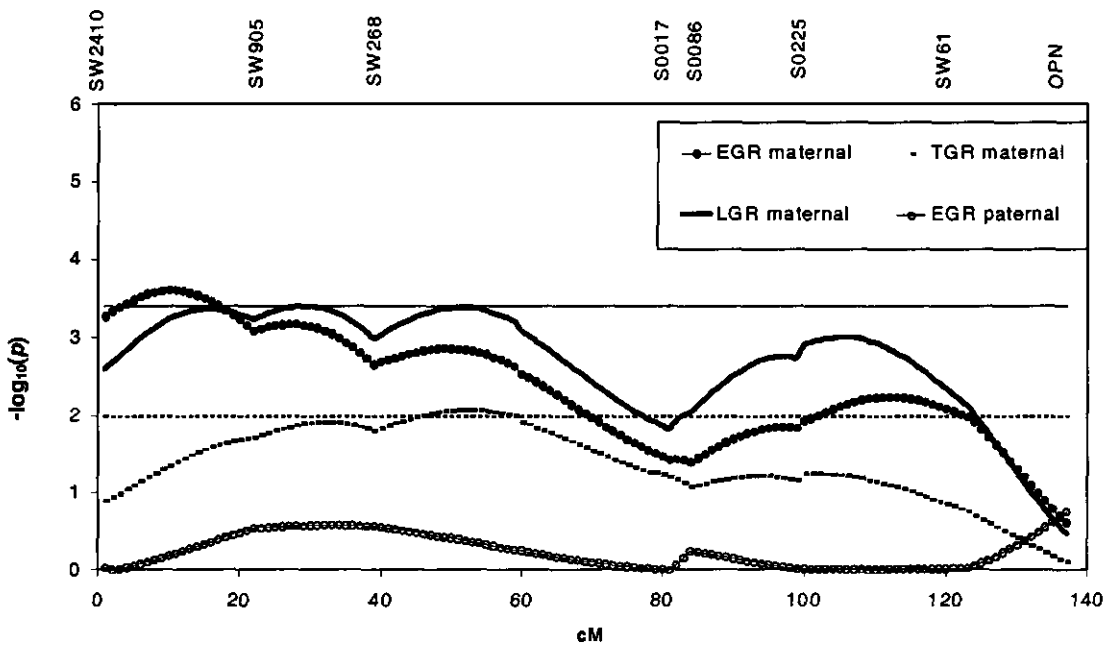

Figure 2. Test-statistic along SSC8 for the maternally expressed QTL affecting early growth (EGR), test growth (TGR), and life growth (LGR). The test statistic of a putative paternally expressed QTL affecting early growth is included for comparison. The dashed and solid horizontal lines denote the thresholds for suggestive and genome-wide significant linkage, respectively. Marker names are given above the graph.

males compared to the females. An F_2 boar carrying an allele originating from the Meishan had on average 2.5 mm more BFT than a boar carrying an allele originating from the Dutch lines. In the F_2 sows, the estimated difference was only 1.5 mm (Table 2).

The strongest suggestive QTL was detected on SSC4 (P = 0.13) with an estimated effect of 0.35 mm lower BFT for the Meishan allele, indicating a second cryptic allele. This QTL maps to a different marker interval than the QTL affecting the three growth traits on SSC4 (Figure 1). On SSC5, a suggestive QTL maps to the same interval as a suggestive QTL affecting LGR. However, the QTL affecting BFT is paternally expressed whereas maternal expression was inferred for the QTL affecting LGR. The suggestive QTL at 91 cM is maternally expressed and maps to a different region than any of the putative QTL affecting the growth traits on SSC6.

Results for reproduction traits. The best QTL affecting litter size are summarized in Table 4. The line-cross analyses revealed one suggestive QTL affecting LS1 and three suggestive QTL affecting LS2. It must be noted that the number of F_2 animals with phenotypic data available for these traits is very low compared to the performance traits

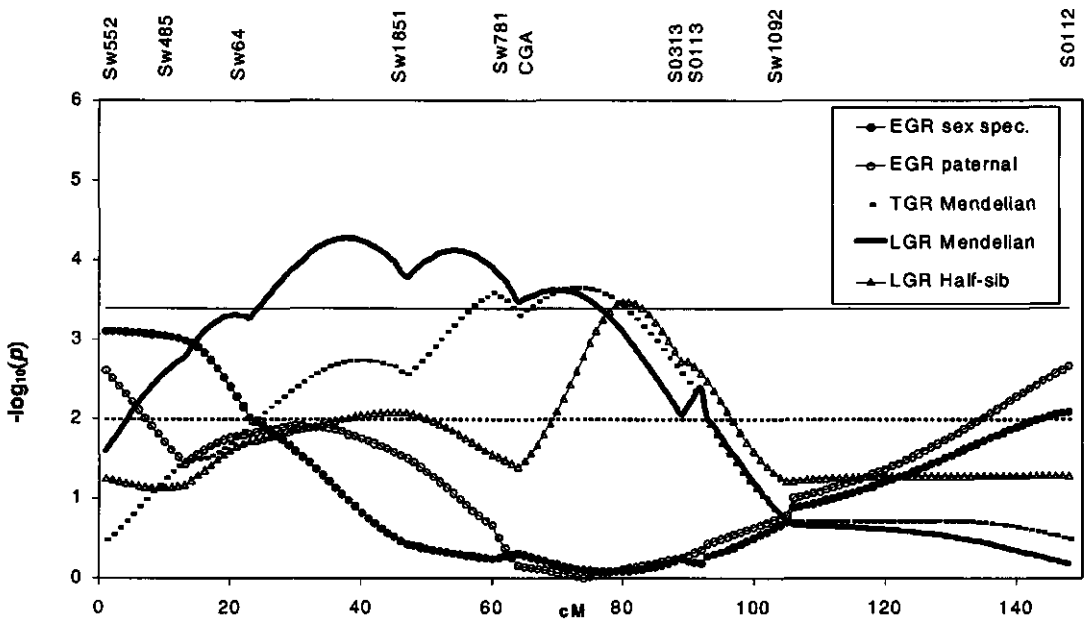

Figure 3. Test-statistic along SSC1 for QTL affecting early growth (EGR), test growth (TGR), or life growth (LGR) for the inferred genetic models. The dashed and solid horizontal lines denote the thresholds for suggestive and genome-wide significant linkage, respectively. Marker names are given above the graph.

(Table 1). For LS1, a suggestive QTL was detected on SSC7 with maternal expression. The estimated effect was 1.5 piglet more for a sow inheriting a maternal Meishan allele instead of an allele originating from the Dutch lines. For LS2, three suggestive QTL were detected on SSC12, SSC14, and SSC17. The suggestive QTL on SSC12 was over-dominant with an estimated dominance effect of 1.7 piglet and an estimated additive effect of 0.3 piglet. The suggestive QTL on SSC14 was maternally expressed with an estimated effect of similar magnitude as the suggestive QTL affecting LS1 on SSC7, but with opposite sign (Table 4). The suggestive QTL on SSC17 showed some slight over-dominance with estimated additive and dominance effects of –0.9 and 1.4 piglet respectively. Because Meishan pigs have on average larger litters than commercial lines, the QTL effects on SSC14 and SSC17 are opposite the expectation, with the Meishan allele giving a lower litter size.

Discussion

For production traits, a number of genome scans have been performed and many candidate genes have been evaluated. This study has revealed several QTL affecting growth and fat deposition in pigs and added interesting new insight into their mode of expression. Given the large number of QTL

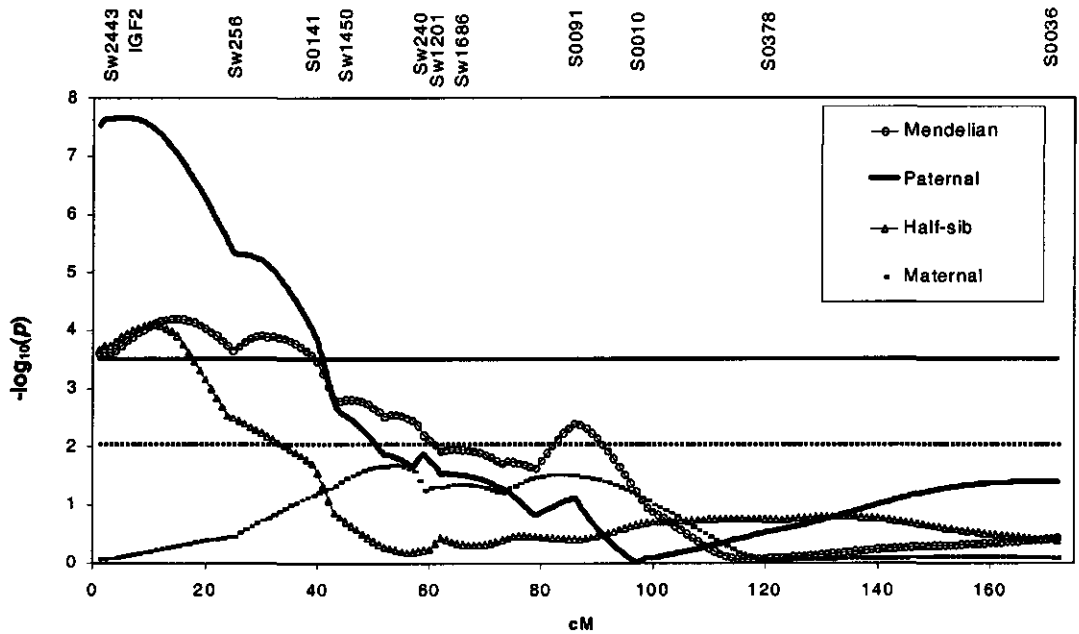

Figure 4. Test-statistic along SSC2 for the presence of a QTL affecting ultrasonic backfat thickness under four different models. The dashed and solid horizontal lines denote the thresholds for suggestive and genome-wide significant linkage, respectively. Marker names are given above the graph.

identified in the present study, we will not discuss all the identified QTL in detail. In the following paragraphs we will first discuss some findings for specific traits and subsequently interesting results across traits and models.

Backfat thickness. For BFT, the pre-adjustment of the trait values was done with body weight at end of test as a covariable. This is analogous to the analysis carcass backfat thickness measured with the Hennessy grading probe (BF-HGP) by DE KONING *et al.* (1999, 2000), where carcass weight was included as a covariable. In the segregation analyses of BFT on the population described in this study, JANSS *et al.* (1997) did not include body weight as a covariable. Fitting a correlated trait as a covariable, reduces the variance of the trait under study. This should increase the power to detect QTL for this trait, except for QTL affecting both traits either by pleiotropy or close linkage. To test the effect of trait definition, we also analyzed BFT without pre-adjustment for body weight. Under the model without adjustment for body weight, the genome-wide significant QTL affecting BFT on SSC14 was only suggestive while the QTL on SSC2, SSC7, and SSCX remained genome-wide significant albeit with lower test statistics than those reported in Table 2. Fitting a correlated trait as a covariable, reduces the variance of the trait under study. This should increase the power to

detect QTL for this trait, except for QTL affecting both traits either by pleiotropy or close linkage. In contrast, the QTL on SSC4 was genome-wide significant ($P = 0.01$) under the model without adjustment for body weight while it was only suggestive ($P = 0.13$) under the model presented in Table 2. This can be explained by the QTL affecting growth, also on SSC4 (Table 2, Figure 1). Given the negative effect of the Meishan allele for the QTL on SSC4 for all performance traits, the estimated QTL effect for BFT absorbs part of the effect of the growth QTL on SSC4, when not adjusting for body weight.

The paternally expressed QTL affecting BFT on SSC2 is in line with earlier investigations for QTL affecting BF-HGP on this experimental populations (DE KONING *et al.*, 2000; RATTINK *et al.*, 2000). However, the position of the imprinted QTL affecting BFT in the present study (5 cM) is different from the QTL affecting BF-HGP (36 cM) and closer to the *IGF2* region, for which an imprinted QTL has been reported earlier (JEON *et al.*, 1999; NEZER *et al.*, 1999). Using only animals that had observations for both ultrasonic and backfat thickness at slaughter (n = 774) showed that the different position for BFT in the present study could not be attributed to the larger number of animals in the present study (data not shown). The phenotype for slaughter backfat consisted of a single measurement on the carcass (DE KONING *et al.*, 1999) whereas the BFT phenotype in the present study was the average of 4-8 measurements at different positions along the spine. The estimated genetic correlation between the two traits was 0.93 using MTDFREML (BOLDMAN *et al.*, 1995), which means that genetically the two traits are very similar but not identical. The test statistic for a Mendelian QTL along SSC2 shows a very broad peak, indicating that, beside the paternally expressed QTL near the *IGF2* region, there might be additional QTL affecting BFT on SSC2.

The QTL affecting BFT on SSC7 is in agreement with findings for BF-HGP that were reported in literature and discussed by DE KONING *et al.* (1999, 2000) and RATTINK *et al.* (2000), for the same experimental population as in the present study. The QTL on SSCX has also been reported and discussed in the analyses of BF-HGP (HARLIZIUS *et al.*, 2000).

Growth. This study provides strong evidence for QTL affecting growth on SSC1. However, there is some variation in the most likely position and the genetic model of the QTL for the different growth traits (Table 2). Also the profiles of the test statistics in Figure 3 do not point towards a single QTL affecting all three growth traits. Especially the broad peak for LGR suggests that there are multiple QTL affecting growth on SSC1, although a model fitting two Mendelian QTL did not give a significant improvement. Support for additional growth QTL comes from PASZEK *et al.* (1999) who report a QTL affecting growth in a region comparable to the marker interval SW1092-S0112 on SSC1. One of the candidate genes on SSC1 is the melanocortin-4 receptor (*MC4R*) for which KIM *et al.*

(2000) presented significant associations with growth and fatness traits.

To our knowledge, this is the first study reporting a significant QTL affecting growth on SSC8. This is not surprising because the QTL shows exclusive maternal expression. Under a Mendelian model, which is applied in most studies to date, the QTL was only suggestive. The QTL was genome-wide significant for EGR, strongly suggestive for LGR, suggestive for TGR, and imprinted throughout. It seems that the effect of this QTL is strongest for earlier growth as can also be seen in Figure 2. It can also be seen from Figure 2 that the test statistics for EGR and LGR are significant but rather flat. This means that, although there is significant evidence for an imprinted QTL affecting growth on SSC8, its position cannot be estimated very accurately in the present study. A possible explanation could be the presence of two or more QTL affecting growth on SSC8.

Across traits and models

Imprinting. In the present study, imprinting was only inferred if a model with only a single parental component was not significantly worse compared to a model with both parental components and a dominance component. For some loci, like the QTL affecting growth on SSC8, this is true for all the examined traits. For other loci, like the growth QTL on SSC4, we inferred an imprinted QTL for TGR and a Mendelian QTL for EGR at similar positions. When looking at the contributions of the three components across a range of traits the classification as Mendelian or imprinted is not that unequivocal. Table 5 shows the F ratios for the three components for a number of traits on SSC4 and SSC7. When looking across traits, there is a range in parent-of-origin effects from Mendelian expression to uni-parental expression rather than a clear division between Mendelian and imprinted QTL. For the QTL affecting EGR on SSC4 all three components are contributing equally (Table 5). The QTL affecting BFT was inferred as Mendelian, although the paternal component is negligible and only the maternal and dominance components are significant (Table 5). The QTL affecting TGR shows only a significant maternal component and the QTL affecting LGR shows the combined action of the QTL affecting EGR and TGR (Table 5). For SSC7 the QTL affecting LGR has comparable contributions for the paternal and maternal component, whereas for BF-HGP, TGR and BFT the paternal component is still highly significant but considerably less extreme than the maternal component. For muscle depth measured with Hennessy grading probe (MD-HGP), the difference between the paternal and maternal component was so extreme that a maternally expressed QTL was inferred (DE KONING *et al.*, 2000). This range of parent-of-origin effects provides support for the hypothesis that QTL might be imprinted during specific stages of development and show Mendelian expression at other stages.

Chromosome 4. Many studies have focussed on SSC4 since the first report of a significant QTL affecting growth and backfat

thickness in an experimental cross between Wild Boar and Large White by ANDERSSON *et al.* (1994). The QTL was confirmed within descendants of this experimental population by MARKLUND *et al* (1999), while KNOTT *et al.* (1998) showed a significant imprinting effect on SSC4 in the same population described by ANDERSSON *et al.* (1994). WALLING *et al.* (1998) showed significant QTL on SSC4 affecting fatness and growth in an F_2 cross between Meishan and Large White. The most likely positions of the QTL were very comparable between WALLING *et al.* (1998) and the present study. Although in both studies the Meishan allele is associated with lower growth, the effect of the Meishan allele on backfat thickness is different. WALLING *et al.* (1998) reported an increased backfat thickness for the Meishan allele whereas in the present study the Meishan allele gives lower backfat.

Part of the animals of the present study (n = 586) were included in a joint analysis for SSC4 of pig data from six countries described by WALLING *et al.* (2000). Although no significant effects were found in the individual analysis of the Dutch data, the direction of the estimated QTL effects were in agreement with the present study (WALLING *et al.*, 2000). The results from the present study, illustrated by Figure 1, and the results from WALLING *et al.* (1998, 2000) point toward multiple linked QTL affecting growth and fatness on SSC4, rather than a single QTL. A possible dissection of these QTL does not only require additional markers but also software that can fit multiple QTL with different genetic models.

Chromosomes 6 and 7. In the present study six suggestive QTL mapped to different regions of SSC6 with maternal expression (7, 91, and 191 cM), Mendelian expression (31 and 33 cM), or an effect under a paternal half-sib design (191 cM) (Tables 2, 3). Together with the two significant imprinted QTL affecting intramuscular fat content presented by DE KONING *et al.* (2000), the present study provides further evidence for imprinting on SSC6. However, the new evidence from this study gives no indication for specific regions on SSC6 being either paternally or maternally expressed.

The cryptic allele of the QTL affecting backfat thickness on SSC7 has been reported earlier by DE KONING *et al.* (1999, 2000) and is a confirmation of earlier findings by ROHRER and KEELE (1998) and WANG *et al.* (1998). The present study also confirms a much more recent finding by ROHRER (2000) of a cryptic allele for growth on SSC7. Considering the cryptic nature of the Meishan allele for both traits and the similar positions of the best QTL (Tables 2, 3, and 5) for all traits points toward a single QTL affecting both growth and backfat thickness. To test this, the correlation of the estimated QTL effects for the individual families under the half-sib analysis can be compared to the expected correlation if a QTL only affects one of the traits (SCHROOTEN *et al.*, submitted). Another alternative would be a multivariate QTL analysis that was recently proposed for an outbred F_2 design by KNOTT and HALEY

(2000). The beneficial effect of the Meishan allele on both growth and backfat thickness make this QTL an interesting candidate for marker assisted introgression. For this purpose it is not required to distinguish between a single pleiotropic QTL or closely linked QTL.

Conclusion

This study has confirmed QTL affecting growth and backfat thickness on SSC2, SSC4, SSC7, and SSCX. New significant QTL affecting growth have been detected on SSC1 and SSC8, and a new QTL affecting backfat thickness was detected on SSC14. Furthermore, new genome-wide significant imprinting effects were found for SSC4, SSC8, and SSC14. Several suggestive QTL also showed imprinting effects, but need to be confirmed in other populations or for other traits. So far imprinting has only been tested in a limited number of studies, which hampers the comparison across experiments. The power to detect QTL affecting litter size was low due to the small number of F_2 sows with reproduction data.

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On the detection of imprinted quantitative trait loci in experimental crosses between outbred species.

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Abstract – In QTL detection, imprinting effects can be estimated when parental origin of alleles is tractable. In this paper, the quantitative genetic aspects of imprinted genes and statistical properties of methods to detect imprinted QTL are studied. Different models to detect imprinted QTL and to distinguish between imprinted and Mendelian QTL were compared in a simulation study. Mendelian and imprinted QTL were simulated in an F_2 design and analyzed under Mendelian and imprinting models. Mode of expression was evaluated against the H_0 of a Mendelian QTL as well as the H_0 of an imprinted QTL. An imprinting model with a paternal, maternal, and dominance component was tested against: a) a Mendelian model, and b) an imprinting model with a single parental effect. It was shown that imprinted QTL might remain undetected when only analyzing the genome with Mendelian models. Compared to testing against a Mendelian QTL, using the H_0 of an imprinted QTL gave a higher proportion of correctly identified imprinted QTL, but also gave a higher proportion of false inference of imprinting for Mendelian QTL. When QTL were segregating in the founder lines, spurious inference of imprinting became more prominent under both tests, especially for designs with few F_1 sires.

Parental genomes undergo modifications during gametogenesis, resulting for some genes in parent-of-origin-specific expression in the offspring. This phenomenon of genomic imprinting, as a form of epigenetic gene regulation, has been shown to influence several genes and traits in animals (MORISON *et al.*, 2001) as well as plants (ALLEMAN and DOCTOR, 2000) and insects (LOYD *et al.*, 1999). Different approaches have been used over time to identify imprinted areas in the genome. These include mice with uni-parental disomy for portions of the genome (BEECHY, 1999), chromosomal anomalies associated with imprinted diseases in humans (NICHOLLS *et al.*, 1998), and molecular genetic approaches looking at methylation patterns (PETERS *et al.*, 1999).

Genome scans that have been carried out for many species have revealed a number of genes or Quantitative Trait Loci (QTL) contributing to genetic variation. Genome scans can also be used to search for imprinted QTL (KNOTT *et al.*, 1998) but these opportunities have not been exploited systematically. Genome scans in plants or animals are often performed on experimental crosses between divergent lines. For most livestock species, inbred lines are not available and therefore existing breeds that are divergent for the phenotype of interest are crossed to detect QTL underlying the phenotypic differences. For the detection of

Table 1. Values of genotypes for a bi-allelic gene with exclusive uni-parental expression

Genotypes	A- (=AA +AB)	B- (= BB +BA)
Frequencies	p	q
Assigned values	a_i	$-a_i$
Genotypic value[a]	$a_i-a_i(p-q)$	$-a_i-a_i(p-q)$
Breeding value[a]	$2qa_i$	$-2pa_i$
	$2\alpha 1$	$2\alpha 2$

[a] The genotypic and breeding value for the non-imprinted sex, for the imprinted sex these values are zero (for proof see Appendix A).

Mendelian QTL, such designs are less powerful compared to inbred species, because markers are not fully informative (HALEY *et al.*, 1994) and the lines that are crossed might share QTL alleles for the traits of interest (ALFONSO AND HALEY, 1998). An advantage of crossing outbred lines is that the parental origin of alleles can often be traced back from the F_2 to the F_1 parents, which is a pre-requisite to test for of parent-of-origin effects such as genomic imprinting (KNOTT *et al.*, 1998). This pre-requisite excludes crosses between completely inbred lines because all F_1 parents will be heterozygous for the same alleles. This can be circumvented by using reciprocal backcrosses, as demonstrated by CLAPCOTT *et al.* (2000) who found a parent-of-origin effect for a trypanosomiasis susceptibility locus in inbred mice.

Methods to detect imprinted QTL have been described by KNOTT *et al.* (1998) and successfully applied to genome scans by JEON *et al.* (1999) and in a modified form by De KONING *et al.* (2000). NEZER *et al.* (1999) used a maximum likelihood algorithm to detect QTL with specific LOD scores for imprinted QTL against Mendelian QTL, but their methods are not described in detail. The quantitative genetics of imprinted QTL and the statistical properties of tests to detect imprinted QTL and distinguish between Mendelian and imprinted QTL have not been described in great detail. In the present study we will first outline some of the quantitative genetic aspects of an imprinted gene compared to that of a gene with Mendelian expression. Subsequently, we will describe the results of a comprehensive simulation study on the detection of imprinted and Mendelian QTL in outbred F_2 designs.

