Individual and epistatic genetic effects of quantitative trait loci affecting growth, feed intake, body composition and meat quality in pigs

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Declaration

I declare that this thesis is my own composition. I wrote the manuscript and all analyses were conducted by myself. The work has not been submitted for any other degree or professional qualification except as specified.

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Abstract

Selection of pigs has focussed on the improvement of lean growth with simultaneous reduction in fat tissue, due to the high economic importance of these traits. As a consequence, a large number of quantitative trait loci (QTL) have been reported for these traits. In contrast, very few QTL have been reported for chemical body composition (protein and lipid). Knowledge about the deposition rates of these components is important to accurately predict the nutritional requirements of pigs and to determine selection objectives for optimal development of body tissues and feed intake capacity. Therefore, the principle aims of this thesis were to investigate the genomic regulation of physical and chemical body composition as well as feed intake, feed efficiency and meat quality in a commercial pig population.

Data for all analyses were derived from a three generation full-sib design created by crossing Pietrain sires with a crossbred dam line. In total, 386 animals were genotyped for 96 molecular markers covering 11 chromosomes. Phenotypic data were available for 315 F_2 animals for carcass characteristics measured at slaughter weight, chemical body composition measured at different target weights throughout growth, feed intake measured throughout growth, and meat quality traits collected post-slaughter.

Individual QTL analyses of several autosomes and chromosome X uncovered a large number of QTL in different regions of the genome for physical body composition traits as well as novel QTL for chemical body composition and deposition. Associations between QTL for chemical and physical body composition were also detected. The results highlighted that different stages of growth are under different genomic regulation. Further QTL were detected for feed intake and feed efficiency and interesting causative biological reasons for QTL of feed efficiency were derived in associations with QTL for body composition and growth. Epistatic QTL analyses were performed to investigate the contribution of interactions (epistasis) to the genomic regulation of physical and chemical body composition as well as growth and feed intake. Epistasis

was found to contribute to the entire growth period, however, different epistatic QTL pairs contributed to different stages of growth. Epistatic QTL pairs mostly accounted for higher proportions of the phenotypic variance than QTL detected from individual QTL analyses. A large number of QTL were identified, which could not be detected from individual QTL analyses, mainly because these QTL did not express individually significant additive or dominance effects and only expressed their effects through interactions with other QTL. Individual and epistatic QTL analyses uncovered numerous QTL as well as epistatic interactions influencing meat quality traits, including pH, meat colour and conductivity, traits which influence the quality of pork.

The work of this thesis gives substantial insight into the genomic regulation of economically important traits of pigs. The research highlights that the genomic regulation of growth and body composition, feed intake and meat quality is complex, involving numerous QTL located in different regions of the genome, controlled partly by imprinting effects, as well as a complex network of interactions between QTL. The results obtained in this study can be used in pig breeding to optimise breeding programmes and for marker assisted selection.

Acknowledgements

Publications

Refereed Papers

Duthie, C., Simm, G., Doeschl-Wilson, A., Kalm, E., Knap, P. W. and Roehe, R. (2008). Quantitative trait loci for chemical body composition traits in pigs and their positional associations with body tissues, growth and feed intake. *Animal Genetics* **39** (2), 130-140 (Chapter 2).

Conference Papers

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Duthie, C., Simm, G., Knap, P., Wilson, A., Kalm, E. and Roehe, R. (2008). Genomic scan for quantitative trait loci of pig chemical and physical body composition and growth traits on chromosome X. *Proceedings of the British Society of Animal Science*, 67. Southport, UK. April 08 (based on Chapter 3).

Duthie, C., Simm, G., Perez-Enciso, M., Doeschl-Wilson, A., Kalm, E., Knap, P. and Roehe, R. (2008). Novel QTL on the sex chromosome of pigs including the pseudoautosomal region. *3rd International Symposium on Animal functional Genomics. Hosted by ARK-Genomics,* ISAFG-P92. Edinburgh International Conference Centre, Edinburgh, UK. April 08 (based on Chapter 3).

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List of abbreviations

a	Additive
AA and aa	Additive-by-Additive
ACSL4	*Acyl-CoA synthetase long-chain 4*
AD and ad	Additive-by-Dominance
AF	AutoFom
BLUP	Best Linear Unbiased Predictor
cM	Centimorgan
CT	Computerised Tomography
d	Dominance
DA and da	Dominance-by-Additive
DD and dd	Dominance-by-Dominance
DFI	Daily Feed Intake
DG	Daily Gain
DNA	Deoxyribonucleic acid
FABP3	*Heart fatty acid binding protein 3*
FBXO32	*F-BOX protein 32*
FCR	Food Conversion Ratio
FFS	Fat-Free Substance
FFS_{EB}	Fat-Free Substance of the Empty Body
FOM	Fat-O-Meter
H	Heterozygosity
i	Imprinting
IGF2	*Insulin-like growth factor*
IGF1R	*Insulin-like growth factor 1 receptor*
IMF	Intramuscular Fat
LAR	Lipid Accretion Rate
LCEB	Lipid Content of the Empty Body
LR	Likelihood Ratio

MC4R	*Melanocortin-4 receptor*
ME	Metabolisable Energy
MJ	Mega Joule
M.l.t.l.	*Musculus longissimus thoracis et lumborum*
MTP	*Microsomal triglyceride transfer protein large subunit*
PAR	Protein Accretion Rate
PCEB	Protein Content of the Empty Body
PCFFS	Protein Content of the Fat-Free Substance
PIC	Polymorphic Information Content
PSE	Pale, Soft, Exudative
PSME1	P*roteasome (prosome, macropain) activator subunit 1 (PA28 alpha)*
PSME2	*Proteasome (prosome, macropain) activator subunit 2 (PA28 beta)*
QTL	Quantitative Trait Loci
RN	*Rendement napole*
RYR1	*Ryanodine receptor 1*
SD	Standard Deviation
SDHD	*Succinate dehydrogenase complex, subunit D*
SE	Standard Error
SHP	*Small heterodimer partner*
SNPs	Single Nucleotide Polymorphisms
SSC	*Sus scrofa* Chromosome
TGFBR3	*Transforming growth factor, beta receptor III*
USDA	Unites States Department of Agriculture

Chapter 1

General introduction

1.1 Genetic improvement of livestock

Animal breeding has effectively changed the genomic composition of livestock species for centuries. Artificial selection of superior animals, based on their phenotypes, has brought about considerable progress in the genetic improvement of animals for economically important traits, such as growth, body composition and carcass characteristics. This genetic improvement has occurred without knowledge of the underlying genetic regulation of these traits (Andersson 2001; Georges 2001; Weller 2001; Dekkers and Hospital 2002). Selection of superior animals as parents for subsequent generations has brought about an increase in the frequency of favourable alleles as well as allelic combinations (Georges 2001).

One main aim in pig breeding programmes has been to increase lean content and reduce fatness, as fat is a tissue associated with high feed energy cost and a low commercial return (Quiniou *et al.* 1999; Nagamine *et al.* 2003). The latter is largely due to consumer demand for lean pork meat (de Koning *et al.* 1999). Intensive artificial selection has seen an increase in loin muscle area and a reduction in backfat thickness, particularly in the last 50 years, an indication that body composition traits have high genetic determination (Andersson 2001; Roehe *et al.* 2003).

However, this genetic improvement of pigs for high lean tissue has had some undesirable side effects. One of the most important side effects is the undesirable reduction in feed intake capacity of pigs, which may limit further genetic improvement of growth. Feed intake capacity is an important trait, particularly in the early stages of growth, as reduced feed intake capacity may prevent pigs from reaching their true genetic potential for growth (Schulze *et al.* 2002). Furthermore, the change in deposition of protein and lipid during growth may be the underlying cause of change in feed intake capacity (Schulze *et al.* 2002). Another side effect is that selection for increased lean tissue has been unfavourably associated with meat eating quality characteristics and subsequent consumer acceptability (Schwab *et al.* 2006). Therefore, in many pig

breeding lines, it has been recognised that the optimum fat content may have been reached and further reduction in backfat should be avoided, as it is likely to decrease feed intake capacity further and produce undesirable effects on meat eating quality and subsequently lower consumer acceptability (Kanis and De Vries 1992; Roehe *et al.* 2003).

1.2 Genome research

Qualitative traits, such as coat colour and eye colour, are controlled by a small number of genes, each with large effect. However, quantitative traits which are of economic interest to livestock breeders, such as growth and body composition, are more complex and controlled by only few genes of large effect but mostly by a large number of genes with small effect (Hayes and Goddard 2001). Such quantitative traits are complex in both their biochemical and physiological properties (Geldermann 1975; Andersson 2001).

Genome research in farm animals is of great interest for our understanding of the genetic control of economically important traits (Andersson 2001). At present, our knowledge of the genetic regulation of quantitative traits is very limited (Nagamine *et al.* 2003). This means there is little knowledge of the number of genes involved, the effect of alleles at the genes, or the mode of inheritance of the genes (Casas-Carrillo *et al.* 1997; Nagamine *et al.* 2003). As genome research proceeds in livestock, it will lead to a better understanding of genetic regulation and physiological functions of quantitative traits (Clamp *et al.* 1992; Andersson 2001; Dekkers and Hospital 2002).

One of the main aims within genome research of livestock is to identify regions of the genome which are associated with traits of economic interest (Knott *et al.* 1998; Wang *et al.* 1998; Andersson 2001; Roehe *et al.* 2003). This information can be incorporated into breeding programmes through marker assisted selection in order to improve product

quality and production efficiency (Walling *et al.* 1998; Andersson 2001; Ovilo *et al.* 2002). The application of genomic research results may change the genetic improvement programmes of livestock in the future (Kappes 1999; Georges 2001; Malek *et al.* 2001a). This is expected to increase the efficiency of production and the quality of the market product (Andersson 2001).

1.3 Quantitative trait loci

Considerable progress has been made within molecular genetics in the past decade which has allowed for the detection of genes or segments of DNA, known as quantitative trait loci (QTL), which contribute to variation in quantitative traits (Andersson 2001; Hayes and Goddard 2001). In order to detect these QTL, mapping methods have been developed (e.g. Lander and Botstein 1989; Haley *et al.* 1994; Knott and Haley 1998). Such methods of scanning the genome for QTL have been made possible through the development of genetic markers and linkage maps (Rohrer *et al.* 1994; Archibald *et al.* 1995; Rohrer *et al.* 1996). These maps provide a powerful tool to detect regions of the genome associated with variation in quantitative traits (Wada *et al.* 2000; Milan *et al.* 2002). These methods of QTL mapping have been implemented in different software packages such as QTL Express (Seaton *et al.* 2002), which adopts a least squares regression method of QTL mapping, and QxPak (Perez-Enciso and Misztal 2004), which adopts a maximum likelihood method of QTL mapping. Both software packages have been used in the studies reported in this thesis.

1.3.1 Interval mapping

In this thesis, QTL analysis was carried out using the interval mapping approach of QTL mapping, also known as flanking-marker analysis (Lynch and Walsh, 1998). In this type of analysis the intervals between each pair of flanking markers are investigated

for evidence of QTL at each putative position within the marker-bracket. Interval mapping offers an increase in power and more precise estimates of QTL effects and position compared to single marker analysis (Lynch and Walsh, 1998). Interval mapping was originally implemented using a maximum likelihood approach (Lander and Botstein, 1989). Regression based approaches to interval mapping have also been developed and have been shown to provide very similar estimates of QTL location and effect to those obtained using a maximum likelihood approach (Haley and Knott, 1992).

1.3.2 QTL Express

The least squares regression method of QTL mapping implemented in QTL Express was developed for inbred lines by Haley and Knott (1992) and extended for outbred lines by Haley *et al.* (1994). The analysis of QTL Express proceeds in two stages. In the first stage the data on marker positions as well as marker genotypes are used to calculate the probabilities of individuals inheriting one or two grandpaternal or grandmaternal alleles at positions throughout the genome and the parent-of-origin probability of the alleles. These probabilities are combined into additive and dominance coefficients in order to observe the information contents of the markers along the chromosome as well as segregation distortion. In the second stage, at every putative QTL position, least squares is used to regress the phenotypic value for each individual onto their individually calculated additive and dominance coefficients which then provides estimates of additive and dominance for that position. This is then repeated at each defined position on the chromosome and the best estimate of the QTL effects and position are obtained (at the position in which the residual sum of squares is minimised) where the F statistic is highest and estimates for additive and dominance effects are calculated at this position (Seaton *et al.* 2002).

1.3.3 QxPak

In the software QxPak, QTL analysis is implemented using mixed model equations and a maximum likelihood approach in order to estimate the location and effects of QTL. The analysis of QxPak proceeds in two main stages. In the first stage the probabilities of alleles being identical-by-descent are calculated using a Monte Carlo Markov Chain algorithm. In the second stage, the mixed model equations are built and the QTL estimates are obtained using a maximum likelihood approach (Perez-Enciso and Misztal 2004). Using maximum likelihood trial values are assigned to the unknown parameters and an iterative procedure is used to find the likelihood for each value. The trial values which maximise the likelihood are therefore the maximum likelihood estimates of the unknown parameters (Falconer and Mackay 1996). In the QxPak software the maximum likelihood estimates are obtained via the expectation-maximisation algorithm. At each putative position the likelihood ratio is computed and the estimates for the parameters are those where the likelihood is highest. In this analysis the significance is tested with a likelihood ratio test which consists of computing minus twice the difference in log-likelihoods between the alternative and the null models (Perez-Enciso and Misztal 2004).

1.3.4 QTL analysis in pigs

QTL mapping studies have successfully identified QTL across the pig genome for a wide variety of traits. Of all species of livestock, the pig is one of the species where large genomic information has been reported (Hayes and Goddard 2001). The pig has numerous advantages for QTL mapping in comparison to other species of livestock, as diverse breeds exist, three generation resource populations for studies can be produced in a relatively short time period, and the porcine linkage map is well developed (Archibald *et al.* 1995; Rohrer *et al.* 1996; Walling *et al.* 1998). The first genome-wide scan for QTL in pigs was carried out by Andersson *et al.* (1994) in order to detect QTL

for growth and fatness using an F_2 population derived from crosses of the European Wild Boar and the domesticated Large White. This study was the first to adopt the least squares regression method of interval mapping for outbred lines developed by Haley *et al.* (1994). Following on from the study by Andersson *et al.* (1994), interest in this area has increased substantially, and now results from QTL mapping experiments in the pig are widely available in the literature. To date, most QTL experiments have been performed using crosses of domestic breeds with either, the Meishan, Wild Boar or Iberian breed (Andersson-Eklund *et al.* 1998; Rohrer and Keele 1998ab; Rohrer 2000; Ovilo *et al.* 2002). This is because the power to detect QTL is much greater in crosses between largely divergent populations than crosses of commercial populations (Andersson 2001; Nagamine *et al.* 2003; Nagamine *et al.* 2004). Using divergent populations, there is a higher likelihood of segregation of alleles with large effects, the information content is higher due to the high marker heterozygosity, and higher heterozygosity at the QTL increases the statistical power (Andersson-Eklund *et al.* 1998; Andersson 2001). Commercial populations comprise of elite outbred populations which are produced through intense selection on performances defined in the breeding goal such as muscularity and lean content (e.g. Pietrain) and reproductive performance (e.g. Large White) (Nezer *et al.* 2002; Nagamine *et al.* 2003). Therefore, QTL detected from diverse 'non-commercial' populations may not be segregating within commercial breeds because of their selection history (Andersson-Eklund *et al.* 1998; Nagamine *et al.* 2004). Additionally, QTL identified in crosses of these divergent breeds may not be directly utilised in pig breeding due to the poor performance of the exotic breeds for traits of commercial interest (Malek *et al.* 2001a). It is therefore important to detect QTL using commercial populations in order to exploit the merit of these QTL within pig breeding programmes. Only more recently, QTL studies have started to incorporate the use of commercial breeds (e.g. Grindflek *et al.* 2001; Malek *et al.* 2001ab; Evans *et al.* 2003; Thomsen *et al.* 2004; Karlskov-Mortensen *et al.* 2006). QTL detected within commercial populations can be utilised within practical pig breeding through marker assisted selection (Walling *et al.* 1998; Karlskov-Mortensen *et al.* 2006).

1.4 QTL for physical body composition

The earliest studies in pigs have focused around the identification of QTL for growth and fatness characteristics (e.g. Andersson *et al.* 1994; Casas-Carrillo *et al.* 1997; Marklund *et al.* 1999; Bidanel *et al.* 2001; Malek *et al.* 2001a). This is because of the high economic importance of improving growth and reducing fatness but also because these traits have shown moderate to high heritablities and therefore it is more likely to detect QTL for these traits than for traits with low heritability. A large number of QTL have been identified across the genome for valuable carcass cuts such as weights of ham, loin, and shoulder cuts (e.g. Milan *et al.* 2002; Beeckmann *et al.* 2003a; Cepica *et al.* 2003a; Geldermann *et al.* 2003; Lee *et al.* 2003a; Yue *et al.* 2003a). In addition, QTL have been identified for dissected lean and fat tissue characteristics (e.g. Milan *et al.* 2002; Beeckmann *et al.* 2003ab; Cepica *et al.* 2003ab; Dragos-Wendrich *et al.* 2003a; Geldermann *et al.* 2003; Lee *et al.* 2003a; Yue *et al.* 2003a; Karlskov-Mortensen *et al.* 2006). These studies have already provided substantial information about the genomic control of carcass characteristics.

The most important factors that influence carcass value are the lean and fat tissue content. It is important to have detailed information regarding the lean composition of specific carcass cuts in order to determine primal cut value (Tholen *et al.* 2003). Different techniques have been adopted in the past for measurement of the lean content of carcasses. The most common method is to use an optical probe to measure fat and lean meat depths and use these measurements to calculate lean meat percentage. This method is invasive, likely to cause damage and may increase the risk of contamination. Examples of manual grading equipments are the Fat-O-Meter device, and the Hennessy Grading Probe. Due to the risks associated with these methods and advances in technology, there is interest in identifying and incorporating non-invasive methods for grading of carcasses, such as electrical conductivity and ultrasound. In addition, computerised tomography (CT) and nuclear magnetic resonance can be used in reference studies as a replacement for dissection (Busk *et al.* 1999; Meat and Livestock

Commission 2004). Norsvin International are currently utilising CT within pig breeding in order to maximise progress in carcass traits, growth and feed efficiency. At Norsvin, data provided using CT is being utilised in BLUP-based breeding values. Furthermore, the expectations are to widen the application of this technique to different traits, not just for the optimisation of lean content (Norsvin International 2008; www.norsvin.com).

One of the new and most promising methods of carcass grading is the AutoFom system, which is already well established in leading pig producing countries such as Denmark, Germany and the USA. The AutoFom carcass grading system is a non-invasive and fast method of grading carcasses and adopts a fully automatic ultrasound scanning technique to produce a three-dimensional image of the carcass. This device consists of 16 ultrasound transducers which are positioned in a U-shaped frame 25mm apart. Individual pigs are passed over this U-shaped cradle with optimal contact with the transducers. Carcasses do not need to be position in the centre of the U-shaped cradle as transducers can easily determine the midline and position of the carcass (see Figure 1.1). Transducers send out waves which facilitate the measurement of carcass traits such as percentage of lean meat and lean meat content of primal cuts ham, loin, neck and shoulder. This device takes measurements at every 5mm of the carcass and every transducer can provide up to 200 measurements each; therefore a maximum of 3200 measurements of the carcass can be provided. These measurements make it possible to form a three-dimensional image of the carcass. The data are processed for orientation of the carcass and noise reduction. After calibration, predicted measures of both fat and muscle depth are recorded. From the measurements, 127 variables are sufficient to describe the composition of the carcass and are then used in a regression model to predict carcass grading information such as percentage of lean, fat thickness and weights of primal cuts (Brondum *et al.* 1998; Busk *et al.* 1999; Tholen *et al.* 2003; Meat and Livestock Commission 2004). This is a fast method of carcass grading with the ability to measure 1250 carcasses per hour (Busk *et al.* 1999).
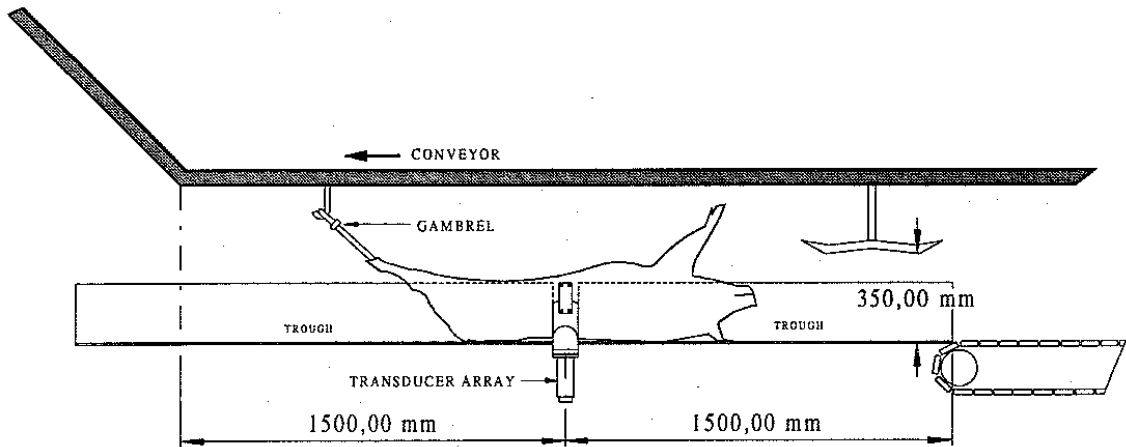
Figure 1.1
Representation of the AutoFom carcass grading system. Source: Busk *et al.* (1999).

It has been reported that the performance of this system is better than that of existing systems (Brondum *et al.* 1998). There are a number of benefits associated with the AutoFom method of carcass grading. Busk *et al.* (1999) has shown that AutoFom measurements can be used to predict the lean meat percentage of the carcass with accuracy. This is important and can assist producers to optimise the physical performance of their pigs allowing for optimal production and breeding goals. The detailed information regarding individual carcass composition provided by AutoFom allows for monitoring of desirable characteristics such as carcass quality. As a result, breeding companies can assess the performance of genotypes more accurately and better selection strategies can be developed in order to optimise the desired traits (Meat and Livestock Commission 2004). Furthermore, the AutoFom device measures depths of fat and meat at the start of the slaughter process, thereby eliminating errors that can arise from differences in the slaughter procedure. Operator error is also removed as the system is fully automatic and requires minimal human intervention (Busk *et al.* 1999).

It is important to incorporate phenotypic measurements taken from current carcass grading systems, such as the AutoFom device, into QTL mapping experiments as this information can be used directly to improve carcass quality. In the present thesis

phenotypic measurements of the carcass at slaughter weight were recorded by dissection as well as using the AutoFom device.

1.5 QTL for chemical body composition

In contrast to the numerous QTL that have been identified for physical body composition traits, there is much less information in the literature for QTL associated with chemical body composition traits, such as protein and lipid deposition, or for the change in deposition of these components during growth (Roehe *et al.* 2003). QTL studies in this area may provide insight into the physiological and biological regulation of chemical body composition, by determining QTL affecting protein and lipid accretion and also other traits affected by these identified QTL (Rohrer and Keele 1998a). It is likely that protein and lipid deposition are regulated by a large number of genes in different regions on the genome and regulated differently at different stages of growth (Roehe *et al.* 2003). Using the phenotypic data of this study, a previous analysis of four chromosomes has reported QTL for protein and lipid contents as well as accretion rates of these components (Mohrmann *et al.* 2006a). QTL in different regions of the genome were found to be associated with chemical body composition and deposition at different growth stages. This indicates that chemical body composition is regulated by different QTL throughout growth.

Reports of QTL for chemical body composition traits are limited because of the difficulty of collecting data on protein, lipid and ash contents in live animals. Most studies have used data collected from serial slaughter trials where pigs are slaughtered at specific weights or ages (Gu *et al.* 1992; Quiniou and Noblet 1995; Schinckel *et al.* 1996; Wagner *et al.* 1999). Data collected from serially slaughtered animals does not give an accurate representation of the change in deposition of protein and lipid throughout growth. Phenotypic data of chemical body composition in the present study were measured in live animals with the deuterium dilution technique, an *in vivo* method

of determining chemical body composition based on body water. This technique determined the empty body water content, from which the percentage of the fat-free substance of the empty body was estimated. Protein and ash contents of the empty body were estimated from the percentage of the fat-free substance. Lipid content was the difference of the fat-free content from 1.0. The accuracy of the deuterium dilution technique has been verified using chemical analysis of serially slaughtered animals (Landgraf *et al.* 2006a) and magnetic resonance imaging (Mohrmann *et al.* 2006b). For estimating these chemical body components, equations were developed by Langraf *et al.* (2006b).

The identification of QTL associated with both physical and chemical body composition of the pig is of substantial economic interest. It is important to know rates of deposition of these components to accurately estimate nutritional requirements of the pig and to optimise efficiency of pig production (Quiniou and Noblet 1995; Schinckel and de Lange 1996; Emmans and Kyriazakis 1997; Wagner *et al.* 1999). Furthermore, it is important to optimise the efficiency of nutrient utilisation in order to decrease the cost of food per unit gain as feed is one of the largest cost factors involved in pig production (Quiniou and Noblet 1995; Quiniou *et al.* 1999). Knowledge of both physical and chemical body composition is important to optimise the entire production system, characterise the population of interest, and within breeding systems to optimise food intake in relation to the deposition of chemical body components.

1.6 QTL for feed intake and feed efficiency

Fewer QTL have been reported in the literature for feed intake and feed efficiency, compared to carcass traits. Feed efficiency has a very high economic value in pig production and it is therefore one of the most important traits in pig breeding programmes. The undesirable reduction in feed intake capacity of pigs due to intensive selection on lean content makes it necessary to improve this trait in those lines in order

to obtain further improvement in growth rate. Additionally, the optimisation of feed intake capacity can be used to improve feed efficiency and body composition of pigs indirectly. More recently, however, interest in these traits has increased. The first QTL identified for feed intake was on SSC1 close to the *melanocortin-4 receptor* gene (*MC4R)* (Kim *et al.* 2000). A mutation in this gene is associated with feed intake as well as fatness and growth. Following on from this, QTL for feed intake have been detected throughout the genome (Beeckmann *et al.* 2003a; Cepica *et al.* 2003abc; Dragos-Wendrich *et al.* 2003a; Geldermann *et al.* 2003; Lee *et al.* 2003b; Pierzchala *et al.* 2003; Houston *et al.* 2005). At present, only a small number of QTL across the genome have been identified for food conversion ratio (Beeckmann *et al.* 2003c; Cepica *et al.* 2003b; Dragos-Wendrich *et al.* 2003b; Yue *et al.* 2003a; Houston *et al.* 2005).