Theory

Quantitative genetics of an imprinted gene: For a Mendelian gene with additive effect a and dominance effect d, with frequency p for the positive allele of a, and q for the negative allele the population mean under random mating is (FALCONER AND MACKAY, 1996, p.118):

$$M = a(p-q) + 2pqd \quad (1)$$

The average effect of allele substitution α is:

$$\alpha = a + d(q-p) \quad (2)$$

The single gene variance is (FALCONER AND MACKAY, 1996, p.126-127):

$$V_G = 2pq[a + d(q-p)]^2 + (2pqd)^2 \quad (3)$$

Now consider a biallelic, imprinted gene with exclusive uni-parental expression under

random mating. There is no dominance effect because only one allele is expressed. The heterozygous individuals (AB and BA) have the same expected phenotypic value as either the AA or BB individuals, depending on the type of imprinting and from which parent they inherited the A and the B allele. If the gene is paternally expressed and the first allele denotes the paternally inherited allele, the AB individuals have the same genotypic value as the AA individuals. The uni-parental gene effect is denoted by a_i, with frequencies p and q within the non-imprinted sex (i.e. the sex from which alleles will be expressed in the offspring). The population mean M becomes:

$$M = a_i(p - q) \quad (4)$$

The average effects $\alpha 1$ and $\alpha 2$ for alleles A and B, respectively, become:

$$\begin{aligned} \alpha 1 &= qa_i \\ \alpha 2 &= -pa_i \end{aligned} \quad (5)$$

The average allele substitution effect is the difference between these average effects :

$$\alpha = \alpha 1 - \alpha 2 = a_i \quad (6)$$

These components are summarized in Table 1. The single gene variance V_{Gi} for an imprinted gene is (Table 1):

$$V_{Gi} = p(2qa_i)^2 + q(-2pa_i)^2 = 4pqa_i^2 \quad (7)$$

The Appendix shows the derivations for a partially imprinted gene, where both parental alleles contribute to the phenotype, but not equally. Whether genes can be partially imprinted is unclear but it is realistic to assume that some genes are only imprinted in specific tissues or during specific stages of development, resulting in a phenotype that appears to be partially imprinted.

Detection of imprinted QTL in outbred F_2 designs: While there is an abundance of tools for the QTL analyses of crosses between inbred lines (MANLY AND OLSEN, 1999), the analyses of crosses between outbred species are mainly based on the line-cross methodology proposed by HALEY *et al.* (1994). Under the line-cross model it is assumed that the two founder lines, although they may segregate at the marker loci, are fixed for alternative alleles at the QTL affecting the traits of interest. For every F_2 individual, the probabilities that it inherited two alleles from line 1 (P_{11}), two alleles from line 2 (P_{22}), or one from each line (P_{12} or P_{21}, different subscripts according to parental origin; first subscript is paternally inherited allele) are inferred at fixed (e.g.1 cM) intervals across the genome. Under the traditional line-cross approach (Mendelian inheritance), an additive effect (a) and a dominance effect (d) are estimated using the regression of the phenotypes on $P_a=P_{11}-P_{22}$ and $P_d=P_{12}+P_{21}$:

$$y_j = m + ap_{a_j} + dp_{d_j} + e_j \quad (8)$$

Where y_j is the trait score of individual j, m is the population mean, a and d are the estimated additive and dominant effect of a putative QTL at the given location, p_{a_j} is the conditional probability of animal j to carry two alleles of line 1, p_{d_j} the conditional

probability of animal j to be heterozygous and e_j is the residual error. The calculation of these probabilities and QTL effects are described in more detail by HALEY *et al.* (1994) and applications to crossbred populations are numerous (e.g., ANDERSSON *et al.*, 1994; KNOTT *et al.*, 1998).

For imprinted genes, individuals that are heterozygous with respect to the line origin of their marker alleles are expected to differ in their phenotypes, depending on the parental origin of their alleles. To test for imprinting, KNOTT *et al.* (1998) added the contrast between the two types of heterozygous individuals as an additional imprinting component to model (8):

$$y_j = m + ap_{a_j} + dp_{d_j} + ip_{i_j} + e_j \quad (9)$$

Variables are as in (8), with the extension that i is the estimated imprinting effect and $p_{i_j} = P_{12}\text{-}P_{21}$, the probability that individual j is heterozygous and inherited the line 1 allele through its sire, instead of through its dam. DE KONING *et al.* (2000) proposed a re-parameterization of (9) by introducing the conditional probabilities that an individual inherited a line 1 allele through its sire P_{pat} or through its dam P_{mat}:

$$p_{pat} = (p_{11} + p_{12}) - (p_{22} + p_{21}) = p_a + p_i$$
$$p_{mat} = (p_{11} + p_{21}) - (p_{22} + p_{12}) = p_a - p_i \quad (10)$$

Model (9) can be re-written with a specific maternal and paternal QTL component:

$$y_j = m + a_{pat} p_{pat_j} + a_{mat} p_{mat_j} + dp_{d_j} + e_j \quad (11)$$

Where a_{pat} is the paternally inherited QTL effect and a_{mat} is the maternally inherited QTL effect. Model (9) and (11) are identical in terms of total variance explained by the model. KNOTT *et al.* (1998) propose to test for imprinting by finding the best QTL under model (9) and comparing the variance explained by this QTL under the full model [either (9) or (11)] against the variance explained by the best QTL under a Mendelian model (8). This test is an F test with 1 d.f. in the numerator and (n-4) d.f. in the denominator with H_0: $i = 0$ ($a_{pat} = a_{mat}$, i.e. the QTL is Mendelian). DE KONING *et al.* (2000) proposed to scan the genome with reduced imprinting models with exclusive paternal or maternal expression:

$$y_j = m + a_{pat} p_{pat_j} + e_j$$
$$y_j = m + a_{mat} p_{mat_j} + e_j \quad (12)$$

The best QTL under these reduced models cannot be tested directly against a Mendelian model because the reduced model is of lower rank than the Mendelian model. DE KONING *et al.* (2000) evaluated the genetic model for a putative QTL by fitting the full model (11) to the best position of a QTL and calculating the F ratios for the individual components. Imprinting was inferred if only one of the parental contributions was significant and no dominance was present. Rather than looking

at the F ratios of individual components, imprinting can be tested with a single F ratio of the full model (11) against the relevant reduced model with uni-parental expression. Imprinting is inferred when the full model does not explain significantly more variance than the reduced model at the position of the best QTL under the reduced model. This is an F test with 2 d.f. in the nominator and (n-4) d.f. in the denominator. The H_0 of this test is that the QTL is imprinted (e.g. H_0: $a_{mat} = d = 0$ when evaluating a model with exclusive paternal expression).

Simulation Study

The objective of the simulation study was twofold: 1) Empirically determine the power for detection of imprinted QTL in outbred F_2 designs under Mendelian or imprinting models. 2) Quantify the risk of spurious detection of imprinted QTL under different tests.

Simulation details: The outline of the simulation study is comparable to that of ALFONSO AND HALEY (1998), who investigated the effect of mating design and segregation of QTL alleles in the founder lines on the power of detecting Mendelian QTL. F_1 individuals were generated by random mating of 20 sires from line 1 to 80 different dams (4 dams / sire) from line 2, each having five offspring. For most of the simulations 20 F_1 sires and 80 F1 dams (4 dams / sire), were randomly mated to produce 400 F_2 offspring (5 offspring / dam). We also simulated an extreme design, where only two F_1 sires were mated to 80 F_1 dams (40 dams / sire). Marker data were simulated for all animals for a 100 cM chromosome with 11 evenly spaced markers. To have fully informative markers with regard to line origin as well as optimal distinction of parental origin for the marker alleles in the F_2, eight alleles were simulated for every marker, with four line-specific alleles segregating at equal frequencies in the two founder lines. An additive, a dominant ($a = d$), a paternally expressed, or a maternally expressed bi-allelic QTL was simulated at 46 cM. Founder lines were either fixed for alternative QTL alleles or segregating at a frequency of 0.80 and 0.20 for the positive allele, respectively. For all scenarios, QTL effects were varied between 1, 0.75, 0.50 and 0.25. Imprinted QTL were simulated with exclusive uni-parental expression and no dominance (i.e. complete imprinting). For specific scenarios, a larger range of QTL effects was considered, especially in the range between 0.50 and 0.25. The phenotype of an individual was further determined by ten unlinked bi-allelic QTL, with an effect of 0.25 and segregating at a frequency of 0.5 in both founder lines, giving an expected additive genetic variance of 0.31 (ALFONSO AND HALEY, 1998). An additional environmental component was sampled from a normal distribution with a variance of 0.47 and added to the genetic (QTL) value of an individual to obtain the phenotype (ALFONSO AND HALEY, 1998). For an additive, dominant, or imprinted QTL, an effect of 1 was approximately equivalent to respectively 0.44, 0.61, or 0.75 phenotypic S.D. Thousand replicates were simulated and analyzed for every combination

of design, QTL model, and QTL effect. For every mating design, simulations were also performed without a QTL to validate the use of chromosome-wide 5% thresholds.

Analyses: For every replicate, coefficients P_{11}, P_{12}, P_{21}, and P_{22} were estimated following HALEY *et al.* (1994). For every replicate, the best Mendelian and imprinted QTL were found using (8) and both the paternal and maternal model of (12). For these three models, a chromosome-wide 5% threshold against the H_0 of no QTL was imposed to claim a significant QTL. These thresholds were obtained by permutation tests (CHURCHILL AND DOERGE, 1994) with 10,000 permutations for every 20th replicate and subsequent averaging over the 50 thresholds. For the significant replicates of both reduced imprinting models (12), imprinting was tested in the following manner: Alternative a) It was tested whether, at the best position of an imprinted QTL for that reduced model, a full model (9,11) explained significantly more variance than a Mendelian QTL (8). This test, which will be referred to as F_{Mend} was described by KNOTT *et al.* (1998), with the exception that here F_{Mend} is carried out against a Mendelian QTL at the position of the best imprinted QTL, which is not necessarily the best position of the Mendelian QTL. Alternative b) It was tested, at the position of the best imprinted QTL, whether the specific reduced model (12) was not significantly worse than the full model (9, 11) at that position. This test will be referred to as F_{Red}. For both a) and b), a tabulated F value corresponding to $P = 0.05$ was imposed to respectively infer (a) or reject (b) imprinting. Alternative c) Imprinting was only inferred if both a) and b) pointed towards imprinting.

Results

Detection of imprinted QTL: The results of the analyses of data simulated with an imprinted QTL with Mendelian and imprinting models are summarized in Table 2. Under fixation of founder lines, the results for paternally and maternally expressed QTL were very similar and therefore only the results for paternal expression are given in Table 2. Under this scenario, all replicates showed significant QTL under both the Mendelian and the correct imprinting model for QTL effects of 0.50 or larger (Table 2). However, for a QTL effect of 0.25, only 83% of the replicates showed significant QTL under a Mendelian model while under the imprinting model all replicates showed significant QTL. The estimated QTL position was unbiased under both models although the Mendelian model showed consistently larger empirical standard deviations (Table 2). The estimates of the QTL effect were unbiased under both the Mendelian and imprinting model, for QTL effects of 0.50 or larger. For the QTL effect of 0.25, the estimate of the effect was biased upwards under the Mendelian model (Table 2). For effects of 0.50 or larger, F_{Mend} pointed towards imprinting for all replicates, while F_{Red} pointed towards imprinting in 96% of the replicates (Table 2). For the QTL effect of 0.25, imprinting was inferred for 97%, 95%,

Table 2. Empirical power, QTL position, and estimated effects for simulated imprinted QTL, analyzed under Mendelian (Mend.) and imprinting (Imp.) models for 400 F_2 individuals with different designs, QTL effects, and allele frequencies.

Simulation details		Power [b]		Estimated effects[c]		QTL position[c]		Imprinting inferred		
No. males / females F_1	QTL effect[a] (freq.)	Mend	Imp.[d]	Mend. $\hat{a} \pm$ s.d[e]	Imp.[d] $\hat{a} \pm$ s.d[e]	Mend. cM ± s.d	Imp.[d] cM ± s.d	F_{Mend}[e]	F_{Red}[f]	Both [g]
20/80	P .75 (1.0/0.0)	1.0	1.0	.75 ±.07	.75 ±.06	46 ±2.4	46 ±1.4	1.0	.96	.96
	P .50 (1.0/0.0)	1.0	1.0	.50 ±.07	.50 ±.05	46 ±4.2	46 ±2.1	1.0	.96	.96
	P .25 (1.0/0.0)	.83	1.0	.28 ±.05	.25 ±.05	46 ±11.3	46 ±6.8	.97	.95	.92
	P .75 (0.8/0.2)	.95	.99	.46 ±.11	.45 ±.11	46 ±7.1	46 ±4.2	.98	.94	.93
	P .50 (0.8/0.2)	.82	.98	.33 ±.08	.31 ±.08	46 ±10.3	46 ±6.6	.94	.93	.90
	P .25 (0.8/0.2)	.33	.74	.22 ±.06	.18 ±.04	47 ±17.4	46 ±12.9	.61	.70	.59
	M .75 (0.8/0.2)	.97	1.0	.46 ±.10	.45 ±.09	47 ±8.1	46 ±3.6	.99	.94	.94
	M .50 (0.8/0.2)	.85	.99	.33 ±.07	.30 ±.06	46 ±11.4	46 ±6.3	.97	.93	.91
	M .25 (0.8/0.2)	.33	.74	.22 ±.06	.18 ±.04	47 ±17.3	46 ±13.3	.59	.70	.56
2/80	P .75 (0.8/0.2)	.84	.85	.51 ±.29	.50 ±.29	46 ±6.7	46 ±4.0	.85	.80	.80
	P .50 (0.8/0.2)	.76	.84	.36 ±.20	.34 ±.19	46 ±9.4	46 ±5.5	.82	.79	.77
	P .25 (0.8/0.2)	.45	.69	.24 ±.10	.20 ±.10	47 ±15.1	46 ±11.0	.60	.64	.56
	M .75 (0.8/0.2)	.99	1.0	.46 ±.10	.45 ±.08	46 ±7.0	46 ±3.5	1.0	.95	.95
	M.50 (0.8/0.2)	.88	.99	.32 ±.07	.30 ±.06	47 ±10.4	46 ±5.8	.97	.94	.93
	M .25 (0.8/0.2)	.37	.78	.22 ±.06	.18 ±.04	48 ±19.2	46 ±13.4	.64	.74	.62

[a] P, paternally expressed QTL effect; M, maternally expressed QTL effect (frequency of positive QTL allele in F_0). [b] Proportion of replicates significant at the 5% chromosomewise level against the H_0 of no QTL. [c] Estimates and empirical standard deviations, calculated with the replicates that exceed the 5% chromosomewise significance level [d] Analyzed under the appropriate reduced model (equation 12).[e] Proportion of replicates significant at the 5% chromosomewise level and for which a full model explains significantly more variance ($P < 0.05$) than a Mendelian QTL at the position of the best QTL under the respective model .[f] Proportion of replicates significant at the 5% chromosomewise level, for which a full model does not explain significantly more variance ($P < 0.05$) than a QTL with a single parental effect at the position of the best imprinted QTL. [g] Proportion of replicates where both a test of full vs. Mendelian (F_{Mend}) and Reduced vs. full (F_{Red}) indicate imprinting.

or 92%, depending whether F_{Mend}, F_{Red}, or both tests were applied (Table 2).

When founder lines are segregating for the positive QTL allele at 0.80 and 0.20, respectively, the results for maternally expressed QTL were very similar to the results for paternally expressed QTL under the design with 20 F_1 sires and 80 F_1 dams (Table 2). The differences in power to detect imprinted QTL between Mendelian and imprinting models became larger compared to fixation of founder lines, up to a difference of 40% in power for imprinted QTL with an effect of 0.25 (Table 2). The estimated QTL

Table 3. Empirical power and inferred models for simulated Mendelian QTL, analysed under Mendelian (Mend.) and imprinting (Mat. / Pat.) models for 400 F_2 animals with different designs, QTL effects, and allele frequencies.

Simulation details		Power[b]			Imprinting inferred					
No. males / females F_1	QTL effect[a] (freq.)	Mend	Mat.	Pat.	Maternal			Paternal		
					F_{Mend}[c]	F_{Red}[d]	Both[e]	F_{Mend}[c]	F_{Red}[d]	Both[e]
20/80	No QTL	0.05	0.05	0.05	0.03	0.05	0.03	0.03	0.05	0.03
	A 0.75 (1.0/0.0)	1.0	1.0	1.0	0.05	0.0	0.0	0.05	0.0	0.0
	A 0.50 (1.0/0.0)	1.0	1.0	1.0	0.06	0.01	0.0	0.06	0.01	0.0
	A 0.25 (1.0/0.0)	0.85	0.64	0.62	0.07	0.28	0.06	0.06	0.27	0.05
	D 0.75 (1.0/0.0)	1.0	1.0	1.0	0.05	0.0	0.0	0.05	0.0	0.0
	D 0.50 (1.0/0.0)	1.0	0.99	1.0	0.05	0.01	0.0	0.05	0.0	0.0
	D 0.25 (1.0/0.0)	0.96	0.61	0.60	0.06	0.15	0.04	0.06	0.13	0.04
	A 0.75 (0.8/0.2)	0.99	0.94	0.91	0.11	0.11	0.06	0.08	0.07	0.02
	A 0.50 (0.8/0.2)	0.91	0.72	0.71	0.07	0.24	0.06	0.08	0.22	0.06
	A 0.25 (0.8/0.2)	0.37	0.25	0.38	0.05	0.19	0.05	0.06	0.22	0.06
	D 0.75 (0.8/0.2)	0.98	0.88	0.87	0.09	0.09	0.03	0.10	0.06	0.02
	D 0.50 (0.8/0.2)	0.9	0.66	0.67	0.08	0.19	0.06	0.08	0.17	0.05
	D 0.25 (0.8/0.2)	0.39	0.24	0.25	0.07	0.17	0.06	0.07	0.19	0.06
2/80	No QTL	0.05	0.05	0.04	0.03	0.04	0.03	0.03	0.04	0.03
	A 0.50 (1.0/0.0)	1.0	0.99	0.99	0.06	0.01	0.0	0.05	0.01	0.0
	A 0.75 (0.8/0.2)	0.92	0.94	0.82	0.33	0.17	0.13	0.28	0.05	0.03
	A 0.50 (0.8/0.2)	0.83	0.77	0.71	0.18	0.24	0.13	0.21	0.17	0.1
	A 0.25 (0.8/0.2)	0.40	0.28	0.32	0.07	0.21	0.07	0.09	0.24	0.09
	D 0.75 (0.8/0.2)	0.89	0.82	0.77	0.35	0.15	0.12	0.32	0.07	0.04
	D 0.50 (0.8/0.2)	0.83	0.65	0.67	0.19	0.19	0.12	0.21	0.12	0.06
	D 0.25 (0.8/0.2)	0.47	0.30	0.32	0.09	0.20	0.08	0.09	0.20	0.07

[a] A, additive QTL; D, dominant QTL with a=d (frequency of positive QTL allele in F_0). [b] Proportion of replicates significant at the 5% chromosomewise level against the H_0 of no QTL. [c] Proportion of replicates significant at the 5% chromosomewise level and for which a full model explains significantly more variance ($P < 0.05$) than a Mendelian QTL at the position of the best QTL under the respective model. [d]Proportion of replicates significant at the 5% chromosomewise level and for which a full model does not explain significantly more variance ($P < 0.05$) than a QTL with a single parental effect at the position of the best imprinted QTL. [e] Proportion of replicates where both a test of full vs. Mendelian (F_{Mend}) and Reduced vs. full (F_{Red}) indicate imprinting.

effects are slightly larger under the Mendelian model, and the estimated QTL positions had larger empirical standard deviations, compared to the imprinting models. The estimated QTL effects were smaller than the simulated effects as a result of segregation of founder lines. The estimated effects follow approximately the following relationship:

$$\hat{a} = \Delta f * a \qquad (13)$$

Where $\hat{a}$ is the estimated QTL effect, Δf is the difference in allele frequency between the founder lines, and a is the simulated QTL effect. For QTL effects between 0.50 and 0.75, F_{Mend} gave the highest proportion of detected imprinted QTL while for the smaller effect of 0.25, F_{Red} gave the highest proportion of imprinted QTL. Further simulations under this design with different

Table 4. Estimated QTL position and effects for simulated Mendelian QTL for 400 F_2 animals with different designs, QTL effects, and allele frequencies.

No. males / females F_1	QTL effect[a] (frequency)	cM ± s.d.[b]	â ± s.d.[b]	$\hat{d}$ ± s.d.[b]
20/80	No QTL	48 ±32.4	0.0 ±0.16	0.0 ±0.23
	A 0.75 (1.0/0.0)	46 ±2.1	0.75 ±0.07	0.0 ±0.10
	A 0.50 (1.0/0.0)	46 ±3.8	0.50 ±0.07	0.0 ±0.10
	A 0.25 (1.0/0.0)	46 ±13.9	0.26 ±0.07	0.0 ±0.11
	D 0.75 (1.0/0.0)	46 ±1.4	0.75 ±0.07	0.75 ±0.10
	D 0.50 (1.0/0.0)	46 ±2.4	0.50 ±0.07	0.50 ±0.10
	D 0.25 (1.0/0.0)	46 ±7.7	0.26 ±0.06	0.26 ±0.10
	A 0.75 (0.8/0.2)	46 ±6.1	0.46±0.10	0.0 ±0.11
	A 0.50 (0.8/0.2)	46 ±9.6	0.32±0.07	0.0 ±0.10
	A 0.25 (0.8/0.2)	47 ±19.1	0.23 ±0.05	0.0 ±0.15
	D 0.75 (0.8/0.2)	46 ±5.8	0.45 ±0.10	0.28 ±0.14
	D 0.50 (0.8/0.2)	46 ±9.6	0.31 ±0.07	0.20 ±0.13
	D 0.25 (0.8/0.2)	47 ±17.8	0.21 ±0.06	0.14 ±0.14
2/80	No QTL	50 ±32.7	0.05 ±0.14	0.04 ±0.23
	A 0.50 (1.0/0.0)	46 ±3.7	0.50±0.06	0.0 ±0.10
	A 0.75 (0.8/0.2)	46 ±6.8	0.49±0.13	0.0 ±0.11
	A 0.50 (0.8/0.2)	46 ±10.0	0.35 ±0.09	0.0 ±0.11
	A 0.25 (0.8/0.2)	47 ±16.4	0.23 ±0.06	0.0 ±0.15
	D 0.75 (0.8/0.2)	46 ±6.0	0.49 ±0.17	0.30 ±0.21
	D 0.50 (0.8/0.2)	47 ±9.0	0.35±0.10	0.22 ±0.15
	D 0.25 (0.8/0.2)	47 ±14.4	0.22±0.07	0.14 ±0.14

[a] A, additive QTL; D, dominant QTL with a = d (frequency of positive QTL allele in F_0). [b] Estimates and empirical standard deviations, determined on the replicates that exceed the 5% chromosomewise significance level.

QTL effects showed that for QTL effects smaller than 0.40 F_{Red} has higher power to distinguish imprinted QTL than F_{Mend} (data not shown).

Under the extreme design with two F_1 sires, there were strong differences between the results for maternally and paternally expressed QTL. Under the Mendelian model, the power to detect maternally expressed QTL was larger than that for paternally expressed QTL, for QTL effects larger than 0.50. For a QTL effect of 0.25, the power under the Mendelian model was higher for the paternally expressed QTL. For the imprinting models, there was consistently more power to detect maternally expressed QTL compared to paternally expressed QTL, across all effects under this design (Table 2). The power to detect paternally expressed QTL, with the correct imprinting model, under this design was lower compared to the design with 20 sires, while the power to detect maternally expressed QTL was comparable between the two designs. Also the empirical standard deviations of the estimated QTL effect for the Mendelian and imprinting model, were larger for paternally

Table 5. Empirical power and inference of genetic model for simulated additive, Mendelian QTL, analyzed under Mendelian and imprinting models for 800 (20/160) and 400 (5/80) F_2 animals with different designs, QTL effects, and allele frequencies.

Simulation details		Power[b]			Imprinting inferred					
No. males /	QTL effect[a]	Mend.	Mat.	Pat.	Maternal			Paternal		
Females F_1	(frequency)				F_{Mend}[c]	F_{Red}[d]	Both[e]	F_{Mend}[c]	F_{Red}[d]	Both[e]
20/160	0.50 (1.0/0.0)	1.0	1.0	1.0	0.06	0.0	0.0	0.06	0.0	0.0
	0.25 (1.0/0.0)	0.99	0.90	0.91	0.07	0.12	0.04	0.05	0.12	0.04
	0.15 (1.0/0.0)	0.67	0.49	0.45	0.08	0.32	0.08	0.06	0.25	0.05
	0.50 (0.8/0.2)	0.99	0.94	0.90	0.10	0.10	0.05	0.07	0.06	0.02
	0.25 (0.8/0.2)	0.66	0.47	0.47	0.08	0.26	0.07	0.07	0.26	0.07
5/80	0.75 (0.8/0.2)	0.97	0.84	0.83	0.20	0.18	0.11	0.15	0.05	0.03
	0.50 (0.8/0.2)	0.87	0.74	0.68	0.12	0.26	0.10	0.10	0.20	0.07
	0.25 (0.8/0.2)	0.41	0.27	0.30	0.06	0.21	0.06	0.06	0.23	0.06

[a] Additive QTL (frequency of positive QTL allele in F_0). [b] Proportion of replicates significant at the 5% chromosomewise level against the H_0 of no QTL. [c] Proportion of replicates significant at the 5% chromosomewise level, for which a full model explains significantly more variance ($p < 0.05$) than a Mendelian QTL at the position of the best QTL under the respective model. [d] Proportion of replicates significant at the 5% chromosomewise level, for which a full model does not explain significantly more variance ($p < 0.05$) than a QTL with a single parental effect at the position of the best imprinted QTL. [e] Proportion of replicates where both a test of full vs. Mendelian (F_{Mend}) and reduced vs. full (F_{Red}) indicate imprinting.

expressed QTL under the design with 2 F_1 sires, compared to the design with 20 F_1 sires (Table 2). As before, F_{Mend} had the highest power to distinguish between imprinted and Mendelian QTL for larger QTL effects, while F_{Red} had higher power to distinguish imprinted QTL for a QTL effect of 0.25.

Detection of Mendelian QTL: The results of the analyses of simulated Mendelian QTL are summarized in Table 3. Table 3 also includes the results of the analyses where no QTL effects were simulated under two designs. This shows that using the 5% chromosomewise thresholds for the H0 of no QTL was sufficient to keep the type I error below 5% for all models that were applied, under both designs (Table 3).

When founder lines are fixed for different QTL alleles, all replicates showed significant QTL for QTL effects larger than 0.50 under both the Mendelian and imprinting models. For a QTL effect of 0.25 the Mendelian model had higher power for the dominant QTL compared to additive QTL. For the QTL effect of 0.25, about 60 % of the replicates were significant against the H0 of no QTL under the imprinting models (Table 3). The tests for imprinting performed generally well in distinguishing the simulated QTL as Mendelian for QTL effects of 0.50 and 0.75. However, for a QTL effect of 0.25 F_{Mend} indicated significant imprinting for up to 7 % of the replicates, while F_{Red} pointed towards imprinting for up to 28 % of the replicates (Table 3). The proportion of spuriously

imprinted QTL was higher for purely additive QTL compared to dominant QTL (Table 3). Applying both thresholds restricted the spurious detection of imprinting to 5 % of the replicates or less.

When founder lines were segregating for the positive QTL allele at 0.80 and 0.20, respectively, the power to detect QTL was reduced (Table 3). In contrast to the situation where founder lines were fixed, there was comparable power to detect additive and dominant QTL under the Mendelian model. The imprinting models had more power to detect additive QTL than dominant QTL (Table 3). There was little difference in power between the paternal and maternal imprinting models. The spurious detection of imprinting was 11 % for F_{Mend} and 22 % for F_{Red} (Table 3). Imposing both tests to infer imprinting kept the level of spurious imprinting below 6 %. Analyses with QTL effects between 0.50 and 0.25 revealed that spurious detection of imprinting, when only applying F_{Red}, was as high as 29 % of the replicates for a QTL effect of 0.35 (Data not shown). For smaller QTL effects, the proportion of spurious imprinted replicates decreased as a result of decreased power to detect any QTL effect under the imprinting models.

For the extreme design, with only two F_1 sires, the results under fixation of founder lines were very similar to those for the design with 20 F_1 sires. For comparison, the results for a QTL effect of 0.50 are included in Table 3. When founder lines were segregating under this design, the power to detect QTL under the Mendelian model was lower than for the design with 20 F_1 sires, for effects of 0.50 and 0.75, but slightly higher for the QTL effect of 0.25 (Table 3). The maternal imprinting model showed higher power to detect additive QTL compared to the paternal model for effects of 0.75 and 0.50. For smaller effects and for dominant QTL the paternal and maternal imprinting model showed comparable power. Under the imprinting models, F_{Mend} gave levels of spurious imprinting up to 35 %, whereas F_{Red} indicated imprinting for 24% of the replicates (Table 3). Even when both tests were imposed, spurious imprinting was detected for up to 13 % of the replicates under the model with maternal expression and up to 10% under the model with paternal expression (Table 3). This clearly demonstrates the effect of the design of the experiment on the spurious detection of imprinted QTL when founder lines are not fixed for alternative QTL alleles.

The estimates of the QTL position and effects under the Mendelian model are given in Table 4. Under fixation of founder lines, the estimates for additive and dominance effects under the Mendelian model were unbiased but the empirical standard deviation was slightly larger for the dominance effects (Table 4). When founder lines were segregating for the positive QTL allele at 0.80 and 0.20, respectively, the estimated dominance effects were much smaller than the estimated additive effects although the simulated values were identical (Table 4). Figure 1 shows the relationship between the simulated and estimated QTL effects. The estimates of the additive effect follow

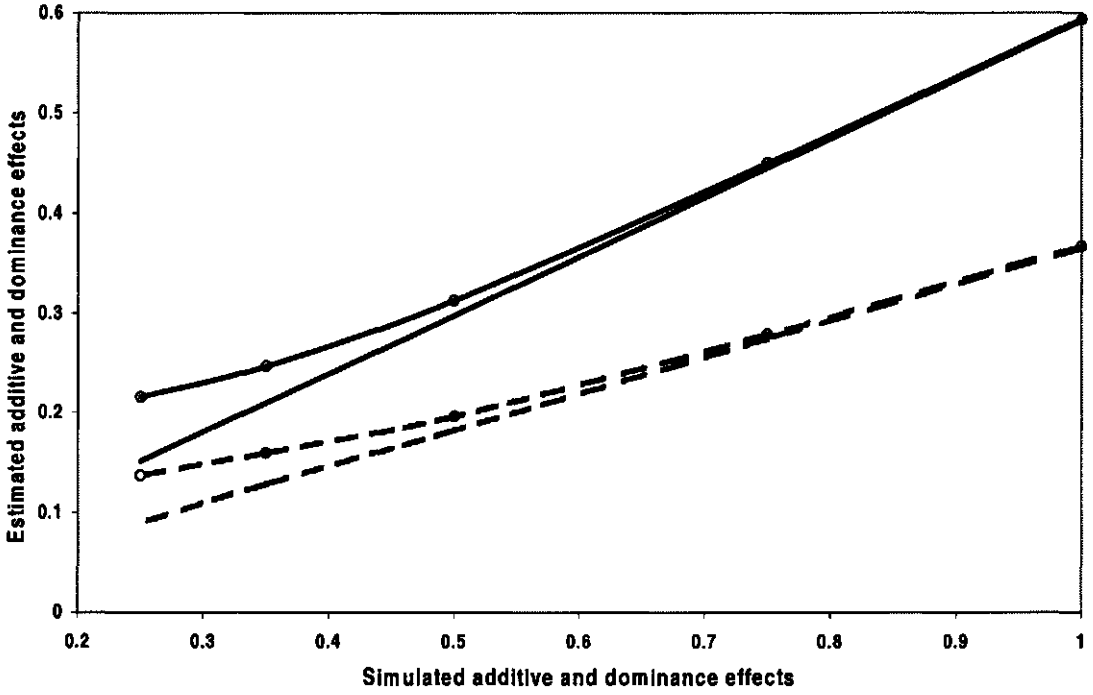

Figure 1. Relationship between simulated and estimated QTL effects when founder lines are segregating for the positive allele of the QTL at 0.8 and 0.2, respectively. The solid lines represent the estimates for the additive effect and the dashed lines represent the estimates for the dominance effect. The lines with circles are averaged over the significant replicates among 1,000 simulations, while the other lines are averaged over all replicates.

equation (13) while the estimated dominance effects were empirically shown to be proportional to the squared difference in allele frequency between the founder lines:

$$\hat{d} = \Delta f^2 * d \qquad (14)$$

Where $\hat{d}$ is the estimated QTL effect, Δf is the difference in allele frequency between the founder lines, and d is the simulated dominance effect. This clearly shows that the power to detect significant dominance effects is compromised severely when founder lines are not fixed. Under the design with only two F_1 sires with segregating founder lines, the estimates of the QTL effects were slightly larger and had larger empirical standard deviations compared to the design with 20 F_1 sires (Table 4).

Further analyses: The results of the analyses of Mendelian QTL under imprinting models showed that false detection of imprinting, when QTL are actually Mendelian, is a greater concern than the detection of imprinted QTL. More simulations were performed with purely additive QTL,

because they gave the largest proportion of replicates, that were falsely identified to be imprinted. The effect of population size was investigated by simulating additive QTL for 800 F_2 individuals, obtained by mating 20 sires to 8 dams each, under fixation of founder lines and with the segregation of the positive allele at 0.80 and 0.20, respectively. To further investigate the effect of design when founder lines are not segregating, additive QTL were simulated in a design where 400 F_2 individuals were obtained by mating 5 sires to 16 dams each. The results of these additional analyses are summarized in Table 5.

As expected, there was better power to detect smaller QTL effects for the design with 800 F_2 individuals, both under fixation and segregation of founder lines compared to a design with 400 F_2 individuals (Table 5). For QTL effects between 0.25 and 0.75, with fixation of founder lines, there was considerably less spurious imprinting compared to the same QTL effects in the with 400 F_2 individuals (Table 3, 5). However, for a QTL effect of 0.15, up to 32 % of the replicates showed spurious imprinting under the model with maternal expression. It is not clear why the model with maternal expression gave a higher proportion (0.32 vs. 0.25) of spurious imprinting compared to the model with paternal expression (Table 5). Under segregation of founder lines, there was considerable spurious imprinting for a QTL effect of 0.25, indicating that also for larger F_2 populations spurious detection of imprinting can be problem.

For the design with five F_1 sires, the proportion of spuriously detected imprinted QTL was lower compared to the design with two F_1 sires, but still considerably higher compared to the design with 20 F_1 sires. Imposing both tests for imprinting kept the proportion of spuriously imprinted QTL below 11 % (Table 5). The proportion of spuriously detected imprinted QTL was higher under the model with maternal expression.

Discussion

Detection of imprinted QTL: For smaller QTL effects and when founder lines are segregating for the same QTL alleles, it was demonstrated that the reduced imprinting models had higher power to detect imprinted QTL than standard Mendelian models (Table 2). Consequently, it is not surprising that performing additional QTL analyses with reduced imprinting models reveals imprinted QTL that remained undetected under a Mendelian model (DE KONING *et al.*, 2001).

For larger QTL effects, F_{Mend}, the test suggested by KNOTT *et al.* (1998), has slightly higher power to distinguish imprinted QTL compared to F_{Red}. However, for smaller QTL effects, F_{Red} gives the highest proportion of correctly identified imprinted QTL (Table 2).

For the design with 20 F_1 sires there is no difference in power to detect maternally or paternally expressed QTL. For the extreme design with two F_1 sires and the QTL allele segregating in the founder lines, there is considerably less power to detect paternally expressed QTL compared to maternally expressed QTL. With only two F_1 sires, there

is an increased risk that one or both F_1 sires are homozygous for their QTL alleles or have a different phase between line origin and QTL effect. When founder lines are segregating for the positive QTL allele at a frequency of 0.80 and 0.20, respectively, 32 % of the F_1 individuals are expected to be homozygous for the QTL, and 68 % are expected to be heterozygous (i.e. informative), under random mating. However, for 4 % of the F_1 individuals, the positive QTL allele is coming from line 2, while the remaining 64% heterozygous F_1 individuals have the positive QTL coming from line 1. Offspring of these 4% F_1 individuals with opposite phase between line origin and QTL allele will have QTL effects with opposite effects compared to offspring of the other 64% of F_1 individuals. This affects the detection of both Mendelian and imprinted QTL.

When simulating imprinted and Mendelian QTL with the same QTL effects (a_i or a), there was more power to detect imprinted QTL compared to Mendelian QTL (Table 2, 3). This is not surprising because even for a completely dominant Mendelian QTL the genetic variance explained by the QTL (3) is only ~0.56 of the variance explained by an imprinted QTL of the same magnitude (7). For an imprinted gene, only one allele is expressed while for a Mendelian gene both alleles are expressed. It could therefore be argued that, on average, the effects of imprinted genes are expected to be smaller than for Mendelian genes. At present, there is not enough empirical data on imprinted genes affecting quantitative traits to compare the distribution of imprinted gene effects to that of Mendelian genes.

Detection of Mendelian QTL: ALFONSO AND HALEY (1998) performed an extensive simulation study on the detection of Mendelian QTL in F_2 designs. They investigated the effect of mating design of the F_0 and F_1, as well as the effect of avoidance or preferential mating of sibs in the F_1. In the present study we have simulated random mating throughout and used the same design for the F_0 across all scenarios. The estimated power and QTL effects in Table 3 and Table 4 correspond generally well with those reported by ALFONSO AND HALEY (1998). The estimated QTL effects reported by ALFONSO AND HALEY (1998) follow approximately the expectations denoted in (12) and (13), for some of the mating designs. It can be seen from Figure 1 that the relationship is clearer when averaging across all replicates, because the estimates for the significant replicates are larger than predicted by (12) and (13). Under segregation of founder lines, the estimated additive effect is proportional to the difference in QTL allele frequency between the two lines, while the estimated dominance effect is proportional to the squared value of this difference. As a result, a completely dominant QTL might appear to be only partially dominant or even completely additive, when founder lines are segregating. This is important to take into account when looking at results of QTL analyses of crosses between outbred lines.

Detection of spurious imprinted QTL: The simulations of the Mendelian QTL show that spurious detection of imprinting is a serious problem for smaller QTL effects and when founder lines are segregating (Table 3). For most scenarios, the test of KNOTT *et al.* (1998) is more conservative, while F_{Red}, similar to DE KONING *et al.* (2000), is more liberal and can give higher rates of spurious imprinting. However, for larger QTL effects and segregation of founder lines, under the extreme design with two F_1 sires, F_{Mend} gave spurious imprinting for up to 35 % of the replicates (Table 3). This clearly shows that both tests have their flaws, although F_{Mend} performs better on average, for the scenarios considered in this study. For smaller QTL effects, the H_0 of F_{Red} appears to be too robust against a purely additive Mendelian QTL. Imposing both tests to infer imprinting kept the level of spurious imprinting below 6 % for the design with 20 F_1 sires. This could be an ad-hoc solution to control the spurious detection of imprinting, but better alternatives should be investigated (LEE *et al.*, 2001). Imposing both tests to the simulations with imprinted QTL resulted in a proportion of correctly identified imprinted QTL that was close or equal to the smallest of the two proportions identified by the individual tests (Table 2). This indicates that the power to detect imprinted QTL would not be greatly affected by imposing both tests.

When simulating 800 F_2 individuals, the problem of spurious imprinting seemed less apparent compared to the simulations with 400 F_2 individuals (Tables 3 and 5). However, when moving to smaller QTL effects, the proportion of spuriously imprinted QTL was also very high for 800 F_2 individuals (Table 5). The risk of spurious detection of imprinted QTL shows an 'optimum' around a design specific QTL effect. This is probably the combined result of the power to detect any QTL effect, and the power to distinguish between Mendelian and imprinted QTL. It is postulated here that the risk of spurious detection of imprinting does not depend on the number of F_2 individuals. For every number of F_2 individuals there is probably a range in QTL effects, where the risk of spurious detection of imprinted QTL is prominent. However, as the risk of spurious detection shifts to smaller QTL effects for designs with more F_2 individuals, the proportion of spuriously imprinted QTL among all detected QTL is expected to be lower for larger designs.

The design with only two F1 sires resulted in very high proportions of spuriously imprinted QTL, even when both tests were imposed (Table 3). Although the detection of imprinted QTL was reasonable compared to the design with 20 F_1 sires, the results for the Mendelian QTL clearly indicate that this design is unsuitable for the detection of imprinted QTL when founder lines are segregating for the QTL alleles. Using a design with 5 F_1 sires was slightly better but still gave high proportions of spuriously imprinted QTL (Table 5). It is not straightforward to provide a yardstick for the minimum number of F_1 parents of each sex, that should be used to circumvent the risks of

spurious detection of imprinting. However, the results here indicate that with only two or five F_1 parents from one sex, not only the power to detect QTL is affected, but also the risk of spurious detection of imprinting is increased. Obviously, design is not an issue when founder lines are completely fixed for their QTL alleles but for experimental crosses in livestock this is not very likely. Although this study focussed on the effect of mating design in the F_1, the results are also applicable for the mating design of the F_0. When founder lines are segregating for QTL alleles, it is important to use enough F_0 parents. If many F_1 parents are used, but only a few F_0 parents, then a large proportion of the F_1 might be homozygous for the QTL, giving the same loss of power and risk of spurious imprinting compared to the situation where only a few F_1 parents are used. In practice, it might seem cost-effective to restrict the number of F_0 and F_1 parents to the number that is necessary to obtain the desired number of F_2 individuals. The present study indicates that this is not the best strategy when the objectives of a study also include testing for imprinting effects.

The effect of the null hypothesis: The H_0 of F_{Men} is that of a Mendelian QTL whereas the H_0 of F_{Red} is that of an imprinted QTL. The results of the simulation study indicate a confounding between the power of the design to detect QTL and the power to discriminate between Mendelian and imprinted QTL. When the power to detect QTL reduces, both F_{Men} and F_{Red} favor the acceptance of respective their H_0, leading to different conclusions, depending on the H_0 of the test. MALÉCOT (1999) demonstrated that the choice of the null hypothesis is never subjective, but a result of experiences and ideas of a researcher, or a group of researchers. When testing for imprinting, the H_0 of the test clearly affects the conclusion. The null hypothesis that genes, and hence QTL, show Mendelian expression may be the most reasonable H_0 when you are the first researcher to study a new genetic phenomenon. It could however be argued that this is partly because most, if not all, genetical research of the 20th century was based on the Mendelian principles. The Mendelian principles provide no explanation for reciprocal differences that are observed in crossbreeding and that may be attributable to genomic imprinting. Genomic imprinting has only been studied during the last decade and is still considered a rare phenomenon.

Furthermore, it could be argued whether the inference of the mode of expression of a QTL should be tested with the same stringent criteria as the existence of that QTL. In other words, is spurious inference of imprinting for a Mendelian QTL (or vice versa) just as serious as spurious detection of a QTL? The discrepancies between the test as a result of different H_0 make it unlikely that the issue of testing the mode of expression of a QTL can de solved in a classical testing framework. An appealing alternative is to adopt a Bayesian approach (MALÉCOT, 1999), where QTL get prior probabilities to show Mendelian or uni-parental expression, based on knowledge about the proportion of imprinted genes among identified genes.

As science progresses, and new observations accumulate, the effect of the subjective parts (i.e. the assumptions and H_0) is expected to diminish (MALÉCOT, 1999). With regard to the detection of imprinted QTL, the new information should not only come from independent replicates of QTL studies, but especially from expression studies that can provide proof for imprinting at the molecular level.

Implications: The simulation study showed that, compared to detecting Mendelian QTL, the successful detection and inference on mode of inheritance of a QTL puts more demands on both the design of the experiment as well as the interpretation of the results. Because the possibility to test for imprinting effects in QTL experiments was only described by KNOTT *et al.* in 1998, most QTL mapping experiments to date are not optimized to detect imprinted QTL.

Using a test very similar to F_{Red} in the present study, DE KONING *et al.* (2000) reported four imprinted QTL in pigs. When applying also the test suggested by KNOTT *et al.* (1998) to the best positions of these QTL, the conclusion remained that these QTL were imprinted. Only for a paternally expressed QTL at chromosome 6 the F ratio of the test of a full against a Mendelian model was not conclusive ($p = 0.068$). However, this p value was still strongly suggestive and a graphical comparison of maternal, paternal, and Mendelian models showed that there was no maternal expression at the position of the paternal QTL. Furthermore, this QTL was genome-wide significant under the imprinting model, and only suggestive under the Mendelian model. The design used by DE KONING *et al.* (2000) comprises almost 800 F_2 animals obtained from 38 F_1 sires and 264 F_1 dams. The F_0 consisted of 19 sires and 126 dams. Given the large number of parents, this design should not suffer from spurious detection of imprinting as a result of having a too small number of parents when founder lines are segregating.

KNOTT *et al.* (1998) demonstrated the possibility to test for imprinting on an experimental cross between Wild Boar and Large White pigs. This cross was obtained by crossing two wild boars with eight Large White sows. From the F_1, four boars were mated to a total of 22 sows. According to the simulation study, this design appears far from optimal for the detection of imprinted QTL, because of the small number of F_0 and F_1 parents. Nevertheless, JEON *et al.* (1999) reported a paternally expressed QTL in the region of the *IGF2* region in Chromosome 2 for the same experimental population. This imprinted QTL was corroborated by NEZER *et al.* (1999) for a cross between Large White and Piétrain pigs with 1,032 F_2 animals. NEZER *et al.* (1999) also confirmed the exclusive paternal expression for the *IGF2* locus at the molecular level. This clearly demonstrates that, although the design used by JEON *et al.* (1999) was not optimal for the detection of imprinted QTL, it was extremely valuable to detect the imprinting effect for *IGF2*. Furthermore, the Wild Boar is genetically more distant to the Large White breed than the Meishan or Piétrain breeds,

diminishing the risk of segregation of the same QTL alleles within both founder lines.

It is shown in Table 2 that the imprinting models can detect up to 40 % of QTL that are not detected under a Mendelian model. However, for up to 14 % of the replicates that are significant against the H_0 of no QTL, the test against a Mendelian model does not point towards imprinting. This is also seen in the analyses of real data, where several QTL only exceed the significance threshold under a reduced imprinting model, but are not imprinted following F_{Mend}. This raises the question whether testing against a Mendelian model is necessary when the Mendelian model does not detect the QTL. We have not studied what proportion of Mendelian QTL show higher significance under an imprinting model. Although intuitively unlikely, this could happen because the Mendelian model always includes a dominance component, even when a purely additive QTL was simulated.

It is recommended that researchers include tests for imprinting whenever possible, but critically reflect upon their results with regard to the design of the experiment and the probability of segregation of QTL alleles within founder lines. This not only holds for F_2 crosses between outbred species but also for making strategic backcrosses to test for imprinting effects following CLAPCOTT *et al.* (2000). This strategy relies on finding a QTL in a certain backcross and not in the reciprocal backcross. This is no problem when using completely inbred mice strains but when it is not completely sure that all F_1 individuals will be heterozygous for the QTL, the design must be optimized to minimize the spurious detection of imprinted QTL.