1.7 Biological growth models and body composition

The importance of biological growth models is widely described in the literature (De Vries and Kanis 1992; Schinckel and de Lange 1996; Emmans and Kyriazakis 1997; Wagner *et al.* 1999; de Lange *et al.* 2003; Knap *et al.* 2003; Moughan 2003; Pomar *et al.* 2003; van Milgen and Noblet 2003). Biological growth models are important for predicting the response to changes in management and nutrition, for evaluation of optimal slaughter weights and for the development of appropriate selection strategies. Using a biological growth model, De Vries and Kanis (1992) outlined the importance of optimising feed intake of pigs to improve feed efficiency. They developed a biological growth model to examine the economic values of feed intake and outlined the influence of feed intake on production costs. Sub optimal feed intake increases production costs as pigs do not reach their full potential for protein deposition and therefore have to grow over a longer period which increases management and husbandry costs. Feed intake above optimum results in increased deposition of lipid tissue, which is associated with high feeding costs and represents a tissue of low commercial value. The implementation of pig growth models into breeding schemes is limited due to the lack of economical and

accurate methods to obtain the information required by growth models (Schinckel and de Lange 1996; Schinckel *et al.* 1996; Wagner *et al.* 1999). The importance of acquiring accurate estimates of the animals' potential for protein accretion for pig growth models has been outlined (Schinckel and de Lange 1996). Doeschl-Wilson *et al.* (2007) developed a computational method of obtaining these estimates for a population of pigs based on phenotypic information about growth and backfat measures in an F_1 population. This method was based on population values rather than individual values.

The need to optimise the feed intake curve with respect to lean tissue growth is an important goal in genetic improvement programmes. At present, feed intake during the early stages of growth is generally not sufficient to meet the pigs' potential for protein deposition, whereas feed intake at later stages of growth is often too high and consequently results in the increased deposition of lipid tissue. The feed intake curve needs to be adapted by aiming to increase feed intake at early stages of growth, at which pigs are most efficient at lean tissue growth and should be limited at the later stages of growth to prevent extensive fat accretion (Schulze *et al.* 2002). One of the most important goals in pig breeding in the future should therefore be to optimise feed intake in relation to protein and lipid deposition. It has been suggested that breeding strategies should place emphasis on an optimal feed intake capacity depending on protein deposition considering a minimum fat/protein ratio (Kanis and De Vries 1992). QTL for feed intake and chemical body composition could provide additional information for these types of models.

1.8 QTL and imprinting effects

Genomic imprinting is a relatively uncommon genomic feature of placental mammals. Epigenetic effects of imprinting causes parent-of-origin-specific effects in the offspring, where the expression of imprinted genes depend on the parental origin of that gene i.e. whether it is inherited maternally or paternally, rather than by the DNA sequence.

Parent-of-origin-specific effects arise from modifications of the parental genomes during gametogenesis (de Koning *et al.* 2002; Hunter 2007). Genomic imprinting is controlled by sequence elements called control regions. At these regions, there is DNA methylation on one of the two parental alleles. In the majority of cases, the DNA methylation occurs during female gametogenesis and only at some control regions during spermatogenesis. The methylation marks at control regions are maintained throughout development and mediates the allelic expression of imprinted genes (Reik and Walter 2001; Delaval and Feil 2004; Feil and Berger 2007).

The phenomenon of imprinting had been largely overlooked because of the influence of Mendel's laws of inheritance, which maintain that the phenotype of an individual is determined by the underlying alleles and independent of other parental or environmental aspects (Hunter 2007). The discovery of imprinting complicates the subject of inheritance.

In pigs, a region at the telomeric end of the p arm of chromosome 2 has been well characterised to contain imprinting effects. A paternally expressed (maternal imprinting) QTL affecting muscle growth and fatness has been mapped to be *insulin-like growth factor 2* (*IGF2*) locus (Jeon *et al.* 1999; Nezer *et al.* 1999). Van Laere *et al.* (2003) showed that this QTL is caused by a nucleotide substitution in intron 3. Although not as well characterised as the *IGF2* locus, other regions of the pig genome have been found to harbour maternal and paternal imprinting effects (e.g. de Koning *et al.* 2001a; Milan *et al.* 2002; Quintanilla *et al.* 2002; Thomsen *et al.* 2004; Rohrer *et al.* 2005).

In other species of livestock, reports of QTL with significant imprinting effects are limited in the literature. In chickens, QTL with imprinting effects have been identified for body weight and conformation score (Rowe *et al.* 2009), white meat percentage, growth and carcass traits (McElroy *et al.* 2006), as well as egg production traits body weight and feed intake (Tuiskula-Haavisto *et al*. 2004). To date relatively few imprinting effects have been identified in cattle and sheep. In cattle imprinting effects

have been implicated in milk production traits (Kuehn *et al.* 2007), mammalian growth and development (Zhang *et al.* 2004) and ovulation rate (Allan *et al.* 2008). In sheep imprinting has been implicated in ovulation rate (Davis *et al.* 2001) as well as growth and development (Feil *et al.* 1998; McLaren and Montgomery 1999). An interesting parent-of-origin effect has been identified in sheep at a mutation in the callipyge gene. This mutation has been well characterised to influence carcass composition, muscle development and meat quality. The extreme muscling phenotype is caused by a parent-of-origin effect called polar overdominance, where the phenotype only occurs in heterozygous individuals where the mutant allele is inherited from the sire and a normal allele is inherited from the dam. However, animals that inherit two copies of the mutant allele do not show extreme muscling (Freking *et al.* 2002; Georges *et al.* 2003). In contrast to livestock species there is much more evidence of genes with imprinting effects in humans as well as mice which can be seen in the geneimprint (www.geneimprint.com/site/genes-by-species) and MRC Harwell databases (http://www.har.mrc.ac.uk/mousebook/?search=chrregion~). These databases provide information about the location and effect of imprinted genes in human and mice.

It is important to consider imprinting within QTL mapping studies, as some QTL may remain undetected. Identifying imprinting effects will provide a greater understanding of the genetic regulation of traits and can be used for specific crossbreeding systems. For example, the large effect of the paternally expressed *IGF2* mutation on leanness makes it desirable to be exploited within pig breeding programmes. At this mutation, the allele inherited from the dam will have no effect on the phenotype of the offspring, as only the allele inherited from the sire is expressed. The advantage of this gene is therefore at the terminal sire (Buys 2003). Within breeding, imprinting effects can be exploited to develop sire lines which have extreme lean tissue content. These sire lines can be crossed with dam lines with average lean tissue content, as leanness is negatively associated with reproduction, to produce offspring with optimal lean content (Roehe *et al.* 2003).

1.9 Challenges associated with QTL studies

QTL studies have provided some information about the genomic regulation of economically important traits. However, most studies have not accounted for the unique features provided by the sex chromosome. Furthermore, epistasis (gene interactions) has been largely ignored in these studies. Gene interactions are likely to play an important role in the genomic regulation of quantitative traits and therefore it is important to account for epistasis within QTL studies.

1.9.1 Sex-linked QTL

The majority of QTL have been identified on autosomes with fewer QTL reported on the sex chromosomes. One reason for this may be that the genomic analysis of the sex chromosomes is more challenging in methodology and modelling. Because of this, much less attention has been paid to the analysis of pig chromosome X.

The sex chromosomes behave differently to autosomes. Males and females of mammals differ in their sex chromosomes, where females carry two X chromosomes and males carry one X and one Y chromosome. Consequently, female cells contain twice as many copies of X chromosome genes in comparison to male cells (Alberts *et al.* 2002). The mammalian X chromosome is much larger than the Y chromosome and contains many more genes. In humans, the X chromosome contains around 1000 genes, whereas the Y contains less than 100 genes (Alberts *et al.* 2002; Graves *et al.* 2006; Heard and Disteche 2006). There is limited homology between chromosome X and Y, except for a small region called the pseudoautosomal region (Alberts *et al.* 2002; Perez-Enciso *et al.* 2002). Mammals have developed a mechanism to equalise the dosage of the X chromosome gene products between sexes, called the dosage compensation phenomenon. This is achieved by X-inactivation, a process whereby one of the two X chromosomes in female somatic cells is inactivated. The choice of which X chromosome is inactivated, whether

it has been inherited from the maternal or paternal parent, appears to be random (Alberts *et al.* 2002; Heard and Disteche 2006).

Due to the lack of software implementing appropriate methodology and models for the QTL analysis of the sex chromosomes, most QTL studies have analysed males and females separately, thus decreasing the power to detect QTL (e.g. Knott *et al.* 1998; de Koning *et al.* 2001a; Geldermann *et al.* 2003). This approach does not allow for simultaneous estimation of the sex chromosomes, does not consider the pseudoautosomal region and does not account for the dosage compensation phenomenon.

Methodology and models which accounts for the special challenges associated with the analysis of sex chromosomes has been incorporated into the software QxPak (Perez-Enciso *et al.* 2002; Perez-Enciso and Misztal 2004). This increases accuracy and power for detecting QTL on chromosome X in comparison to single sex analysis. This methodology, based on mixed model techniques, is much more flexible. It includes all pedigree information and uses the maximum likelihood method to estimate the QTL effects. The methodology accounts for the heterogeneity of sex chromosomes, considers the pseudoautosomal region and accounts for the dosage compensation phenomenon.

1.9.2 Epistasis

To date, most QTL mapping studies have focussed on identifying the individual effects of QTL (additive, dominance and imprinting) in the absence of interactions between QTL (epistasis). It is hence assumed that the genetic background at other loci have no impact on the phenotypic expression of QTL (Lander and Botstein 1989; Carlborg and Haley 2004). Quantitative traits are in fact controlled by many QTL as well as a complex network of interactions between QTL, as has been identified in studies of mice and chickens (e.g. Routman and Cheverud 1997; Brockmann *et al.* 2000; Carlborg *et al.*

2003; Carlborg *et al.* 2004; Yi *et al.* 2004a; Wolf *et al.* 2006; Yi *et al.* 2006; Le Rouzic *et al.* 2008). The effect of a genotype at a particular locus on phenotypic expression is dependent on the genetic background at other loci. Therefore the phenotype of a given genotype cannot be determined from the sum of its single locus effects (Phillips 1998). In the simplest case, epistasis can be described as the interaction between a pair of loci, where the effect of one locus on a particular genotype depends on the genotype at a second locus. However, the situation is probably more complex, such that the effect of one locus on a particular phenotype is likely to depend on the genotypes at several other loci.

Epistasis may cause the individual QTL effects (additive and dominance) to decrease or even totally cancel each other. QTL displaying this type of epistasis are hard to find using standard mapping (Carlborg 2006). Individual loci can remain undetected and the estimated effects of the detected QTL could be severely biased if epistatic interactions are not properly accounted for. Inaccurate QTL estimates can lead to invalid interpretations of the importance of these identified QTL, and also to problems of confirmation of QTL effects in further crosses. When attempts are made to use the QTL, for example in marker assisted selection, this will result in lower response and economic gain (Carlborg 2006). Even though epistatic QTL are difficult to identify, the interest in investigating epistasis is increasing. By accounting for gene-gene interactions within QTL mapping studies we can improve the power to detect novel loci which mainly exhibit their actions through interactions with other loci and provide greater insight into the biology of the traits under study (Carlborg 2006).

The degree to which epistasis contributes to variation in complex traits is not known at present, but results from studies in different organisms indicates that epistasis is likely to be an important component of the genetic variance (Flint *et al.* 2004). Carlborg *et al.* (2003) found that epistasis was particularly important for early growth in an intercross between Jungle Fowl and White Leghorn chickens. This is the stage of development where the foundation for growth is established by the development of internal organs.

They found epistasis to be less important for later growth, which is the stage which involves the main deposition of body tissues. In a cross between a White Leghorn line and a commercial broiler line, Carlborg *et al.* (2004) found that epistasis was an important contributor to the genetic variance of growth, with the largest effects on body weight at 6 weeks of age and growth between 3 and 6 weeks of age. Carlborg *et al.* (2004) also reported evidence that genetic regulation of early and late growth in the chicken differs by identifying a discrete set of interacting loci involved in early growth. In addition to chickens, a large number of studies in mice indicate an important role of epistasis in the genomic regulation of growth and body composition. Routman and Cheverud (1997) identified a contribution of epistasis to the genomic control of adult body weight in mice. Brockmann *et al.* (2000) identified epistatic effects for serum concentrations of leptin, insulin and *Insulin-like growth factor 1*, body weight, abdominal fat weight and muscle weight in mice. This study provided evidence of co-coordinated regulation of body and muscle weight by the interaction of two pairs of loci, which may contribute to the high correlation between muscle and body weight. In the same species, epistasis has also been reported to play an important role in controlling obesity (Yi *et al.* 2004a). In their study, the authors found that different groups of traits were influenced by different genetic architecture. Moreover, there are reports of epistatic QTL in mice for abdominal fat, body weight, kidney weight, spleen weight (Carlborg *et al.* 2005) as well as organ weights and limb length traits (Wolf *et al.* 2006). Yi *et al.* (2006) also reported that epistasis influenced fatness and organ weights in mice. In terms of growth traits, Yi *et al.* (2006) reported that epistasis had a more pronounced effect for body weight at later stages of growth in mice, which is a contrast to the results of Carlborg *et al.* (2003; 2004) in chickens. However, Ishikawa *et al.* (2005) identified that epistasis was more important in early stages of growth in mice. In other species evidence of epistatic QTL are extremely limited. In cattle, Barendse *et al.* (2007) investigated the importance of epistasis between putative causative mutations at the *Calpain 1* gene and the *Calpastatin* gene influencing meat tenderness. These genes were chosen as their role in the post mortem tenderization of meat has been well characterised. From this study, significant epistasis was identified between SNPs at these genes. Further epistatic

interactions influencing fertility traits in cattle have been reported by Khatib *et al.* (2009) between genes involved in the POU1F1 pathway. In sheep there are currently no reports of epistatic QTL influencing economically important traits. At present, there are only a few reports of epistatic QTL in pigs. Epistatic effects have been reported for reproduction traits (Bidanel 1993; Rodriguez *et al.* 2005; Noguera *et al.* 2006), coat colour (Hirooka *et al.* 2002), meat quality traits (meat colour and intramuscular fat content) (Ovilo *et al.* 2002; Szyda *et al.* 2006) and muscle fibre traits (Estelle *et al.* 2008). No epistatic QTL have been reported in pigs for body composition, such as entire carcass cuts, lean tissue and fat tissue characteristics or growth traits.

1.10 Genomic effects on meat quality

Intense selection for increased productivity has brought about an undesirable reduction in the meat eating quality characteristics and subsequent consumer acceptability (Schwab *et al.* 2006). Meat quality can be an important factor in the economics of pig production as the quality and consumer acceptability influences the price of the market product. Therefore, breeding goals should incorporate selection for meat quality as well as productivity (van Wijk *et al.* 2005).

The quality of meat is influenced by a number of characteristics. Consumer satisfaction is influenced by traits associated with eating quality, such as texture, tenderness, flavour and juiciness as well as traits associated with appearance including colour, leanness, fatness, and amount of intramuscular fat tissue (Sellier 1998; Kanis *et al.* 2005). Intramuscular fat content has a favourable influence on the colour, flavour, tenderness and juiciness of the meat, however substantial levels can have detrimental effects on the aesthetic value and thus detrimental to consumer acceptability (de Koning *et al.* 1999; Ciobanu *et al.* 2004). In order to preserve meat quality the possibility of reducing subcutaneous fat without reducing, or more desirably, with an increase in intramuscular fat content has been suggested (Roehe *et al.* 2003). More technological aspects of meat

quality include properties such as water-holding capacity (e.g. drip loss during storage), intensity and homogeneity of colour, firmness, shelf-life, cooking loss and various processing yields (Sellier 1998; Otto *et al.* 2004; Kanis *et al.* 2005; Otto *et al.* 2006). Commonly used indicators of meat quality include measurements of pH at 45-60 minutes post-mortem as well as 24 hours after slaughter, conductivity, reflectance values and meat colour scores.

The unfavourable associations of breeding for leanness with meat eating quality has sparked an interest in understanding the genetic regulation of meat quality (Karlsson *et al.* 1993; De Vries *et al.* 1994; Knapp *et al.* 1997; Oksbjerg *et al.* 2000; Kanis *et al.* 2005; Aaslyng *et al.* 2007). Because of the economic importance of these traits, reports of QTL for meat quality traits are increasing (de Koning *et al.* 2001b; Grindflek *et al.* 2001; Ovilo *et al.* 2002; Nii *et al.* 2005; Vidal *et al.* 2005), and are likely to gain further attention in the near future (Otto *et al.* 2007a).

1.11 Value and benefit of genome research

Genome research in livestock has already provided some information which has had important practical implications. For example, the diagnostic test for the *ryanodine receptor* gene *(RYR1)* and *rendement napole (RN)* mutations have been widely used in practical pig breeding (Andersson 2001). Information obtained from QTL mapping studies may have additional input in breeding programmes by marker assisted selection, using superior genomic regions affecting traits of economic importance. At present, however, marker assisted selection has not had the success that was expected. The main focus of this research is on the basic genomic regulation of economically important traits in pig breeding. Information about QTL and epistatic interactions between QTL can be used to build up an understanding of the genomic networks influencing a biological system (Carlborg and Haley 2004) which can be used for breeding purposes. Pig breeding is driven by improvement in technologies and genetic selection based on

complex traits is becoming increasingly important (Lander and Kruglyak 1995; Knap *et al.* 2001). QTL identified for physical body composition traits can be used directly to improve carcass quality traits for the market of interest. As a result, there is potential to breed for different market demands at lower cost. QTL determined for chemical body composition traits may be used to optimise protein and lipid deposition as well as feed intake with the aim to decrease the amount (cost) of food per gain. This is economically beneficial, as food is the largest cost factor associated with pig production. It is important to determine the association between QTL influencing both physical and chemical body composition traits as well as meat quality in order to exploit the merit of all QTL effects efficiently thereby avoiding detrimental effects in a trait through selection for another. Therefore, knowledge of associations between QTL can be used in breeding programmes to maximise the overall improvement of all economically important traits.

There is a requirement for further QTL studies, in order to increase information relating to the change in both physical and chemical body composition during growth. It is important to consider carcass information obtained from carcass grading systems, like the AutoFom system, within genome research as the information obtained from such studies can be directly used to improve the grading of carcasses. Furthermore, it is of great benefit to use commercial populations within QTL mapping studies in order to determine whether QTL detected in crossings of exotic and commercial breeds are also segregating in crossings of purely commercial breeds. The advantage of the use of the latter crossbred resource family is that information about QTL identified in commercial populations can be directly used in breeding programmes. Once the QTL is identified it is also important to identify the gene which is responsible for the effect (Rohrer 2000). The number of genes identified in livestock is still small compared to that of the human. However, several genes have already been identified that directly or indirectly affect body composition such as the *RYR1* gene, also known as the halothane gene, which is responsible for malignant hyperthermia and porcine stress syndrome and is associated with increased lean content (Fujii *et al.* 1991), the porcine *MC4R* gene which is

associated with fatness, growth, and feed intake (Kim *et al.* 2000), and the *RN* mutation which is associated with increased glycogen content in the muscle of live animals, which results in substantial post-mortem degradation of glycogen and thus detrimental effects on pork quality such as pH, water holding capacity, colour and processing yield (Andersson 2001). It is not essential to identify genes in order to utilise QTL within livestock breeding programmes; however, it is much more efficient to use information of genes and mutations explaining substantial variation of economically important traits for selection. With decrease in cost of genotyping, it is likely that marker analysis will become a large scale industry (Kappes 1999; Andersson 2001). The development of large collections of single nucleotide polymorphisms (SNPs) is expected to more efficiently facilitate the identification of causative mutations underlying QTL. These can be identified using genome-wide association analyses with dense single SNP marker chips. This involves searching the genome for SNPs which are associated with a particular trait (Andersson 2008). Furthermore, it is important to gain further information about the interactions between QTL (epistatic effects) in order to gain a more accurate understanding of the genetic regulation of important traits. Furthermore, high density SNP chips can be used for genomic selection (Meuwissen *et al.* 2001; Goddard and Hayes 2007).

1.12 Thesis outline

The overall aim of this study is to provide a better understanding of the genomic regulation of growth and body composition of pigs as well as feed intake, feed efficiency and meat quality traits in a commercial population. The objectives of the work presented in this thesis are:

- to investigate QTL for chemical body composition and the associations with QTL for body tissues, growth and feed intake traits,
- to investigate the role of chromosome X in the genomic regulation of growth and body composition as well as feed intake and feed efficiency using appropriate methodology of QTL mapping for this chromosome,
- to explore the contribution of epistasis to the genomic regulation of physical and chemical body composition traits as well as feed intake and feed efficiency,
- to investigate the genomic effects on meat quality traits including the contribution of epistasis.

1.13 Project data

The work of this thesis is based on data from a three generation full-sib design outlined in Figure 1.2. In the founder population (F_0), seven unrelated Pietrain sires were mated to 16 sows from a crossbred dam line (Leicoma × (Landrace × Large White)) to produce 160 animals of the F_1 generation. Of these animals, 116 animals were used to test the methodologies of measuring chemical body composition in live animals. From the F_1 generation 8 boars were mated to 40 sows, whilst avoiding inbreeding, to produce two litters of the F_2 generation comprising 315 animals in total.
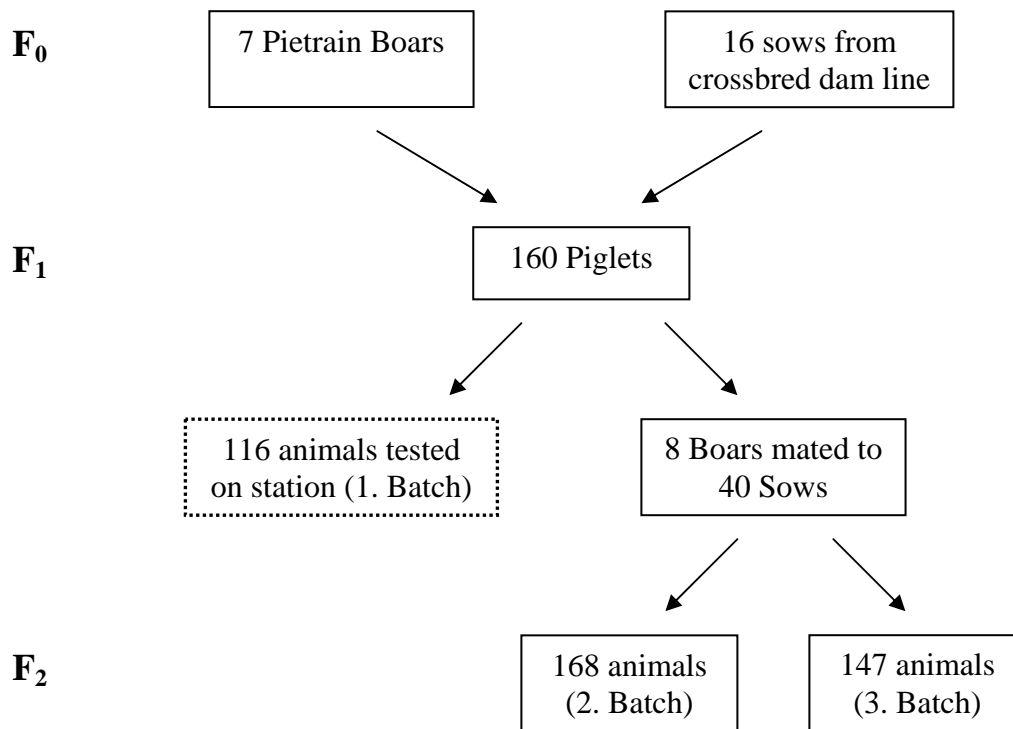
Figure 1.2
Diagram representing the three generation full-sib design of this project.

Phenotypic data was available for physical body composition traits, including weights of valuable carcass cuts, lean tissue and fat tissue, collected from pigs slaughtered in a commercial abattoir at 140 kg body weight. These traits were collected by two methods, the AutoFom carcass grading system and by dissection of the right side of the carcass. This information allowed for the investigation into the genomic regulation of the lean and fat tissue composition of the carcass. In order to gain an accurate representation of the change in body composition throughout growth, phenotypic information of chemical body composition (protein and lipid content) was collected in live animals using the deuterium dilution technique at target body weights of 30, 60, 90, 120 and 140 kg. Information about the accretion rates of protein and lipid tissue were available for the

growth stages 30-60, 60-90, 90-120 and 120-140 kg body weights. Using this data, the genomic regulation of chemical body composition at different stages of growth and associations with physical body composition traits were investigated. Feed intake capacity and particularly feed efficiency traits are of great economic importance. Phenotypic measurements were available in this study for these traits measured at different growth stages (60-90, 90-120 and 120-140 kg body weights). Moreover, phenotypic measurements were available for a number of traits associated with meat quality collected at different times post-slaughter.

The data were recorded within the research projects of Landgraf (2004) and Mohrmann (2005) at the official performance test station L.P.A. Achterwehr (Landwirtschaftskammer Schleswig-Holstein, Germany) in co-operation with the University of Kiel, Germany. The genotyping was carried out in two batches by Dr. Van Haeringen Laboratorium B.V., The Netherlands in 2003 and 2004. In the first batch chromosomes SSC1, SSC6, SSC7 and SSC13 were genotyped and in the second batch chromosomes SSC2, SSC4, SSC8, SSC9, SSC10, SSC14, SSCX were genotyped.

The first stage of individual QTL analysis using the phenotypic data of the present study was carried out by Mohrmann *et al.* (2006) using genotypic data of four chromosomes, including SSC1, SSC6, SSC7 and SSC13. Individual QTL analysis of the present study considered seven different chromosomes, including SSC2, SSC4, SSC8, SSC9, SSC10, SSC14, SSCX in Chapters 2 and 3. Genotypic information of all eleven chromosomes was combined in the epistatic QTL analysis of Chapters 4 and 5 and the meat quality analysis of Chapter 6.