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Table A1. Genetic values, allele substitution effects, and expected breeding values of a partially imprinted gene

Genotypes	AA	AB	BA	BB	mean	α
Frequencies	p^2	pq	pq	q^2		
Values	a	d+i	d-i	-a		
♂A	p	q			ap+dq+iq	α1♂= q[a+i+d(q-p)]
♂B			p	q	-aq+dp-ip	α2♂= -p[a+i+d(q-p)]
♀A	p		q		ap+dq-iq	α1♀= q[a-i+d(q-p)]
♀B		p		q	-aq+dp+ip	α2♀= -p[a-i+d(q-p)]

	AA	AB	BA	BB
Genotypic values	2q(a-pd)	a(q-p)+i+d(1-2pq)	a(q-p)-i+d(1-2pq)	-2p(a+qd)
Breeding value ♂	2q α♂	(q-p)α♂	(q-p) α♂	-2pα♂
Breeding value ♀	2q(α♂-2i)	(q-p)(α♂-2i)	(q-p)(α♂-2i)	-2p(α♂-2i)

Appendix A

Consider a biallelic gene with partial paternal expression under random mating. The additive gene effect is a, dominance d and frequencies p and q as before. The imprinting value i is defined such that the expected deviation for AB individuals is $d + i$ and for BA individuals the expected deviation is $d - i$. An overview of genotypic values is given in Table A1. The population mean becomes:

$$M = ap^2 + dqp + ipq + dpq - ipq - aq^2 = a(p-q) + 2dpq \quad \text{(A.1)}$$

This is identical to the population mean of a Mendelian QTL. The allele substitution effects have to be calculated for the sexes separately because the value of an allele is dependent on the parent through which it is transmitted. The specific allele substitution effects for the separate sexes are given in Table A1. The average allele substitution effects for the separate sexes becomes:

$$\alpha♂ = \alpha1♂ - \alpha2♂ = a + i + d(q-p)$$
$$\alpha♀ = \alpha1♀ - \alpha2♀ = a - i + d(q-p)$$
$$= \alpha♂ - 2i \quad \text{(A.2)}$$

The single gene variance becomes:

$$V_G = [p^2a^2 + pq(i+d)^2 + pq(d-i)^2 + q^2a^2] - [a(p-q)+2dpq]^2$$
$$= 2pq[a^2 + i^2 - 2ad(p-q) + p^2d^2 + q^2d^2] \quad \text{(A.3)}$$

In case of complete imprinting ($i=a$ and $d=0$), α♀ becomes zero and (A.3) reduces to (7):

$$V_G = 4pqa^2$$

Mapping of multiple quantitative trait loci by simple regression in half-sib designs

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Abstract – Detection of quantitative trait loci (QTL) in outbred half-sib family structures has mainly been based on interval mapping of single QTL on individual chromosomes. Methods to account for linked and unlinked QTL have been developed, but most of them are only applicable in designs with inbred species or pose great demands on computing facilities. This study describes a strategy that allows for rapid analysis, involving multiple QTL, of complete genomes. The methods combine information from individual analyses after which trait scores for a specific linkage group are adjusted for identified QTL at other linkage groups. Regression methods are used to estimate QTL positions and effects; permutation tests are used to obtain empirical threshold values. The description of the methods is complemented by an example of the combined analysis of 28 bovine chromosomes and their associations with milk yield in Finnish Ayrshire cattle. In this example, the individual analysis revealed five suggestive QTL affecting milk yield. Following the strategy presented in this paper, the final combined analysis showed eight significant QTL affecting milk yield. This clearly demonstrates the potential gain of using the combined analysis. The use of regression methods, with low demands on computing resources, makes this approach very practical for total genome scans.

In livestock species, where artificial insemination (AI) is common, and in tree breeding, large half-sib family structures are common. NEIMANN-SORENSEN and ROBERTSON (1961) and WELLER *et al.* (1990) introduced respectively the daughter and granddaughter designs to analyze linkage between a single marker and a QTL in a half-sib design. KNOTT *et al.* (1996) and GEORGES *et al.* (1995) developed methods for interval mapping in outbred half-sib designs. These methods do not take possible QTL on other chromosomes into account.

JANSEN (1993, 1994) and ZENG (1994) proposed methods to account for linked and unlinked QTL by fitting markers as cofactors. These methods are developed for inbred line cross experiments and only recently JANSEN *et al.* (1996, 1998) and KAO *et al.* (1999) describe methods for multiple QTL mapping in outcrossing species.

Half-sib studies in livestock often include families with only 30 or 40 animals (GEORGES *et al.*, 1995; SPELMAN *et al.*, 1996; VILKKI *et al.*, 1997). Conditioning on unlinked QTL by cofactors (e.g. JANSEN, 1993, 1994; ZENG, 1994) in a half-sib design means that these cofactors should be fitted within families. For an analysis across families the maximum number of cofactors is restricted by the size of the smallest family. The number of parameters in the model should not exceed twice the

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square root of the number of observations (SAKAMOTO *et al.*, 1986). This limits the number of cofactors to 15 for a family of around 50 animals, which is not sufficient to condition on the entire genome.

The objective of this research was to develop a strategy for simultaneous analysis of multiple chromosomes in an outbred half-sib design. The methods account for identified QTL and resemble the strategy to obtain residual empirical thresholds as described by DOERGE and CHURCHILL (1996). These methods are demonstrated with the combined analysis of 28 bovine chromosomes in a granddaughter design.

Material and Methods

The procedure consists of three stages. First, the chromosomes are analyzed individually to identify candidate regions. At the second stage the best candidate positions are chosen as cofactors and their effects are re-estimated jointly with multiple linear regression. Third, the phenotypic data are adjusted for the effects of cofactors and the linkage groups are re-analyzed by interval mapping. If this reveals new or better candidate regions the set of cofactors can be modified and the effects re-estimated. A graphical representation of the analyses is given in Figure 1.

The methods will now be described in detail for an analysis across several half sib families. The first step is analysis of the individual linkage groups using the multimarker approach for interval mapping as described by KNOTT *et al.* (1996). In short, for each offspring the probability of inheriting the parent's first haplotype of a linkage group is calculated at fixed intervals (e.g., 1 cM) conditional on its marker genotype. Subsequently, a QTL is fitted at the fixed intervals along the linkage group by regression of phenotype on the probability of inheriting the first haplotype of the parent. The analysis is nested within families and the residuals are pooled across families to calculate a test statistic. This 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 DE KONING *et al.* (1998). Fitting the QTL within families is necessary because of the random assignment of the first haplotype, different QTL genotypes between parents, and different phases between markers and QTL between parents. The regression model for every chromosome is:

$$Y_{ij} = a_i + b_i X_{ij} + e_{ij} \qquad [1]$$

where Y_{ij} is the trait score of individual j, half-sib offspring from parent i, a_i is the polygenic effect for half-sib family i, b_i is the regression coefficient within family i (i.e., allele substitution effect for a putative QTL); X_{ij} is the conditional probability for individual j of inheriting the first haplotype from parent i, and e_{ij} is the residual effect.

For every linkage group the most likely position of a QTL is calculated.

In the second step, candidate regions are identified based on significance levels from permutation tests on the individual chromosomes as described by CHURCHILL and DOERGE (1994) and applied to several half-sib

studies (SPELMAN *et al.*, 1996; VILKKI *et al.*, 1997). Following suggestions made by SPELMAN *et al.* (1996), QTL that exceed a given threshold are the cofactors in the further analyses. The threshold for inclusion of a QTL as a cofactor should be less stringent than the threshold for claiming genome-wide significance for a QTL, as can be seen from the example.

For every half-sib offspring, the transmission probabilities of the parent's first haplotype at the positions of the cofactors are taken as "virtual markers" (DE KONING *et al.*, 1998). Subsequently the effects of all cofactors are re-estimated by multiple linear regression following [2]:

$$Y_{ij} = a_i + \sum_{k=1}^{n} b_{ik} X_{ijk} + e_{ij} \qquad [2]$$

Variables are the same as in [1] except b_{ik} is the substitution effect within half-sib family i for cofactor k, X_{ijk} is the conditional probability for individual j of inheriting parent i's first haplotype at the position of cofactor k and *n* is the number of cofactors in the analysis.

Using transmission probabilities as virtual markers is a convenient alternative to fitting marker scores as cofactors since it allows any position on a linkage group to be included as a cofactor. Furthermore, transmission probabilities are calculated with multiple marker methods (KNOTT *et al.*, 1996) and use all marker information whereas individual markers are usually not informative in all families.

In the third step, the original phenotypic data are adjusted for the estimated effects of the cofactors. The phenotypic data are adjusted separately for every linkage group, only adjusting the data for the effects of those cofactors that reside on other linkage groups. One of the reasons for this is that fitting an effect on a linkage group under study reduces the power to find additional QTL on that linkage group (ZENG, 1994; DOERGE and CHURCHILL, 1996). Furthermore, conditioning on unlinked QTL only, allows a re-evaluation of the cofactors (i.e., identified QTL) themselves rather than considering them fixed after they are identified. The formula for obtaining the adjusted phenotypes is:

$$Z_{hij} = Y_{ij} - \sum_{k=1}^{n} b_{ik} X_{ijk} \qquad [3]$$

Variables are as in [2] with the extension that Z_{hij} is the adjusted phenotype for animal j of parent i with regard to chromosome h. A cofactor is excluded when located on chromosome h by putting its estimated substitution effect (b_{ik}) to zero.

Subsequently all linkage groups are analyzed by interval mapping following [1] with the exception that the adjusted phenotype Z_{hij} is used rather than Y_{ij}. If this reveals additional QTL, a new set of cofactors is selected. Cofactors can also be dropped from the analysis if their significance drops below the pre-specified threshold or their position can change. This step is repeated until no new QTL are identified and estimated locations of identified QTL are stable.

Significance thresholds are determined empirically by permutations (CHURCHILL and DOERGE, 1994; DOERGE and CHURCHILL, 1996). For a linkage group the phenotypes,

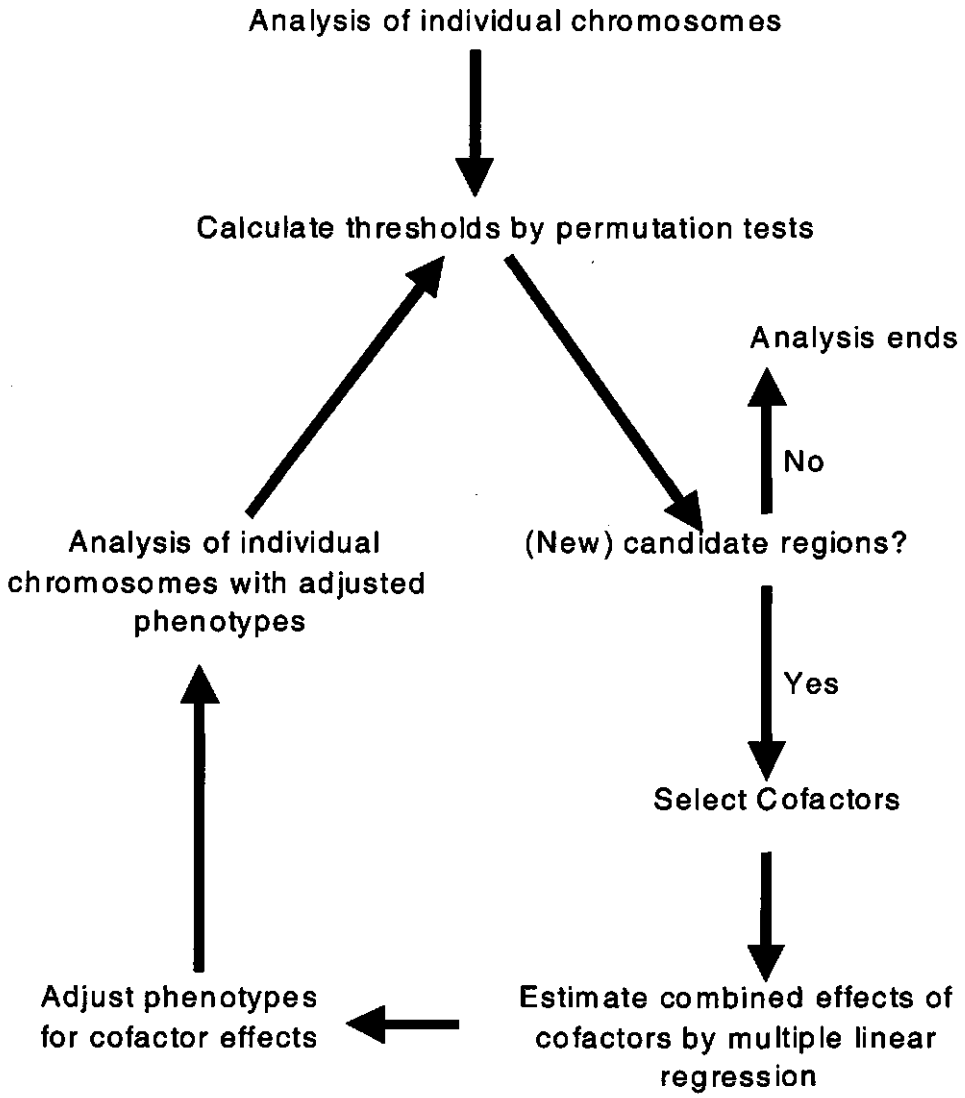

Figure 1. Flow diagram for the combined analysis of multiple chromosomes.

which are adjusted for the estimated cofactor effects, are shuffled within half-sib families while the marker genotypes are retained. This way any associations between markers on that linkage group and trait values are distorted while those for the unlinked cofactors are kept intact. The permuted data are analyzed and the best test statistic is stored. This procedure is repeated N (e.g. 10,000) times to obtain an empirical distribution of the test statistic under the null hypothesis of no QTL associated with the linkage group under study. This provides a

specific test for the chromosome under study rather than a test for the complete multiple QTL model. The desired threshold α can be obtained by taking the (1- α) percentile of the sorted test statistics. These chromosome-wise risk levels might be adjusted by Bonferroni correction for multiple testing of the whole genome to obtain genome-wide significance levels (LANDER and KRUGLYAK, 1995).

Example

Experimental population. We applied the method to a granddaughter design (WELLER *et al.*, 1990) consisting of 12 extensively used AI bulls and their half-sib sons from the Finnish Ayrshire cattle breed. The number of sons ranged from 21 to 82 per grandsire with a total of 493. For every son the Estimated Breeding Value (**EBV**) for milk yield was obtained from the national animal model evaluation of 1998. These EBV's were based on records from 105 up to over 3,000 daughters per son. Since the EBV is not purely based on offspring performance, it would be more appropriate to use daughter yield deviations (VAN RADEN and WIGGANS, 1991). However, with the number of daughters exceeding 100 for all sons, the information coming from other relatives was very small. Genotypes were obtained for 142 informative microsatellite markers covering 28 bovine chromosomes (only BTA 3 not included), spanning a total of 2,585 cM (Haldane). For more details see earlier reports on this experimental population (VILKKI *et al.*, 1997; ELO *et al.*, 1999; VELMALA *et al.*, 1999). The results of a complete scan for production traits will be presented by MOISIO *et al.* (in preparation).

Thresholds and cofactor selection. Chromosome-wide thresholds (based on 10,000 permutations) were converted to genome-wide risk levels by a Bonferroni correction for testing all 29 autosomes following DE KONING *et al.* (1998). A putative QTL was included as a cofactor when it exceeded the level of 5% chromosome-wise linkage. Significant linkage was inferred when a QTL exceeded the 5% genome-wide risk level (LANDER and KRUGLYAK, 1995). An additional threshold is that of suggestive linkage, where one false positive is expected to occur in a whole genome scan (LANDER and KRUGLYAK, 1995). Calculating these thresholds for five chromosomes, with and without cofactors, showed that including cofactors in the analysis had little effect on the thresholds. A test statistic of 2.3 was used as a robust threshold for suggestive linkage and a test statistic between 3.0 and 3.1 for significant linkage.

Results and Discussion

Figure 2 shows the development of the best test statistics of the individual chromosomes during the process of selecting and fitting cofactors. The initial analysis of the individual chromosomes revealed five QTL affecting milk yield exceeding the level of 5% chromosome-wise linkage. Including these as cofactors provided two additional QTL in the next round of analysis. The following round, with seven cofactors, revealed another QTL. By the final round, the initial number of five

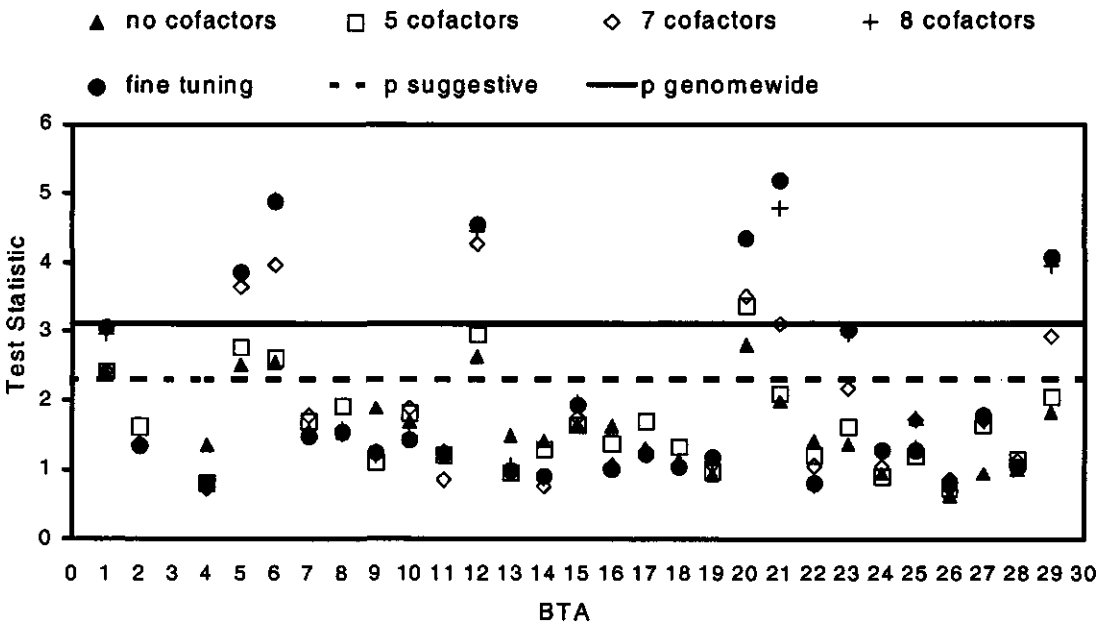

Figure 2. Development of the test statistic for 28 chromosomes during the combined analysis. The different symbols indicate the highest test statistic for the linkage groups during the process of fitting cofactors and re-analyzing the data. In the final 'fine tuning' round no additional cofactors were added but the most likely positions of the QTL were refined.

suggestive QTL had increased to eight significant QTL. Table 1 compares the results of the eight relevant chromosomes for models without cofactors and with the final set of eight cofactors. This table not only shows the changes in test statistic but also in best QTL position for some chromosomes. These eight QTL explained 43% of the variance in breeding values for milk yield when fitted jointly with a multiple linear regression. Figure 2 shows that including cofactors revealed additional QTL on some chromosomes while the test statistics of other chromosomes were hardly affected by the cofactors.

Figure 3 shows the test statistic along the linkage group for BTA6, which was directly included as a cofactor, and BTA21, which showed evidence for a QTL affecting milk yield only after inclusion of cofactors. The curve on BTA6 is not only higher but also steeper, which will reduce the confidence interval of the QTL. The curve for BTA21 is reasonably flat because only three markers were typed on this chromosome.

The evidence for a QTL in a half-sib design comes from the joint results of the individual half-sib families. Table 2 illustrates the effect of cofactor analyses on the within-family test statistics. When fitting cofactors, the number

Table 1. Comparison between results without cofactors and with the final set of cofactors

BTA[a]	Individual chromosomes		Cofactor analysis	
	Test statistic[b]	Position (cM)	Test statistic[b]	Position (cM)
1	2.40[s]	145	3.05[g]	145
5	2.51[s]	107	3.86[g]	117
6	2.54[s]	76	4.89[g]	78
12	2.63[s]	31	4.55[g]	27
20	2.80[s]	14	4.35[g]	36
21	1.98	33	5.18[g]	34
23	1.36	19	3.02[g]	14
29	1.82	63	4.07[g]	48

[a] *Bos Taurus* autosome

[b] Superscripts g and s denote genome-wide suggestive and significant linkage, respectively.

of families that appear to be informative for the QTL is higher for all chromosomes except BTA20 compared to the analysis without cofactors. Remarkable changes were observed in family 2 where for BTA6, BTA12, BTA21, and BTA23 the test statistic improved from non significant to significant ($P < 0.01$) while for BTA 20 the test diminished from 7.0 to 0.35 (Table 2). Such findings warrant extra scrutiny of the family data, both at the phenotypic and the marker level before any conclusions can be drawn. Also for family 3 the test statistic increased from non-significant to significant for five chromosomes when cofactors were fitted. Analyses of other families also showed remarkable increase in test statistics for three or fewer chromosomes when cofactors were fitted (Table 2).

The combined analyses showed increases in the test statistics for identified QTL and for detection of additional QTL. These results imply a larger power to detect QTL in the combined analyses, which is partly caused by the decrease in the residual variance by taking into account variance that is explained by the cofactors. In this example all cofactors were eventually genome-wide significant QTL. This is a coincidence and there are also situations where after the final round of analysis some of the cofactors are significant QTL and others only exceed the lower threshold for inclusion as a cofactor. It should be noted that the test statistic for a putative QTL tends to increase considerably once that QTL is included as a cofactor. This is because the putative QTL becomes part of the complete model where all cofactor effects are estimated jointly to give the best fit of the data.

For convenience, we used the same threshold for significant linkage across all chromosomes, with and without cofactors. Although thresholds were very consistent in this particular example, this is not generally the case. We therefore advise to estimate separate thresholds for individual chromosomes for a specific set of cofactors and a specific trait.

Interval mapping methods for half-sib designs (KNOTT *et al.*, 1996) in which chromosomes are analyzed individually, assume that alleles of QTL on linkage groups

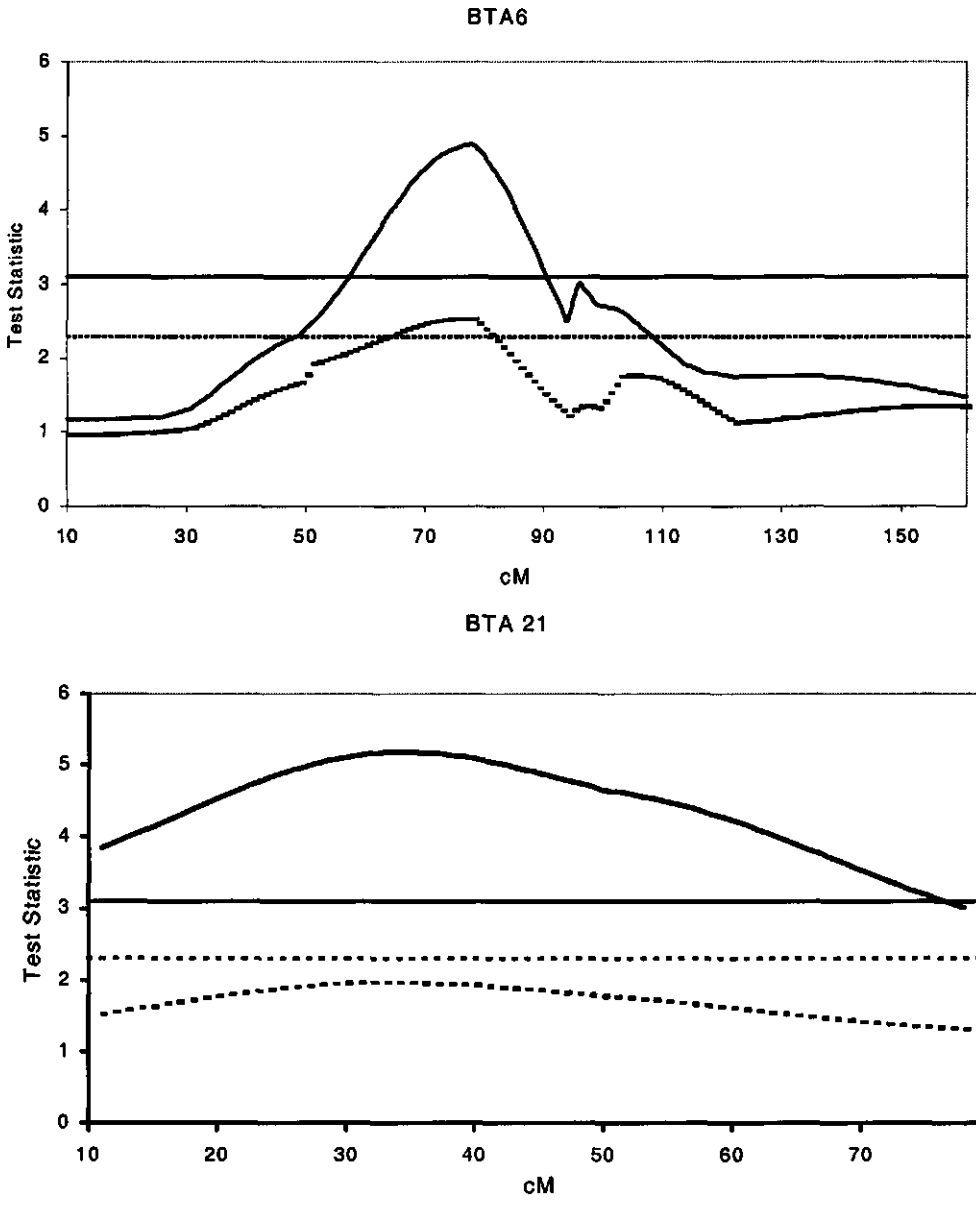

Figure 3. Individual and combined analyses of two chromosomes. In both graphs the dashed curve shows the values of the test statistic for positions along the chromosomes in the individual analyses and the solid curve shows the test statistic when all other QTL are included as cofactors. The horizontal lines denote the suggestive (dashed) and genome-wide 5% (solid) significance threshold, respectively.

other than the chromosome under study are randomly distributed within a family. However, in small families some "co-segregation" of QTL alleles on different chromosomes might occur by chance and might lead to bias in estimation of QTL effects and significance. Co-segregation of unlinked QTL in dairy cattle can be explained by the extensive linkage disequilibrium in dairy cattle that has recently been demonstrated by FARNIR *et al.* (2000). Evidence for co-segregation of QTL alleles in the example can be seen in Table 2. For several families the individual test statistics for several linkage groups changed dramatically in the combined analyses. The proposed strategy accounts for co-segregation of unlinked QTL, which can also potentially contribute to extra power of the combined analysis.

The described strategy resembles that proposed by DOERGE and CHURCHILL (1996) to obtain residual empirical thresholds (**RET**). One of the differences is that with the methods of DOERGE and CHURCHILL (1996) the position and effect of an identified QTL remains fixed throughout the analyses whereas in the methods described here identified QTL are re-evaluated in every round of selecting and fitting QTL as cofactors.

Typical for an outbred design is that not all families are informative (heterozygous) for a QTL. For every family, an allele substitution effect for the QTL is estimated, including families that are homozygous for the QTL. It is not clear how adjusting for a, non-existent, QTL effect in homozygous families affects the analyses of other chromosomes for these families. An alternative is to make the cofactor adjustments only within the families that are informative for a given cofactor. However, the distribution of the within family F ratio's for an identified QTL do not show a sharp division between informative and non-informative families (Table 2). Therefore, discriminating between informative and uninformative families is cumbersome, if not infeasible. It is assumed that adjusting non-informative families for their estimated cofactor effects is adding noise rather than bias to the analysis.

The strategy presented here is not suitable to dissect multiple linked QTL. Ghost QTL, demonstrated by MARTINEZ and CURNOW (1992), are not recognized as such. Including ghost positions as cofactors instead of the actual QTL that give rise to the ghost position will have little effect on the analysis of other chromosomes. For analyzing multiple linked QTL on the same linkage group the grid search performed by SPELMAN *et al.* (1996) and VELMALA *et al.* (1999) can be used while correcting for unlinked QTL by cofactors. However, with the relatively sparse linkage maps used for genome scans in livestock, analysis with more than two linked QTL is unrealistic (DE KONING *et al.*, 1998).

One issue that remains to be solved is what should be the threshold for a candidate region to be included as a cofactor. In the example the threshold for 5% chromosome-wise linkage was used as a criterion for a QTL to be included or excluded as a cofactor. Other strategies using less stringent thresholds

have not been evaluated. Fitting non significant effects as cofactors could add noise to the analyses and might result in loss of power rather than gain. The problem of proper selection of cofactors is not unique to the methods described here. JANSEN (1994) and ZENG (1994) suggest respectively backward elimination and stepwise regression to select the cofactors using an ad-hoc threshold based on nominal significance levels. In the analysis

Table 2. Test statistics for individual families for individual chromosome and cofactor analyses

	BTA1		BTA5		BTA6		BTA12	
Fam	F[a]	Fc[b]	F	Fc	F	Fc	F	Fc
1	2.6	2.5	2.4	3.8	0.0	1.1	0.3	0.8
2	0.5	1.8	0.8	3.3	0.0	8.0	0.1	8.7
3	0.5	0.2	4.4	6.8	3.8	6.6	5.1	8.6
4	0.1	0.9	4.2	4.8	6.1	18.4	0.2	2.5
5	0.1	0.1	0.1	0.0	0.1	0.1	5.1	6.2
6	1.0	1.0	3.0	3.0	0.6	0.6	0.8	1.1
7	8.7	9.1	0.0	0.0	15.6	15.1	17.0	21.2
8	0.9	0.9	1.8	1.1	0.1	0.1	3.3	2.6
9	1.4	3.8	0.6	3.1	1.0	0.4	0.0	0.1
10	2.9	6.1	2.6	8.2	2.3	3.0	0.0	1.3
11	10.0	11.1	8.3	6.8	0.4	1.3	3.9	3.4
12	1.5	0.7	2.9	6.2	1.0	3.3	0.0	1.6
N_{QTL}[c]	2	3	3	5	2	4	3	4
	BTA20		BTA21		BTA23		BTA29	
Fam	F	Fc	F	Fc	F	Fc	F	Fc
1	0.4	2.5	0.2	1.5	0.4	1.3	0.6	0.2
2	7.0	0.3	4.3	11.9	3.8	13.4	9.8	15.7
3	0.1	0.0	3.3	12.8	3.8	7.6	1.2	1.6
4	0.7	3.7	0.3	0.1	0.0	1.5	5.9	22.1
5	4.2	5.7	0.1	0.5	3.7	4.5	0.2	0.0
6	1.2	0.2	1.0	1.0	0.9	1.0	0.2	0.6
7	0.2	2.3	0.5	0.2	0.1	1.8	3.3	4.5
8	0.0	0.0	0.9	0.9	0.1	0.1	0.0	0.0
9	7.6	13.7	11.4	23.5	0.3	0.0	0.8	0.8
10	0.2	0.1	0.4	0.	3.5	4.7	0.0	0.3
11	4.9	4.5	0.8	7.4	1.0	2.9	0.1	3.9
12	8.0	18.0	1.5	4.0	0.4	1.0	0.1	0.0
N_{QTL}[c]	5	4	2	5	0	4	2	3

[a] F ratio for individual families at best position across families without cofactors. The nominal thresholds are between 4 and 4.3 for $p = 0.05$ and between 7.0 and 8.0 for $p = 0.01$, depending on family size. F values exceeding the threshold for $p = 0.05$ are in bold .[b] F ratio for individual families at best position across families with eight cofactors. [c] Number of families that are informative for the QTL.

of an outbred F_2 design, KNOTT *et al.* (1998) start by jointly fitting the most significant marker locations for every individual chromosome. Subsequently, they drop the non-significant cofactors by backward elimination. In such a strategy, the initial number of cofactors equals the number of autosomes, which would be too large for most half-sib designs. KAO *et al.* (1999) also used stepwise regression and acknowledged the complexity of choosing the appropriate threshold for a QTL to be included as a cofactor in their multiple interval mapping (MIM) analysis. As with the 5% chromosome-wise threshold used in the example here, the thresholds used in the MIM methods of KAO *et al.* (1999) are based on the model for one QTL, which might not be appropriate for the multiple QTL model. In the present study, permutation was used to determine thresholds, which might circumvent some of the problems related to hypothesis testing of multiple QTL.

From the more advanced methods to map multiple QTL in outbred species the methods of JANSEN *et al.* (1998) and KAO *et al.* (1999) could be applicable to livestock designs. Although developed for complex designs, JANSEN *et al.* (1998) implemented their methods only for analyses of a single linkage group in a half-sib design. The feasibility of analyzing a complete genome with such a computer intensive method has not been demonstrated yet. The MIM methods by KAO *et al.* (1999) have been used for the analysis of 12 linkage groups in pine for a backcross design but not for other designs or complex pedigrees.

We have implemented some of the ideas of DOERGE and CHURCHILL (1996) in the analysis of multiple chromosomes in outbred half-sib designs. The proposed strategy allows for fast screening of complete genomes. Identified QTL can subsequently be investigated by more sophisticated methods to allow better estimation of the QTL effect and allele frequencies (HOESCHELE *et al.*, 1997). The use of regression methods allows for empirical determination of significance levels by permutation. Extension to different models and other designs where regression methods are often applied such as with F2 line crosses (HALEY *et al.*, 1994), is straightforward.

Implications

Although new statistical tools become rapidly available, the analysis of genome scans in half-sib designs is often based on the analysis of individual chromosomes. In this study, a strategy was developed that allows for efficient combined QTL analysis of complete genomes in a half-sib family structure. Compared to analysis of individual chromosomes, the strategy results in additional power to detect QTL while maintaining the speed and robustness of regression methods.

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The friendly cow all red and white,
I love with all my heart:
She gives me cream with all her might;
to eat with apple tart.
Robert Louis Stevenson (1850-1894)

Multiple QTL and Major Genes, Results for Intramuscular Fat Content and Backfat Thickness

Abstract – The multiple QTL strategy for half-sib designs was modified and implemented for the line-cross analyses. The suggestive QTL from the individual chromosomes were taken as the starting point. Subsequently, effects of a QTL and cofactors were re-estimated jointly until convergence was reached. The analyses accommodated for imprinted QTL, X-linked QTL, and QTL with sex interaction. Furthermore, a permutation approach was introduced for testing two linked QTL against a single QTL. For the best two QTL, it was tested whether both QTL together explained significantly more variance than the single best of the two QTL. The distribution of this test statistic was obtained by permutations of the coefficients for the second QTL, while keeping the coefficients of the best QTL. The models were applied to backfat thickness (BFT) and intramuscular fat content (IMF). The multiple QTL analyses for BFT revealed no new QTL, but a suggestive over-dominant QTL on SSC1 disappeared under the multiple QTL model. The paternally expressed QTL on SSC2 became much more significant and the Mendelian QTL on SSC7 showed significant sex-interaction. For IMF, a suggestive QTL on SSC4 became highly significant under the multiple QTL analyses. The permutation approach for two linked QTL revealed suggestive evidence for an additional QTL affecting BFT on SSC7, and confirmed the two imprinted QTL affecting IMF on SSC6. Using all QTL exceeding suggestive linkage, it was subsequently tested whether the joint QTL effects could explain the major gene effects that were found by segregation analyses. Estimators for all QTL were included as covariables in segregation analyses for IMF and BFT. For both traits, the estimates of the major genes were very similar compared to analyses without QTL. Including the QTL showed a decrease in residual variance but the variance associated with the major gene was only marginally affected. It was concluded that the major genes affecting IMF and BFT could not be explained by the joint effect of the identified QTL. Finally, it was demonstrated that the estimated QTL effects showed little difference when using pre-adjusted phenotypes compared to estimating the QTL effects simultaneously with the systematic effects on unadjusted phenotypes.

A strategy to detect multiple QTLs in half-sib designs was presented in Chapter 7.

For the line-cross model, KNOTT et *al.* (1998) describe a strategy were cofactors are first selected for the individual chromosomes, and subsequently selected across chromosomes by backward elimination. In the first part of this chapter we will implement the strategy proposed in Chapter 7 for the line–cross model, while accommodating for different modes of expression of the QTL. The analysis will be demonstrated by the analysis of intramuscular fat content (IMF) and Backfat thickness (BFT). Subsequently, a strategy to test for two linked QTL will be outlined and also applied to IMF and BFT. In Chapter 2, none of the identified QTL for IMF and BFT represented the major genes that

were described by JANSS *et al.* (1997). Using multiple QTL information, it was investigated whether the joint QTL effects that have been identified for IMF and BFT, could explain the major genes for these traits that were identified by JANSS *et al.* (1997). In our studies, we have pre-adjusted the phenotypic data for the effects of systematic environmental factors, prior to the QTL analysis. In the last section, we evaluated the effect of pre-adjustment of phenotypic data for systematic effects prior to QTL analyses.

Multiple QTL analyses

Methodology: The general strategy of the multiple QTL analysis is analogous to that described for half-sib analyses (Chapter 7), with the extension that in the strategy for line-cross models the QTL and cofactors can have different modes of expression.

First, the chromosomes are analyzed individually to identify candidate regions under models with Mendelian, imprinted (paternal and maternal) or sex-specific expression. The statistical models for these analyses and the inference of the mode of expression have been described in earlier Chapters (Chapters 3-5). The X chromosome is analyzed following the procedures described by KNOTT *et al.* (1998) and implemented as in Chapters 4 and 5. Following the results of Chapter 6, imprinting is evaluated in two manners. I) If the QTL exceeds the threshold of suggestive linkage under both a Mendelian and an imprinting model, a full imprinting model is tested against both a Mendelian and a reduced imprinting model. II) If a QTL is not detected under a Mendelian model (i.e. not exceeding suggestive linkage), the test against a Mendelian model is not used. Sex-specific QTL expression is tested with a standard F test ($p < 0.05$) against a model with sex-equal effects at the position of the best sex-specific QTL.

In the second stage, all QTL that exceed the threshold for suggestive linkage are chosen as cofactors and their effects are re-estimated jointly with multiple linear regression, under the mode of expression that was inferred for each QTL. Thirdly, the phenotypic data are adjusted for the effects of cofactors and the linkage groups are re-analyzed by interval mapping. For a chromosome under study, the phenotypes are only adjusted for cofactors that are on other chromosomes. If this reveals new candidate regions, or different mode of expression of a cofactor, the set of cofactors is modified and the effects re-estimated. This process is repeated until no new QTL are identified and positions of QTL are stable. As before, suggestive and genome-wide thresholds were determined by chromosome-wide permutations and subsequent Bonferroni correction for the genome-wide significance levels (Chapters 2-5). Apart from the initial round of analyses without cofactors, permutations were performed with phenotypes that were adjusted for the cofactor effects, rather than with the original phenotypes (Chapter 7).

Table 1. Results for backfat thickness and intramuscular fat content for single QTL analysis and after the final round of the multiple QTL analysis. For QTL with non-Mendelian expression, an F test against a Mendelian model is included in the results for the multiple QTL analysis (F Mend.).

		Single QTL			Multiple QTL			
SSC	Genetic model	Pos.	F ratio[a]	*p* Gen.[b]	Pos.	F ratio[a]	*p* Gen.[b]	F Mend.[c]
Backfat thickness								
1	Mendelian	148	5.28	0.49	148	2.46	NS	-
2	Paternal	36	23.21	<0.001	34	31.12	<0.001	6.40*
6	Maternal	1	6.22	NS	97	7.50	0.45	9.02**
7	Sex-spec.	56	21.54	<0.001	60	22.57	<0.001	5.91**
14	Mendelian	51	6.88	0.12	60	6.29	0.20	-
X	X-linked	60	21.94	<0.001	62	25.03	<0.001	-
Intramuscular fat content								
2	Maternal	150	4.18	NS	151	8.08	0.43	1.84
4	Mendelian	65	7.67	0.08	69	16.65	<0.001	-
6	Maternal	23	14.42	0.01	24	13.95	0.02	6.13*
6	Paternal	117	14.68	0.01	118	16.72	<0.001	3.43†
8	Sex-spec.	123	4.18	0.23	123	5.03	0.06	13.8***
13	Maternal	53	10.28	0.12	51	8.48	0.31	0.99
X	X-linked	69	12.38	<0.001	56	11.90	<0.001	-

[a] F ratio against the H_0 of no QTL. [b] Empirical, genome-wide *p* values against the H_0 of no QTL, all QTL exceeded the thresholds for suggestive linkage, except those indicated NS. [c] F ratio against the H_0 of a Mendelian QTL with 1 and 2 d.f. in the nominator when testing an imprinted or sex-specific QTL, respectively. † $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (based on tabulated F values).

Results and Discussion: The results of the multiple QTL analyses are summarized in Table 1. The individual analyses for BFT showed significant QTL on SSC2, SSC7, and the X chromosome, and suggestive QTL on SSC1 and SSC14. Including these as cofactors revealed a suggestive QTL on SSC6, while the suggestive, over-dominant QTL on SSC1 was no longer suggestive and therefore dropped from the analyses. After two more rounds of cofactor fitting, the positions of the cofactors were stable. The significance thresholds did not differ much between the individual and the multiple QTL analyses and there was no clear trend. For BFT, all QTL were more significant under the multiple QTL analysis compared to the single QTL analyses, except for the QTL on SSC14. The largest increase in significance was observed for the paternally expressed QTL on SSC2. The difference between the single and the multiple QTL analyses is illustrated for SSC2 in Figure 1. Following the parameterizations proposed in Chapter 5, the test statistics were transformed by $-\log_{10}(P)$, where P is the tabulated value of the F distribution with the appropriate d.f. Comparing the test statistics in Figure 1, with and without cofactors, shows

Table 2. Estimated QTL effects for single and multiple QTL analyses. Estimates are given for the genome-wide significant QTL affecting backfat thickness (BFT) and intramuscular fat content (IMF).

	Single QTL[a]		Multiple QTL[a]	
SSC[b]	a (S.E)[c]	d (S.E)[c]	a (S.E)[c]	d (S.E)[c]
BFT (mm)				
2_{Pat}	.94 (.19)	.	1.00 (.18)	.
7_{ss} ♂	-1.94 (.33)	-.07 (.45)	-1.85 (.31)	-.08 (.42)
♀	-2.94 (.41)	.32 (.51)	-2.74 (.38)	.71 (.48)
X ♂	1.44 (.25)	.	1.43 (.23)	.
♀	1.02 (.32)	.	1.03 (.30)	.
IMF (%)				
4	.18 (.05)	-.01 (.07)	.19 (.04)	.23 (.06)
6_{Mat}	.14 (.04)	.	.14 (.04)	.
6_{Pat}	-.13 (.03)	.	-.13 (.03)	.
X ♂	.21 (.05)	.	.19 (.04)	.
♀	.13 (.06)	.	.10 (.05)	.

[a] For the positions of the QTL in the corresponding columns in Table 1. [b] Subscripts ss, Pat, and Mat denote sex-specific, paternal and maternal expression, respectively. [c] Estimated additive and, where appropriate, dominance effects. The additive effect is expressed as the deviation of the Meishan allele.

a higher test statistic for the cofactor model between 1 and 100 cM. The QTL became more significant but the test statistic still shows a very broad peak, giving little extra resolution to refine the QTL position. Table 1 shows that for BFT all imprinted QTL explain significantly more variance than a standard Mendelian QTL at that position.For IMF, the individual analyses revealed two significant, imprinted QTL on SSC6 and a significant QTL on the X chromosome. Suggestive QTL were detected on SSC4, SSC8, and SSC13. Including these six QTL as cofactors revealed an additional suggestive QTL on SSC2. After one more round of cofactor fitting the positions of the QTL were stable. The most striking result was found for SSC4, where the QTL went from strongly suggestive to highly significant under the multiple QTL analyses (Table 1, Figure 2). Figure 2 also shows a sharper peak of the test statistic under the cofactor analyses, giving a more reliable indication of the QTL position. Also the sex-specific QTL on SSC8 increased from a genome-wide *P* value of 0.23 to a level of 0.06 (Table 1). The maternally expressed QTL on SSC13 and the X-linked QTL show a small decrease in significance under the multiple QTL model, but they remained significant at respectively, the suggestive and genome-wide level. Figure 3 shows the test statistic along the X chromosome, for both IMF and BFT, with and without cofactors. The maternally expressed QTL on SSC6 was significant against a Mendelian model (Table 1). The maternally expressed QTL on SSC2 and SSC13 were not significant against a Mendelian model, but these QTL were not

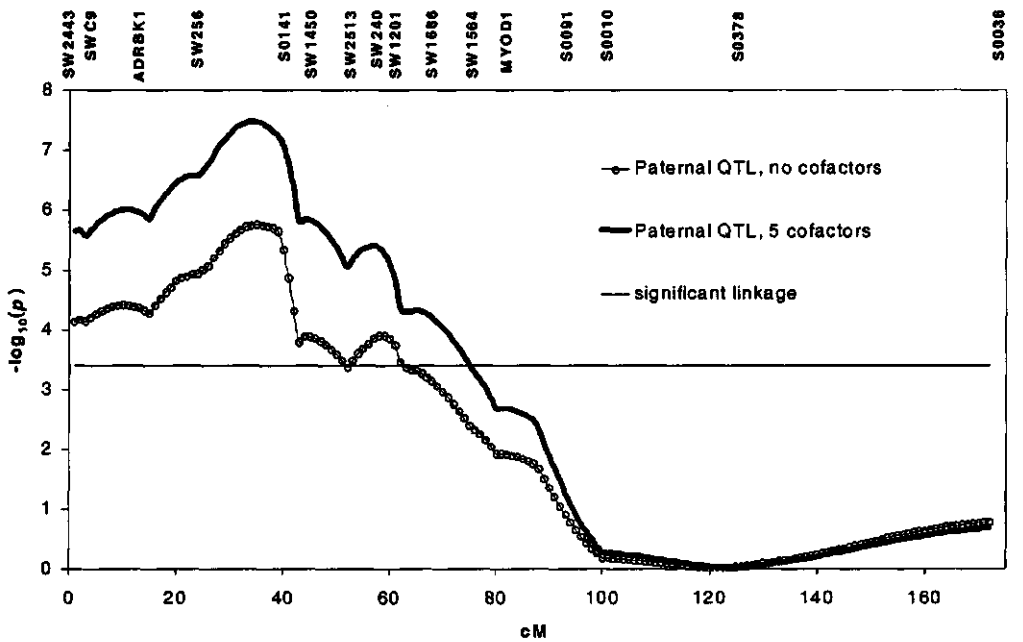

Figure 1. Test statistic along SSC2 for a paternally expressed QTL affecting backfat thickness under single and multiple QTL analysis. Marker names are indicated above the graph.

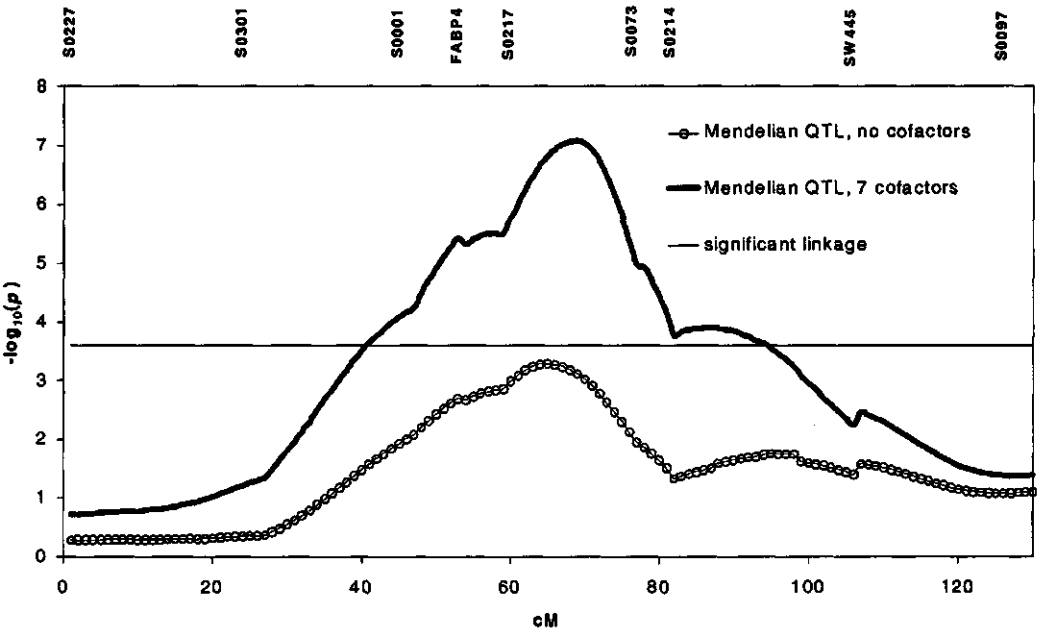

Figure 2. Test statistic along SSC4 for a Mendelian QTL affecting intramuscular fat content under single and multiple QTL analysis. Marker names are indicated above the graph.

detected under a Mendelian model. The significant, paternally expressed QTL affecting IMF on SSC6 was not significant against a Mendelian model (P = 0.064), but under the imprinting model, the QTL was much more significant than under the Mendelian model (Chapters 2 and 6).