Chapter 2

Quantitative trait loci for chemical body composition traits in pigs and their positional associations with body tissues, growth and feed intake

Abstract

In this study, quantitative trait loci (QTL) for chemical and physical body composition, growth and feed intake in pigs were identified in a three generation full-sib population, developed by crossing Pietrain sires with a commercial dam line. Phenotypic data from 315 F_2 animals were available for protein and lipid deposition measured in live animals by the deuterium dilution technique at 30, 60, 90, 120 and 140 kg body weight. At 140 kg body weight, carcass characteristics were measured by the AutoFOM grading system and after dissection. Three hundred and eighty six animals from 49 families were genotyped for 51 molecular markers covering chromosomes SSC2, SSC4, SSC8, SSC9, SSC10, and SSC14. Novel QTL for protein (lipid) content at 60 kg body weight and protein (lipid) accretion from 120 to 140 kg were detected on SSC9 near several previously detected QTL for lean and fat tissue in neck, shoulder and ham cuts. Another QTL for lipid accretion was found on SSC8, closely associated with a QTL for intramuscular fat content. QTL for daily feed intake were detected on SSC2 and SSC10. The favourable allele of a QTL for food conversion ratio (FCR) on SSC2 was associated with alleles for increased lean tissue and decreased fat tissue. Because no QTL for growth rate were found in the region, the QTL for FCR is most likely due to change in body composition. These QTL provide insight into the genomic regulation of chemical or physical body composition and its association with feed intake, feed efficiency and growth.

2.1 Introduction

At present, a large number of quantitative trait loci (QTL) in pigs have been detected for physical body composition, which are associated with lean and fat tissue characteristics (e.g. Bidanel *et al.* 2001; Milan *et al.* 2002; Geldermann *et al.* 2003). In contrast, QTL associated with protein and lipid deposition and their change during growth have only been reported in one study analysing chromosomes 1, 6, 7 and 13 (Mohrmann *et al.* 2006a). Knowledge of the deposition rates of chemical components is necessary to accurately estimate nutritional requirements of pigs during growth, to determine selection objectives for optimal development of body tissue growth and feed intake capacity and more generally, to provide parameters of a pig growth model that can be used to improve the efficiency of the entire pig production system (e.g. Schinckel and de Lange 1996; de Lange *et al.* 2003; Knap *et al.* 2003). Optimising the efficiency of nutrient utilisation is important to decrease the cost of food per unit gain, as feed is one of the largest cost factors involved in pig production (Quiniou and Noblet 1995; Quiniou *et al.* 1999). Additionally, the market price of the final product is based on carcass quality. Therefore, the association between chemical and physical body composition is of great economic interest.

Most QTL studies have been based on crosses of domestic breeds with the Meishan, Wild Boar or Iberian breed (e.g. Andersson-Eklund *et al.* 1998; Rohrer and Keele 1998ab; Rohrer 2000). Favourable QTL alleles found in these less-improved breeds cannot be directly exploited within pig breeding due to the poor performance of these exotic breeds for traits of commercial interest. Alternatively, there is potential to integrate QTL identified in commercial populations into existing pig breeding programmes.

Information in the literature indicates that pig chromosomes 2, 4, 8, 9, 10 and 14 are associated with lean and fat tissue growth (e.g. Andersson *et al.* 1994; Malek *et al.* 2001ab; Geldermann *et al.* 2003). These chromosomes were chosen in this study for

QTL analysis of physical and chemical body composition as well as feed intake, food conversion ratio (FCR) and growth rate in commercial breeds.

2.2 Materials and methods

2.2.1 Design and data

QTL mapping was based on data from a three generation full-sib design. The resource family was created by mating seven unrelated Pietrain grandsires to 16 unrelated grandams from a crossbred dam line (Leicoma × (Landrace × Large White)). Pietrain sires were all heterozygous (Nn) at the *ryanodine receptor 1 (RYR1)* locus. Eight F_1 boars and 40 F_1 sows were mated to produce 315 F_2 pigs of 49 families across two litters. Of these F_2 animals, 48 gilts and 46 barrows were housed individually in straw-bedded pens. These pigs were fed manually, and feed consumption was recorded weekly. The remaining 117 gilts and 104 barrows were housed in straw-bedded pens in groups of up to 15 pigs of both sexes. Food was supplied to these pigs by an electronic feeding station (ACEMA 48), which recorded feed consumption at each visit. Pigs were provided with one of three pelleted diets containing 13.8 MJ ME/kg and 1.2% lysine, 13.8 MJ ME/kg and 1.1% lysine, or 13.4 MJ ME/kg and 1.0% lysine for weight ranges 30-60, 60-90 and 90-140 kg body weight, respectively. Pigs were able to reach maximal protein deposition by providing *ad libitum* access to diets, which were formulated slightly above requirement. For a more detailed description of the management of this project see Landgraf *et al.* (2006ab) and Mohrmann *et al*. (2006ab).

2.2.2 Physical body composition

Phenotypic measurements of physical body composition were collected from pigs slaughtered in a commercial abattoir at 140 kg body weight. Measurements of valuable carcass cuts were obtained using the AutoFOM device, which uses an automatic ultrasound scanning technique to produce a three-dimensional image of the pig (Brondum *et al.* 1998). Using this device, measurements were obtained for average fat thickness, belly weight, lean content, lean content of the belly and weights of entire and trimmed shoulder, loin and ham without bones. The right carcass side of each pig was then dissected into primal carcass cuts neck, shoulder, loin, ham and belly weights. The former four carcass cuts were further dissected into lean and fat tissue. Moreover, weights of jowl, thick rib, flank, front as well as hind hock, tail and claw were recorded. Additional measurements were obtained from the cold left carcass side including carcass length; sidefat thickness; loin eye area, fat area and thinnest fat measure (fat degree B) at the $13^{th}/14^{th}$ rib interface; fat content and area of the belly. Additional information about the dissection of carcasses is presented by Landgraf *et al.* (2006b).

2.2.3 Chemical body composition

Protein, lipid and ash content of the empty body was determined at target body weights of 30, 60, 90, 120 and 140 kg using deuterium dilution technique, an *in vivo* method of determining chemical body composition based on body water. The accuracy of this technique has been verified in previous studies using magnetic resonance imaging on live animals (Mohrmann *et al.* 2006b) and chemical analysis of serially slaughtered animals (Landgraf *et al.* 2006a). The deuterium dilution method determined the empty body water content, from which the percentage of fat-free substance of the empty body was estimated. Protein and ash content of the empty body were estimated based on the percentage of fat-free substance. Lipid content was the deviation of the fat-free content from one. The equations for estimating these chemical components were developed in

the study of Landgraf *et al.* (2006a) using the same data that was analysed here. Accretion rates of protein and lipid were calculated as the difference between lipid or protein composition at two consecutive target weights divided by days of growth between target weights. Protein content of loin and intramuscular fat content (IMF) were measured in the *musculus longissimus thoracis et lumborum* using near-infrared reflectance spectroscopy. Mean values and standard deviations of traits analysed in the present study are shown in Tables 2.1 and 2.2.

Table 2.1 Means and standard deviations (SD) of carcass characteristics measured on pigs of the F_2 generation

Trait	Mean	SD	Number of Records
AutoFOM traits			
AF average fat thickness (mm)	22.295	4.989	313
AF entire shoulder weight (kg)	6.176	0.406	313
AF shoulder lean meat weight (kg)	4.577	0.408	313
AF entire loin weight (kg)	6.265	0.396	313
AF loin lean meat weight (kg)	3.764	0.352	313
AF entire ham weight (kg)	13.573	0.814	313
AF ham lean meat weight (kg)	9.511	1.052	313
AF entire belly weight (kg)	9.168	0.548	313
AF lean content (%)	50.509	6.403	313
AF lean content of belly (%)	43.741	7.891	313
Carcass characteristics – dissected carcass cuts			
Entire neck weight (kg)	5.316	0.505	306
Neck weight without external fat (kg)	4.160	0.430	306
External neck fat weight (kg)	1.156	0.285	306
Entire shoulder weight (kg)	8.452	0.564	307
Shoulder weight without external fat (kg)	5.910	0.584	307
External shoulder fat weight (kg)	1.403	0.261	307
Entire loin weight (kg)	9.163	0.730	308
Loin weight without external fat (kg)	6.650	0.624	308
External loin fat weight (kg)	2.513	0.645	308
Entire ham weight (kg)	16.908	0.997	310
Ham weight without external fat (kg)	11.568	1.087	310
External ham fat weight (kg)	2.566	0.493	310
Belly weight (kg)	6.461	0.655	308
Jowl weight (kg)	1.914	0.284	306
Thick rib (kg)	1.441	0.217	307
Flank weight (kg)	1.789	0.407	308
Front hock weight (kg)	1.139	0.189	307
Hind hock weight (kg)	1.430	0.141	310
Tail weight (kg)	0.429	0.134	310
Hind claw (kg)	0.914	0.122	310
Carcass characteristics – standard performance test			
Carcass length (cm)	107.947	49.296	310
Sidefat thickness[1] (cm)	3.847	0.866	315
Thinnest fat measure[1] (cm)	1.725	0.552	314
Loin eye area *M.l.t.l.*[1,2] (cm^2)	54.160	6.767	314
Fat area *M.l.t.l.*[1,2] (cm^2)	24.514	5.884	314
Fat content of belly (%)	53.508	8.272	306
Fat area of belly (cm^2)	23.789	6.782	306

[1] collected at the 13th/14th rib interface.

[2] measured on *musculus longissimus thoracis et lumborum*.

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Table 2.2 Means and standard deviations (SD) of chemical body composition, accretion rates, daily gain, daily feed intake and food conversion ratio measured on pigs of the F_2 generation

Trait	Mean	SD	Number of Records
Chemical body composition			
Intramuscular fat content (%)	1.343	0.542	313
Protein content of loin (%)	24.215	2.066	313
Protein content of FFS, 30 kg (%)	18.656	0.524	299
Protein content of FFS, 60 kg (%)	20.115	0.419	305
Protein content of FFS, 90 kg (%)	21.209	0.426	311
Protein content of FFS, 120 kg (%)	21.960	0.506	302
Protein content of FFS, 140 kg (%)	22.359	0.543	302
Protein content of empty body, 30 kg (%)	16.643	0.065	310
Protein content of empty body, 60 kg (%)	16.477	0.047	305
Protein content of empty body, 90 kg (%)	16.359	0.045	311
Protein content of empty body, 120 kg (%)	16.282	0.051	302
Protein content of empty body, 140 kg (%)	16.242	0.053	302
Lipid content of empty body, 30 kg (%)	10.845	2.920	310
Lipid content of empty body, 60 kg (%)	18.045	2.000	305
Lipid content of empty body, 90 kg (%)	22.832	1.773	311
Lipid content of empty body, 120 kg (%)	25.813	1.926	302
Lipid content of empty body, 140 kg (%)	27.308	1.987	302
Chemical accretion rates			
DG, 30-60 kg (kg/day)	0.677	0.114	315
DG, 60-90 kg (kg/day)	0.838	0.138	312
DG, 90-120 kg (kg/day)	0.779	0.140	313
DG, 120-140 kg (kg/day)	0.718	0.193	313
PAR, 30- 60 kg (kg/day)	0.110	0.018	300
PAR, 60-90 kg (kg/day)	0.135	0.023	300
PAR, 90-120 kg (kg/day)	0.125	0.022	299
PAR, 120-140 kg (kg/day)	0.115	0.031	292
LAR, 30-60 kg (kg/day	0.168	0.040	300
LAR, 60-90 kg (kg/day)	0.271	0.060	300
LAR, 90-120 kg (kg/day)	0.274	0.069	301
LAR, 120-140 kg (kg/day)	0.267	0.099	293
Feed intake and food conversion traits			
DFI 60-90 kg (kg/day)	2.467	0.361	312
DFI 90-120 kg (kg/day)	2.818	0.376	313
DFI 120-140 kg (kg/day)	2.815	0.496	313
FCR 60-90 kg (kg feed/kg gain)	2.975	0.379	312
FCR 90-120 kg (kg feed/kg gain)	3.678	0.517	313
FCR 120-140 kg (kg feed/kg gain)	4.214	1.975	313

Definition of symbols: FFS, fat free substance; DG, daily gain; PAR, protein accretion rate; LAR, lipid accretion rate; DFI, daily feed intake; FCR, food conversion ratio.

2.2.4 Genotypic data

Blood samples were collected from F_0, F_1 and F_2 animals from the *vena jugularis,* and genomic DNA was isolated. All animals were genotyped for 51 informative microsatellite markers selected from the published USDA linkage map (http://www.marc.usda.gov; Rohrer *et al.* 1996), of which 9, 9, 8, 9, 9 and 7 genomic markers were located on SSC2, SSC4, SSC8, SSC9, SSC10 and SSC14, respectively (Table 2.3). Average distance between markers was 16.5, 16.3, 18.4, 17.3, 16.0, and 17.4 cM and the largest gap between markers was 25.2, 26.5, 23.1, 21.7, 20.8, and 23.6 cM on SSC2, SSC4, SSC8, SSC9, SSC10 and SSC14, respectively.

Table 2.3 Markers used in the present QTL mapping project, their relative map position using USDA pig map, number of different alleles and the information contents for the additive (a) and dominance (d) F_2 coefficients and heterozygosity in the F_1 generation (H)

Marker	SSC	Position (cM)	H	Number of alleles	Information content of coefficients	
					a	d
SWR2516	2	0.0	0.67	5	0.59	0.47
SW2623	2	9.8	0.68	5	0.53	0.32
SWR783	2	23.7	0.51	3	0.40	0.23
SW240	2	42.0	0.84	7	0.78	0.62
SW1026	2	60.6	0.47	6	0.48	0.25
SW1370	2	74.8	0.91	8	0.78	0.69
SWR2157	2	89.2	0.78	8	0.59	0.47
SWR345	2	114.4	0.87	8	0.86	0.73
S0036	2	132.1	0.85	7	0.80	0.73
SW2404	4	0.0	0.91	10	0.78	0.74
SW489	4	8.0	0.66	5	0.71	0.42
S0301	4	27.1	0.72	6	0.42	0.40
S0001	4	41.8	0.66	6	0.50	0.26
SW839	4	62.3	0.44	4	0.41	0.21
S0214	4	79.3	0.80	6	0.56	0.48
SW445	4	105.8	0.91	10	0.80	0.76
MP77	4	120.0	0.87	8	0.77	0.61
SW856	4	130.1	0.98	14	0.81	0.74
SW2410	8	-1.3	0.42	4	0.61	0.25
SW905	8	20.8	0.71	6	0.57	0.40
SWR1101	8	38.3	0.88	12	0.15	0.04
SW444	8	52.5	0.85	7	0.79	0.83
S0086	8	62.2	0.69	6	0.64	0.50
SW374	8	82.8	0.88	5	0.77	0.67
SW1551	8	105.9	0.75	6	0.68	0.43
S0178	8	127.7	0.54	7	0.58	0.34
SW983	9	4.0	0.81	6	0.72	0.50
SW21	9	15.1	0.65	5	0.45	0.16
SW911	9	36.8	0.75	7	0.63	0.38
SW2401	9	57.1	0.71	6	0.65	0.34
SW2571	9	73.3	0.46	6	0.55	0.32
S0019	9	86.4	0.75	6	0.58	0.33
SW2093	9	103.6	0.90	6	0.89	0.75
SW174	9	122.9	0.81	3	0.73	0.55
SW1349	9	142.5	0.81	7	0.50	0.42
SW830	10	0.0	0.67	7	0.54	0.31
SWR136	10	7.6	0.77	6	0.64	0.45
SW1894	10	23.2	0.65	4	0.58	0.35
SW2195	10	44.0	0.48	3	0.34	0.16
SW173	10	56.1	0.35	4	0.28	0.17
SW1041	10	67.5	0.46	3	0.26	0.10
SW2043	10	87.7	0.56	5	0.50	0.27
SW1626	10	108.0	0.79	11	0.76	0.56
SW2067	10	128.0	0.81	7	0.09	0.03
SW857	14	7.4	0.87	9	0.81	0.87
S0089	14	14.0	0.67	7	0.42	0.20
SW245	14	32.0	0.77	7	0.63	0.50
SW342	14	53.2	0.79	7	0.64	0.48
SW1081	14	72.1	0.87	6	0.80	0.68
SW1557	14	87.9	0.64	4	0.61	0.35
SWC27	14	111.5	0.45	8	0.27	0.26

2.2.5 Statistical analysis

The QTL analysis was performed with QTL Express (http://qtl.cap.ed.ac.uk; Seaton *et al.* 2002) using line-cross least squares multi-marker regression interval mapping for outbred lines (Haley *et al.* 1994). The analysis of QTL Express proceeds in two stages. In the first stage the data on marker positions as well as marker genotypes are used to calculate the probabilities of individuals inheriting one or two grandpaternal or grandmaternal alleles at positions throughout the genome and the parent-of-origin probability of the alleles. These probabilities are combined into additive and dominance coefficients in order to observe the information contents of the markers along the chromosome as well as segregation distortion. In the second stage, at every 1cM QTL position, least squares is used to regress the phenotypic value for each individual onto their individually calculated additive and dominance coefficients which then provides estimates of additive and dominance for that position. This is then repeated at each defined position on the chromosome and the best estimate of the QTL effects and position are obtained (at the position in which the residual sum of squares is minimized) where the F statistic is highest and estimates for additive and dominance effects are calculated at this position (Seaton *et al.* 2002).

In this analysis, the additive estimate is defined as half of the difference between pigs homozygous for alleles from the grandpaternal sire line and pigs homozygous for alleles from the grandmaternal dam line. A positive additive genetic value indicates that the allele originating from the grandpaternal sire line (Pietrain) showed a higher effect than the allele from the grandmaternal dam line and *vice versa*. The dominance effect is defined as deviation of heterozygous animals from the mean of both types of homozygous animals. A positive dominance value indicates an increase in the trait of interest as a result of a heterozygous genotype and *vice versa*. Moreover, traits were tested for QTL expressing paternal or maternal imprinting. In this analysis imprinting is defined as the difference between heterozygous genotypes when the Pietrain allele is

inherited from parents of the opposite sex. The individual QTL analysis was performed with the following models:

Carcass characteristics measured at slaughter:

$$y_i = sex_i + MHS_i + batch_i + \beta slwt_i + C_a a + C_d d + e_i,$$ [1]

Chemical body composition at each target weight:

$$y_i = sex_i + MHS_i + batch_i + \beta wt_i + C_a a + C_d d + e_i,$$ [2]

Chemical accretion rates (protein and lipid accretion):

$$y_i = sex_i + MHS_i + batch_i + \beta stwt_i + \beta endwt_i + C_a a + C_d d + e_i,$$ [3]

Feed intake and food conversion ratio:

$$y_i = sex_i + MHS_i + batch_i + ht_i + \beta stwt_i + \beta endwt_i + C_a a + C_d d + e_i,$$ [4]

where y_i is the i-th individual phenotype. Fixed effects of *sex*, ryanodine receptor genotype (*MHS*) and *batch* were fitted in the model for all traits. In addition, the effect of housing (*ht: housing type)* was significant for feed intake and FCR traits. For carcass characteristics and chemical body composition, linear regression on body weight at slaughter (*slwt*) and at each target weight (*wt*), respectively, was included in the model. Protein and lipid accretion, daily gain (DG), feed intake and FCR were adjusted for the small differences between target and actual body weight at the start (*stwt*) and end (*endwt*) of the considered weight range. C_a and C_d represent the additive and dominance coefficients, respectively, and *a* is the additive effect and *d* is the dominance effect. Traits were analysed individually and thresholds to determine chromosome-wide statistical significance levels were obtained by permutation test (Churchill and Doerge 1994) under 10 000 iterations.

2.3 Results

In the genomic analysis, five QTL were identified for entire carcass characteristics, 13 for lean tissue characteristics, seven for fat tissue characteristics, seven for chemical body composition and deposition and two each for DG, daily feed intake (DFI) and FCR (Table 2.4). QTL with significant imprinting effects were identified for 32 traits, of which 19 traits showed novel QTL not previously detected using the additive and dominance model (Table 2.5).

Table 2.4 Evidence for quantitative trait loci (QTL) for AutoFOM (AF) grading characteristics, carcass cuts, growth, feed intake and chemical body composition or deposition

SSC	Trait	Results of the present study					Other studies confirming the QTL
		F-ratio	Pos[1]	% Var[2]	a ± SE[3]	d ± SE[3]	References[4]
Carcass characteristics (lean and fat)							
8	Hind hock weight (kg)	5.29*	3.7	3.5	**0.044 ± 0.014**	0.011 ± 0.025	-
8	Entire ham weight (kg)	11.43***	11.7	7.2	**0.351 ± 0.082**	**0.340 ± 0.149**	de Koning *et al.* (2001a); Quintanilla *et al.* (2002)
9	Entire shoulder weight (kg)	9.03**	65	5.8	**0.182 ± 0.043**	0.002 ± 0.077	-
9	AF entire shoulder weight (kg)	7.53**	68	4.8	**0.099 ± 0.030**	**-0.113 ± 0.053**	-
14	AF entire shoulder weight (kg)	5.15*	64.4	3.3	**-0.100 ± 0.031**	0.055 ± 0.050	-
Lean tissue characteristics							
2	Loin weight without external fat (kg)	5.7*	10	3.7	**0.166 ± 0.051**	0.060 ± 0.077	Andersson-Eklund *et al.* (1998); Geldermann *et*
2	Ham weight without external fat (kg)	6.66*	15	4.3	**0.333 ± 0.091**	-0.018 ± 0.149	*al.* (2003); Lee *et al.* (2003a)
2	Shoulder weight without external fat (kg)	5.75*	92	3.8	0.014 ± 0.047	**-0.252 ± 0.074**	Geldermann *et al.* (2003); Lee *et al.* (2003a)
4	AF lean content (kg)	5.62*	33	3.6	**-1.213 ± 0.420**	-1.262 ± 0.737	Cepica *et al.* (2003a); Geldermann *et al.* (2003); Edwards *et al.* (2006)
8	AF loin lean meat weight (kg)	9.59**	5.7	6.0	**0.089 ± 0.030**	**0.179 ± 0.055**	Andersson-Eklund *et al.* (1998); de Koning *et al.*
8	Loin weight without external fat (kg)	6.96*	15.7	4.5	0.106 ± 0.054	**0.297 ± 0.093**	(2001a); Quintanilla *et al.* (2002); Kim *et al.*
8	Ham weight without external fat (kg)	8.02**	12.7	5.2	**0.304 ± 0.091**	**0.376 ± 0.163**	(2005)
8	Loin eye area m.l.t.l.[5] (cm[2])	6.21*	11.7	4.0	**1.734 ± 0.611**	**2.397 ± 1.105**	
8	Protein content of loin (%)	5.14*	37.7	3.3	**-0.212 ± 0.076**	0.210 ± 0.124	Beeckmann *et al.* (2003c); Geldermann *et al.* (2003)
9	Shoulder weight without external fat (kg)	8.63**	73	5.6	**0.184 ± 0.044**	0.003 ± 0.075	-
9	Neck weight without external fat (kg)	7.13*	86	4.7	**0.115 ± 0.032**	-0.072 ± 0.053	
10	Protein content of loin (%)	6.17*	94	4.0	**-0.193 ± 0.072**	**-0.279 ± 0.120**	-
14	AF ham lean meat weight (kg)	6.24*	68.4	4.0	**-0.273 ± 0.083**	0.174 ± 0.128	Dragis-Wendrich *et al.* (2003b); Geldermann *et al.* (2003)
Fat tissue characteristics							
2	External neck fat weight (kg)	7.07*	6	4.6	**-0.089 ± 0.027**	-0.071 ± 0.041	de Koning *et al.* (2001a); Milan *et al.* (2002);
2	External ham fat weight (kg)	7.42**	11	4.8	**-0.128 ± 0.041**	**-0.133 ± 0.063**	Kim *et al.* (2005); Sanchez *et al.* (2006);
8	Intra muscular fat content (%)	5.19*	48.7	3.4	**0.129 ± 0.046**	-0.114 ± 0.069	Rohrer & Keele (1998a)
9	Fat area of belly (cm[2])	6.57*	30	4.3	-0.834 ± 0.573	**3.440 ± 1.047**	Rohrer & Keele (1998a)
9	External ham fat weight (kg)	6.81*	86	4.4	**-0.131 ± 0.036**	0.064 ± 0.060	Karlskov-Mortensen *et al.* (2006)
9	External shoulder fat weight (kg)	7.48**	86	4.9	**-0.047 ± 0.021**	**0.111 ± 0.034**	
14	AF average fat thickness (cm)	5.53*	69.4	3.6	**0.930 ± 0.391**	**-1.436 ± 0.599**	Malek *et al.* (2001a); Dragos-Wendrich *et al.* (2003a); Geldermann *et al.* (2003)

Table 2.4 continued

SSC	Trait	Results of the present study					Other studies confirming the QTL
		F-ratio	Pos[1]	% Var[2]	a ± SE[3]	d ± SE[3]	References[4]
Chemical body composition and deposition							
8	LAR 60-90 kg (kg/day)	5.42*	49.7	3.7	**0.015 ± 0.005**	0.005 ± 0.007	Rohrer & Keele (1998a); de Koning *et al.* (2001a); Malek *et al.* (2001a)
9	PAR 120-140 kg (kg/day)	6.22*	92	4.3	-0.003 ± 0.003	**0.014 ± 0.004**	-
9	LAR 120-140kg (kg/day)	5.37*	93	3.8	-0.017 ± 0.009	**0.042 ± 0.015**	-
9	Protein cont empty body, 60 kg (%)	5.64*	115	3.7	0.002 ± 0.003	**0.018 ± 0.006**	-
9	Protein cont FFS$_{EB}$, 60 kg (%)	5.63*	116	3.7	-0.017 ± 0.029	**-0.161 ± 0.048**	-
9	Lipid cont empty body, 60 kg (%)	5.63*	115	3.7	-0.072 ± 0.142	**-0.785 ± 0.235**	-
10	PAR 90-120 kg (kg/day)	5.25*	4	3.6	**-0.006 ± 0.002**	0.001± 0.003	-
Daily gain, feed intake and food conversion ratio							
2	FCR 90-120 kg (kg feed/kg gain)	5.95*	3	3.9	**-0.143 ± 0.044**	-0.076 ± 0.068	-
2	DFI 120-140 kg (kg/day)	6.59*	79	4.3	-0.062 ± 0.040	**0.214 ± 0.063**	Rohrer (2000); Lee *et al.* (2003a)
4	FCR, 90-120 kg (kg feed/kg gain)	6.07*	20	4.0	**0.149 ± 0.043**	0.013 ± 0.074	-
9	DG 120-140 kg (kg/day)	6.5*	89	4.2	-0.020 ± 0.015	**0.087 ± 0.025**	-
10	DFI 60-90 kg (kg/day)	7.3**	44	4.7	**-0.108 ± 0.028**	0.011 ± 0.046	Knott *et al.* (1998)
10	DG 90-120 kg (kg/day)	5.08*	4	3.3	**-0.037 ± 0.012**	0.000 ± 0.018	-

m.l.t.l., *musculus longissimus thoracis et lumborum*; LAR, lipid accretion rate; PAR, protein accretion rate; FFS$_{EB}$, fat-free substance of the empty body; FCR, food conversion ratio calculated as kg feed / kg gain; DFI, daily feed intake; DG, daily gain.

*, **, and *** implies significance at the 5%, 1% or 0.1% chromosome-wise levels, respectively.

[1]Positions of the QTL in cM.

[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual sum of squares due to the QTL effect on the residual sum of squares excluding the QTL effect: (residual sum of squares of reduced model – residual sum of squares of full model)/residual sum of squares of reduced model.

[3]Estimated additive (a) and dominance (d) effects and their standard errors (SE).

[4]References of other studies reporting QTL for similar traits in similar regions of the genome.

[5]Measured at the 13th/14th rib interface.

Values in bold represent significant additive or dominance effects.

Table 2.5 Evidence for quantitative trait loci (QTL) expressing imprinting effects on AutoFOM (AF) grading characteristics, carcass cuts, growth, feed intake and chemical body composition or deposition

SSC	Trait	F-ratio	Pos[1]	% var[2]	a ± SE[3]	d ± SE[3]	i ± SE[3]
Carcass characteristics (lean and fat)							
2	Entire ham weight[5] (kg)	4.44*	10	4.3	**0.165 ± 0.078**	-0.188 ± 0.116	**0.182 ± 0.070**
4	Thick rib[5] (kg)	5.27*	13	5.2	-0.025 ± 0.018	**-0.075 ± 0.031**	**0.059 ± 0.020**
9	Entire shoulder weight (kg)	7.33**	73	7.0	**0.159 ± 0.041**	0.052 ± 0.069	**0.099 ± 0.045**
10	Tail weight[5] (kg)	4.29*	88	4.2	0.014 ± 0.013	-0.001 ± 0.020	**0.040 ± 0.012**
14	AF entire shoulder weight (kg)	5.79**	73.4	5.5	**-0.086 ± 0.029**	0.041 ± 0.044	**0.081 ± 0.028**
Lean tissue characteristics							
2	AF shoulder lean meat weight[5] (kg)	8.36***	0	7.8	0.007 ± 0.034	-0.041 ± 0.050	**0.158 ± 0.032**
2	AF lean content[5] (%)	5.52*	0	5.3	0.590 ± 0.411	0.148 ± 0.604	**1.451 ± 0.387**
2	Loin eye area m.l.t.l.[4,5] (cm^2)	7.81***	0	7.3	0.749 ± 0.540	-0.281 ± 0.792	**2.316 ± 0.507**
2	Loin weight without external fat (kg)	11.01***	2	10.2	**0.153 ± 0.049**	-0.002 ± 0.074	**0.216 ± 0.046**
2	Ham weight without external fat (kg)	9.73***	10	9.0	**0.307 ± 0.083**	-0.074 ± 0.124	**0.297 ± 0.075**
8	AF lean content of belly[5] (%)	4.38*	1.7	4.2	0.453 ± 0.619	**3.300 ± 1.125**	**1.332 ± 0.655**
8	AF loin lean meat weight (kg)	7.78***	7.7	7.3	**0.090 ± 0.030**	**0.191 ± 0.056**	**0.061 ± 0.030**
9	Loin eye area m.l.t.l.[4,5] (cm^2)	5.25*	71	5.0	**1.049 ± 0.519**	-1.199 ± 0.908	**1.698 ± 0.579**
9	Shoulder weight without external fat (kg)	8.52***	73	8.1	**0.176 ± 0.044**	0.006 ± 0.074	**0.135 ± 0.048**
9	AF lean content[5] (%)	5.58**	115	5.3	0.493 ± 0.390	**1.846 ± 0.641**	**1.093 ± 0.411**
10	Shoulder weight without external fat[5] (kg)	4.46*	84	4.4	0.102 ± 0.052	-0.087 ± 0.086	**0.132 ± 0.048**
14	AF lean content of belly[5] (%)	5.4**	68.4	5.2	**-1.591 ± 0.580**	1.361 ± 0.901	**1.407 ± 0.562**
14	AF ham lean meat weight (kg)	6.19**	71.4	5.9	**-0.260 ± 0.078**	0.151 ± 0.116	**0.188 ± 0.076**
14	AF shoulder lean meat weight[5] (kg)	4.44*	75.4	4.3	**-0.090 ± 0.034**	0.060 ± 0.054	**0.081 ± 0.034**

Table 2.5 continued

SSC	Trait	F-ratio	Pos[1]	% var[2]	a ± SE[3]	d ± SE[3]	i ± SE[3]
	Fat tissue characteristics						
2	External ham fat weight (kg)	11.28***	0	10.3	**-0.090 ± 0.038**	-0.008 ± 0.056	**-0.188 ± 0.036**
2	External loin fat weight[5] (kg)	6.63**	0	6.4	-0.064 ± 0.052	-0.036 ± 0.076	**-0.206 ± 0.049**
2	Thinnest fat measure[4,5] (cm)	8.54***	0	7.9	-0.067 ± 0.044	-0.009 ± 0.064	**-0.196 ± 0.041**
2	Fat area m.l.t.l.[4,5] (cm^2)	6.2**	0	5.9	-0.670 ± 0.486	-0.461 ± 0.713	**-1.824 ± 0.456**
2	Fat area of belly[5] (cm^2)	5.64**	0	5.5	-0.567 ± 0.556	0.264 ± 0.808	**-2.029 ± 0.517**
9	External loin fat weight[5] (kg)	7.62***	75	7.3	-0.080 ± 0.048	-0.007 ± 0.084	**-0.234 ± 0.054**
9	External ham fat weight (kg)	5.94**	86	5.7	**-0.132 ± 0.036**	0.061 ± 0.059	**-0.077 ± 0.038**
9	Fat area of belly (cm^2)	4.63*	87	4.6	**-1.217 ± 0.516**	-0.097 ± 0.843	**-1.587 ± 0.543**
9	Sidefat thickness[4,5] (cm)	6.12**	67	6.3	**-0.149 ± 0.067**	0.136 ± 0.122	**-0.238 ± 0.075**
10	External loin fat weight[5] (kg)	4.46*	0	4.4	-0.049 ± 0.050	-0.106 ± 0.080	**-0.153 ± 0.049**
14	AF average fat thickness (mm)	5.27*	69.4	5.1	**0.934 ± 0.389**	**-1.382 ± 0.596**	**-0.810 ± 0.377**
	Chemical body composition and deposition						
9	LAR, 120-140 kg (kg/day)	5.4*	87	5.6	-0.018 ± 0.009	**0.036 ± 0.014**	**-0.023 ± 0.009**
	Daily gain, feed intake and food conversion ratio						
9	DG, 120-140 kg (kg/day)	6.83**	86	6.5	-0.022 ± 0.015	**0.082 ± 0.025**	**-0.044 ± 0.016**

m.l.t.l., *musculus longissimus thoracis et lumborum*; LAR, lipid accretion rate; DG, daily gain.

*, **, and *** implies significance at the 5%, 1% or 0.1% chromosome-wise levels, respectively.

[1]Positions of the QTL in cM.

[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual sum of squares due to the QTL effect on the residual sum of squares excluding the QTL effect: (residual sum of squares of reduced model – residual sum of squares of full model)/residual sum of squares of reduced model.

[3]Estimated additive (a), dominance (d) and imprinting (i) effects and their standard errors (SE).

[4]Measured at the 13th/14th rib interface.

[5]New QTL only identified when the imprinting effect is included in the model.

Values in bold represent significant additive, dominance or imprinting effects.

2.3.1 Carcass characteristics (lean and fat)

QTL were identified for valuable carcass cuts on SSC8, SSC9 and SSC14. The QTL with the highest *F*-ratio significant at the 0.1% chromosome-wide level was identified on SSC8 for entire ham weight at 11.7 cM between *SW2410* and *SW905* and explained 7.2% of the phenotypic variance. The additive genetic effect of the allele originating from the Pietrain grandpaternal breed was associated with 351 g higher ham weight and heterozygous animals showed 340 g higher ham weight due to dominance effects. In a similar region (3.7 cM), a QTL was identified for hind hock weight; it explained 3.5% of the phenotypic variance, but only showed significant additive genetic effects.

QTL were detected on SSC9 between *SW2401* and *SW2571* for entire shoulder weight measured by the AutoFOM device (68 cM) and by dissection (65 cM), explaining 4.8% and 5.8% of the phenotypic variance, respectively. The allele originating from the Pietrain founder breed showed higher shoulder weight. A further QTL for shoulder weight measured by the AutoFOM system was detected on SSC14 at 64.4 cM between *SW342* and *SW1081*. In this case, the Pietrain allele was associated with decreased shoulder weight. Furthermore, two QTL reaching the 5% chromosome-wide significance level were detected for entire shoulder weight measured by dissection on SSC9 at 23 cM and by the AutoFOM system on SSC14 at 37.4 cM (not shown in Table 2.4).

2.3.2 Lean tissue characteristics

QTL for lean tissue characteristics were detected on SSC2, SSC4, SSC8, SSC9, SSC10 and SSC14. On SSC2, QTL were identified for carcass cuts loin, ham, and shoulder without external fat explaining 3.7%, 4.3% and 3.8% of the phenotypic variance, respectively. QTL for weights of trimmed carcass cuts loin and ham were located at 10 and 15 cM respectively, close to *SW2623*. The allele originating from Pietrain founder parents was associated with higher lean tissue weights of both carcass cuts. The QTL for

trimmed shoulder weight was located in a different region at 92 cM, close to *SWR2157*. Heterozygous animals were associated with significantly lower lean meat of the shoulder.

On SSC4 a single QTL was identified for lean content measured by the AutoFOM system accounting for 3.6% of the phenotypic variance. Additive genetic effects at this QTL indicate that alleles from the grandpaternal Pietrain breed were associated with decreased lean content.

QTL were identified on SSC8 for loin lean meat measured by the AutoFOM carcass grading system (5.7 cM) and dissection (15.7 cM), ham lean meat (12.7 cM) and loin eye area (11.7 cM). These QTL explained 6.0%, 4.5%, 5.2% and 4.0% of the phenotypic variance, respectively, and were located in the same region as QTL for entire ham weight and hind hock weight between *SW2410* and *SW905* (Figure 2.1). Dominance and additive effects for these QTL indicate that heterozygous animals and Pietrain alleles were associated with higher loin and ham lean meat weight and higher loin eye area.
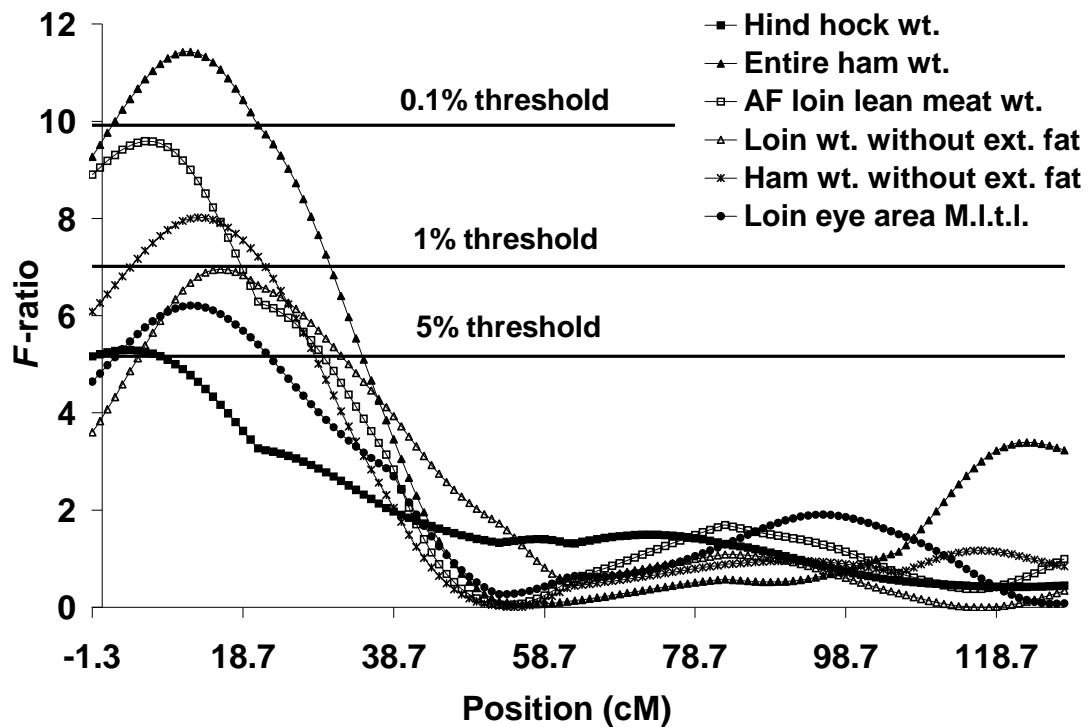
Figure 2.1
F-ratio curves for evidence of quantitative trait loci for carcass, AutoFOM (AF) and lean traits on SSC8. Horizontal lines indicate the chromosome-wide significance levels.

In a different region of SSC8 (37.7 cM), a QTL for protein content of loin was identified with additive effects only.

On SSC9, QTL were detected for lean meat of the shoulder and neck cuts close to *SW2571*, explaining 5.6% and 4.7% of the phenotypic variance, respectively. At these QTL, the favourable allele originated from the Pietrain founder population.

A QTL for protein content of loin was detected on SSC10, showing both additive and dominance effects. An additional QTL was detected for ham lean meat weight measured by the AutoFOM carcass grading system on SSC14 between *SW342* and *SW1081*. The Pietrain allele for this QTL was associated with lower ham lean meat weight.

2.3.3 Fat tissue characteristics

QTL were identified for fat tissue characteristics on SSC2, SSC8, SSC9 and SSC14. QTL were identified for external fat weights of ham and neck cuts explaining 4.8% and 4.6% of the phenotypic variance, respectively, in the same region of SSC2 as QTL identified for lean weights of loin and ham cuts. The allele originating from the Pietrain grandpaternal breed was associated with significantly less external fat in both cuts. A QTL was identified on SSC8 for IMF at 48.7 cM between *SWR1101* and *SW444*. The Pietrain allele at this QTL was associated with higher IMF. On SSC9, a QTL was identified for fat area of the belly at 30 cM, between *SW21* and *SW911*, explaining 4.3% of the phenotypic variance. Heterozygous animals showed 3.44 cm^2 larger fat area of the belly. In a different region of SSC9 (86 cM) close to *S0019*, QTL were identified for external fat weights of ham and shoulder cuts explaining 4.4% and 4.9% of the phenotypic variance, respectively. At these QTL, the Pietrain allele was associated with significantly less external fat in both cuts, but only the shoulder showed significantly more external fat in heterozygous animals. These QTL were in the same region as QTL for lean tissue characteristics. An additional QTL reaching 5% chromosome-wide significance was identified for external fat weight of the shoulder at 12 cM on SSC9 (not shown in Table 2.4).

A QTL was detected for average fat thickness measured by the AutoFOM device in the same region as QTL on SSC14 for entire shoulder weight and ham lean meat weight measured by the AutoFOM device. Heterozygous animals showed a dominance effect of 1.4 mm less average fat thickness, whereas the additive genetic effect of the Pietrain allele yielded in 0.9 mm higher average fat thickness than that from the crossbred dam line.

2.3.4 Chemical body composition and deposition

QTL for protein accretion rate (PAR) from 90 to 120 kg was identified on SSC10. QTL for PAR for a later growth period (120-140 kg) was found on SSC9 at 92 cM explaining 4.3% of the phenotypic variance. In the same region of SSC9, a QTL for lipid accretion rate (LAR) was identified for the same growth period. These were located in the same region of SSC9 as QTL for lean and fat tissue (Figure 2.2).
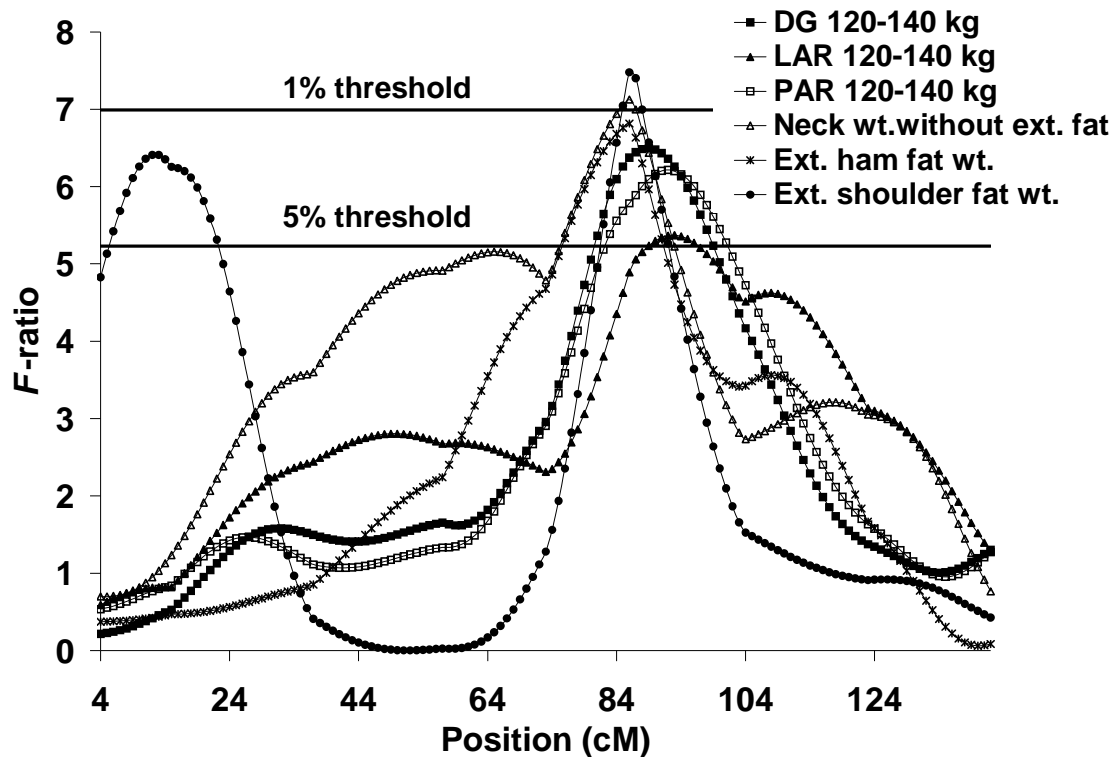
Figure 2.2
F-ratio curves for evidence of quantitative trait loci for chemical body composition, lean and fat tissue on SSC9. Horizontal lines indicate the chromosome-wide significance levels. DG, daily gain; LAR, lipid accretion rate; PAR, protein accretion rate.

A second QTL for LAR for an earlier growth period (60-90 kg) was identified on SSC8 close to *SW444*, positioned very close to the QTL for IMF (Figure 2.3). Alleles from the Pietrain breed are associated with higher LAR between 60 and 90 kg and decreased PAR from 90 to 120 kg. Heterozygous animals were associated with higher PAR and LAR at the later growth stage (120-140 kg).
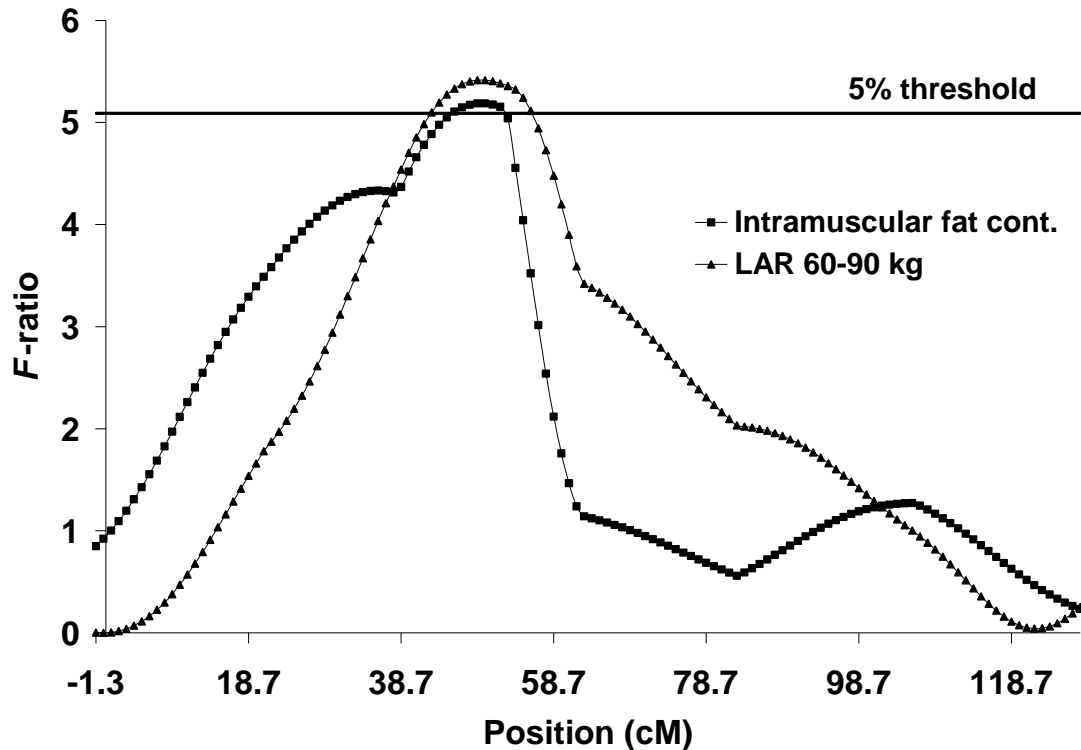
Figure 2.3
F-ratio curves for evidence of quantitative trait loci for intramuscular fat content and lipid accretion 60-90 kg on SSC8. Horizontal line indicates the chromosome-wide significance level. LAR, lipid accretion rate.

QTL for protein and lipid content of the empty body and protein content of the fat-free substance at 60 kg body weight were identified on SSC9 between *SW2093* and *SW174*. Heterozygous animals were associated with significantly higher protein content of the

empty body and significantly lower protein content of the fat-free substance and lipid content of the empty body at 60 kg body weight.

2.3.5 Feed intake, daily gain and food conversion ratio

A QTL for FCR from 90 to 120 kg was detected on SSC2 in the same region as QTL for lean and fat tissue characteristics between *SWR2516* and *SW2623* (Figure 2.4). The Pietrain allele was associated with higher feed efficiency, i.e. 143 g less food per one kg gain.
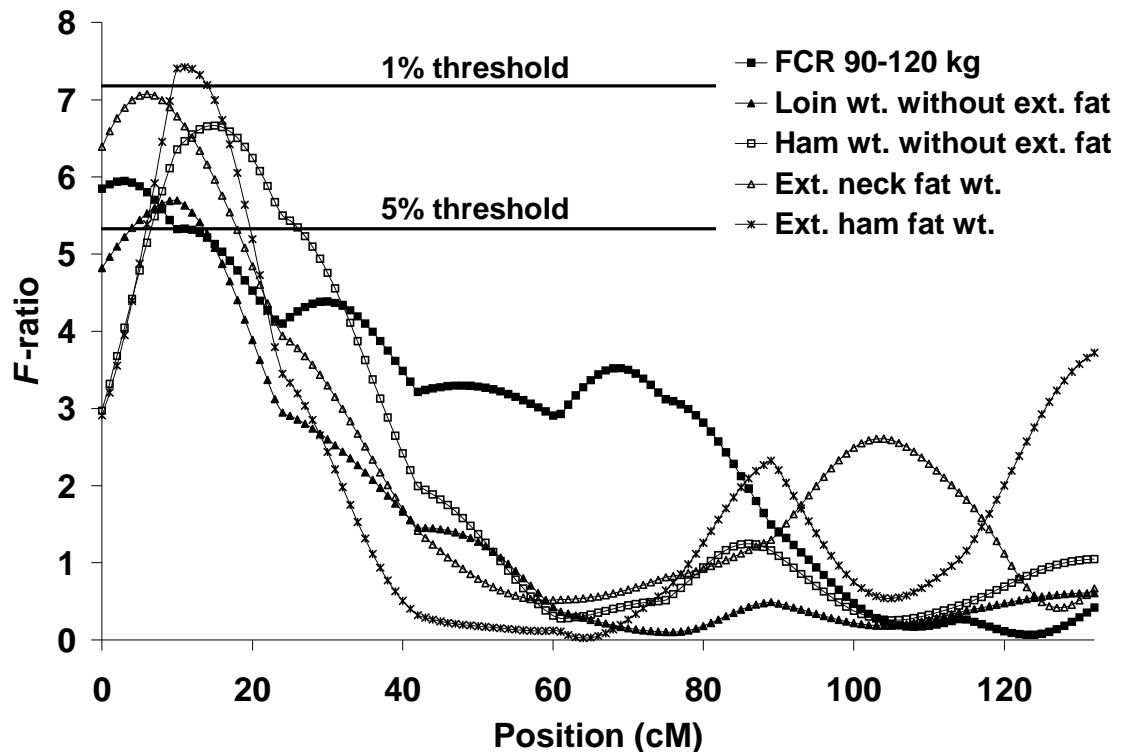
Figure 2.4
F-ratio curves as evidence of quantitative trait loci for food conversion ratio, lean and fat tissue on SSC2. Horizontal lines indicate the chromosome-wide significance levels. FCR, food conversion ratio.