The estimated QTL effects under the single and multiple QTL analyses are summarized in Table 2 for the genome-wide significant QTL affecting IMF and BFT. Comparing these estimates, it can be seen that for BFT the estimate of the QTL effect on SSC2 increased slightly for the multiple QTL analysis, while for the X chromosome they did not change. For SSC7, the estimates became smaller in the sense that the negative effects were closer to zero for the multiple QTL analysis. For IMF, there is a remarkable increase in the estimated dominance effect for SSC4, under the multiple QTL analysis (Table 2). The estimated effects for the X chromosome are slightly smaller under the multiple QTL analyses, while the estimates for the imprinted QTL on SSC6 remain the same. There seems to be little differences between the individual and the multiple QTL analyses.

In Chapter 7, application of a multiple QTL analysis resulted in the detection of a larger number of QTL as well as considerable changes in estimated QTL effects. We did not see a clear increase in number of detected QTL here. A possible explanation is that for QTL that contribute to the phenotypic differences between two lines, the line cross approach is more powerful than the half-sib approach (Chapter 2). Using cofactors, one additional suggestive QTL was identified for BFT and one over-dominant QTL was dropped. For IMF, multiple QTL analyses revealed one additional suggestive QTL and a considerable increase in significance for two other QTL. The implementation of this multiple QTL strategy is very straightforward and also computation time is only marginally longer compared to the analyses and permutation testing of individual chromosomes. Application of these methods in QTL detection experiments is recommended because it extracts more information from the experiment by adding little extra complexity.

Multiple linked QTL

Background: In outbred populations, mapping multiple QTL on the same linkage group is more complicated compared to inbred lines, because markers are rarely fully informative. As a result, probabilities of line origin or parental origin in linked marker intervals are not independent. Fitting linked markers as cofactors (JANSSEN, 1994; ZENG, 1994), is expected to reduce the power to identify QTL on the linkage group under study. As an alternative, SPELMAN *et al.* (1996) and KNOTT *et al.* (1998) proposed to fit combinations of two QTL across a linkage group, for a half-sib and line-cross design, respectively. If the two QTL model was significant against a model with no QTL, both SPELMAN *et al.* (1996) and KNOTT *et al.* (1998) tested subsequently whether the two QTL explained significantly more variance than the best QTL from the single QTL

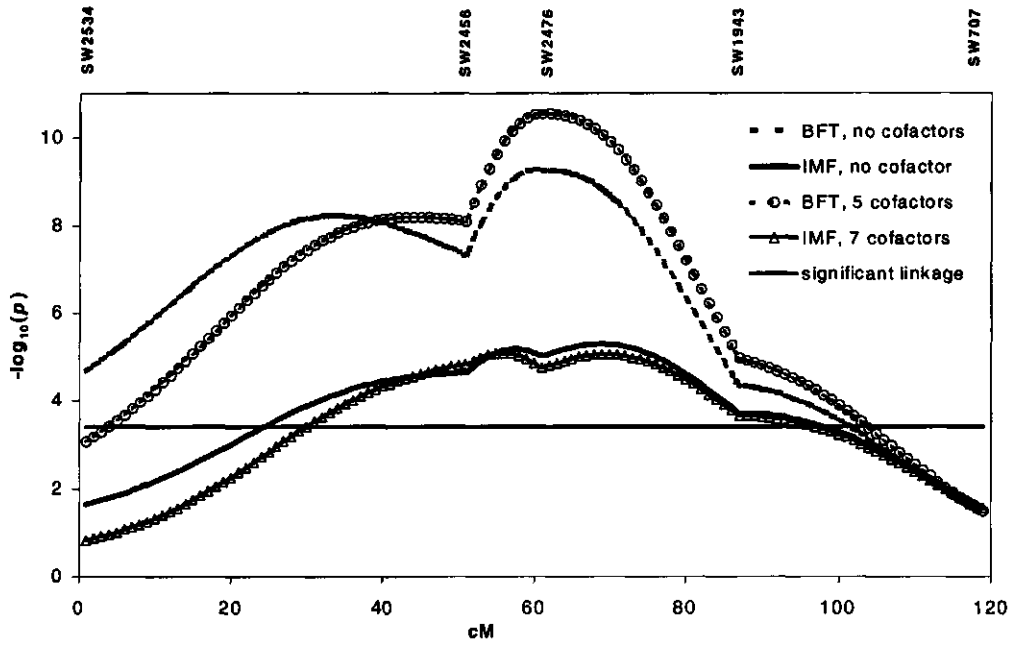

Figure 3. Test statistic along SSCX for QTL affecting backfat thickness (BFT) and intramuscular fat content (IMF) under single and multiple QTL analysis. Marker names are indicated above the graph.

analysis. KNOTT *et al.* (1998) use a tabulated *F* distribution, while SPELMAN *et al.* (1996) use the empirical thresholds that were obtained for the permutation test of a single QTL against the H_0 of no QTL. This raises two issues: I) If the best single QTL is a result of the joint action of the two linked QTL (i.e. a ghost QTL), then testing against the best single QTL is conservative, because the best single QTL explains variance of both linked QTL. II) Because all combinations of QTL positions are evaluated, there is clearly a multiple testing issue. This means that using a tabulated *F* distribution is probably too liberal. It is, however, not obvious that the distribution of the test of two against one QTL, is comparable to that of a single QTL against an H_0 of no QTL as assumed by SPELMAN *et al.*, (1996). Here, we propose a strategy where the significance of both QTL is tested against a model with only the more significant of the two QTL. Thresholds for this test are obtained empirically by an alternative permutation strategy.

Methodology: A chromosome where a QTL has been detected in earlier analyses, is analyzed fitting all combinations of two QTL, with the restriction that there should be at least one empty marker bracket between the two QTL. Each QTL can be Mendelian or imprinted, resulting in a total of six combinations. For the best two QTL from each of these six models, it is first evaluated whether the genetic model of each QTL is

appropriate (i.e. whether the best imprinted QTL is actually imprinted), following the same procedures as applied for single QTL (Chapters 3-6). For the best two-QTL model it is subsequently determined which of the two QTL is most significant. A test statistic is calculated, testing both QTL against the most significant of the two QTL. This test is calculated as an F ratio with 1 or 2 d.f. in the nominator when the least significant of the two QTL is imprinted or Mendelian, respectively. The distribution of this test statistic is obtained empirically by permutation tests (CHURCHILL AND DOERGE, 1994). In contrast to "standard" permutation, genotype probabilities for the second QTL are randomized, while phenotypes and the genotype probabilities for the most significant QTL are retained. Each replicate is analyzed under the inferred two-QTL model, where the position of the first QTL is fixed using the non-permuted values, while the remainder of the chromosome is tested for the second QTL with the permuted genotype. For each replicate, the test of two QTL against the best of the two QTL is calculated for the best combination of two QTL and stored. These *F* values are sorted to provide the empirical distribution of the test statistic under the H_0 that only one of the two QTL is significant. These chromosome-wide values can be adjusted to genome-wide values by a Bonferroni correction. Because the position of the best QTL is fixed throughout the permutations, the analysis is effectively one-dimensional and computationally just as efficient as permutations for a single QTL.

The methods were applied to the linkage groups where genome-wide significant QTL were identified for BFT (SSC2 and SSC7) and IMF (SSC4 and SSC6), using all QTL on other chromosomes (Table 1) as cofactors. The X chromosome was not analyzed for multiple linked QTL because it had molecular data for only five markers. The analyses fitted two QTL at a 2 cM grid search, with at least 20 cM between the two QTL. For the two best positions it was verified whether there was at least one empty marker bracket between the two QTL positions. Ten thousand replicates were used for the permutation tests.

Results: The thresholds for the test of both QTL against the best of the two QTL, were very similar to those obtained for the test of a single QTL against the H_0 of no QTL by permutations. If this is generally the case, then thresholds from the permutation tests for single QTL could be used, as applied by SPELMAN *et al.* (1996), rather than performing additional permutations. However, it is not clear whether this holds in general and given that the permutations do not take much time, it is recommended to perform the permutations for the specific model as described.

Results for BFT: The analyses for SSC2 showed the best results for two combinations, that were very similar in terms of total variance explained: 1) A paternally expressed QTL at 33 cM and a Mendelian QTL at 87 cM. 2) A paternally expressed QTL at 1 cM and another paternally expressed QTL at 39 cM. The model with a paternally expressed QTL and a Mendelian QTL had an *F* ratio against a model with only the paternal QTL of

3.10, corresponding to a tabulated *P* value of ~0.04. The model with two paternal QTL had an *F* ratio against a model with the single paternal QTL at 39 cM of 3.78, corresponding to a tabulated *P* value of ~0.05. However, the 5% chromosome-wide threshold from the permutation test was at 5.3 for the model with a Mendelian and paternal QTL, and 7.4 for the model with two paternal QTL. Applying these thresholds, neither of the two QTL models did explain significantly more variance than the best single QTL. The paternally expressed QTL affecting BFT around 34 cM, was the most significant QTL under all two-QTL models, indicating that this QTL was not a ghost QTL (i.e. the result of two linked QTL). For SSC2, other studies (JEON *et al.*, 1999; NEZER *et al.*, 1999; Chapter 5) found QTL for backfat thickness that mapped to the *IGF2* region. The present analyses provide support for an additional paternally QTL around the *IGF2* region as well as an additional Mendelian QTL around 87 cM, but the available information does not allow discrimination between these models.

For SSC7, the best model showed two Mendelian QTL at 61 and 109 cM. The empirical chromosome-wide *P* value for the test against the single Mendelian QTL at 61 cM was 0.02 ($P_{\text{genome-wide}} = 0.25$). This means that these analyses identified a second, suggestive, QTL affecting BFT on SSC7. It must be noted that here a standard Mendelian QTL was fitted for both positions, while the best single QTL on SCC7 was sex-specific. To limit the number of combinations, the two-QTL analysis does not accommodate sex-specific QTL.

Results for IMF: For SSC4, there was no combination of two QTL that explained significantly more variance than the single best QTL. For SSC6, a maternally and a paternally expressed QTL were already detected in previous analyses, by fitting alternative single QTL models (Chapter 3). When applying the grid search, the best combination was that of a maternally and a paternally expressed QTL at 23 and 117 cM, respectively. The empirical chromosome-wide *P* value for the test against the single paternal QTL at 117 cM was 0.002 ($P_{\text{genome-wide}} = 0.023$). This confirms the presence of two genome-wide significant imprinted QTL on SSC6. Also the positions of the two QTL are the same as those reported in Chapter 3.

Major Genes in retrospective

Background: On the data from this experiment, JANSS *et al.* (1997) identified major genes for both IMF and BFT by segregation analyses. This was one of the incentives to start the molecular genetic research described in this thesis. From the initial analyses for IMF and BFT (Chapter 2), there was no strong indication that any of the identified QTL represented the major genes reported by JANSS *et al.* (1997). In Chapter 3, imprinted QTL were described for both IMF and BFT, while HARLIZIUS *et al.* (2000) found significant QTL for both traits on the X chromosome. Here it is tested whether the joint effects of the Mendelian, imprinted, and X-linked QTL, detected under the multiple

Table 3. Marginal posterior means (mpm) and standard deviations (mpsd) for polygenic variance (σ^2_u), error variance (σ^2_e), major gene variance (σ^2_w) and major gene estimates (a, d) for segregation analyses of BFT and IMF, with (QTL) and without (no QTL) identified QTL as covariables. Results are averaged over three chains of 300,000 iterations, each.

	σ^2_u		σ^2_e		σ^2_w		a		d	
Trait	mpm	mpsd	mpm	mpsd	mpm	mpsd	mpm	mpsd	mpm	mpsd
BFT, QTL	4.52	1.72	9.93	1.71	6.53	2.13	4.42	0.67	-4.10	1.10
BFT, no QTL	3.42	1.96	13.65	1.85	8.52	2.44	4.45	0.68	-4.21	1.17
IMF, QTL	0.144	0.049	0.212	0.037	0.306	0.095	1.08	0.09	-1.01	0.14
IMF, no QTL	0.134	0.048	0.254	0.038	0.361	0.109	1.15	0.09	-1.09	0.13

QTL model, could explain the major gene that was identified for each trait. In order to test this, we include all QTL as covariables in the segregation analysis. When this removes the effect of the major gene, it will subsequently be tested whether this is due to a single QTL or a combination of QTL.

Methodology: For both IMF and BFT, segregation analyses were performed with the original phenotypes, while including company, sex, and slaughter day as fixed effects, and carcass weight as a covariable (JANSS *et al.*, 1997). Subsequently, the segregation analyses were repeated, including all QTL that exceeded the threshold for suggestive linkage (Table 1), as covariables. If one of the QTL or a combination of QTL represents the major gene effect, the major gene is expected to disappear when all QTL are included in the model. Segregation analyses were performed with the MAGGIC package described by JANSS *et al.* (1995).

For each model, three chains of 300,000 Gibbs iterations were used. The first 500 results were omitted for burn-in and every fifth sample was stored to estimate marginal posterior densities for the polygenic variance (σ^2_u), error variance (σ^2_e), major gene variance (σ^2_w), major gene effects, and QTL effects if they were included in the model. The major gene variance was calculated following FALCONER and MACKAY (1996) $[2pq(a + d(q-p))^2+(2pqd)^2]$.

Results: The results of the segregation analyses are summarized in Table 3. For segregation analyses without QTL as covariables, the estimates for the major gene variance and the major gene effects were very comparable to those reported by JANSS *et al.*, (1997). After the inclusion of identified QTL, there was still evidence for a major gene for both traits (Table 3). Under the segregation analyses with the QTL as covariables, there was a decrease in residual variance (σ^2_e) for both IMF and BFT (Table 3). The polygenic variances (σ^2_u), for both IMF and BFT increased in the analyses with QTL as covariables. In an outbred situation, it is expected that the QTL absorb part of the polygenic variance. However, in an F_2 design, the additive genetic variance is estimated by the variance between F_1 families. Following the assumption of fixation under the line-cross analyses, the QTL effects are assumed equal for all families, so they have little effect on the variance between F_1 families. Therefore, the

QTL effects are expected to affect the variance within families, resulting in the reduced residual variance (σ^2_e). This does not explain why the polygenic variance (σ^2_u) actually increased under the models with QTL (Table 3). A possible explanation is that the imprinted, sex-specific, and X-linked QTL do not follow the standard rules for the expected covariance between relatives, that are based on the additive genetic relationship between those relatives. Including these non-Mendelian components in the analyses may have resulted in a better fit for the "real" polygenic effects. The estimates for the major gene variance (σ^2_w) were slightly smaller for the analyses with QTL as covariables, compared to the segregation analyses without QTL, but the differences were well within the marginal posterior standard deviations. When accounting for identified QTL, the estimates of the major genes were only marginally affected. This indicates that the major genes for IMF and BFT, reported by JANSS *et al.*, (1997) cannot be explained by a linear combination of QTL that have been identified for these traits up to now.

One aspect that was not investigated was the possibility that the major genes represent a combination of two epistatic loci. A post-hoc analysis, fitting additive x additive, dominance x additive, and dominance x dominance interactions for every combination of two identified QTL (Table 1), revealed no significant epistatic effects. However, we have not performed two-dimensional genome scans for epistatic loci as described by CARLBORG *et al.* (2000), so epistatic QTL might have remained undetected.

Failure of the QTL analyses to detect the major genes that were identified by the segregation analyses could be attributed to differences in genetic model or methods to find the best statistical fit between the two analyses.

The QTL analyses under the line cross model assume fixation of founder lines whereas the segregation analyses estimates major gene frequencies in the founder lines. However, JANSS *et al.* (1997) concluded that at least one the alleles of the major genes affecting BFT and IMF was unique for one of the founder lines. This would suggest that a line cross model should have sufficient power to detect the major gene. However, the variance explained by the major gene under a line-cross QTL model, would never equal the single gene variance of the segregation analysis. Even if founder lines were segregating for the QTL alleles, the half-sib analyses should have sufficient power to detect the major gene, given the size of the estimated major gene effects.

The QTL analyses use regression methods to find the most likely QTL position and effects whereas the segregation analyses use a Bayesian framework. The regression methods find an optimum by minimizing the error term whereas the Bayesian analyses maximizes the posterior probability of the parameters, given the data and the prior probabilities. Effectively, the QTL analyses using regression methods rely on the phenotypic means of different genotype classes whereas

Table 4. Estimated QTL effects for multiple QTL analyses with pre-adjusted phenotypes, and estimated jointly with fixed effects and covariates on original data using a least squares model (SAS) or a segregation analysis (MAGGIC). Estimates are given for the genome-wide significant QTL affecting backfat thickness (BFT) and intramuscular fast content (IMF).

	QTL analysis[a]		Least squares[b]		MAGGIC[c]	
SSC[d]	a (S.E)[e]	d (S.E)[e]	a (S.E)[e]	d (S.E)[e]	a (mpsd)[f]	d (mpsd)[f]
BFT (mm)						
2_{Pat}	1.00 (.18)	.	1.06 (.19)	.	.98 (.17)	.
7_{ss} ♂	-1.85 (.31)	-.08 (.42)	-2.04 (.32)	-.23 (.49)	-2.04 (.30)	-.44 (.45)
♀	-2.74 (.38)	.71 (.48)	-2.80 (.40)	.86 (.62)	-2.86 (.38)	.68 (.58)
X ♂	1.43 (.23)	.	1.45 (.24)	.	1.25 (.22)	.
♀	1.03 (.30)	.	0.98 (.31)	.	.85 (.29)	.
IMF (%)						
4	.19 (.04)	.23 (.06)	.20 (.05)	.05 (.07)	.16 (.04)	-.00 (.05)
6_{Mat}	.14 (.04)	.	.15 (.04)	.	.11 (.03)	.
6_{Pat}	-.13 (.03)	.	-0.13 (.03)	.	-.09 (.03)	.
X ♂	.19 (.04)	.	.20 (.04)	.	.14 (.04)	.
♀	.10 (.05)	.	.11 (.06)	.	.08 (.04)	.

[a] Multiple QTL analyses with pre-adjusted phenotypes. [b] A linear model which included sex, company, and slaughter day as fixed affects, carcass weight as covariable, and all QTL that exceeded suggestive linkage in the multiple QTL analysis as covariables. [c] A segregation analyses including all the components of the least squares model as well as a polygenic component and a major gene component. [d] Subscripts ss, Pat, and Mat denote sex-specific, paternal, and maternal expression, respectively. [e] Estimated additive and, where appropriate, dominance effects. The additive effect is expressed as the deviation of the Meishan allele. [f] Marginal posterior means for a and d with their marginal posterior standard deviations (mpsd).

maximum likelihood or Bayesian methods also utilize the distributional properties of the phenotypes. However, for QTL detection KAO (2000) showed little extra power to detect QTL using maximum likelihood instead of regression algorithms.

Although these differences between QTL analysis and segregation analyses may explain the discrepancies between the results of the two methods, the failure to detect QTL that were representing the major genes was unexpected. The conclusion of JANSS *et al.*, (1997), that this experimental population was very promising for QTL analysis, was proven right in this thesis, but the major gene issue remains to be resolved.

QTL effects

Throughout this thesis, the phenotypes were pre-adjusted for systematic effects prior to the QTL analyses. Other studies (fi. KNOTT *et al.*, 1998), used original phenotypes and included the fixed effects and covariables in the QTL model. Here, we investigated the effect of pre-adjustment of phenotypes in our data by comparing estimated QTL effects when using pre-adjusted phenotypes in the QTL analysis to using the original phenotypes under a least squares and a mixed inheritance model.

Methodology: QTL effects were estimated for BFT and IMF in the QTL analyses, on phenotypes that were pre-adjusted for slaughter day, sex, company, and carcass weight, as described in Chapter 2. The coefficients for the QTL exceeding suggestive linkage under the multiple QTL analyses (Table 1) were used to re-estimate the QTL effect under two models: I) The QTL effects we re-estimated with the systematic effects and the original phenotypes, using a least squares model (SAS). II) The QTL effects were re-estimated on the original phenotypes with the systematic effects under a segregation analyses, as described in the "Major genes in retrospective " section of this Chapter. Beside the QTL and systematic effects, this model included a polygenic and a major gene component.

Results: The estimates of the QTL effects under the three models are summarized in Table 4, for the genome-wide significant QTL affecting IMF and BFT. The estimates of the multiple QTL effects, using the original phenotypes in a least squares model, were very comparable to those obtained for the multiple QTL analyses on adjusted phenotypes. The largest difference was found for the dominance effect for IMF on SSC4, which was negligible in the least squares model with the original data. The estimates using the original phenotypes under a segregation analysis, were comparable or slightly smaller compared to those obtained for the least squares model and the original phenotypes. Also here, the estimated dominance effect for IMF on SSC4 was not significantly different from zero. The pre-adjustment of phenotypes, prior to the QTL analyses, seems to have little or no effect on the magnitude of the estimated QTL effects in our data.

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Implications of Imprinting

Abstract – This chapter gives an overview of the imprinted QTL that have been detected on the experimental cross between Meishan pigs and commercial lines. In total, nine chromosomes showed imprinted QTL that exceeded a genome-wide threshold of 0.10. From these, chromosome 2 showed imprinting for three uncorrelated traits in the same chromosomal region. The other chromosomes showed a single imprinted QTL or large distances between the imprinted QTL. The possibilities for implementation in a pig breeding program are illustrated by an example. Using the same founder lines, application of imprinted and X-linked QTL allows a diversification in the slaughter pigs for different markets.

Many imprinted QTL have been described in this thesis as well as QTL on the X chromosome. HIROOKA *et al.* (2001a,b) described additional imprinted QTL while HARLIZIUS *et al.* (2000) described the detection of additional QTL on the X chromosome. The imprinted QTL that have been detected in this experimental population, will be summarized and discussed briefly. A possible application of the identified imprinted and X-linked QTL in a commercial pig breeding program is described.

Overview of imprinted QTL

For the traits described in this thesis, imprinted QTL are reported in Chapters 3-5. Besides the traits described in this thesis, HIROOKA *et al.* (2001a,b) analysed the same experimental population for QTL affecting teat number and loci affecting coat color. For teat number, HIROOKA *et al.* (2001a) detected four QTL, of which three were imprinted. These included a paternally expressed QTL that mapped to the *IGF2* region on SSC2, for which imprinting has been reported for other traits (Chapter 5; NEZER *et al.*, 1999; JEON *et al.*, 1999). In the same region, HIROOKA *et al.* (2001b) detected a locus affecting the black coat color in pigs. In contrast to previous imprinted QTL in the *IGF2* region, this locus showed exclusive maternal expression. Table 1 gives an overview of imprinted QTL that have been detected on this experimental population, across all the traits that were investigated. Only loci that exceeded a genome-wide threshold of 10% against the H_0 of no QTL were included in Table 1. Imprinted QTL were detected on nine chromosomes. However, seven of these chromosomes only showed imprinting for a single trait, when ignoring additional suggestive imprinted QTL on these chromosomes (Chapters 4 and 5). The most striking evidence for imprinting was obtained for the *IGF2* region on SSC2, where imprinted loci were found for three traits that show no obvious correlation.