An additional QTL for FCR from 90 to 120 kg was detected on SSC4 between *SW489* and *S0301*. In contrast, the Pietrain allele was associated with lower feed efficiency at this QTL. On SSC10, QTL for DFI for 60 to 90 kg was identified at the same position as *SW2195*, and for a later growth stage (120-140 kg) on SSC2 between *SW1370* and *SWR2157*. Pietrain alleles were associated with 108 g less DFI at 60 to 90 kg body weight and heterozygous animals were associated with 214 g higher DFI at heavier weights. A QTL for DG (120-140 kg) was detected on SSC9 in the same region as QTL for PAR, LAR, lean and fat tissue (Figure 2.2). An additional QTL was detected on SSC10 for DG 90-120 kg at the same position as the QTL detected in this study for PAR at the same stage of growth.

2.3.6 Imprinting

For several carcass cuts, lean tissue and fat tissue characteristics, QTL expressing maternal imprinting effects were identified on SSC2 close to *SWR2516* and *SW2623*. This indicates that only the paternal allele is expressed at these QTL. On SSC8, QTL expressing maternal imprinting were detected for lean tissue traits at 1.7 cM and 7.7 cM between *SW2140* and *SW905*. SSC9 harboured a large number of QTL showing maternal imprinting for carcass characteristics, lean tissue, fat tissue, LAR and DG between *SW2401* and *S0019* or 67 cM and 87 cM. An additional QTL showing maternal imprinting effects for lean content was identified in a different region of SSC9 (115 cM). Furthermore, QTL with maternal imprinting effects associated with carcass characteristics and lean tissue were detected on SSC10 close to *SW2043* and for fat tissue at the same position as *SW830*. For carcass characteristics, lean tissue and fat tissue measured by the AutoFOM device, QTL expressing paternal imprinting effects were detected on SSC14 close to *SW1081*. This indicates that only the maternal allele is expressed at these QTL.

2.4 Discussion

Numerous QTL were identified in this study for important carcass cuts, lean and fat tissue characteristics and chemical body composition. QTL for chemical body composition and their associations with QTL for physical body composition will provide a better understanding of growth and body composition.

QTL for important carcass cuts have high economic value and were identified in the present study on SSC8, SSC9 and SSC14. The most significant QTL detected in this study at the 0.1% chromosome-wide level was for entire ham weight on SSC8. QTL have been reported in the literature around this location for early growth rate and body weight (cited in Table 2.4). QTL for entire shoulder weight identified in this study on SSC9 and SSC14 have not been reported before in the literature.

QTL have been reported for lean weights of carcass cuts shoulder, loin and neck as well as lean meat content measurements in the same region of SSC2 as the QTL detected for loin and ham lean meat weight in the present study (cited in Table 2.4). Additionally, QTL have been reported in this region for weight gain (Lee *et al.* 2003a; Thomsen *et al.* 2004) as well as for carcass cuts and lean tissue characteristics around 0 cM (Milan *et al.* 2002; Nezer *et al.* 2002; Sanchez *et al.* 2006). In a different region of SSC2, there is evidence in the literature (cited in Table 2.4) supporting the QTL identified in the present study for shoulder lean meat weight. Additional QTL have been reported in this region for weight gain (Malek *et al.* 2001a; Lee *et al.* 2003a).

Numerous QTL were identified in this study between 5.7 cM and 15.7 cM on SSC8 for lean tissue characteristics in the same region as QTL were detected in the present study for entire ham weight and hind hock weight (Figure 2.1). This is supported with reports in the literature (cited in Table 2.4) for loin eye area, average daily gain, body weight and growth rate as well as bone/lean meat ratio in ham. A single QTL for protein content of loin was identified in a different region of SSC8 (37.7 cM). This QTL is supported by

reports in the literature for loin, neck, ham and shoulder meat weights (cited in Table 2.4). QTL were also identified in this study for shoulder and neck lean meat weight in a region of SSC9 where no QTL for lean tissue have been reported. There is evidence in the literature (cited in Table 2.4) for QTL associated with lean meat of shoulder, loin, neck and ham in the same region of SSC14 as the QTL identified in the present study for ham lean meat weight measured by the AutoFOM device.

Surprisingly, in the present F_2 population only one QTL was detected for lean tissue on SSC4. In the literature, a large number of QTL have been reported for growth and fatness on SSC4 (e.g. Andersson *et al.* 1994; Marklund *et al.* 1999; Cepica *et al.* 2003a). Most QTL from these studies have been detected in F_2 populations at least partly derived from exotic breeds. Therefore, these QTL may not be segregating within the commercial lines used in the present study. Nonetheless, there is evidence in the literature (cited in Table 2.4) to support the location of the QTL identified in the present study on SSC4 for lean content.

QTL were identified for fat weights of carcass cuts neck and ham in the same region of SSC2 as QTL identified for lean meat weights of important carcass cuts in the present study. There is substantial evidence in the literature (cited in Table 2.4) for a QTL influencing fat tissue in this area. Additionally, a large number of QTL have been identified for backfat around 0 cM (e.g. Knott *et al.* 1998; Milan *et al.* 2002). In a similar region an imprinted QTL has been mapped to the *insulin-like growth factor 2 (IGF2)* locus with large effects on fat deposition and muscle mass (Nezer *et al.* 1999).

A novel QTL for IMF on SSC8 was identified in the present study. Although no QTL have been reported before for intramuscular fat in this genomic region, QTL have been reported for fat tissue (cited in Table 2.4). The Pietrain allele for this QTL is associated with increased IMF. This may have implications for meat quality as IMF is a major factor affecting meat quality and consumer satisfaction.

QTL were identified in the present study for external fat weights of carcass cuts ham and shoulder in the same region as QTL identified for lean meat weights of neck and shoulder on SSC9 (Figure 2.2). There is limited information supporting a QTL in this region for growth and fatness, however, a QTL has been reported for weight of fat in ham (cited in Table 2.4). In a different region of this chromosome, a QTL for fat area of the belly was identified in the present study in the same region as a QTL previously reported in the literature for leaf fat weight (cited in Table 2.4). Additionally, a QTL for average fat thickness measured by the AutoFOM system was identified in the present study in the same region of SSC14 as for ham lean meat weight and entire shoulder weight measured by the same device. There is a lot of evidence in the literature for a QTL influencing fatness in this region of SSC14 (cited in Table 2.4). As discussed, several genomic regions contained QTL for both leanness and fatness (SSC2, SSC9 and SSC14), indicating their close relationships when animals are slaughtered at almost the same finishing weight.

From the present study, it was found that the allele originating from Pietrain founder parents was generally associated with increased lean and decreased fat as expected for a breed, which has been intensively selected for lean content. This was not the case for the QTL on SSC14 where the Pietrain allele (cryptic) was associated with decreased weight of ham lean meat and higher average fat thickness. It is also surprising to find that Pietrain alleles were associated with decreased lean content at the QTL identified on SSC4.

The present study is the first to report QTL for PAR on SSC9 and SSC10 for different stages of growth. In conjunction with the study by Mohrmann *et al.* (2006a), QTL for PAR have now been detected for all observed growth periods (30-60 kg, SSC1; 60-90 kg, SSC13; 90-120 kg, SSC1 and SSC10; 120-140 kg, SSC9). Novel QTL for LAR from 60 to 90 kg was identified on SSC8. A further QTL for LAR was identified on SSC9 around the same location as the QTL for PAR for the same growth period (120-140 kg). The QTL for PAR and LAR on SSC9 were identified around numerous QTL for lean

and fat tissue (Figure 2.2) where the Pietrain breed is associated with increased leanness and reduced fatness. Therefore, the reason for these QTL for PAR and LAR is likely to be change in body composition. The QTL for PAR on SSC10 was located around no other QTL than for DG, suggesting that the reason for this QTL is likely to be growth rate *per se.* In contrast to the QTL on SSC9 for LAR which was identified around QTL for subcutaneous fat, the QTL on SSC8 for LAR was positioned at the same location as the QTL for IMF detected in this study (Figure 2.3). QTL have been previously reported in this region for fat tissue, growth, weight gain and carcass weight (cited in Table 2.4). Additionally, this study is the first to report QTL for protein and lipid content on SSC9. In a previous study by Mohrmann *et al.* (2006a), QTL for protein and lipid content at early stages of growth (30, 60, and 90 kg) were detected in a different genomic region (SSC6). As QTL for chemical body composition and accretion rates were identified in different genomic regions for different growth stages, it is likely that these components are regulated by more than one genomic region and regulated differently throughout growth.

QTL for FCR, DFI and DG were identified in the present study on SSC2, SSC4, SSC9 and SSC10. The QTL for FCR identified on SSC2 is probably caused by a change in body composition, because it is positionally associated with QTL in which the Pietrain allele resulted in an increase in lean tissue and a decrease in fat tissue, and in this region, no growth QTL were detected (Figure 2.4). In contrast, the QTL for FCR from 60 to 90 kg on SSC13 identified in the previous study by Mohrmann *et al.* (2006a) is probably caused by a QTL associated with protein accretion, which was located at the same chromosomal position. No reports confirm the QTL identified in the present study for FCR on SSC4, although QTL have been reported in a different region of SSC4 for food consumption and FCR (Cepica *et al.* 2003a). QTL for early body weight and weight gain have been reported in the same genomic region as the QTL identified in the present study for DFI on SSC2 (cited in Table 2.4). QTL have not been reported for DFI in this region, however significant and suggestive QTL for this trait have been found in two other genomic regions by Houston *et al.* (2005), one of which was located at the same

position as a QTL for DG reported by Lee *et al.* (2003a). No QTL have been reported confirming the QTL for DFI on SSC10; however, a QTL has been reported for daily gain in a similar region (cited in Table 2.4). At these QTL in the present study, Pietrain alleles are associated with lower DFI and heterozygous animals are associated with higher DFI. This is likely to be a result of long-term selection of the Pietrain breed for increased lean content and reduced backfat, known to have an unfavourable genetic association with feed intake (e.g. Roehe *et al.* 2003). At the QTL for DG on SSC9, heterozygous animals showed significantly higher daily gain (87 g/day) due to dominance, which is important because growth of purebred Pietrain are often restricted due to limited feed intake capacity (Roehe 2006). In contrast, the QTL identified on SSC10 for DG showed additive effects where Pietrain alleles are associated with decreased DG.

In the present study, QTL for physical body composition traits were identified at the slaughter weight of 140 kg body weight, which is higher than would occur within commercial pig production. In this study animals were grown to a higher body weight in order to investigate QTL affecting growth traits beyond commercial slaughter weight. The QTL identified in the present study for physical body composition were in most cases confirmed by reports in the literature from animals slaughtered at lower commercial weights and therefore the QTL identified in the present study are of interest for breeding purposes.

A large number of QTL with significant imprinting effects were identified in the present study, of which some QTL were not detected using an additive and dominance model. For several lean and fat tissue traits, QTL expressing maternal imprinting (paternal expression) were identified in the same region of SSC2 where an imprinted QTL has been mapped to the paternally expressed *IGF2* locus (Nezer *et al.* 1999). Therefore the *IGF2* locus is the most probable candidate for the effects detected in this study. A maternally expressed QTL for early growth has been detected close to the QTL showing maternal imprinting effects identified in the present study on SSC8 for lean tissue

characteristics (de Koning *et al.* 2001a). Milan *et al*. (2002) reported a QTL on SSC9 expressing imprinting for (ham + loin)% near the QTL showing maternal imprinting in the present study for carcass characteristics, lean tissue, fat tissue, LAR and DG. Additionally on SSC9, QTL with imprinting effects for live weight, average daily gain and belly weight have been reported in the same region as the QTL for lean content showing maternal imprinting identified in the present study (Milan *et al.* 2002; Quintanilla *et al.* 2002). de Koning *et al*. (2001a) found a paternally expressed QTL for early growth rate on SSC10 in the same region as the QTL showing maternal imprinting detected in the present study for tail weight and shoulder lean meat weight. Thomsen *et al*. (2004) reported maternally expressed QTL on SSC10 for fat tissue and meat quality traits and a paternally expressed QTL for lean tissue in the same region as the paternally expressed QTL detected in the present study for external loin fat weight. On SSC14, a paternally expressed QTL for growth was detected by de Koning *et al*. (2001a) close to the QTL detected in the present study showing paternal imprinting effects for carcass, lean tissue and fat tissue characteristics. Additionally, Rohrer *et al*. (2005) identified paternally expressed QTL in this region for meat quality traits. Therefore, imprinting effects are likely to play an important role in the regulation of physical and chemical body composition.

In the present study a large number of QTL with significant imprinting effects were identified. To date, there is limited evidence of imprinting effects in pigs and therefore many of the imprinting effects identified in the present study have not been reported before. On the other hand, there is a lot of evidence for imprinting effects in humans (see geneimprint, www.geneimprint.com). Investigation of the comparative regions of the human genome revealed imprinting effects which provide support for the imprinted QTL of the present study. Regions of human chromosomes 5 and 11 correspond to the region of SSC2 where QTL with significant maternal imprinting (paternal expression) effects were identified in the present study. Genes in these corresponding regions also show maternal imprinting, including *Transmembrane protein 157* (Luedi *et al.* 2007) on human chromosome 5 and *Insulin-like growth factor 2*, *Insulin-like growth factor 2*

antisense, Insulin and *Transient receptor potential cation channel, subfamily M, member 5* on human chromosome 11 (Chao and D'Amore 2008, Prawitt *et al.* 2000). There is evidence for genes on human chromosome 8 which show paternal imprinting (maternal expression) including *Potassium channel, subfamily K, member 9* and *Glutamic-pyruvate transaminase* (Luedi *et al.* 2007). This region of human chromosome 8 is comparative to the region of SSC4 where a QTL was identified for thick rib showing paternal imprinting effects in the present study. Maternally imprinted genes have been identidied on human chromosome 11, including *Kelch repeat and BTB domain containing 3* (Luedi *et al.* 2007) and *Succinate dehydrogenase complex, subunit D, integral membrane protein* (Hensen *et al.* 2004) which is comparative to the region of SSC9 where maternal imprinting effects were identified for lean and fat tissue. Furthermore, there is evidence for a maternally imprinted gene on human chromosome 4 (*Spondin 2, extracellular matrix protein*) (Luedi *et al.* 2007) which is comaparative to the region of SSC8 where a maternally imprinted QTL has been identified in the present study for lean tissue.

An important point to note is that in many cases the dominance and imprinting effects identified in the present study are higher than the additive effect and therefore the results should be interpreted with care. Therefore, these effects need to be confirmed in further studies.

Chapter 3

Genomic scan for quantitative trait loci of chemical and physical body composition and deposition on pig chromosome X considering the pseudoautosomal region

Abstract

QTL analysis of pig chromosome X (SSCX) was carried out using a methodology which accounts accurately for the features of sex chromosomes such as their heterogeneity, pseudoautosomal region and dosage compensation phenomenon. A three-generation full-sib population of 386 animals was created by crossing Pietrain sires with a crossbred dam line. Phenotypic data for 72 traits were available for at least 292 and up to 315 F_2 animals, for chemical body composition measured in live animals at five target weights from 30 to 140 kg, daily gain and feed intake measured throughout growth, and carcass characteristics obtained at slaughter weight (140 kg body weight). In the pseudoautosomal region, QTL were identified for entire loin weight, which showed paternal imprinting. A suggestive QTL for feed intake was detected closely linked to *SW2456*, at which Pietrain alleles were associated with higher feed intake. This is unexpected for a breed known for its low feed intake capacity. At the telomeric end of the q arm of SSCX, QTL were identified for jowl weight and lipid accretion. Furthermore, suggestive QTL for chemical body composition at 30 kg body weight were identified. The results indicate that SSCX is important for physical and chemical body composition and accretion as well as feed intake regulation.

3.1 Introduction

To understand the genetic control of economically important traits in pigs, a large number of studies have investigated quantitative trait loci (QTL) that contribute to variation in these traits (e.g. Rohrer and Keele 1998ab; Milan *et al.* 2002; Geldermann *et al.* 2003). Most QTL have been identified on autosomes with fewer QTL reported on the sex chromosomes. One reason may be that the sex chromosomes are of less importance for the genomic regulation of these traits. Another likely reason may be the previous lack of available software to model the specific features of the sex chromosome more appropriately. These features are due to the fact that the mammalian X chromosome is considerably larger than the Y and richer in gene content (Graves 2006; Graves *et al.* 2006). In humans, for example, there are 1 250 known genes on the X chromosome and only 147 on the Y chromosome (Hubbard *et al.* 2007). As a result, female cells, which carry two copies of the X chromosome, contain twice as many X-linked genes than males. Mammals have developed a mechanism to equal the dosage of the X chromosome gene products between sexes, called the dosage compensation phenomenon (Alberts *et al.* 2002; Heard and Disteche 2006). Furthermore, there is limited homology between chromosomes X and Y, except for a small pseudoautosomal region (Graves *et al.* 1998).

Due to previous unavailability of QTL mapping software accounting for the features of chromosome X, most studies have adopted a regression based approach analysing males and females separately, which decreases the power to detect QTL (e.g. Knott *et al.* 1998; de Koning *et al.* 2001a; Geldermann *et al.* 2003). Recently, Perez-Enciso and Misztal (2004) developed software using mixed model methodology and maximum likelihood approach, which enables modelling of the specific features of the X chromosome in a QTL analysis.

Therefore, the aim of the present study was to investigate QTL on pig chromosome X (SSCX) for chemical and physical body composition and deposition using a

methodology which accounts accurately for the features associated with this chromosome.

3.2 Materials and methods

3.2.1 Animal resources

This study was based on data from a three-generation full-sib design, developed from crossing seven unrelated Pietrain grandsires, all heterozygous (Nn) at the *ryanodine receptor 1 (RYR1)* locus, to 16 unrelated grand-dams from a three-way cross of Leicoma boars with Landrace x Large White dams. From the F_1 generation, eight boars were mated to 40 sows to produce the F_2 generation, comprising 315 pigs of 49 families across two litters. Forty eight gilts and 46 barrows of the F_2 generation were housed in straw-bedded pens individually and fed manually with feed consumption recorded weekly. The remaining animals (117 gilts and 104 barrows) were housed in mixed sex groups of up to 15 pigs in straw-bedded pens. Group housed animals were fed with an electronic feeding station (ACEMA 48), which recorded feed consumption at each visit. One of three pelleted diets were provided for weight ranges 30-60, 60-90 and 90-140 kg body weight, containing 13.8 MJ ME/kg and 1.2% lysine, 13.8 MJ ME/kg and 1.1% lysine, or 13.4 MJ ME/kg and 1.0% lysine, respectively. Maximal protein deposition was reached by providing pigs with *ad libitum* access to diets, which were formulated slightly above requirement. For a more detailed description of the project management see Landgraf *et al*. (2006ab) and Mohrmann *et al*. (2006ab).

3.2.2 Physical body composition

Pigs were slaughtered at 140 kg body weight in a commercial abattoir. Phenotypic measurements of 37 traits relating to physical body composition were collected by two methods, the AutoFOM device and dissection. The AutoFOM device used an automatic ultrasound scanning technique to produce a three-dimensional image of the pig (Brondum *et al.* 1998). Using this device, measurements of valuable carcass cuts were obtained, including average fat thickness, belly weight, lean content, lean content of the belly and weights of entire and trimmed shoulder, loin and ham without bones. The right side of each carcass was dissected into weights of the primal cuts, neck, shoulder, loin, ham and belly. The former four cuts were dissected into lean and fat tissue. Furthermore, records were obtained for weights of jowl, thick rib, flank, front as well as hind hock, tail and claw. From the cold left carcass side, further measurements were obtained for carcass length; sidefat thickness; fat content and area of the belly; as well as loin eye area, fat area, and thinnest fat measure (fat degree B) measured at the $13^{th}/14^{th}$ rib interface. Further information about the dissection of carcasses is presented by Landgraf *et al.* (2006b).

3.2.3 Chemical body composition

In total, phenotypic information was available for 25 traits relating to chemical body composition and deposition. Protein content of the loin and intramuscular fat content were measured by near-infrared reflectance spectroscopy in the *musculus longissimus thoracis et lumborum.* Using the deuterium dilution technique, an *in vivo* method of determining chemical body composition based on body water; protein, lipid and ash content of the empty body were determined at target body weights of 30, 60, 90, 120, and 140 kg. The accuracy of this technique has been verified in previous studies using magnetic resonance imaging on live animals (Mohrmann *et al.* 2006b) and chemical analysis of serially-slaughtered animals (Landgraf *et al.* 2006a). This method determined

the water content of the empty body, from which the percentage of fat-free substance of the empty body was estimated. Based on the percentage of the fat-free substance, protein and ash content of the empty body were estimated, and lipid content was the deviation of the fat-free content from one. The equations for estimating these chemical components were developed by Landgraf *et al*. (2006a) using the data of the F_1 generation of the three generation full-sib population analysed in the present study. Protein and lipid accretion rates of four stages of growth were calculated as the difference between protein or lipid composition at two consecutive target weights divided by days of growth between the target weights. Furthermore, daily gain, feed intake and food conversion ratio were recorded at different stages of growth. Means and standard deviations of the 72 traits analysed in the present study are presented in Tables 3.1 and 3.2.

Table 3.1 Means and standard deviations (SD) of carcass characteristics measured on pigs of the F_2 generation

Trait	Mean	SD	Number of Records
AutoFOM traits			
AF average fat thickness (mm)	22.295	4.989	313
AF entire shoulder weight (kg)	6.176	0.406	313
AF shoulder lean meat weight (kg)	4.577	0.408	313
AF entire loin weight (kg)	6.265	0.396	313
AF loin lean meat weight (kg)	3.764	0.352	313
AF entire ham weight (kg)	13.573	0.814	313
AF ham lean meat weight (kg)	9.511	1.052	313
AF entire belly weight (kg)	9.168	0.548	313
AF lean content (%)	50.509	6.403	313
AF lean content of belly (%)	43.741	7.891	313
Carcass characteristics – dissected carcass cuts			
Entire neck weight (kg)	5.316	0.505	306
Neck weight without external fat (kg)	4.160	0.430	306
External neck fat weight (kg)	1.156	0.285	306
Entire shoulder weight (kg)	8.452	0.564	307
Shoulder weight without external fat (kg)	5.910	0.584	307
External shoulder fat weight (kg)	1.403	0.261	307
Entire loin weight (kg)	9.163	0.730	308
Loin weight without external fat (kg)	6.650	0.624	308
External loin fat weight (kg)	2.513	0.645	308
Entire ham weight (kg)	16.908	0.997	310
Ham weight without external fat (kg)	11.568	1.087	310
External ham fat weight (kg)	2.566	0.493	310
Belly weight (kg)	6.461	0.655	308
Jowl weight (kg)	1.914	0.284	306
Thick rib (kg)	1.441	0.217	307
Flank weight (kg)	1.789	0.407	308
Front hock weight (kg)	1.139	0.189	307
Hind hock weight (kg)	1.430	0.141	310
Tail weight (kg)	0.429	0.134	310
Hind claw (kg)	0.914	0.122	310
Carcass characteristics – standard performance test			
Carcass length (cm)	107.947	49.296	310
Sidefat thickness[1] (cm)	3.847	0.866	315
Thinnest fat measure[1] (cm)	1.725	0.552	314
Loin eye area *M.l.t.l.*[1,2] (cm^2)	54.160	6.767	314
Fat area *M.l.t.l.*[1,2] (cm^2)	24.514	5.884	314
Fat content of belly (%)	53.508	8.272	306
Fat area of belly (cm^2)	23.789	6.782	306

[1] collected at the $13^{th}/14^{th}$ rib interface.
[2] measured on *musculus longissimus thoracis et lumborum*.

Table 3.2 Means and standard deviations (SD) of chemical body composition, accretion rates, daily gain, daily feed intake and food conversion ratio measured on pigs of the F_2 generation

Trait	Mean	SD	Number of Records
Chemical body composition			
Intramuscular fat content (%)	1.343	0.542	313
Protein content of loin (%)	24.215	2.066	313
Protein content of FFS, 30 kg (%)	18.656	0.524	299
Protein content of FFS, 60 kg (%)	20.115	0.419	305
Protein content of FFS, 90 kg (%)	21.209	0.426	311
Protein content of FFS, 120 kg (%)	21.960	0.506	302
Protein content of FFS, 140 kg (%)	22.359	0.543	302
Protein content of empty body, 30 kg (%)	16.643	0.065	310
Protein content of empty body, 60 kg (%)	16.477	0.047	305
Protein content of empty body, 90 kg (%)	16.359	0.045	311
Protein content of empty body, 120 kg (%)	16.282	0.051	302
Protein content of empty body, 140 kg (%)	16.242	0.053	302
Lipid content of empty body, 30 kg (%)	10.845	2.920	310
Lipid content of empty body, 60 kg (%)	18.045	2.000	305
Lipid content of empty body, 90 kg (%)	22.832	1.773	311
Lipid content of empty body, 120 kg (%)	25.813	1.926	302
Lipid content of empty body, 140 kg (%)	27.308	1.987	302
Chemical accretion rates			
DG, 30-60 kg (kg/day)	0.677	0.114	315
DG, 60-90 kg (kg/day)	0.838	0.138	312
DG, 90-120 kg (kg/day)	0.779	0.140	313
DG, 120-140 kg (kg/day)	0.718	0.193	313
PAR, 30- 60 kg (kg/day)	0.110	0.018	300
PAR, 60-90 kg (kg/day)	0.135	0.023	300
PAR, 90-120 kg (kg/day)	0.125	0.022	299
PAR, 120-140 kg (kg/day)	0.115	0.031	292
LAR, 30-60 kg (kg/day	0.168	0.040	300
LAR, 60-90 kg (kg/day)	0.271	0.060	300
LAR, 90-120 kg (kg/day)	0.274	0.069	301
LAR, 120-140 kg (kg/day)	0.267	0.099	293
Feed intake and food conversion traits			
DFI 60-90 kg (kg/day)	2.467	0.361	312
DFI 90-120 kg (kg/day)	2.818	0.376	313
DFI 120-140 kg (kg/day)	2.815	0.496	313
FCR 60-90 kg (kg feed/kg gain)	2.975	0.379	312
FCR 90-120 kg (kg feed/kg gain)	3.678	0.517	313
FCR 120-140 kg (kg feed/kg gain)	4.214	1.975	313

Definition of symbols: FFS, fat free substance; DG, daily gain; PAR, protein accretion rate; LAR, lipid accretion rate; DFI, daily feed intake; FCR, food conversion ratio.

3.2.4 Genotypic data

Blood samples were collected from F_0, F_1 and F_2 animals from the *vena jugularis* and DNA was isolated. SSCX was chosen for genotyping because of the likely associations with lean and fat carcass characteristics, indicated by reports in the literature (e.g. Rohrer and Keele 1998ab; Bidanel *et al.* 2001; Milan *et al.* 2002; Geldermann *et al.* 2003; Perez-Enciso *et al.* 2005). All animals were genotyped for eight informative microsatellite markers. Markers and their distances were taken from the published USDA linkage map (http://www.marc.usda.gov; Rohrer *et al.* 1996), which provided all information relating to their positions and alleles (Table 3.3). The average distance between markers was 18.3 cM and the largest gap was 22.4 cM.

Table 3.3 Markers used in the present QTL mapping project, their relative map position based on the USDA pig map, number of different alleles, heterozygosity in F_1 generation (H) and polymorphic information content in the F_2 generation (PIC)

Marker	Position (cM)	Number of alleles	H	PIC
SW949	0.0	6	0.65	0.53
SW980	11.9	7	0.87	0.80
SW1903	33.0	5	0.87	0.70
SW2456	55.4	6	0.81	0.67
SW259	74.4	5	0.89	0.70
SW1943	87.4	5	0.70	0.70
SW707	107.9	4	0.49	0.59
SW2588	128.4	4	0.25	0.37

3.2.5 Statistical analysis

QTL mapping was carried out using the software QxPak version 2.16 (Perez-Enciso and Misztal 2004). This program uses mixed models and the maximum likelihood method to estimate the QTL location and effects. The analysis of QxPak proceeds in two main stages. In the first stage the probabilities of alleles being identical-by-descent are calculated using a Monte Carlo Markov Chain algorithm. In the second stage, the mixed model equations are built and the QTL estimates are obtained using a maximum likelihood approach via the expectation-maximisation algorithm. At each putative position the likelihood ratio is computed and the estimates for the parameters are those where the likelihood is highest. In this analysis the significance is tested with a likelihood ratio test which consists of computing minus twice the difference in log-likelihoods between the alternative and the null models (Perez-Enciso and Misztal 2004).

A fixed effects model was chosen for the QTL analysis, estimating additive and dominance effects. In cases where the dominance effect was not significant, the analysis was repeated with an additive only model. Maternal and paternal imprinting were tested for only in the pseudoautosomal region. In this analysis, the additive estimate is defined as half of the difference between animals homozygous for alleles from the grandpaternal sire line and those homozygous for alleles from the grandmaternal dam line. A positive additive genetic value indicates that the allele originating from the grandpaternal sire line (Pietrain) showed an increasing QTL effect compared to the allele from the grandmaternal dam line and *vice versa*. The dominance effect is defined as the deviation of heterozygous animals from the mean of both types of homozygous animals. In this analysis imprinting tested with models where only the allele of paternal origin is expressed (maternal imprinting) and only the allele of the maternal origin is expressed (paternal imprinting) i.e. setting the maternal or paternal coefficients to zero. The individual QTL analysis was applied with the following models:

Carcass characteristics measured at slaughter:

$$y_i = sex_i + MHS_i + batch_i + \beta slwt_i + C_a a + C_d d + e_i,$$ [1]

Chemical body composition at each target weight:

$$y_i = sex_i + MHS_i + batch_i + \beta wt_i + C_a a + C_d d + e_i,$$ [2]

Chemical accretion rates (protein and lipid accretion):

$$y_i = sex_i + MHS_i + batch_i + \beta stwt_i + \beta endwt_i + C_a a + C_d d + e_i,$$ [3]

Feed intake and food conversion ratio:

$$y_i = sex_i + MHS_i + batch_i + ht_i + \beta stwt_i + \beta endwt_i + C_a a + C_d d + e_i,$$ [4]

where y_i is the i-th individual phenotype. Fixed effects and covariates were fitted in the models depending on their significance for the trait. *Sex*, *RYR1* (*MHS*) genotype and *batch* were included in the model for all traits. In addition, housing (*ht: housing type)* was included as a fixed effect for feed intake and food conversion ratio traits. Body weight at slaughter (*slwt*) was fitted in the model as a covariate for carcass characteristics measured at slaughter. For chemical body composition traits measured at different target weights, body weight at that target weight (*wt*) was fitted in the model as a covariate. Protein and lipid accretion, daily gain, feed intake and food conversion ratio were adjusted for the small differences between target and actual body weight at the start (*stwt*) and end (*endwt*) of the considered weight range. The additive (*a*) and dominance (*d*) effects were estimated by consideration of the coefficients of C_a and C_d, respectively. The coefficient C_a was calculated for each individual and position as the probability of the individual being homozygous for alleles of the grandpaternal sire line (QQ) minus the probability of pigs being homozygous for alleles from the grandmaternal dam line (qq). The coefficient C_d is the probability of the individual being at the chromosomal position heterozygous (Qq).

The analysis provides the likelihood ratios under the models tested and associated nominal P-values. A previous study by Perez-Enciso *et al.* (2000) showed that nominal P-values 0.005 and 0.001 correspond to 5% and 1% chromosome-wide significant P-values, respectively, based on the chi-squared distribution with two degrees of freedom. Therefore, in the present study, nominal P-values <0.001, 0.005 and 0.01 were treated as significant at the 1%, 5% and suggestive at the 10% chromosome-wide level, respectively. Table 3.3 provides information for the used markers and their positions, number of alleles, heterozygosity in the F_1 generation and polymorphic information content in the F_2 generation.

3.2.6 Methodology

The mixed model methodology applied in this study includes all pedigree information and uses the maximum likelihood method to estimate the QTL effects. The flexibility of the methodology allowed for the consideration of the pseudoautosomal region and to account for the heterogeneity and dosage compensation phenomenon of sex chromosomes. The methodology applied here is described in Perez-Enciso *et al.* (2002) and implemented in the programme QxPak.

In more detail, the main issues relate to the modelling of the mammalian dosage compensation and computing the p_s, ρ_{sA} and ρ_{sB} coefficients. p_{si} is the average probability for the ith individual of a gene within segment s being of breed A origin. $\rho_{sA(i,i')}$, is the probability of individuals i and i' having received identical-by-descent (IBD) alleles of breed A, and $\rho_{sB(i,i')}$ is the probability of individuals i and i' having received identical-by-descent (IBD) alleles of breed B.

In the differential region of the X chromosome (the non-pseudoautosomal region) the male phenotype is expressed as:

$$\gamma_M = \mu_M + g^2 + e,$$

and the female phenotype as:

$$\gamma_F = \mu_F + \psi^1 g^1 + \psi^2 g^2 + d_{g1,g2} + e,$$

μ is sex mean; g^i, the genetic origin, indicates the haplotype origin, 1 male and 2 female; ψ^h is the dosage compensation effect for hth haplotype allele effect and d is the dominance interaction. Interaction between alleles (dominance) can only be estimated in females in this case. The allele which contributes to the phenotype of the male is always of the mothers origin (g^2). Parameters ψ^1 and ψ^2 should always add up to 1.

The genetic covariances between two crossed individuals are calculated as:

if i and i' are both males

$$Cov(g_i, g_{i'}) = \Pr(g_i^2 \equiv g_{i'}^2 \in A)\sigma_{Ag}^2 + \Pr(g_i^2 \equiv g_{i'}^2 \in B)\sigma_{Bg},$$

if i is a male and i' is a female

$$Cov(g_i, g_{i'}) = \sum_{h=1}^{2} \psi^h [\Pr(g_i^2 \equiv g_{i'}^h \in A)\sigma_{Ag}^2 + \Pr(g_i^2 \equiv g_i^h \in B)\sigma_{Bg}^2],$$

if i and i' are both females

$$Cov(g_i, g_{i'}) = \sum_{h=1}^{2} \sum_{h'=1}^{2} \psi^h \psi^{h'} [\Pr g_i^h \equiv g_i^{h'} \in A)\sigma_{Ag}^2 + \Pr(g_i^h \equiv g_{i'}^{h'} \in B)\sigma_{Bg}^2],$$

where, $\Pr(g_i^h \equiv g_{i'}^{h'} \in A)$ is the probability of alleles g_i^h and $g_{i'}^{h'}$ being IBD and of breed origin A, and $\Pr(g_i^h \equiv g_{i'}^{h'} \in B)$ is the probability of alleles g_i^h and $g_{i'}^{h'}$ being IBD and

of breed origin B. σ_{Ag}^2 is the variance of the gene effects in breed A and σ_{Bg}^2 is the variance of the gene effects in breed B. We define $p_{gi} = \Pr(g_i^2 \in A)$ when i is a male and $p_{gi} = \sum_{h=1}^{2} \psi^h \Pr(g_i^h \in A)$ a female.

In addition the pseudoautosomal region of the X and Y chromosomes has been considered in which males only recombine (Perez-Enciso *et al.* 2002).

3.3 Results

From the genomic analysis, three significant QTL and five suggestive QTL were identified for carcass cuts, lean tissue characteristics, chemical body composition and deposition as well as daily feed intake. The additive and dominance effects of these QTL are presented in Table 3.4. Two QTL were identified with imprinting effects in the pseudoautosomal region which is shown in Table 3.5.

Table 3.4 Evidence for quantitative trait loci (QTL) for carcass cuts, chemical body composition, lipid accretion and feed intake on pig chromosome X

Trait	LR	Pos[1]	% Var[2]	a ± SE[3]	d ± SE[3]
Carcass characteristics – dissected carcass cuts					
Entire loin weight (g)	11.56**	7	3.7	**283.8 ± 82.7**	-
Loin weight without external fat (g)	7.68[a]	11	2.5	**184.5 ± 66.1**	-
Jowl weight (g)	17.91**	128.4	5.8	57.4 ± 37.6	**216.9 ± 74.3**
Chemical body composition and accretion rates					
Lipid cont. empty body, 30 kg (%)	10.38[a]	83	3.3	-0.496 ± 0.259	**1.705 ± 0.532**
Protein cont. empty body, 30 kg (%)	10.43[a]	83	3.8	0.011 ± 0.006	**-0.038 ± 0.012**
Protein cont. FFS$_{EB}$, 30 kg (%)	9.36[a]	82	3.1	**-0.093 ± 0.046**	**0.285 ± 0.095**
LAR 90-120 g (g/day)	9.60*	128.4	3.2	**26.7 ± 8.5**	-
Daily gain, feed intake and food conversion ratio					
DFI 120-140 g (g/day)	7.00[a]	56	2.3	**101.6 ± 38.2**	-

LR, likelihood ratio; FFS$_{EB}$, fat-free substance of the empty body; LAR, lipid accretion rate; DFI, daily feed intake.

[1]Positions of the QTL in cM based on the USDA reference map.

[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no QTL effect - residual variance of model with QTL effect)/residual variance of model with no QTL effect.

[3]Estimated additive (a) and dominance (d) effects and their standard errors (SE).

Values in bold represent significant additive or dominance effects.

[a] implies suggestive at the 10% chromosome-wide level.

* and ** implies significance at the 5%, or 1% chromosome-wide levels, respectively.

Table 3.5. Evidence for quantitative trait loci (QTL) associated with imprinting effects in the pseudoautosomal region

Trait	LR	Pos[1]	% Var[2]	a ± SE[3]	Imprinting
Carcass characteristics (lean and fat)					
Entire loin weight (g)	10.07*	6	3.2	**133.3 ± 41.6**	Paternal
Neck weight without external fat[4] (g)	8.85*	1	2.8	**374.0 ± 124.8**	Maternal

LR, likelihood ratio.
[1]Positions of the QTL in cM based on the USDA reference map.
[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no QTL effect - residual variance of model with QTL effect)/residual variance of model with no QTL effect.
[3]Estimated additive (a) effects and their standard errors (SE).
[4]New QTL only identified when the imprinting effect is included in the model.
Values in bold represent significant additive effects.
* QTL significant at the 5% chromosome-wide level.

3.3.1 Carcass characteristics

A QTL significant at the 1% chromosome-wide level was identified for entire loin weight in the pseudoautosomal region of SSCX at 7 cM between *SW949* and *SW980*, explaining 3.7% of the phenotypic variance. The significant additive effect at this QTL indicates that the grandpaternal Pietrain breed is associated with 284 g higher loin weight. In a similar location within the pseudoautosomal region at 11 cM a suggestive QTL was identified for loin weight without external fat explaining 2.5% of the phenotypic variance (Figure 3.1). The significant additive effect at this QTL indicates that the grandpaternal Pietrain breed is associated with 185 g higher lean meat weight of the loin cut.
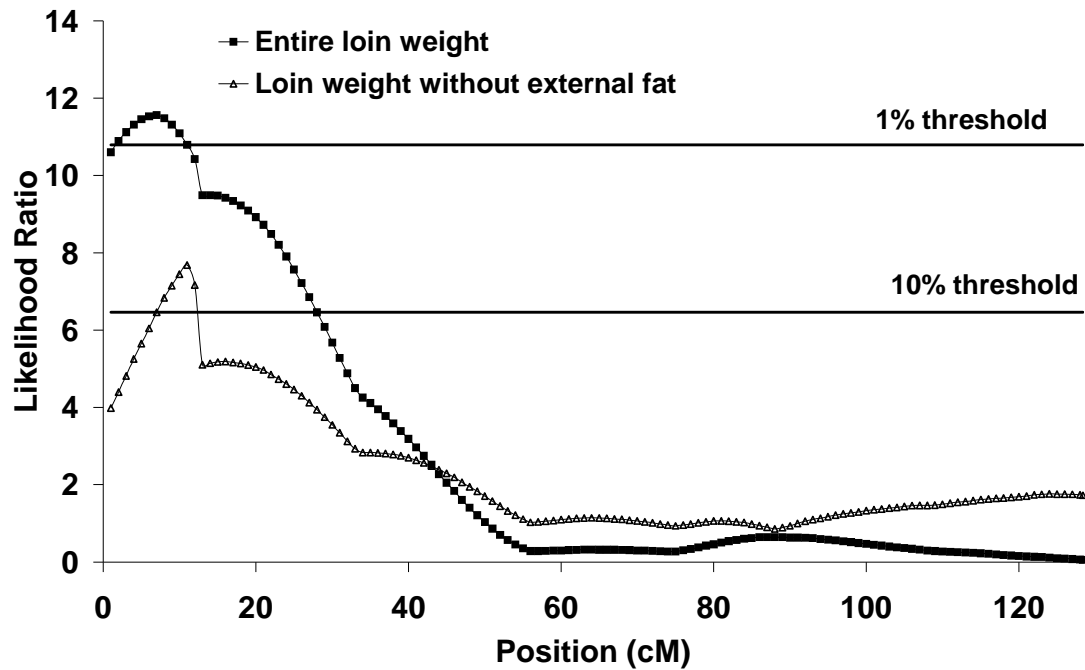
Figure 3.1
Likelihood ratio curves for evidence of quantitative trait loci for entire loin weight and loin lean meat weight in the pseudoautosomal region of SSCX. Horizontal lines indicate chromosome-wide significance levels.

At the telomeric end of the q arm of SSCX, at the same location as *SW2588* (128.4 cM), a QTL significant at the 1% chromosome-wide level was identified for jowl weight accounting for 5.8% of the phenotypic variance. The significant dominance effect at this QTL indicates that heterozygous animals are associated with 217 g higher jowl weight.

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3.3.2 Chemical body composition and accretion

At the telomeric end of the q arm of SSCX, in the same location as the QTL for jowl weight and *SW2588* (128.4 cM), a QTL significant at the 5% chromosome-wide level was identified for lipid accretion rate during the growth period from 90-120 kg (Figure 3.2). This QTL accounts for 3.2% of the phenotypic variance and the significant additive effect indicates that the purebred Pietrain breed is associated with 27 g higher lipid accretion rate at this growth period.
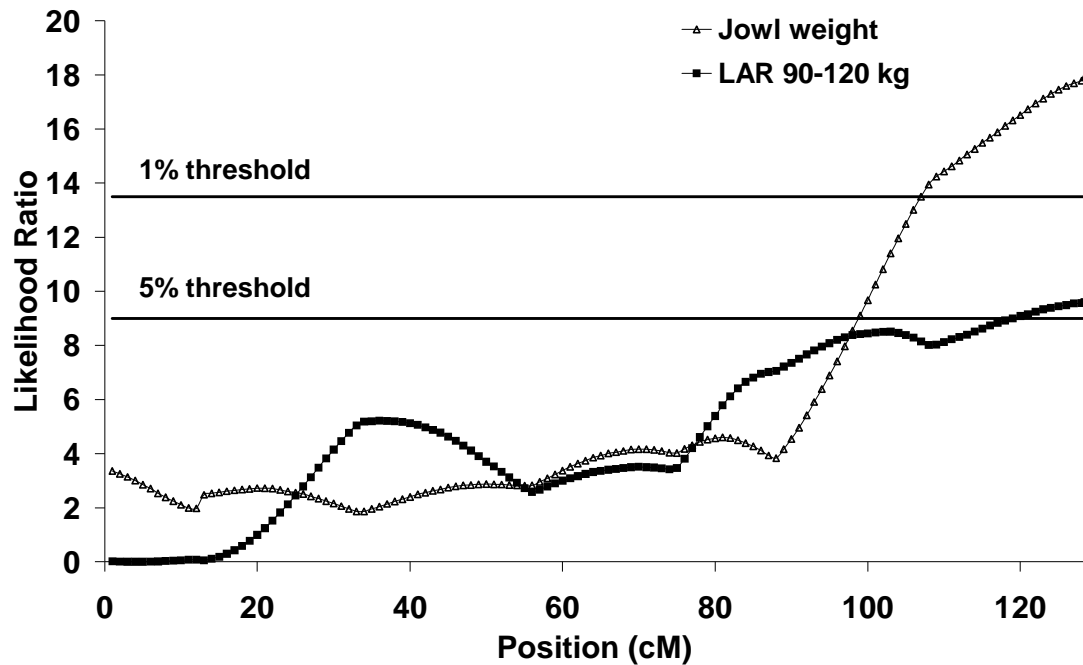
Figure 3.2
Likelihood ratio curves for evidence of quantitative trait loci for lipid accretion rate (LAR) 90-120 kg and jowl weight at the telomeric end of the q arm of SSCX. Horizontal lines indicate chromosome-wide significance levels.

Suggestive QTL for protein content of the fat-free substance and protein and lipid content of the empty body were identified between *SW259* and *SW1943* at 82-83 cM explaining 3.1%, 3.8% and 3.3% of the phenotypic variance, respectively (Figure 3.3). These traits showed similar likelihood ratio profiles as they are closely correlated. At these QTL, Pietrain alleles are associated with decreased additive genetic effects of protein content of the fat-free substance and heterozygous animals showed dominance effects associated with increased lipid content of the empty body, decreased protein content of the empty body and increased protein content of the fat-free substance at 30 kg body weight.
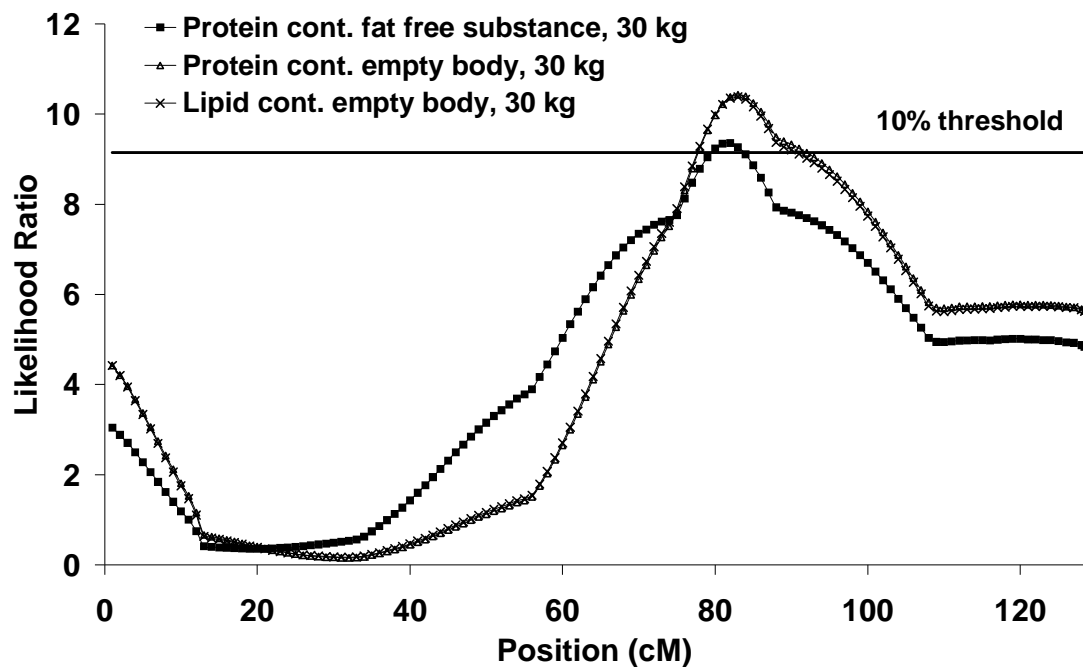
Figure 3.3
Likelihood ratio curve for evidence of quantitative trait loci for chemical body composition at 30 kg body weight. The horizontal line indicates the chromosome-wide significance level.

3.3.3 Daily gain, feed intake and food conversion ratio

A single suggestive QTL was identified for daily feed intake at a late stage of growth (120-140 kg) in a region of SSCX (56 cM) where no other QTL were identified (Figure 3.4). This QTL accounts for 2.3% of the phenotypic variance and the significant additive effect indicates that Pietrain alleles are associated with 102 g/day higher feed intake at this stage of growth.
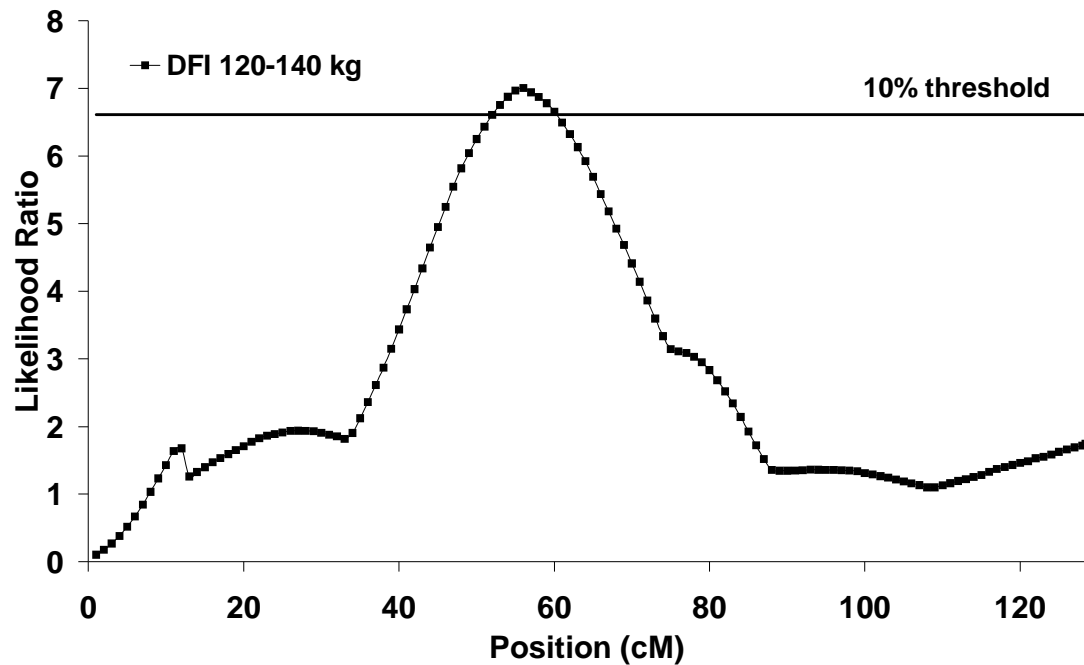
Figure 3.4
Likelihood ratio curve for evidence of quantitative trait loci for daily feed intake at 120-140 kg on SSCX. The horizontal line indicates the chromosome-wide significance level.

3.3.4 Imprinting in the pseudoautosomal region

Two QTL with significant imprinting effects were identified in the pseudoautosomal region (Table 3.5). At 6 cM significant paternal imprinting effects were identified for entire loin weight, indicating that only the maternal allele is expressed at this QTL. A QTL with significant maternal imprinting effects was identified at 1 cM for neck weight without external fat, indicating that only the paternal allele is expressed at this QTL. This QTL for neck weight without external fat was only identified when imprinting was considered in the analysis.

3.4 Discussion

The aim of the present study was to investigate QTL on pig chromosome X for traits of carcass characteristics, chemical and physical body composition and accretion rates as well as daily gain, feed intake and food conversion ratio considering the specific features of the sex chromosomes. There is published evidence in the literature for QTL on pig chromosome X for carcass characteristics, lean tissue, growth and fatness (e.g. Rohrer and Keele 1998ab; Bidanel *et al.* 2001; Milan *et al.* 2002; Cepica *et al.* 2003b; Perez-Enciso *et al.* 2005). In particular, the study by Milan *et al*. (2002) reported QTL on SSCX with the largest effects for leanness and fatness traits based on a cross between the French Large White and the Meishan breeds. In the present study, QTL were identified on SSCX for carcass characteristics (entire carcass cuts and lean tissue), chemical body composition, lipid accretion as well as feed intake. The QTL analysis is based on animals of the F_2 full-sib design of crosses between Pietrain boars and crossbred commercial dams in order to reflect the commercial product of growing-finishing pigs. Therefore, the dam founder QTL alleles may not in all cases be fixed which has to be considered in the interpretation of the results.