Across the 19 traits that have been analyzed to date, most imprinted QTL were found for

Table 1. Overview of all chromosomes and traits for which imprinted QTL have been detected, exceeding a genome-wide threshold of 0.10.

SSC	Position, cM	Genetic model	Trait	Reference
2	2	Paternal	Teat number	HIROOKA *et al.* (2001a)
2	3	Maternal	Black coat color	HIROOKA *et al.* (2001b)
2	5	Paternal	Ultrasonic backfat thickness	Chapter 5
2	36	Paternal	Carcass backfat thickness	Chapter 3
3	90	Maternal	Teat number	HIROOKA *et al.* (2001a)
4	81	Maternal	Test growth (25-90 kg)	Chapter 5
6	23	Maternal	Intramuscular fat content	Chapter 3
6	117	Paternal	Intramuscular fat content	Chapter 3
6	191	Maternal	Early growth (weaning-25 kg)	Chapter 5
7	56	Maternal	Carcass muscle depth	Chapter 3
8	29	Maternal	Life growth	Chapter 5
10	85	Paternal	Early growth (weaning-25 kg)	Chapter 5
12	80	Paternal	Teat number	HIROOKA *et al.* (2001a)
14	30	Maternal	Ultrasonic backfat thickness	Chapter 5

traits related to growth and body composition (Table 1). However, these were also the traits for which the Meishan pigs have the largest phenotypic differences with the commercial lines, giving high statistical power to detect QTL for these traits. This means that based on these results, it cannot be concluded that growth and body composition traits in pigs are more affected by imprinting than other traits.

Imprinted and X-linked QTL in pig breeding

Background: In pig breeding, qualities of different purebred lines are exploited for efficient production of high quality pork. Selection criteria for these breeds include a wide range of traits, including growth, reproduction, and meat quality. Breeding programs are used to improve these purebred lines. An important component of each breeding program is the identification of animals with the highest genetic merit that can be used as parents for the next generation. For many years, people have recognized the need for genetic evaluation of animals and today best linear unbiased prediction (BLUP) has become the most widely accepted method for genetic evaluation of domestic livestock.

The findings that several QTL affecting body composition and growth are under control of genomic imprinting, or located on the sex chromosome, has major implications for the practice of animal breeding.

Identification of imprinted and X-linked QTL opens new perspectives for crossbreeding, which is common practice in pig breeding. In the following paragraphs a scenario will be proposed in which strategic use of the identified imprinted and X-linked QTL allows the final product (slaughter pigs) to be tailored to four different markets, using the same purebred lines.

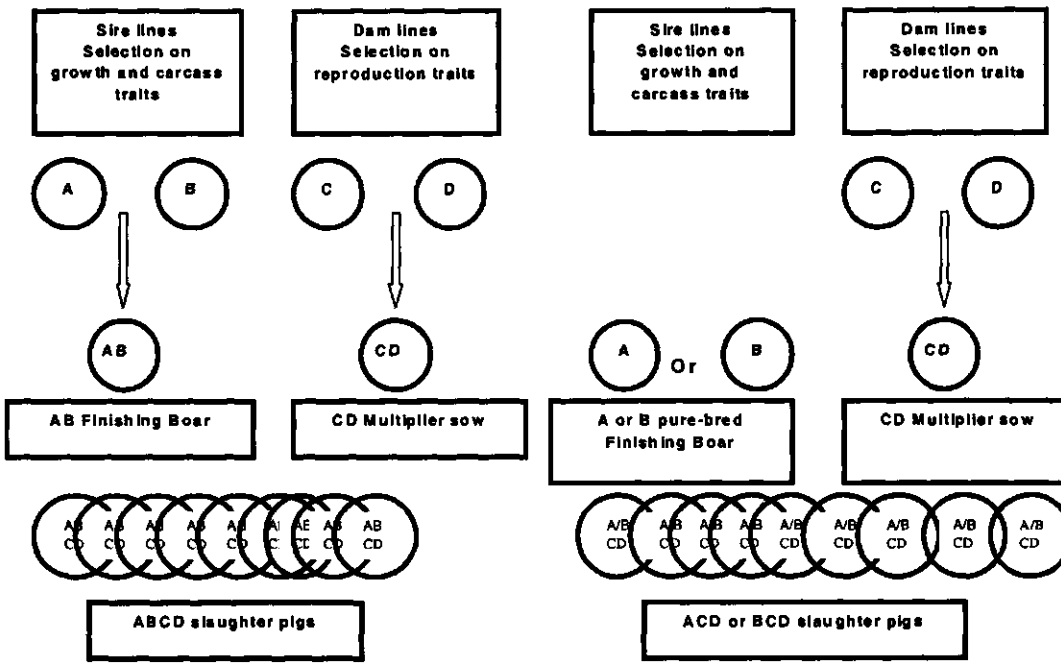

Figure 1. Common crossbreeding schemes in traditional pig breeding using crosses between three (right) or four (left) pure-bred lines. Note that within every pure-bred line sufficient numbers of boars and sows are available to allow sufficient selection intensity.

Pig breeding: At present, pig breeding programs focus on purebred breeding for additive genetic progress and crossbreeding for dominance effects (heterosis) in the final breeding product. This involves first the choice of the breed for the purebred lines that will be used. The choice of breeds is based on the breeding goal within the sire and dam lines and the expected heterosis in the slaughter pigs. The genetic evaluations within the purebred lines are currently based on Mendelian inheritance. In sire lines, selection is generally focussed on production and meat quality traits like daily gain, backfat thickness (BF), muscle depth (MD) and intramuscular fat content (IMF). The generation interval is shorter in the sire lines, enabling larger additive genetic progress. In dam lines, selection is focussed on fertility traits. Selection on growth and carcass traits is hampered in these sow lines because of negative genetic correlations between fertility and production traits. As a result, the genetic potential of a slaughter pig for traits like backfat thickness and daily gain is compromised by the maternally inherited alleles. An overview of common crossbreeding schemes using three or four purebred lines is given in Figure 1.

Scenario for implementation: Applying the imprinted and X-linked QTL described in this thesis and by HARLIZIUS *et al.* (2000), the

Table 2. Desired properties of slaughter pigs for four different markets, standardized to a weight of 100 kg.

	Pork	Bacon[a]	Parma	Japan[a]
Growth g/day	825	850	750	800
Backfat thickness mm	15	20	24	28
Muscle depth mm	59	57	40	57
Intramuscular fat %	2	3	3	4
Slaughter weight (kg)	110	75	130	80

[a] Animals will be slaughtered <100 kg so actual values of backfat and muscle depth will be lower.

same purebred lines can be used to target a variety of markets. After consultation of representatives of the Dutch pig breeding industry, four different pig types were proposed:

1) The Pork pig: Targeting the European market for fresh and processed pork, this pig should have moderate to fast growth. At the slaughter weight of around 110 kg it should have sufficient muscle depth with a low backfat thickness. The IMF can be a little bit lower than for the other products, but should not drop below 2%. Pork for the processing plants can be slightly fatter and less muscular than pork for the fresh meat market. The pork market is at the moment the main market, but this may change in the future.

2) The Bacon pig: Targeting the English bacon market, this pig is characterized by fast early growth, sufficient muscle depth (MD) and high intramuscular fat content (IMF). Because of the low slaughter weight (~ 80 kg) this animal needs relatively high backfat thickness (BF) at young age.

3) The Parma pig: Targeting the Italian Parma ham market, this pig has relatively slow growth, relatively low muscle depth and high backfat thickness. When it is being slaughtered at a body weight >160 kg, it should have accumulated a lot of backfat and the meat should have a high IMF.

4) The Japan pig: Targeting the Japanese meat market, this pig should exhibit very high fatness at a relatively young age. Both the IMF and the BF should be higher than for any other market while muscularity should be sufficient.

The desired trait values for all four products are summarized in Table 2. For most markets a crossbred multiplier sow will be used that has been selected for fertility traits. Preferably she has high fat deposition that will help her to cope with the large energy demand imposed on her by raising large litters of slaughter pigs.

The molecular tools that will allow this diversification comprise the following QTL:

a) SSC2: a paternally expressed QTL affecting BF (Chapters 2, 3, and 5).
b) SSC4: a QTL affecting early growth in a Mendelian fashion and later growth with specific maternal expression (Chapter 5).
c) SSC6: a maternally expressed QTL affecting IMF at 23 cM (Chapter 3).
d) SSC6: a paternally expressed QTL affecting IMF at 117 cM (Chapter 3).
e) SSC7: A QTL affecting growth and BF in a standard Mendelian fashion, at the same locus also a maternally expressed QTL

affecting muscle depth (Chapters 2, 3, and 5).

f) SSC8: a maternally expressed QTL affecting mainly early growth (Chapter 5).

g) SSCX: X-linked QTL affecting both IMF and BF (Chapters 5 and 8, HARLIZIUS *et al.*, 2000)

For all QTL it will be assumed that the purebred lines can be selected for either the high or the low allele of a QTL. For SSC2, we assume that there is a single paternally expressed QTL affecting BF. For SSC7 and SSCX we cannot determine at this stage whether these chromosomes harbor a single pleiotropic QTL or multiple closely linked QTL. For this moment they will both be treated as single loci and the phase between the effects is assumed to be the same as found in our experimental data. This means that for SSC7 there is one allele that gives lower muscle depth and backfat thickness, but an increased growth, and another allele with opposite effects. For SSCX there is one allele that increases both IMF and BF, and one allele that decreases both. Because there is nearly 100 cM between the two imprinted QTL affecting IMF on SSC6, they can be treated as unlinked and any combination of effects can be selected for in the purebred lines.

Based on the desired properties that are summarized in Table 2, for each product the ideal QTL composition was derived. The results are presented in Table 3. To make the scheme practical and to keep genotyping costs to a minimum, molecular typing will only be performed on the purebred lines. The breeds for the pure-bred lines can be pre-selected based on how well their QTL configuration corresponds to the desired configuration. These purebred lines will be selected towards homozygosity for the preferred combination of alleles as indicated in Table 3. Once the purebred lines have reached the desired molecular configuration, no further molecular typing is necessary.

The basic scheme still consists of four purebred lines of which two are selected for growth and meat characteristics (A & B) and two selected for reproduction traits (C & D). All liens will be selected for their QTL configuration following Table 3. The new QTL provided a very good tool for controlling the meat and growth characteristics, allowing sows from lines A and B to be also selected for reproductive capacity and serve as multiplier sows for some of the products. In lines C and D there is molecular selection on growth and slaughter traits, while the phenotypic selection is entirely on reproductive performance. The complete mating scheme for all markets is given in Table 3.

The pork and bacon market make up the largest export segment and the crossbreeding schemes for these markets follow that of a traditional scheme with additional efficiency of Marker Assisted Selection (MAS). For the fresh pork market, the QTL are chosen to increase growth (SSC4 and SSC8), reduce backfat (SSC2 and SSCX), and have sufficient IMF (SSC6). For the bacon market, the female slaughter pigs will get additional

Table 3. Molecular composition of four purebred lines (Figure 1), two crossbred types and four types of slaughter pigs (Table 1). + Indicates selection (purebred lines) or expression (F_1 and slaughter pigs) for the high allele of a QTL, - selection or expression for the low allele of a QTL and 0 indicates no selection (expression) for that QTL in the specified line, or that the inherited allele can be either + or -. For the crossbred animals and the Mendelian QTL in the slaughter pigs both parental alleles are given (first is paternally inherited).

	Boar x Sow	SSC2	SSC4	SSC6[a]	SSC6[b]	SSC7[c]	SSC8	SSCX
Purebred								
A	A x A	-	+	-	-	+--	-	-
B	B x B	-	+	+	+	-++	+	+
C	C x C	+	+	+	+	-++	+	-
D	D x D	+	+	+	+	-++	+	-
F_1 multipliers								
AB	A x B	-/-	+/+	-/+	-/+	+--/-++	-/+	♂+♀-/+
CD	C x D	+/+	+/+	+/+	+/+	-++/-++	+/+	♂-♀-/-
Slaughter pigs								
Pork	A x CD	-	+/+	+	-	+-0/-++	+	♂-♀-/-
Bacon	AB x CD	-	+/+	+	0	000/-++	+	♂-♀+/-
Parma	CD x A	+	+/+	-	+	-+0/+--	-	♂-♀-/-
Japan	CD x B	+	+/+	+	+	-+0/-++	+	♂+♀+/-

[a] Maternally expressed QWTL at 23 cM. [b] Paternally expressed QTL at 117 cM. [c] +-- indicates the maternally inherited allele with higher growth and lower backfat thickness and muscle depth, while -++ indicates the antagonistic allele. +-0 and -+0 are the equivalents of the paternally inherited alleles for this locus, which will have no effect on muscularity.

backfat and IMF through the QTL on the X chromosome. It is expected that boars, that not inherit the allele with higher fatness, will be castrated and hence show increased fatness. For the Parma and Japan pig types, purebred sows from the sire lines will be used as multipliers while crossbred CD boars will be used to sire the slaughter pigs (Table 2). The reproductive performance of these sows will not be as high as that of the sow lines but a slight increase in cost price for the specialized Japan and Parma market is probably acceptable. This type of crossbreeding allows transmission of fatness alleles from the sow lines into the slaughter pigs. A further increase in fatness for the Japanese market is obtained by using a sow from the B line that will transmit her fatness allele on the X chromosome to both female and male offspring.

Remarks: The proposed scenario is only one example out of many possible QTL configurations. It should be clear that it serves mostly as an illustration of the possibilities offered by imprinted and X-linked, rather than a protocol for MAS in pig breeding. In order to give a detailed prediction of the consequences of different strategies, effects of QTL need to be estimated in the commercial lines. It is not unlikely that commercial populations are still segregating for imprinted and X-linked QTL, because allele frequencies at these loci are less affected by standard

(BLUP) selection compared to additive Mendelian loci.

Differentiating between different markets while maintaining only a limited number of purebred lines may be a more promising approach to MAS then using QTL to increase the efficiency of a standard selection program only aimed at the bulk market. A pre-requisite is that QTL positions are known with more precision than currently provided by QTL analyses. For the paternally expressed QTL affecting backfat thickness on SSC2, fine mapping efforts are carried out at Wageningen University (RATTINK *et al.*, 2001)

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Summary

The main theme of this thesis is the analyses of the Wageningen Meishan experiment for quantitative trait loci (QTL) affecting a wide range of production, reproduction, and meat quality traits in pigs. The Wageningen Meishan experiment was initially established to investigate the possibilities of introgression of Meishan genes into the Dutch commercial pig lines. The Wageningen Meishan experiment was initiated by mating 126 purebred females from five breeding companies to 19 boars of the Meishan breed. From the F_1, 39 males and 264 females were randomly selected to produce the F_2 litters. With close to 1200 F_2 animals, this is the largest QTL experiment in pigs.

In Chapter 2, the first QTL analyses on this experimental population are presented for backfat thickness and intramuscular fat content. Approximately half of the experimental population (619 F_2 animals and their parents) was typed for molecular markers covering the entire porcine genome. Linkage 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. 2) A half-sib model where a unique allele substitution effect was fitted within each of the 19 half-sib families. For backfat thickness, both approaches revealed a highly significant QTL on SSC7 and suggestive evidence for a QTL on SSC2. Additional suggestive QTL affecting backfat thickness were detected on SSC1 and SSC6 under the line-cross model. For intramuscular fat content the line- cross model showed suggestive evidence for QTL on SSC2, SSC4, and SSC6 whereas the half-sib analysis showed suggestive linkage for SSC4 and SSC7. The nature of the QTL effects and assumptions underlying both models could explain discrepancies between the findings under the line-cross model and the half-sib model. It was concluded that both approaches can complement each other in the analysis of data from outbred line crosses.

Following the completion of the molecular typing of the entire experimental population, the role of genomic imprinting was investigated for intramuscular fat content, backfat thickness and muscle depth, and described in Chapter 3. Imprinting was tested using a novel statistical model that separated the expression of paternally and maternally inherited alleles. The whole genome scan revealed significant evidence for five QTL affecting body composition, of which four were imprinted. For backfat thickness it was shown that the QTL on SSC2, that was described in Chapter 2, actually represented a paternally expressed QTL. The QTL for backfat thickness that had been identified on SSC7 showed Mendelian expression. In the same region of SSC7, a maternally expressed QTL affecting muscle depth was found. SSC6 harbored a maternally expressed QTL on the short arm and a paternally expressed QTL on the long arm, while in Chapter 2 only a suggestive QTL could

be identified under a standard Mendelian model. The individual QTL explained between 2 and 10% of the phenotypic variance. The known homologies to human and mouse did not reveal positional candidates. In this Chapter, it is concluded that testing for imprinting should become a standard procedure to unravel the genetic composition of multifactorial traits.

In Chapter 4, QTL affecting eight meat quality traits were investigated. QTL analyses were performed using the line-cross approach, including tests for genomic imprinting and sex-specific expression, and a half-sib model, where a unique allele substitution effect was fitted within each of the 38 half-sib families. In total, three genome-wide significant and 26 suggestive QTL were detected. The significant QTL on SSC3, SSC4, and SSC13, all affecting meat color, were only detected under the half-sib model. Failure of the line-cross approach to detect the meat color QTL suggests that the founder lines had similar allele frequencies for these QTL.

Using the same analyses as described in Chapter 4, the QTL analyses for growth traits, ultrasonic backfat thickness, and litter size are described in Chapter 5. For growth and ultrasonic backfat thickness, phenotypes were available on 942 to 1151 animals, while for litter size there were observations on 249 and 206 animals at first and second parity, respectively. For ultrasonic backfat thickness, the analyses revealed significant QTL on SSC2, SSC7, SSC14, and the X chromosome, with significant imprinting for SSC2 and SSC14. However, the paternally expressed QTL affecting ultrasonic backfat thickness mapped to a different region of SSC2 than the paternally expressed QTL affecting backfat thickness at slaughter that was described in Chapter 3. For the different growth traits, significant QTL were detected on SSC1, SSC4, SSC7, and SSC8. Both the QTL on SSC4 and SSC8 showed maternal expression for a specific growth stage. The QTL analyses for litter size revealed one suggestive QTL for first parity and three suggestive QTL for the second parity. Analyses under a half-sib model did not reveal additional significant QTL, but confirmed several of the QTL that were detected under the line-cross models. This Chapter provides confirmation of several QTL affecting growth and fat deposition in pigs and added interesting new insight into their mode of expression.

In Chapter 6, the quantitative genetic aspects of imprinted genes and statistical properties of methods to detect imprinted QTL are studied. Different models to detect imprinted QTL and to distinguish between imprinted and Mendelian QTL were compared in a simulation study. Mendelian and imprinted QTL were simulated in an F_2 design and analyzed under Mendelian and imprinting models. Mode of expression was evaluated against the H_0 of a Mendelian QTL as well as the H_0 of an imprinted QTL. An imprinting model with a paternal, maternal, and dominance component was tested against a) a Mendelian model, and b) an imprinting model with a single parental effect. It was shown that imprinted QTL might remain undetected when only analyzing the genome with Mendelian models. Compared to testing against a Mendelian QTL, using the H_0 of an imprinted QTL gave a higher proportion of correctly

identified imprinted QTL, but also gave a higher proportion of false inference of imprinting for Mendelian QTL. When QTL were segregating in the founder lines, spurious inference of imprinting became more prominent under both tests, especially for designs with few F_1 sires.

The implications of the simulation study are discussed for the Wageningen Meishan experiment as well as for other studies.

Up to Chapter 7, all QTL analyses were restricted to the analyses of a single chromosome for one or two QTL affecting the trait of interest. Chapter 7 describes a strategy for multiple QTL analyses in half-sib designs. The strategy combines information from individual analyses, after which trait scores for a specific linkage group are adjusted for identified QTL at other linkage groups. Regression methods are used to estimate QTL positions and effects; permutation tests are used to obtain empirical threshold values. The description of the methods is complemented by an example of the combined analysis of 28 bovine chromosomes and their associations with milk yield in Finnish Ayrshire cattle. In this example, the individual analysis revealed five suggestive QTL affecting milk yield. Following the multiple QTL strategy presented in this chapter, the final combined analysis showed eight significant QTL affecting milk yield. This clearly demonstrates the potential gain of using the combined analysis. The use of regression methods, with low demands on computing resources, makes this approach very practical for total genome scans.

In Chapter 8, we return to the analyses of the Wageningen Meishan experiment. Inspired by the success of the multiple QTL strategy for half-sib designs in Chapter 7, this approach was implemented for the line-cross analyses. The basic strategy remained that suggestive QTL from the individual chromosomes were included as cofactors and re-estimated jointly. The strategy was extended to accommodate for Mendelian QTL, imprinted QTL, X-linked QTL, and QTL with sex interaction. Subsequently, a permutation approach was introduced for testing two linked QTL against a single QTL. For the best two QTL, it is tested whether both QTL together explain significantly more variance than the single best of the two QTL. The distribution of this test is obtained by permutations for the second QTL, while the best QTL is kept fixed. This model is implemented for all possible combinations of Mendelian and imprinted QTL. The models were demonstrated with the analyses of backfat thickness (BFT) and intramuscular fat content (IMF). The multiple QTL analyses for BFT revealed no new QTL, but a suggestive over-dominant QTL on SSC1, reported earlier in Chapter 2, disappeared under the multiple QTL model. The paternally expressed QTL on SSC2 became much more significant and the Mendelian QTL on SSC7 showed significant sex-interaction. For IMF, a suggestive QTL on SSC4 became highly significant under the multiple QTL analyses. Using all QTL exceeding suggestive linkage, it was subsequently tested whether the joint QTL effects could explain the major gene effects that were found earlier by segregation analyses.

Estimators for all QTL were included as covariables in segregation analyses for IMF and BFT. For both traits, the estimates of the major genes were very similar compared to analyses without QTL. The variance associated with the major gene was slightly smaller under the models with QTL, but still very significant. It was concluded that the major genes affecting IMF and BFT could not be explained by the joint effect of the identified QTL.

Chapter 9 gives an overview of the imprinted QTL that have been detected in the Wageningen Meishan experiment. In total, nine chromosomes showed imprinted QTL that exceeded a genome-wide threshold of 0.10. From these, SSC2 showed imprinting for three uncorrelated traits in the same chromosomal region. The other chromosomes showed a single imprinted QTL or large distances between the imprinted QTL. The possibilities for implementation in a pig-breeding program are illustrated by an example. Using the same purebred lines, application of imprinted and X-linked QTL in a crossbreeding system, allowed a diversification in the slaughter pigs for four different markets.

Epilogue

The analysis of the experimental population, described in this thesis, has provided a wealth of QTL for economically important traits in pigs. Although several QTL studies have been carried out in pigs some of the QTL have only been identified in the Wageningen experiment. This is not only because of the high power to detect QTL, as a result of the large number of F_2 animals involved in the experiment, but also the application of both line-cross and half-sib analyses.

The detection of imprinted QTL affecting body composition and growth (Chapters 3 and 5) has relevance for the whole field of genetical research. Not only did it provide chromosomal regions, for which imprinting has not yet been demonstrated in human or mice, but it also showed that imprinting might be a more common phenomenon than commonly assumed.

The QTL that have been described in Chapters 2-5 provide pig breeders with molecular tools to improve a range of growth, carcass, and meat quality traits in pork production. Especially the imprinted and X-linked QTL are very promising in cross breeding schemes, even more because they may be less affected by traditional selection, compared to Mendelian loci. The experiment has not contributed many QTL for reproduction traits but this could be attributed to the low number with phenotypic observations for litter size (Chapter 5).

The simulation study in Chapter 6, showed some of the pitfalls that might be encountered when testing for imprinting. This will allow better design of mapping experiments and help in the interpretation of imprinting results of ongoing experiments. The multiple QTL strategies that were proposed in Chapters 7 and 8 resulted in additional power for a QTL experiment.

Because pigs are genetically close to humans, QTL that are identified in pigs might provide clues to comparable multi-factorial disorders in human genetics. Fatness QTL that have been

described in this thesis might for instance be relevant for obesity research in humans.