In pigs, the pseudoautosomal region lies at the telomeric end of the p arm of SSCX and is a short section (~ 11 cM) which is homologous to the Y chromosome. In the present study this region showed important associations with entire loin weight and lean meat of the loin cut (Figure 3.1). The purebred Pietrain breed is associated with higher loin weight (284 g) and lean meat weight of the loin cut (185 g). Within the pseudoautosomal region of SSCX, QTL for entire carcass cuts and lean tissue are also reported in the literature (Cepica *et al.* 2003b; Geldermann *et al.* 2003; Perez-Enciso *et al.* 2005). From previous genomic analysis of autosomes using the same phenotypic data as the present study (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapter 2), Pietrain alleles of QTL on SSC2, SSC6, SSC8, SSC9 and SSC13 are also associated with increased weights of carcass cuts and lean tissue. However, this was not the case on SSC14 where the Pietrain allele was associated with decreased weights of these characteristics, suggesting a cryptic gene in a breed selected over a long period for leanness. Pseudoautosomal regions also exist in other mammals; however the length and gene content seem to be variable, such that the mouse and human pseudoautosomal regions are completely non-homologous. This region was considered to have an important role in meiotic pairing and male fertility, however the inconsistent gene contents of this region across species and the absence of this region in marsupials, suggests that this region may not be important for fertility in mammals (Graves *et al.* 1998). The results of the present study confirm that this region in pigs contains genes attributing to carcass characteristics.

In regions of SSCX other than the pseudoautosomal region, there are reports of QTL for lean tissue characteristics (e.g. Rohrer and Keele 1998b; Milan *et al.* 2002; Geldermann *et al.* 2003). In the present study no QTL for lean tissue were identified. This may be because the favourable lean tissue alleles may already be fixed in the populations of this study. It is also surprising that no QTL were identified in the present study for fatness as there are numerous reports in the literature for fatness QTL on SSCX (e.g. Rohrer and Keele 1998a; Bidanel *et al.* 2001; Milan *et al.* 2002; Perez-Enciso *et al.* 2005; Rohrer *et al.* 2005). Most of these studies have been based on crosses of breeds characterised by their high leanness, with the Meishan, Wild Boar or Iberian breeds characterised by their

high fatness. Therefore, the QTL may not be segregating in the population of the present study, which have been selected for leanness over a long-term.

It is likely that the genomic regulation of chemical body components and their accretion is a complex process involving more than one genomic region and regulated differently throughout growth. Measurements of chemical body composition in live animals are expensive. Therefore QTL associated with these traits are limited in the literature to two studies analysing the data of the present population across several autosomes (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapter 2). In the present study, a significant QTL was identified for lipid accretion rate at 90 to 120 kg on SSCX (Figure 3.2). QTL for LAR have only been reported in the study by Duthie *et al*. (2008; Chapter 2) (60-90 kg, SSC8; 120-140 kg, SSC9). Pietrain alleles are associated with increased lipid accretion rate at 60 to 90 kg (SSC8) and 90 to 120 kg (SSCX). A significant dominance effect was identified at the QTL for lipid accretion rate at 120 to 140 kg on SSC9; however no dominance effect was identified on SSCX. In the present study, the QTL for lipid accretion rate was identified in a region of no QTL for fat tissue, unlike the QTL detected from previous analysis, which were identified around numerous QTL for subcutaneous fat (SSC9) and a QTL for intramuscular fat (SSC8). This is surprising as a large number of QTL have been reported in the literature around this region of SSCX for fat tissue traits (Milan *et al.* 2002; Perez-Enciso *et al.* 2002; Rohrer *et al.* 2005). At the same location as the QTL associated with lipid accretion rate a QTL for jowl weight was found (Figure 3.2). Heterozygous animals are associated with higher weight of the jowl cut. A QTL for jowl weight was also detected on SSC1 from previous analysis of the same phenotypic data of this study (Mohrmann *et al.* 2006a). In contrast, at this QTL heterozygous animals are associated with lower jowl weight. Furthermore a significant additive effect indicated that Pietrain alleles were associated with lower jowl weight on SSC1. There are no reports of QTL for this characteristic in the literature.

Suggestive QTL were identified in the present study for chemical body composition at an early growth stage (30 kg body weight), in a region of SSCX where no other QTL

were identified (Figure 3.3). From previous analysis of the data, QTL for chemical body composition for early growth stages were also identified (30 kg, SSC6; 60 kg, SSC6 and SSC9) (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapter 2). At the QTL on SSC6 and SSC9 for chemical body composition at 30 kg and 60 kg, respectively, significant dominance effects indicate that heterozygous animals are associated with decreased protein content of the fat-free substance and lipid content of the empty body, but increased protein content of the empty body. In contrast, on SSCX and SSC6 for chemical body composition at 30 kg and 60 kg, respectively, significant dominance effects indicate that heterozygous animals are associated with increased protein content of the fat-free substance and lipid content of the empty body, and decreased protein content of the empty body. The QTL likelihood ratio profile for protein content of the empty body is almost identical to the QTL for lipid content of the empty body. This is expected for traits changing proportionally in opposite directions. Around these QTL on SSCX for chemical body composition, a large number of QTL have been reported for lean and fat tissue as well as growth (e.g. Milan *et al.* 2002; Cepica *et al.* 2003b; Rohrer *et al.* 2005; Cepica *et al.* 2007). Therefore it is surprising that no QTL were identified in this study for physical body composition traits in this region of SSCX.

Cepica *et al*. (2006) assigned seven genes between markers *SW259* and *SW1943*, within the same region as the QTL for chemical body composition identified in the present study. Based on the location and role, *Acyl-CoA synthetase long-chain 4* gene (*ACSL4*) is a potential positional candidate gene for the QTL for chemical body composition in the present study. *ACSL4* has a key role in the metabolism of fatty acids and thus energy balance (Mercade *et al.* 2006).

A suggestive QTL for daily feed intake for the growth stage 120 to 140 kg was identified in a region of SSCX where no other QTL were identified in the present study (Figure 3.4). In a previous study analysing the data of the present population across several autosomes, significant QTL for daily feed intake were identified for growth periods 60 to 90 kg on SSC6 and SSC10, 90 to 120 kg on SSC6 and 120 to 140 kg on

SSC2 (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapter 2). Pietrain alleles were associated with decreased feed intake at 60 to 90 kg (SSC10), as expected for a breed which has been intensively selected for lean content (Roehe *et al.* 2003; Roehe 2006). However, at the QTL detected on SSCX, Pietrain alleles (cryptic) are associated with 102 g higher feed intake at 120 to 140 kg. This is unexpected, as the Pietrain breed is well known for its low feed intake capacity. Within the same marker bracket (*SW2456-SW259*), Cepica *et al.* (2003b) reported a QTL for food consumption in a population derived from crossing Wild Boar and Meishan breeds.

Imprinting can only be analysed for in the pseudoautosomal region of the X chromosome, where the X and Y chromosomes are homologous. Imprinting analysis is important to achieve a better understanding of the genetic control of important traits and to uncover QTL, which cannot be detected from the analysis considering only additive and dominance effects. In the present study, the QTL for entire loin weight showed paternal imprinting indicating that only the maternal allele is expressed at this QTL. Moreover, a QTL for neck weight without external fat was identified, which was not detected in the analysis without modelling imprinting. At this QTL, maternal imprinting was identified indicating that only the paternal allele is expressed. There is no information in the literature which has reported imprinting within the pseudoautosomal region of SSCX.

The literature is sparse for QTL on chromosome X in other livestock species. In cattle and sheep, no QTL have been reported for similar traits on chromosome X. In cattle, only four QTL have been reported on chromosome X for reproduction and disease resistance traits (Kuhn *et al.* 2003; Zhang *et al.* 2004) and in sheep a single QTL has been reported for parasite resistance (Beraldi *et al.* 2007). There is limited evidence of QTL for production traits in these species and a concentrated effort to detect such QTL in these species is needed. The organization of the sex chromosomes in chickens is different to that of mammals, as females are the heterogametic sex (ZW) and males are the homogametic sex (ZZ) (Ellegren 2000). A large number of QTL for production traits

have been identified on the sex chromosome Z (e.g. Kerje *et al.* 2003; Ikeobi *et al.* 2004; Sasaki *et al.* 2004; Zhou *et al.* 2006).

The results of the present study indicate that pig chromosome X is involved in the genomic regulation of physical and chemical body composition as well as growth and feed intake. Larger numbers of significant QTL were identified from previous analysis of the same data set across several autosomes (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapter 2), indicating that SSCX likely plays a lesser role in the regulation of economically important traits in pig production. The QTL on SSCX did, however, account for similar proportions of the phenotypic variance. In summary, the results of the present study about sex-linked QTL influencing economically important traits, give further insight into their sex-related genomic regulation.

Chapter 4

Epistatic quantitative trait loci affecting chemical body composition and deposition as well as feed intake and feed efficiency throughout the entire growth period of pigs

Abstract

A genomic scan for epistatic QTL was conducted on animals from a three generation full-sib population, created by crossing Pietrain sires with a crossbred dam line. All animals were genotyped for 88 molecular markers covering 10 autosomes. Phenotypic data was available for 315 F_2 animals for chemical body composition measured in live animals, daily gain and feed intake. This study is the first to report epistatic QTL for these traits in pigs. Thirty two significant epistatic QTL pairs were identified contributing to the entire growth period (30 to 140 kg), four of which were between QTL which resided on the same chromosome. Epistatic effects were identified between QTL on all considered chromosomes except SSC9. In this study, different stages of growth were influenced by different pairs of epistatic QTL. The QTL pairs with the highest effect were for daily gain and protein accretion rate at 90 to 120 kg body weight between SSC1 and SSC2. These QTL explained large proportions of the phenotypic variance, at 10.3% and 10.2%, respectively. All types of epistatic effect were identified in this study, with the additive-by-additive effect being the most prevalent. This effect is heritable, providing an opportunity to exploit epistasis within breeding.

4.1 Introduction

Most quantitative trait loci (QTL) mapping methods have focused on identifying the individual QTL effects (additive, dominance and imprinting) in the absence of interactions (epistasis). As a result, the genetic background at other loci has been assumed to have no impact on the phenotypic expression of these QTL (Lander and Botstein 1989; Carlborg and Haley 2004). In the simplest case, epistasis is defined as the interaction between one pair of loci, where the effect of one locus on a particular phenotype depends on the genotype at a second locus (Phillips 1998; Carlborg 2006). However, loci may interact in higher numbers and the interactions may be of different types (Falconer and Mackay 1996). Thus, the contribution of epistasis to the genomic control of complex traits is more complicated to detect than direct, individual gene effects.

Epistasis may cause the individual QTL effects to decrease or even totally cancel each other. QTL displaying this type of epistasis are hard to find using standard mapping and are easily missed (Carlborg 2006). When epistasis is ignored, individual loci could remain undetected and the estimated effects of the detected QTL could be severely biased. Inaccurate QTL estimates can lead to invalid interpretations of the importance of QTL, and to problems of confirmation of QTL in further crosses. When attempts are made to use the QTL, for example in marker assisted selection, this will result in lower response and economic gain than predicted (Carlborg 2006).

In order to gain a more accurate and unbiased understanding of the genetic background of economically important traits, epistatic effects need to be included in QTL mapping studies. Previous studies have already indicated that growth and body composition of pigs is regulated by numerous QTL located throughout the genome (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapters 2 and 3). However, epistasis has been ignored in these studies. The aim of the present study is to investigate epistatic QTL for chemical

body composition traits, growth, feed intake and feed efficiency in a commercial pig population.

4.2 Materials and methods

4.2.1 Design and data

This study was based on data from a resource family created using a three generation full-sib design. The founder generation comprised of seven unrelated Pietrain grandsires, which were all heterozygous (Nn) at the *ryanodine receptor 1* (*RYR1*) locus, which were mated to 16 unrelated grandams from a crossbred dam line (Leicoma × (Landrace × Large White)). From the F_1 generation, whilst avoiding inbreeding, eight boars were mated to 40 sows. The F_2 generation comprised of 315 pigs of 49 families across two litters. From the F_2 generation, 94 animals (49 gilts and 46 barrows) were housed individually in straw-bedded pens and 221 animals (117 gilts and 104 barrows) were housed in straw-bedded pens in groups of up to 15 pigs of mixed sex. Individually housed animals were fed manually with feed consumption recorded weekly. Group housed animals were supplied food by an electronic feeding station (ACEMA 48), which recorded feed consumption at each visit. All animals were provided with one of three diets which contained 13.8 MJ ME/kg and 1.2% lysine, 13.8 MJ ME/kg and 1.1% lysine, or 13.4 MJ ME/kg and 1.0% lysine for weight ranges 30-60 kg, 60-90 kg and 90-140 kg body weight, respectively. Pigs were provided with *ad libitum* access to diets, formulated slightly above requirement, so as to reach maximal protein deposition. The studies of Landgraf *et al.* (2006ab) and Mohrmann *et al.* (2006ab) provide a more detailed description of the management of this project.

4.2.2 Chemical body composition in live animals

At different target body weights throughout growth (30, 60, 90, 120 and 140 kg), protein, lipid and ash content of the empty body was determined using the deuterium dilution technique. This technique is an *in vivo* method of determining chemical body composition based on body water content. Using magnetic resonance imaging on live animals (Mohrmann *et al.* 2006b) and chemical analysis of serially-slaughtered animals (Landgraf *et al.* 2006a), the accuracy of this technique was previously verified. The deuterium dilution technique determined the empty body water content, from which the percentage of fat-free substance of the empty body was estimated. Based on the percentage of the fat-free substance, protein and ash content of the empty body were estimated. Lipid content was the deviation of the fat-free content from one. Using the F_1 data of the full-sib design, the equations for estimating these chemical body components were developed by Landgraf *et al.* (2006a). Protein and lipid accretion rates were calculated as the difference between protein and lipid, respectively, at two consecutive target weights divided by days of growth between these target weights. Means and standard deviations of the traits analysed in the present study are presented in Table 4.1

Table 4.1 Means and standard deviations (SD) of chemical body composition, accretion rates, daily gain, daily feed intake and food conversion ratio measured on pigs of the F_2 generation

Trait	Mean	SD	Number of Records
Chemical body composition			
Protein content of FFS, 30 kg (%)	18.656	0.524	299
Protein content of FFS, 60 kg (%)	20.115	0.419	305
Protein content of FFS, 90 kg (%)	21.209	0.426	311
Protein content of FFS, 120 kg (%)	21.960	0.506	302
Protein content of FFS, 140 kg (%)	22.359	0.543	302
Protein content of empty body, 30 kg (%)	16.643	0.065	310
Protein content of empty body, 60 kg (%)	16.477	0.047	305
Protein content of empty body, 90 kg (%)	16.359	0.045	311
Protein content of empty body, 120 kg (%)	16.282	0.051	302
Protein content of empty body, 140 kg (%)	16.242	0.053	302
Lipid content of empty body, 30 kg (%)	10.845	2.920	310
Lipid content of empty body, 60 kg (%)	18.045	2.000	305
Lipid content of empty body, 90 kg (%)	22.832	1.773	311
Lipid content of empty body, 120 kg (%)	25.813	1.926	302
Lipid content of empty body, 140 kg (%)	27.308	1.987	302
Chemical accretion rates			
PAR, 30- 60 kg (kg/day)	0.110	0.018	300
PAR, 60-90 kg (kg/day)	0.135	0.023	300
PAR, 90-120 kg (kg/day)	0.125	0.022	299
PAR, 120-140 kg (kg/day)	0.115	0.031	292
LAR, 30-60 kg (kg/day	0.168	0.040	300
LAR, 60-90 kg (kg/day)	0.271	0.060	300
LAR, 90-120 kg (kg/day)	0.274	0.069	301
LAR, 120-140 kg (kg/day)	0.267	0.099	293
Daily gain, feed intake and food conversion traits			
DG, 30-60 kg (kg/day)	0.677	0.114	315
DG, 60-90 kg (kg/day)	0.838	0.138	312
DG, 90-120 kg (kg/day)	0.779	0.140	313
DG, 120-140 kg (kg/day)	0.718	0.193	313
DFI 60-90 kg (kg/day)	2.467	0.361	312
DFI 90-120 kg (kg/day)	2.818	0.376	313
DFI 120-140 kg (kg/day)	2.815	0.496	313
FCR 60-90 kg (kg feed/kg gain)	2.975	0.379	312
FCR 90-120 kg (kg feed/kg gain)	3.678	0.517	313
FCR 120-140 kg (kg feed/kg gain)	4.214	1.975	313

Definition of symbols: FFS, fat free substance; PAR, protein accretion rate; LAR, lipid accretion rate; DG, daily gain; DFI, daily feed intake; FCR, food conversion ratio.

4.2.3 Genotypic data

Blood samples were collected from F_0, F_1 and F_2 animals from the *vena jugularis* and their genomic DNA was isolated. Chromosomes SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14 were chosen for genotyping because of their importance for growth and chemical body composition. All animals were genotyped for 88 informative microsatellite markers. Of these genomic markers, 10, 9, 9, 9, 10, 8, 9, 9, 8 and 7 were located on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14, respectively. Genomic markers and their distances were selected from the published USDA linkage map (http://www.marc.usda.gov; Rohrer *et al.* 1996), which provided all information relating to their positions and alleles. The average distance between markers was 16.0, 16.5, 16.3, 20.6, 17.3 18.4, 17.3, 16.0, 18.0 and 17.4 cM and largest gaps between markers were 27.7, 25.2, 26.5, 28.7, 26.2, 23.1, 21.7, 20.8, 24.0 and 23.6 cM on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14, respectively. Information relating to the markers used in the present study as well as their positions and alleles is presented in Table 4.2.

Table 4.2 Markers used in the present QTL mapping project, their relative map position using USDA pig map, number of different alleles, polymorphic information content in the F_2 generation (PIC) and heterozygosity in F_1 generation (H)

Marker	SSC	Position (cM)	H	Number of alleles	PIC
SW1514	1	0.0	0.79	8	0.75
SW1515	1	16.4	0.67	8	0.68
SW1332	1	29.2	0.63	4	0.37
SW1851	1	44.6	0.73	4	0.53
SW1430	1	58.5	0.81	6	0.76
SWR982	1	86.2	0.88	7	0.77
SW1311	1	100.8	0.58	6	0.62
SW1828	1	118.5	0.90	7	0.69
SW1301	1	140.5	0.83	5	0.67
SW2512	1	144.0	0.77	6	0.55
SWR2516	2	0.0	0.67	5	0.48
SW2623	2	9.8	0.68	5	0.63
SWR783	2	23.7	0.51	3	0.30
SW240	2	42.0	0.84	7	0.78
SW1026	2	60.6	0.47	6	0.55
SW1370	2	74.8	0.91	8	0.69
SWR2157	2	89.2	0.78	8	0.68
SWR345	2	114.4	0.87	8	0.75
S0036	2	132.1	0.85	7	0.80
SW2404	4	0.0	0.91	10	0.81
SW489	4	8.0	0.66	5	0.53
S0301	4	27.1	0.72	6	0.56
S0001	4	41.8	0.66	6	0.65
SW839	4	62.3	0.44	4	0.45
S0214	4	79.3	0.80	6	0.74
SW445	4	105.8	0.91	10	0.77
MP77	4	120.0	0.87	8	0.74
SW856	4	130.1	0.98	14	0.84
MP35	6	0.0	0.70	6	0.59
SW2406	6	21.4	0.74	8	0.61
SW1841	6	41.5	0.98	15	0.88
S0087	6	62.8	0.75	5	0.59
SW122	6	83.3	0.85	7	0.69
S0228	6	105.2	0.69	6	0.68
SW1881	6	121.1	0.96	8	0.76
SW322	6	149.8	0.79	8	0.72
SW2052	6	164.6	0.79	9	0.78
SW2564	7	0.0	0.69	5	0.49
SWR1343	7	12.2	0.83	4	0.53
SW2155	7	32.9	0.67	4	0.48
SW1369	7	48.2	0.77	8	0.68
SW1856	7	61.5	0.69	5	0.48
SWR2036	7	78.2	0.81	9	0.77
SW632	7	104.4	0.77	6	0.67
SWR773	7	117.3	0.56	3	0.46
SW2537	7	139.5	0.69	7	0.63
SW764	7	156.0	0.76	5	0.65

Table 4.2 continued

Marker	SSC	Position (cM)	H	Number of alleles	PIC
SW2410	8	-1.3	0.42	4	0.44
SW905	8	20.8	0.71	6	0.71
SWR1101	8	38.3	0.88	12	0.75
SW444	8	52.5	0.85	7	0.76
S0086	8	62.2	0.69	6	0.56
SW374	8	82.8	0.88	5	0.63
SW1551	8	105.9	0.75	6	0.66
S0178	8	127.7	0.54	7	0.68
SW983	9	4.0	0.81	6	0.61
SW21	9	15.1	0.65	5	0.50
SW911	9	36.8	0.75	7	0.68
SW2401	9	57.1	0.71	6	0.68
SW2571	9	73.3	0.46	6	0.61
S0019	9	86.4	0.75	6	0.62
SW2093	9	103.6	0.90	6	0.77
SW174	9	122.9	0.81	3	0.51
SW1349	9	142.5	0.81	7	0.75
SW830	10	0.0	0.67	7	0.64
SWR136	10	7.6	0.77	6	0.72
SW1894	10	23.2	0.65	4	0.50
SW2195	10	44.0	0.48	3	0.42
SW173	10	56.1	0.35	4	0.39
SW1041	10	67.5	0.46	3	0.41
SW2043	10	87.7	0.56	5	0.72
SW1626	10	108.0	0.79	11	0.68
SW2067	10	128.0	0.81	7	0.69
S0282	13	0.0	0.90	8	0.77
SWR1941	13	14.1	0.87	7	0.71
SW1407	13	27.2	0.88	11	0.83
SW864	13	43.1	0.63	5	0.64
S0068	13	62.2	0.78	9	0.72
SW398	13	79.3	0.69	6	0.66
SW2440	13	102.2	0.96	6	0.79
S0291	13	126.2	0.83	8	0.79
SW857	14	7.4	0.87	9	0.74
S0089	14	14.0	0.67	7	0.71
SW245	14	32.0	0.77	7	0.71
SW342	14	53.2	0.79	7	0.71
SW1081	14	72.1	0.87	6	0.65
SW1557	14	87.9	0.64	4	0.49
SWC27	14	111.5	0.45	8	0.41

4.2.4 Statistical analysis

Due to the computational demand of a genomic scan for epistatic QTL, the analysis was performed in two stages, following Estelle *et al.* (2008). In the first stage, a 5 cM scan was carried out across all genomic positions in order to pre-select potential candidate regions with epistatic effects using, depending on the trait, the following models [1-3]:

Chemical body composition measured at target body weights:

$$y_i = sex_i + MHS_i + batch_i + \beta wt_i + C_{aa}I_{aa} + C_{ad}I_{ad} + C_{da}I_{da} + C_{dd}I_{dd} + e_i,$$ [1]

Chemical accretion rates (protein and lipid accretion):

$$y_i = sex_i + MHS_i + batch_i + \beta stwt_i + \beta endwt_i + C_{aa}I_{aa} + C_{ad}I_{ad} + C_{da}I_{da} + C_{dd}I_{dd} + e_i,$$

[2]

Feed intake and food conversion ratio:

$$y_i = sex_i + MHS_i + batch_i + ht_i + \beta stwt_i + \beta endwt_i + C_{aa}I_{aa} + C_{ad}I_{ad} + C_{da}I_{da} + C_{dd}I_{dd} + e_i,$$

[3]

where y_i is the i-th individual phenotype. Fixed effects and covariates were fitted in the model depending on their significance for the trait. *Sex*, *RYR1* genotype (*MHS*) and *batch* were included in the model for all traits. In addition, housing type (*ht: individual or group housed*) was included as a fixed effect for feed intake and food conversion ratio traits. For chemical body composition traits measured at different target weights, *β* is the regression on small differences between body weight and target weight (*wt*). Protein and lipid accretion, daily gain, feed intake and food conversion ratio were adjusted for the small differences between target and actual body weight at the start (*stwt*) and end (*endwt*) of the considered weight range. I_{aa}, I_{ad}, I_{da} and I_{dd} are the additive x additive (AA), additive x dominance (AD), dominance x additive (DA) and dominance x dominance (DD) epistatic effects, respectively. These four epistatic effects were

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estimated, following the Cockerham's decomposition (Cockerham 1954), by regressing on a linear combination of the individual QTL origin probabilities:

$$C_{aa} = P_1(QQ)P_2(QQ) - P_1(QQ)P_2(qq) - P_1(qq)P_2(QQ) + P_1(qq)P_2(qq),$$

$$C_{ad} = P_1(QQ)P_2(Qq) - P_1(qq)P_2(Qq),$$

$$C_{da} = P_1(Qq)P_2(QQ) - P_1(Qq)P_2(qq),$$

$$C_{dd} = P_1(Qq)P_2(Qq),$$

where P_1 and P_2 refers to the probability of QTL at location 1 and 2, respectively, and $P(QQ)$ is the probability of being homozygous for the grandpaternal sire line (Pietrain), $P(qq)$ is the probability of being homozygous for the grandmaternal dam line and $P(Qq)$ is the probability of being heterozygous (Varona *et al.* 2002). These models [1-3] were tested against a null model where no epistatic effects were estimated:

Chemical body composition measured at target body weights:

$$y_i = sex_i + MHS_i + batch_i + \beta wt_i + e_i, \qquad [4]$$

Chemical accretion rates (protein and lipid accretion):

$$y_i = sex_i + MHS_i + batch_i + \beta stwt_i + \beta endwt_i + e_i, \qquad [5]$$

Feed intake and food conversion ratio:

$$y_i = sex_i + MHS_i + batch_i + ht_i + \beta stwt_i + \beta endwt_i + e_i, \qquad [6]$$

Nominal P-values were obtained via maximum likelihood ratio tests. As in the study of Estelle *et al.* (2008), interacting QTL pairs with P-value < 0.001 were selected for further analyses.