This demonstrates that mapping studies in livestock not only elucidate the genetic background of the traits that are studied, but can combine the opportunities that are available to researchers that study model species or human genetics. Like model species, livestock populations offer the possibility of controlled breeding and selection, as well as the possibility to make experimental crosses between genetically divergent lines.

On top of that, livestock populations with well-documented pedigrees also offer the opportunity to study the segregation of important genes in outbreeding family structures like many human genetic studies. These attractive properties of livestock and the expertise in complex quantitative genetic models that has been established in animal breeding research provides ample opportunity for close collaboration between animal breeding and other fields of genetical research.

Samenvatting

Dit hoofdstuk bevat een samenvatting van het proefschrift, geschreven voor een breder publiek met minder oog voor detail dan de rest van het proefschrift. Een wetenschappelijke samenvatting van het proefschrift wordt gegeven in Hoofdstuk 10.

Achtergrond

In het begin van de jaren negentig is door de toenmalige Landbouwuniversiteit in samenwerking met vijf Nederlandse varkensfokkerij organisaties een grootschalig kruisingsexperiment opgezet. Hierbij zijn beren van het Chinese Meishan ras gekruist met zeugen van de commerciële lijnen van de vijf fokkerij organisaties. Het Chinese Meishan ras wordt gekarakteriseerd door een hogere vruchtbaarheid, een lagere groei en een hogere vetheid in vergelijking met de Nederlandse rassen. Uit de eerste generatie dieren van deze kruising (de F_1) zijn 39 beren geselecteerd die elk via kunstmatige inseminatie zijn gepaard met 6-8 zeugen uit de F_1. Aan de ~1200 dieren die hieruit zijn voortgekomen (de F_2) zijn een aantal geboorte- en groei kenmerken gemeten. Ruim 850 van deze dieren zijn geslacht en gekarakteriseerd voor karkas- en vleeskwaliteit. Uit de F_2 populatie zijn ongeveer 250 zeugen aangehouden om gegevens te verzamelen over worpgrootte in de eerste en de tweede pariteit. Van alle dieren uit de drie generaties is materiaal (bloed of weefsel) verzameld om het erfelijk materiaal (DNA) te isoleren.

Dr. Luc Janss heeft, in het kader van zijn promotieonderzoek, het experiment aangestuurd en de kenmerken geanalyseerd. Middels het combineren van afstammings- en kenmerkgegevens, vond hij aanwijzingen dat kenmerken als rugspekdikte, groei en het intramusculaire vetgehalte (vet in de spier) gedeeltelijk aangestuurd worden door individuele genen met grote effecten. Dit proefschrift beschrijft de volgende stap in het onderzoek van deze experimentele populatie: de zoektocht naar de genen die de verschillen in kenmerken (fenotypische variatie) tussen Meishan en commerciële rassen verklaren.

Chromosomen, genen, DNA, merkers en QTLs

Net zoals bij andere dieren en planten, ligt de erfelijke informatie van het varken vast op het genoom wat in elke lichaamscel aanwezig is. Het genoom van het varken bestaat uit 18 paar standaard chromosomen (de zogenaamde autosomen) en één paar geslachts-chromosomen. Elk chromosoom komt in tweevoud voor, één kopie afkomstig van de beer, en één kopie afkomstig van de zeug. Van de geslachtschromosomen is altijd één X

chromosoom afkomstig van de zeug. Een big kan van de beer een X of een Y chromosoom ontvangen wat respectievelijk in een zeugje of een beertje resulteert. Op de chromosomen bevinden zich de circa 30.000 genen van het varken, gecodeerd door het DNA, waaruit de chromosomen zijn opgebouwd. Elk gen heeft net als de chromosomen een kopie van de vader en één van de moeder, aangeduid als de twee allelen van het gen. Het DNA heeft vier verschillende "basen" die fungeren als bouwstenen en aangeduid worden met de letters A, C, T en G. Een combinatie van drie basenparen codeert voor een specifiek aminozuur, waarbij een bepaalde aminozuurvolgorde codeert voor een specifiek eiwit. De totale streng DNA die codeert voor een bepaald eiwit is het gen voor dat eiwit, en de complete basenvolgorde binnen die streng DNA is de sequentie van het gen.

Van al het DNA, codeert naar schatting minder dan 5% voor eiwitten. De rol van de overige 95% is tot op heden nog niet duidelijk. Een gedeelte van dit niet-coderende DNA bestaat uit motieven van 2-6 basenparen die in veelvoud achter elkaar voorkomen. Deze repeterende motieven worden aangeduid als microsatellieten. Het aantal kopieën van elk motief vertoont variatie (polymorfie) tussen individuen die veelal groter is dan de variatie in coderende DNA sequenties. Microsatellieten zijn daarom bij uitstek geschikt als genetische merker om variatie tussen of binnen rassen mee op te sporen. Door de overerving van een groep genetische merkers te volgen binnen een aantal families kun je een zgn. koppelingskaart van deze merkers maken. De koppelingkaart is een schematische weergave van de chromosomen, waarbij de genetische merkers de kilometerpaaltjes langs de chromosomen zijn. Met de ontwikkeling van vele genetische merkers, zijn nu genetische koppelingkaarten beschikbaar voor bijna alle landbouw-huisdieren. De koppelingskaart berust op het principe dat merkers op verschillende chromosomen onafhankelijk van elkaar kunnen overerven. Merkers op hetzelfde chromosoom zullen vaker samen overerven dan op basis van toeval verwacht wordt. De mate van koppeling wordt bepaald door de afstand tussen merkers op hetzelfde chromosoom. Deze afstand wordt uitgedrukt in Morgan (M) of centiMorgan (cM) waarbij een afstand van 1 cM tussen twee merkers (genen) aangeeft dat naar verwachting van de 100 geslachtscellen, er één is waarbij een zogenaamde recombinatie is opgetreden: een overkruising tussen de kopie van de moeder en de kopie van de vader. Het genoom van het varken bestrijkt ongeveer 22 Morgan (of 2200 cM) waarbij de chromosomen verschillen in lengte van 50 cM tot bijna twee Morgan.

Een beperkt aantal kenmerken worden door slechts één of enkele genen aangestuurd. Voorbeelden bij het varken zijn halothaan gevoeligheid dat door één gen wordt veroorzaakt, en de kleur van huid en haar, wat door een klein aantal genen wordt aangestuurd. De meeste kenmerken die voor

de varkensvleesproductie van belang zijn, vertonen een continue verdeling zoals groei, spekdikte, worpgrootte etc. Variatie binnen deze "kwantitatieve" kenmerken wordt veroorzaakt door een complex samenspel van meerdere genen en een aantal omgevingsfactoren. In het laatste decennium is echter aangetoond dat, door het analyseren van de overerving van genetische merkers en (productie-) kenmerken, chromosoom-gebieden kunnen worden aangewezen met een meetbaar effect op een kwantitatief kenmerk. Deze gebieden worden QTL (Quantitative Trait Locus) genoemd. Een QTL is een door genetische merkers gemarkeerd deel van een chromosoom waarin zich één of meerdere genen bevinden met een meetbaar effect op een kwantitatief kenmerk. De schatting van de positie van een QTL is onnauwkeurig: in een QTL regio kunnen wel 3000 genen liggen. Dit onderzoek was gericht op het vinden van QTLs en de wijze van overerving van de gevonden QTLs. Een ander project bij de Leerstoelgroep Fokkerij en Genetica is gericht op de identificatie van de onderliggende genen.

Het zoeken naar QTLs

De zoektocht naar QTLs behelst het combineren van merker data, kenmerkgegevens en familieverbanden. Voor dit onderzoek zijn alle dieren getypeerd voor meer dan 130 genetische merkers, die samen het varkensgenoom voor ongeveer 95% bedekken. Voor het zoeken naar QTLs gebruiken we twee uitgangsmodellen: het lijnkruisingsmodel en het familie-model.

Lijnkruisingsmodel. Binnen het lijnkruisingsmodel gaan we er van uit dat, voor genen met effecten op de kenmerken waar wij naar kijken, de Meishan varkens allemaal het ene type allel (bijv. Q) hebben en de commerciële lijnen allemaal het andere type (q). Uit deze aanname volgt dat elk gen maar twee allelen kan hebben en dat alle F_1 dieren heterozygoot (Qq) zijn voor deze genen. Voor de F_2 is voor elk gen de verwachting dat 25% van de dieren twee Meishan allelen heeft (QQ), 25% twee allelen van de commerciële lijnen (qq) en 50% heterzoygoot met één Meishan allel en één van de commerciële lijnen. We kunnen deze genen niet direct observeren maar wel de genetische merkers. De allelen van de F_2 dieren worden via de F_1 ouders getraceerd naar de grootouders. Hierdoor kunnen we op elke willekeurige locatie op het genoom schatten of een F_2 big twee Meishan allelen heeft, twee allelen van een commerciële lijn, of van elk één. Dit wordt geschat voor alle F_2 dieren, voor alle centiMorgans van het varkensgenoom. Vervolgens wordt op elke genoompositie getoetst, of daar een QTL (één of meerdere genen) voor een bepaald kenmerk zou kunnen liggen. Het additieve effect van een QTL op die positie wordt geschat door de kenmerkgegevens van de dieren met twee Meishan allelen (op die positie) te contrasteren met de kenmerkgegevens van dieren die daar twee allelen van de

commerciële lijnen hebben. Ook wordt geschat of de heterozygote dieren (Qq) qua kenmerkgegevens afwijken van het gemiddelde van de QQ en qq dieren. Deze afwijking van het gemiddelde is de geschatte dominantie van het potentiële QTL. De hoeveelheid variatie in het kenmerk dat wordt verklaard door QTL op die positie, is een maat (toetsingsgrootheid) voor de kans van het daadwerkelijk aanwezig zijn van één of meerdere genen (een QTL) op die positie.. Door deze kans voor elke positie van het chromosoom te berekenen wordt een grafische weergave verkregen van de kans op een QTL op dat chromosoom. De analyse van QTL experimenten bij varkens is tot op heden hoofdzakelijk op gebaseerd op toepassing van dit lijnkruisingsmodel.

Familie-model. Het familie-model maakt geen aannames over het aantal allelen van een gen of de frequenties van deze allelen in de Meishan of de commerciële lijnen. Dit model vindt vooral veel toepassing bij melkvee. Voor dit experiment, worden de F_2 dieren opgedeeld in 38 beer families. Analoog aan het lijnkruisingsmodel, wordt nu voor elke cM binnen elk F_2 dier geschat welke van de twee beer-allelen het dier op die positie heeft geërfd. Ook hier wordt vervolgens op elke positie de effecten van een potentieel QTL geschat. In dit model wordt voor elke F_1 beer een apart effect geschat en de toetsingsgrootheid wordt samengesteld uit de effecten van de afzonderlijke beer families.

Het lijnkruisingsmodel is het meest geschikt om QTLs te vinden die verschillen tussen Meishan en commerciële lijnen verklaren, terwijl het familie-model QTLs op kan pikken die variatie binnen de rassen verklaren

De resultaten van de QTL analyse

In Hoofdstuk 2 is gezocht naar QTLs voor rugspekdikte en intramusculair vetgehalte bij ongeveer de helft van dieren uit het slachtexperiment (420). Het toepassen van het lijnkruisingsmodel alsmede het familie-model resulteerde in overtuigend bewijs voor twee QTLs voor rugspek en suggestief bewijs voor QTLs voor intramusculair vetgehalte. Een groot QTL voor rugspek werd gevonden onder het zowel het lijnkruisingsmodel als het familie-model. De resultaten van de familie analyses lieten zien dat, voor dit QTL, het Meishan allel altijd minder (!) rugspek gaf, maar dat niet alle F_1 beren heterozygoot waren voor dit QTL. De voordelen van het toepassen van meerdere modellen was daarmee al snel duidelijk.

In Hoofdstuk 3 is opnieuw gekeken naar rugspek en intramusculair vetgehalte, alsmede naar spierdikte. Het is onderzocht of het fenomeen van genetische inprenting een effect heeft op deze kenmerken. Bij genetische inprenting is van een bepaald gen slechts het allel van één ouder actief, terwijl het allel van de andere ouder niet in het dier tot expressie komt (ingeprent). Het optreden van inprenting is vooral bestudeerd bij erfelijke

afwijkingen bij muis en mens maar nog nauwelijks onderzocht bij kwantitatieve kenmerken of bij andere diersoorten. Binnen het lijnkruisingsmodel kunnen we onderscheid maken bij de heterozygote dieren (Qq) onderscheid maken tussen dieren die het Meishan allel via de beer of via de zeug hebben geërfd. Door het model opnieuw te formuleren, kunnen we een paternaal en een maternaal QTL effect schatten. Wanneer voor een bepaald QTL het allel, afkomstig van één ouder een duidelijk effect heeft en het effect van de andere ouder en het dominantie effect verwaarloosbaar zijn, is dit een sterke aanwijzing voor inprenting. Voor de drie onderzochte kenmerken werd overtuigend bewijs gevonden voor het bestaan van vijf QTLs, waarvan er maar liefst vier ingeprent bleken. Het QTL voor rugspek op chromosoom 2, dat in Hoofdstuk 2 was opgepikt met een standaard (Mendeliaans) model, bleek uitsluitend paternale expressie te vertonen. Een QTL voor intramusculair vetgehalte op chromosoom 6, waarvoor onder een Mendeliaans model alleen maar suggestief bewijs werd gevonden, bleek overtuigend significant onder een inprenting model met paternale expressie. Op chromosoom 6 werd ook een QTL met maternale expressie voor IMF gevonden. Verder viel een QTL voor spierdikte op chromosoom 7 nagenoeg samen met een QTL voor rugspek. Het QTL voor spierdikte vertoonde echter maternale expressie, terwijl het QTL voor rugspek standaard Mendeliaanse expressie liet zien. Deze resultaten waren een indicatie dat genetische inprenting wellicht een algemener fenomeen is dan tot op dat moment algemeen werd aangenomen. Het werd dan ook geadviseerd om het testen voor inprenting een integraal onderdeel te maken van de genetische analyse van (kwantitatieve) kenmerken.

Voor rugspek en intramusculair vetgehalte zijn verder grote effecten gevonden op het X chromosoom. Dit is niet beschreven in dit proefschrift maar onderzocht als onderdeel van het onderzoek van Dr. Barbara Harlizius bij de leerstoelgroep Fokkerij en Genetica. De rekenregels voor de analyse van het X chromosoom wijken af omdat vanwege het design en het feit dat het X chromosoom niet kan recombineren binnen de beren, alle F_2 zeugjes één kopie hebben van het X chromosoom dat integraal afkomstig is van de commerciële lijnen.

Hoofdstuk 4 beschrijft de resultaten voor de overige kenmerken uit het slachtexperiment. Het betrof vleeskwaliteit kenmerken zoals kleur en zuurgraad (pH) van het vlees, druip- en kookverlies, en de malsheid van het vlees. Het was verassend dat voor deze kenmerken met het lijnkruisingsmodel geen overtuigend bewijs voor QTLs werd gevonden, alleen suggestieve aanwijzingen. Het familie-model bracht overtuigend bewijs voor drie QTLs aan het licht, elk met een effect op vleeskleur. De gevonden QTLs voor vleeskleur verklaren met name variatie binnen de uitgangslijnen.

Hoofdstuk 5 beschrijft de resultaten voor vroege groei (spenen-25 kg), test groei (25-90 kg), levensgroei, ultrasone rugspek dikte (gemiddelde 4-8 metingen) en worpgrootte voor twee pariteiten. Voor de groeikenmerken waren gegevens beschikbaar van 940-1150 dieren, en voor worpgrootte waren gegevens beschikbaar van 200-250 zeugen.

Voort de diverse groeikenmerken werd overtuigend bewijs gevonden voor QTLs op chromosoom 1, 4, 7 en 8. Het QTL op chromosoom 8 was ingeprent voor alle groeitrajecten, terwijl het QTL op chromosoom 4 alleen inprenting liet zien voor testgroei. Voor ultrasoon gemeten rugspek werden de effecten die in hoofdstuk 2 en 3 zijn beschreven voor slacht rugspek bevestigd. Bovendien werd ook overtuigend bewijs gevonden voor een QTL met maternale expressie op chromosoom 14. Voor het QTL met paternale expressie op chromosoom 2 werd een andere positie gevonden dan bij slacht-rugspek. De meest waarschijnlijke verklaring voor dit verschil is het verschil in de manier waarop de twee rugspek metingen worden gedaan. Voor worpgrootte werd geen overtuigend bewijs voor QTLs gevonden. Door het lage aantal zeugen met gegevens voor worpgrootte was de kans op het vinden van een QTL klein.

Andere aspecten van het onderzoek

Omdat voor veel QTLs grote effecten van inprenting werden gevonden, hebben we in Hoofdstuk 6 de methode waarmee inprenting kan worden opgespoord nader bestudeerd. Dit hoofstuk begint met een kwantificering van de effecten van een ingeprent gen in vergelijking met een Mendeliaans gen, omdat daar nog geen aandacht aan is geschonken in de literatuur. Een punt van aandacht is niet alleen het detecteren van ingeprente QTLs, maar ook het correct onderscheiden van ingeprente en Mendeliaanse QTLs. Met behulp van een grote simulatiestudie hebben we gekeken naar het effect van QTL grootte, populatiestructuur en allel-frequenties op de detectie en correcte classificatie van ingeprente en Mendeliaanse QTLs. Uit deze studie kwam naar voren dat: 1) Ingeprente QTLs kunnen verborgen blijven wanneer de analyse alleen wordt gedaan onder Mendeliaanse modellen. 2) Beide toetsen voor het identificeren van inprenting, die in deze studie zijn vergeleken, hebben hun tekortkomingen. 3) Wanneer de QTL allelen in uitgangslijnen niet gefixeerd zijn kunnen Mendeliaanse QTLs zich gemakkelijk voordoen als ingeprente QTLs, met name wanneer het aantal F_1 vaders klein is. In het kader van deze bevindingen zijn de resultaten uit Hoofdstuk 3 en die van andere studies naar inprenting nog eens nader bekeken. Het werd geconcludeerd dat het correct detecteren en identificeren van inprenting meer eisen stelt aan het ontwerp en de analyse van de proef dan het detecteren van Mendeliaanse QTLs. Echter, gezien het grote aantal F_1 beren in het Wageningse Meishan experiment, bleven de

resultaten beschreven in Hoofdstukken 3-5 overeind.

Voor de analyses in de hoofdstukken 2-6, zijn de chromosomen altijd één voor één geanalyseerd, zonder rekening te houden met effecten op andere chromosomen. In Hoofdstuk 7 wordt een strategie voorgesteld om meerdere QTLs tegelijkertijd te modelleren, en zo een hoger onderscheidingsvermogen te verkrijgen. Deze strategie is ontwikkeld in samenwerking met een onderzoeksgroep uit Finland en daarom in eerste instantie uitgewerkt voor een familie-model bij rundvee. Volgens deze strategie wordt de analyse van de individuele chromosomen gevolgd door een meervoudig regressie model waarin alle individuele effecten gelijktijdig worden geschat. Vervolgens worden de chromosomen opnieuw geanalyseerd, waarbij rekening gehouden wordt met de QTLs op de andere chromosomen. Voor melkgift bij rundvee werden bij de individuele analyses vijf aanwijzingen gevonden voor QTLs. Door gebruik te maken van de voorgestelde strategie werd uiteindelijk overtuigend bewijs gevonden voor acht QTLs voor melkgift.

In Hoofdstuk 8 is de meervoudige QTL analyse geïmplementeerd voor het lijnkruisingsmodel en toegepast op intramusculair vetgehalte en rugspekdikte. Het principe is hetzelfde als bij het familie-model, met de uitbreiding dat onder het lijnkruisingsmodel combinaties gemaakt kunnen worden van Mendeliaanse en ingeprente QTLs, alsmede QTLs op het X chromosoom. Voor rugspek bleek een QTL op chromosoom 1, waarvoor eerder suggestief bewijs was gevonden, te verdwijnen onder het gecombineerde model. Het bewijs voor de overige QTLs werd juist sterker door toepassing van de meervoudige analyse. Voor intramusculair vetgehalte leverde de meervoudige analyse overtuigend bewijs op voor een QTL op chromosoom 4 terwijl hiervoor slechts suggestief bewijs gevonden was onder de standaardanalyse.

Een andere verfijning, die in hoofdstuk 8 wordt beschreven, is het modelleren van twee QTLs op hetzelfde chromosoom. Een procedure die in literatuur beschreven is, is uitgebreid door het meenemen van Mendeliaanse en ingeprente QTLs. Ook is een nieuwe benadering voorgesteld om te toetsen of er één of twee QTLs op een chromosoom liggen voor een bepaald kenmerk. Voor rugspek leverde dit suggestief bewijs op voor een tweede QTL op chromosoom 7 en voor intramuscular vetgehalte werd bevestigd dat er twee ingeprente QTLs liggen op chromosoom 6.

Hoewel er veel QTLs gevonden zijn voor rugspek en intramusculair vet gehalte verklaren deze effecten niet de "major genes" die eerder voor deze kenmerken zijn gevonden op basis van de verdeling van kenmerken in bepaalde families (segregatie analyse). Dit kan berusten op verschillen in

genetisch en statistisch model tussen de QTL analyse en de segregatie analyse.

Hoofdstuk 9 begint met een opsomming van de ingeprente QTLs die in de Meishan kruising zijn gevonden. Vervolgens wordt gespeculeerd over een mogelijke toepassing van de gevonden QTLs in een commercieel fokprogramma. Hierbij wordt voorbijgegaan aan de hindernissen die nog moeten worden genomen voordat deze QTLs in de praktijk kunnen worden gebruikt. De nadruk ligt vooral op de mogelijkheden die worden geboden door ingeprente QTLs en QTLs op het X-chromosoom in een fokkerijsysteem waar gebruikt wordt gemaakt van diverse kruisingsschema's. Door het strategisch gebruik van de gevonden QTLs wordt voorgesteld om diverse markten te bedienen met dezelfde uitgangslijnen. De diversificatie tussen varkens voor bijvoorbeeld de Britse bacon en de Italiaanse Parma ham wordt bereikt door het alternatief gebruik van zeugen dan wel beren, waarbij bepaald wordt of ingeprente genen al dan niet tot expressie komen in de mestvarkens.

Het onderzoek heeft niet alleen een bijdrage geleverd aan het ontrafelen van de genetische achtergronden van kenmerken bij varkens, maar verschaft ook inzichten die belangrijk zijn voor het hele onderzoeksveld van de genetica.

Curriculum Vitae

Dirk-Jan de Koning is geboren op 4 september 1970 te Waarder, in het groene hart van Holland. Na het voltooien van het VWO op de Kalsbeek Scholengemeenschap te Woerden, begon hij in 1988 zijn studie zootechniek aan de toenmalige Landbouwuniversiteit in Wageningen. De dienstplicht werd volbracht als Wachtmeester bij de 129e Afdeling Veldartillerie in Havelte van 1991-1992. Na een kortstondige carrière in het distributiecentrum van de Bijenkorf, pakte hij in september 1992 de studie weer op met vernieuwde motivatie. In het kader van zijn stage, werd de winter van 1994-1995 doorgebracht in Finland. Daarna deed hij zijn eerste afstudeervak in het laboratorium van veefokkerij. Het tweede afstudeervak is uitgevoerd bij het Roslin Instituut in Schotland. Na het behalen van de ingenieurs bul in 1996, ging hij als tijdelijk onderzoeker aan de slag in Finland, bij het "Maatalouden Tutkimukeskus". In 1997 keerde hij terug naar Wageningen om als Oio aan de slag te gaan bij de leerstoelgroep Fokkerij en Genetica, resulterend in het proefschrift dat nu voor U ligt. Om de rondgang door Europa weer compleet te maken, gaat hij per 1 oktober als Postdoc onderzoeker aan de slag bij het Roslin Instituut in Schotland, waar hij het genoom van de kip gaat ontrafelen.