In the second stage, a complete epistatic model including the individual QTL effects was applied using a 1 cM scan around the pre-selected positions. This included additive and dominance effects as well as epistatic effects, fixed effects and covariates as outlined in the description of stage one:

Chemical body composition measured at target body weights:

$$y_i = sex_i + MHS_i + batch_i + \beta wt_i + C_{a1}a_1 + C_{a2}a_2 + C_{d1}d_1 + C_{d2}d_2 + C_{aa}I_{aa} + C_{ad}I_{ad} + C_{da}I_{da} + C_{dd}I_{dd} + e_i,$$

[7]

Chemical accretion rates (protein and lipid accretion):

$$y_i = sex_i + MHS_i + batch_i + \beta stwt_i + \beta endwt_i + C_{a1}a_1 + C_{a2}a_2 + C_{d1}d_1 + C_{d2}d_2 + C_{aa}I_{aa} + C_{ad}I_{ad} + C_{da}I_{da} + C_{dd}I_{dd} + e_i,$$

[8]

Feed intake and food conversion ratio:

$$y_i = sex_i + MHS_i + batch_i + ht_i + \beta stwt_i + \beta endwt_i + C_{a1}a_1 + C_{a2}a_2 + C_{d1}d_1 + C_{d2}d_2 + C_{aa}I_{aa} + C_{ad}I_{ad} + C_{da}I_{da} + C_{dd}I_{dd} + e_i,$$

[9]

where a denotes the individual additive genetic effect and C_a represents the difference in probabilities of being homozygous for alleles of the grandpaternal sire line (QQ) and being homozygous for alleles from the grandmaternal dam line (qq). The effect d represents the individual dominance genetic effects and C_d gives the probability of being heterozygous (Qq). These models [7-9] were tested against a null model that contained only the individual QTL effects:

Chemical body composition measured at target body weights:

$$y_i = sex_i + MHS_i + batch_i + \beta wt_i + C_{a1}a_1 + C_{a2}a_2 + C_{d1}d_1 + C_{d2}d_2 + e_i,$$ [10]

Chemical accretion rates (protein and lipid accretion):

$$y_i = sex_i + MHS_i + batch_i + \beta stwt_i + \beta endwt_i + C_{a1}a_1 + C_{a2}a_2 + C_{d1}d_1 + C_{d2}d_2 + e_i,$$ [11]

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Feed intake and food conversion ratio:

$$y_i = sex_i + MHS_i + batch_i + ht_i + \beta stwt_i + \beta endwt_i + C_{a1}a_1 + C_{a2}a_2 + C_{d1}d_1 + C_{d2}d_2 + e_i,$$

[12]

Epistatic interactions were reported as significant if they had a nominal P-value < 0.001. All analyses were performed with QxPak software (Perez-Enciso and Misztal 2004). This program uses mixed models and the maximum likelihood method to estimate the QTL location and effects. The analysis of QxPak proceeds in two main stages. In the first stage the probabilities of alleles being identical-by-descent are calculated using a Monte Carlo Markov Chain algorithm. In the second stage, the mixed model equations are built and the QTL estimates are obtained using a maximum likelihood approach via the expectation-maximisation algorithm. At each putative position the likelihood ratio is computed and the estimates for the parameters are those where the likelihood is highest. In this analysis the significance is tested with a likelihood ratio test which consists of computing minus twice the difference in log-likelihoods between the alternative and the null models (Perez-Enciso and Misztal 2004).

4.3 Results

In total, 32 significant epistatic QTL pairs were identified from the genomic analysis, 11 for growth traits (Table 4.3), six for daily feed intake (DFI) and food conversion ratio (FCR) traits (Table 4.4), and 15 for chemical body composition (Table 4.5). Epistatic interactions were identified between QTL on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC10, SSC13 and SSC14. No epistatic QTL were identified on or with SSC9. Twenty eight of the significant QTL pairs were between QTL which resided on different chromosomes, whereas four were identified between QTL which resided on the same chromosome. All types of epistatic effect were identified (AA, AD, DA and DD), however the AA effect was the most prevalent.

Table 4.3 Evidence of epistatic interactions for growth traits

Trait	LR	P value	Q0 chr (pos)[1]	Q1 chr (pos)[1]	% var[2]	Q0_a ± SE[3]	Q0_d ± SE[3]	Q1_a ± SE[3]	Q1_d ± SE[3]	Q01_aa ± SE[4]	Q01_ad ± SE[4]	Q01_da ± SE[4]	Q01_dd ± SE[4]
Growth period 30-60 kg													
DG	21.39	2.7E-04	7 (79)	14 (89)	6.6	12.77 ± 17.55	-8.28 ± 29.96	-16.42 ± 17.01	-47.55 ± 32.99	**-64.60 ± 16.54**	-56.93 ± 32.16	47.49 ± 28.57	45.97± 52.41
PAR	23.66	9.3E-05	7 (79)	14 (88)	7.6	1.74 ± 2.84	-3.93 ± 4.92	-3.77 ± 2.76	-8.54 ± 5.38	**-10.24 ± 2.68**	-7.89 ± 5.25	**11.46 ± 4.73**	10.84 ± 8.58
Growth period 60-90 kg													
DG	22.33	1.7E-04	2 (2)	8 (94)	6.9	-32.22 ± 25.34	54.64 ± 35.40	2.14 ± 21.70	41.06 ± 45.37	**85.53 ± 21.85**	43.88 ± 44.88	**63.52 ± 30.07**	-112.50 ± 62.64
PAR	21.73	2.3E-04	2 (4)	8 (93)	7.0	-4.47 ± 4.32	6.84 ± 6.31	-1.14 ± 3.73	7.21 ± 7.74	**14.36 ± 3.77**	6.40 ± 7.63	**13.67 ± 5.40**	-15.89 ± 11.04
PAR	19.65	5.9E-04	8 (67)	14 (12.4)	6.3	-4.97 ± 3.87	-6.78 ± 7.08	1.74 ± 4.03	-13.13 ± 7.29	**-11.16 ± 3.80**	**21.23 ± 6.54**	-4.93 ± 6.79	16.65 ± 12.12
Growth period 90-120 kg													
DG	33.68	8.7E-07	1 (119)	2 (46)	10.3	25.20 ± 17.24	**78.74 ± 26.38**	-11.69 ± 17.29	**63.24 ± 26.94**	3.83 ± 17.40	**-121.00 ± 26.79**	48.49 ± 26.27	**-179.27 ± 41.43**
PAR	32.06	1.9E-06	1 (119)	2 (46)	10.2	3.83 ± 2.85	**11.79 ± 4.31**	-2.65 ± 2.85	**9.61 ± 4.46**	1.03 ± 2.89	**-19.59 ± 4.42**	**8.89 ± 4.30**	**-28.61 ± 6.80**
Growth period 120-140 kg													
DG	23.77	8.9E-05	6 (84)	13 (27)	7.4	**70.26 ± 25.11**	38.92 ± 43.31	-28.24 ± 24.09	18.06 ± 37.63	**82.47 ± 23.44**	**-156.10 ± 36.76**	63.66 ± 41.79	-26.91 ± 65.26
PAR	22.25	1.8E-04	6 (84)	13 (27)	7.4	**12.11 ± 4.12**	6.12 ± 7.09	-4.82 ± 3.97	5.08 ± 6.27	**11.98 ± 3.84**	**-27.23 ± 6.13**	9.82 ± 6.86	-6.12 ± 10.78
LAR	18.74	8.8E-04	7 (27)	7 (51)	6.2	-4.71 ± 36.60	-5.43 ± 58.53	-40.46 ± 37.05	-88.80 ± 52.28	**-121.77 ± 35.49**	16.21 ± 50.63	**138.65 ± 56.39**	62.57 ± 78.16
LAR	25.23	4.5E-05	7 (18)	10 (9)	8.3	21.10 ± 15.71	-40.40 ± 28.29	**-33.21 ± 16.37**	**-65.97 ± 24.85**	**-44.45 ± 15.95**	-41.12 ± 23.79	23.98 ± 27.63	**182.74 ± 42.21**

Values in bold represent significant additive, dominance or epistatic effects.

Definition of symbols: LR, likelihood ratio; DG, daily gain; PAR, protein accretion rate; LAR, lipid accretion rate.

[1]Positions of the QTL in cM.

[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no epistatic effects - residual variance of model with epistatic effects)/residual variance of model with no epistatic effects.

[3]Estimated additive (a) and dominance (d) effects and their standard errors (SE) of the individual QTL.

[4]Estimated additive x additive (aa), additive x dominance (ad), dominance x additive (da) and dominance x dominance (dd) effects and their standard errors (SE).

Table 4.4 Evidence of epistatic interactions for daily feed intake (DFI) and food conversion ratio (FCR)

Trait	LR	P value	Q0 chr (pos)[1]	Q1 chr (pos)[1]	% var[2]	Q0_a ± SE[3]	Q0_d ± SE[3]	Q1_a ± SE[3]	Q1_d ± SE[3]	Q01_aa ± SE[4]	Q01_ad ± SE[4]	Q01_da ± SE[4]	Q01_dd ± SE[4]
Growth period 60-90 kg													
FCR	27.67	1.5E-05	7 (49)	13 (2)	8.4	-0.057 ± 0.057	0.011 ± 0.087	-0.037 ± 0.059	-0.027 ± 0.083	**0.244 ± 0.057**	0.066 ± 0.080	**0.201 ± 0.087**	0.014 ± 0.125
FCR	19.58	6.1E-04	8 (1)	7 (107)	6.1	0.096 ± 0.054	**-0.160 ± 0.077**	0.056 ± 0.050	**-0.241 ± 0.097**	**0.192 ± 0.051**	**-0.193 ± 0.097**	-0.005 ± 0.072	0.194 ± 0.138
DFI	23.04	1.2E-04	10 (9)	13 (73)	7.1	**-0.250 ± 0.051**	-0.126 ± 0.087	**0.168 ± 0.052**	-0.081 ± 0.085	0.055 ± 0.050	**0.329 ± 0.079**	**-0.294 ± 0.085**	0.157 ± 0.134
Growth period 90-120 kg													
FCR	26.76	2.2E-05	2 (38)	14 (99)	8.3	**-0.277 ± 0.086**	-0.019 ± 0.155	**0.337 ± 0.086**	0.012 ± 0.160	**-0.275 ± 0.082**	0.301 ± 0.155	**-0.600 ± 0.144**	0.235 ± 0.263
FCR	22.06	2.0E-04	2 (1)	14 (85)	6.9	**-0.325 ± 0.081**	-0.204 ± 0.111	**0.167 ± 0.076**	-0.058 ± 0.146	**-0.328 ± 0.074**	0.358 ± 0.145	-0.167 ± 0.102	0.244 ± 0.196
Growth period 120-140 kg													
DFI	19.52	6.2E-04	13 (7)	2 (6)	6.1	-0.083 ± 0.060	**0.198 ± 0.091**	0.016 ± 0.061	**0.203 ± 0.094**	**-0.152 ± 0.059**	**0.259 ± 0.093**	-0.157 ± 0.092	**-0.302 ± 0.138**

Values in bold represent significant additive, dominance or epistatic effects.

Definition of symbols: LR, likelihood ratio; FCR, food conversion ratio calculated as kg feed/kg gain; DFI, daily feed intake.

[1]Positions of the QTL in cM.

[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no epistatic effects - residual variance of model with epistatic effects)/residual variance of model with no epistatic effects.

[3]Estimated additive (a) and dominance (d) effects and their standard errors (SE) of the individual QTL.

[4]Estimated additive x additive (aa), additive x dominance (ad), dominance x additive (da) and dominance x dominance (dd) effects and their standard errors (SE).

Table 4.5 Evidence of epistatic interactions for chemical body composition traits

Trait	LR	P value	Q0 chr (pos)[1]	Q1 chr (pos)[1]	% var[2]	Q0_a ± SE[3]	Q0_d ± SE[3]	Q1_a ± SE[3]	Q1_d ± SE[3]	Q01_aa ± SE[4]	Q01_ad ± SE[4]	Q01_da ± SE[4]	Q01_dd ± SE[4]
Chemical body composition at 60 kg body weight													
LCEB	19.12	7.4E-04	7 (140)	6 (87)	6.0	**0.681 ± 0.258**	-0.006 ± 0.501	-0.259 ± 0.281	**1.120 ± 0.493**	0.450 ± 0.249	**-1.802 ± 0.459**	-0.234 ± 0.483	-0.160 ± 0.875
PCFFS	19.74	5.6E-04	7 (140)	6 (87)	6.3	**0.137 ± 0.053**	-0.007 ± 0.101	-0.052 ± 0.057	**0.235 ± 0.101**	0.088 ± 0.051	**-0.377 ± 0.094**	-0.051 ± 0.097	-0.039 ± 0.178
PCEB	18.93	8.1E-04	7 (140)	6 (87)	8.3	**-0.016 ± 0.006**	0.004 ± 0.012	0.006 ± 0.007	**-0.026 ± 0.012**	-0.010 ± 0.006	**0.043 ± 0.011**	0.005 ± 0.011	0.004 ± 0.021
Chemical body composition at 90 kg body weight													
LCEB	22.82	1.4E-04	4 (130.1)	7 (100)	7.1	0.141 ± 0.247	0.567 ± 0.355	0.054 ± 0.226	0.136 ± 0.421	**-1.080 ± 0.226**	-0.019 ± 0.432	0.022 ± 0.324	-0.701 ± 0.666
PCFFS	23.45	1.0E-04	4 (130.1)	7 (100)	7.3	0.035 ± 0.058	0.132 ± 0.083	0.012 ± 0.053	0.027 ± 0.099	**-0.259 ± 0.054**	-0.007 ± 0.102	0.008 ± 0.076	-0.163 ± 0.156
PCEB	23.15	1.2E-04	4 (130.1)	7 (100)	7.3	-0.004 ± 0.006	-0.014 ± 0.009	-0.001 ± 0.006	-0.003 ± 0.011	**0.027 ± 0.006**	0.0004 ± 0.011	-0.001 ± 0.008	0.018 ± 0.017
LCEB	24.84	5.4E-05	8 (105)	6 (32)	7.7	0.325 ± 0.343	0.440 ± 0.699	0.326 ± 0.354	1.153 ± 0.689	**-1.515 ± 0.309**	-0.557 ± 0.613	-0.919 ± 0.645	-1.579 ± 1.181
PCFFS	24.51	6.3E-05	8 (105)	6 (32)	7.5	0.074 ± 0.082	0.088 ± 0.168	0.068 ± 0.085	0.262 ± 0.165	**-0.360 ± 0.074**	-0.124 ± 0.147	-0.201 ± 0.155	-0.359 ± 0.283
PCEB	24.57	6.1E-05	8 (106)	6 (32)	7.7	-0.008 ± 0.009	-0.009 ± 0.017	-0.007 ± 0.009	-0.027 ± 0.017	**0.038 ± 0.008**	0.014 ± 0.015	0.021 ± 0.016	0.036 ± 0.029
Chemical body composition at 120 kg body weight													
LCEB	21.20	2.9E-04	2 (43)	2 (63)	6.8	**1.416 ± 0.682**	-0.783 ± 0.906	-0.857 ± 0.682	-0.022 ± 0.877	-0.440 ± 0.684	**-3.204 ± 0.878**	0.903 ± 0.899	0.694 ± 1.100
PCFFS	21.10	3.0E-04	2 (43)	2 (63)	6.7	**0.379 ± 0.180**	-0.196 ± 0.240	-0.235 ± 0.180	0.019 ± 0.231	-0.105 ± 0.181	**-0.857 ± 0.232**	0.255 ± 0.238	0.158 ± 0.291
PCEB	20.99	3.2E-04	2 (43)	2 (63)	6.3	**-0.037 ± 0.018**	0.021 ± 0.024	0.023 ± 0.018	0.0002 ± 0.023	0.012 ± 0.018	**0.085 ± 0.023**	-0.025 ± 0.024	-0.018 ± 0.029
Chemical body composition at 140 kg body weight													
LCEB	18.83	8.5E-04	4 (121)	14 (92)	6.0	-0.179 ± 0.322	**-1.079 ± 0.482**	0.042 ± 0.285	-0.987 ± 0.596	**-0.817 ± 0.287**	0.796 ± 0.610	0.051 ± 0.434	**2.937 ± 0.884**
PCFFS	18.80	8.6E-04	4 (121)	14 (91)	6.0	-0.049 ± 0.086	**-0.284 ± 0.129**	0.018 ± 0.077	-0.259 ± 0.158	**-0.217 ± 0.077**	0.213 ± 0.162	0.003 ± 0.117	**0.789 ± 0.235**
PCEB	18.88	8.3E-04	4 (121)	14(95)	5.0	0.005 ± 0.009	**0.033 ± 0.014**	-0.001 ± 0.008	0.027 ± 0.018	**0.023 ± 0.008**	-0.021 ± 0.018	-0.003 ± 0.012	**-0.086 ± 0.026**

Values in bold represent significant additive, dominance or epistatic effects.

Definition of symbols: LR, likelihood ratio; LCEB, lipid content of the empty body; PCEB, protein content of the empty body; PCFFS, protein content of the fat-free substance.

[1] Positions of the QTL in cM.

[2] Percentages of F_2 variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no epistatic effects - residual variance of model with epistatic effects)/residual variance of model with no epistatic effects.

[3] Estimated additive (a) and dominance (d) effects and their standard errors (SE) of the individual QTL.

[4] Estimated additive x additive (aa), additive x dominance (ad), dominance x additive (da) and dominance x dominance (dd) effects and their standard errors (SE).

4.3.1 Growth

Epistatic interactions were identified across all growth stages for protein accretion rate (PAR) and daily gain (DG). Epistatic interactions were identified for PAR and DG for the earliest growth stage from 30 to 60 kg body weight, between 79 cM of SSC7 and 88-89 cM of SSC14 (AA effects for PAR and DG; DA effects for PAR). For the growth period from 60-90 kg, an interaction was identified between the telomeric end of the p arm of SSC2 at 2-4 cM and in the region of 93-94 cM of SSC8 for DG and PAR (AA and DA effects for DG and PAR). A further interaction influenced PAR for the same growth period, between SSC8 and SSC14 (AA and AD effects). For the growth period 90-120 kg body weight, an interaction between SSC1 at 119 cM and SSC2 at 46 cM was identified for DG and PAR (AD and DD effects for DG and PAR; DA effects for PAR). At the late growth period of 120-140 kg body weight, an interaction was identified between SSC6 at 84 cM and SSC13 at 27 cM for DG and PAR (AA and AD effects for DG and PAR). Epistatic QTL were only identified for the final growth period (120-140 kg) for lipid accretion rate (LAR). Interactions were identified in two different locations. The first interaction was identified between QTL at two locations of SSC7 (AA and DA effects). The second interaction was identified between SSC7 at 18 cM and SSC10 at 9 cM (AA and DD effects).

4.3.2 Daily feed intake and food conversion ratio

For FCR at 60 to 90 kg body weight, two epistatic QTL pairs were identified. The first interaction was identified between QTL on SSC7 at 49 cM and the telomeric end of the p arm of SSC13 (AA and DA effects). The second pair was identified between the telomeric end of the p arm of SSC8 and SSC7 at 107 cM (AA and AD effects). For the same growth period a significant epistatic pair was identified for DFI between SSC10 at 9 cM and SSC13 at 73 cM (AD and DA effects). For a later stage of growth from 90 to 120 kg body weight, two epistatic pairs were identified between QTL on SSC2 and

SSC14 for FCR. The first interaction was between a QTL at 38 cM of SSC2 and 99 cM of SSC14 (AA and DA effects) and the second between the telomeric end of the p arm of SSC2 (1 cM) and 85 cM of SSC14 (AA and AD effects). For the final growth period (120-140 kg) a single epistatic pair was identified between the telomeric p arms of SSC13 and SSC2 for DFI (AA, AD and DD effects).

4.3.3 Chemical body composition

For chemical body composition traits (protein and lipid content of the empty body and protein content of the fat free substance), significant epistatic QTL pairs were identified in different genomic locations for different target body weights. No significant epistatic QTL pairs were identified for chemical body composition at 30 kg body weight. At 60 kg body weight, a significant interaction was identified between QTL on SSC7 at 140 cM and on SSC6 at 87 cM (AD effects). At 90 kg body weight significant epistatic interactions were identified in two genomic locations. The first interaction was between QTL at the telomeric end of the q arm of SSC4 (130.1 cM) and at 100 cM on SSC7 (AA effects). The second interaction was identified between QTL on SSC8 at 105-106 cM and SSC6 at 32 cM (AA effects). At 120 kg body weight, a significant interaction was identified between QTL in two different locations of SSC2, at 43 cM and 63 cM (AD effects). For the final target weight of 140 kg body weight, a significant epistatic QTL pair was identified on SSC4 at 121 cM and SSC14 between 91 and 95 cM (AA and DD effects).

4.4 Discussion

The present study is the first report to estimate epistatic interactions for chemical body composition (protein and lipid content measured in live animals), growth (daily gain, protein and lipid deposition), feed intake and feed efficiency in pigs. The results of this study confirmed the existence of previously detected QTL but also point to new loci of influence.

Much research has focused on the identification of QTL and causative genes influencing complex traits. As a result large numbers of QTL have been reported for economically important traits in the pig, such as growth and body composition (e.g. Bidanel *et al.* 2001; Milan *et al.* 2002; Geldermann *et al.* 2003; Karlskov-Mortensen *et al.* 2006; Sanchez *et al.* 2006). Studies of QTL for chemical body composition measured in live animals are limited in the literature because of the expense and difficulty associated with collecting such data. QTL for chemical body composition measured in live animals have been reported in two studies, which analysed the data of the present study (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapter 2) as well as Chapter 3 of this thesis. The hypothesis underlying the present study was that these economically important traits are not only influenced by numerous major QTL located throughout the genome, but by many interactions between QTL at two or more loci as well as many minor genes.

Epistasis has been largely neglected when trying to dissect the genetic architecture of complex traits, with studies only considering the individual QTL effects (additive, dominance and imprinting), and not the interactions between QTL. Genomic analysis for epistatic QTL is computationally demanding, which is probably one reason for this neglect.

By accounting for epistasis a large number of QTL were identified in the present study, which were not identified from previous QTL analysis on the same dataset which only considered the individual QTL effects. The majority of these "new" QTL did not show

significant additive or dominance effects and therefore mainly exhibit their effects through interactions with other QTL. However, three of the epistatic QTL had been identified from previous analysis. The QTL identified on SSC6 for chemical body composition at 60 kg body weight, was previously identified from individual QTL analysis of this data by Mohrmann *et al.* (2006a). This QTL showed a significant dominance effect in both analyses. The QTL identified at the telomeric end of the p arm of SSC2 for FCR from 90 to 120 kg was previously identified in the study of Duthie *et al.* (2008; Chapter 2). In both analyses this QTL showed significant additive effects. The QTL identified on SSC1 for DG and PAR from 90 to 120 kg was previously identified by Mohrmann *et al.* (2006a). However in the present study this showed significant dominance effects, which was a contrast to the analysis of Mohrmann *et al.* (2006a) which showed significant additive effects. Furthermore, epistatic QTL pairs identified in the present study generally accounted for slightly higher proportions of the phenotypic variance than QTL identified from the individual QTL analyses. The proportions of phenotypic variance explained here by epistatic QTL pairs were approximately double that of the individual QTL identified from the individual QTL analysis (Duthie *et al.* 2008; Chapter 2) and therefore may be simply the combination of two QTL.

From previous analysis of the data used in the present study, it was particularly surprising that no QTL were identified on SSC7 and a few QTL on SSC4. These chromosomes have been shown to harbour many QTL associated with growth and body composition (e.g. Walling *et al.* 1998; Marklund *et al.* 1999; Walling *et al.* 2000; Cepica *et al.* 2003a; Yue *et al.* 2003b; Mercade *et al.* 2005; Sanchez *et al.* 2006). By accounting for epistasis, QTL have however been identified on these chromosomes, particularly SSC7. In the majority of cases, the QTL did not have significant additive or dominance effects, indicating why they were not identified from previous analysis.

In pigs, epistatic QTL have been reported so far for reproduction traits (Bidanel 1993; Rodriguez *et al.* 2005; Noguera *et al.* 2006), coat colour (Hirooka *et al.* 2002), meat

quality traits (meat colour and intramuscular fat content) (Ovilo *et al.* 2002; Szyda *et al.* 2006) and muscle fibre traits (Estelle *et al.* 2008).

4.4.1 Chemical body composition

From previous analysis of the data of this study, QTL have been identified for chemical body composition for early to mid stages of growth at body weights 30 kg (SSC6) and 60 kg (SSC6 and SSC9) (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapter 2). In the present study, epistasis was found to contribute to the genomic control of chemical body composition at all target weights except for 30 kg body weight, the earliest stage considered in this study. Different epistatic QTL pairs were identified at each target weight, indicating that different epistatic QTL pairs contribute to different stages of growth. The QTL identified on SSC6 for chemical body composition measured at 60 kg body weight was previously reported by Mohrmann *et al.* (2006a) in this population and in both studies this QTL had a significant dominance effect. A large number of QTL have been reported in this location for lean and fat tissue characteristics (cited in Table 4.6). Around this region of SSC6 a number of candidate genes are located, including the *RYR1* gene (Rohrer *et al.* 1996), the *heart fatty acid binding protein (FABP3)* gene and the *small heterodimer partner (SHP)* gene (Arnyasi *et al.* 2006). A mutation at the *RYR1* locus is associated with malignant hyperthermia syndrome (Fujii *et al.* 1991) and *RYR1* is significantly associated with production traits in pigs (Kadarmideen 2008). *FABP3* is important for fatty acid transport to sites of fatty acid utilisation. This gene is mainly expressed in skeletal and cardiac muscle (Veerkamp and Maatman 1995). *SHP* represses and inhibits the activity of the *liver X receptor* and *retinoid X receptor* both of which are involved in lipid homeostasis (Chawla *et al.* 2001; Brendel *et al.* 2002). *Liver X receptor* has been shown to influence endocrine homeostasis, lipid metabolism as well as protein metabolism (Stulnig *et al.* 2002). Therefore *SHP* is a candidate gene for lipid as well as protein deposition. The QTL identified on SSC7 for chemical body composition measured at 60 kg body weight was not identified from previous individual QTL

analysis; therefore, this QTL has only expressed its effects through AD interactions with SSC6. Around the same location of SSC4, at the telomeric end of the q arm, where QTL were identified for chemical body composition measured at 90 kg body weight, QTL have been previously reported for average daily gain and carcass weight (cited in Table 4.6). In the marker interval of this QTL the *transforming growth factor, beta receptor III (TGFBR3)* gene is located. Transforming growth factor-beta is encoded by several genes including *TGFBR3* and is involved in tissue development and repair processes (Johnson *et al.* 1995). Around the location of the QTL on SSC7 for the same body weight, QTL have been reported for belly percentage, carcass length and loin weight (cited in Table 4.6). In this region of SSC7, the *proteasome (prosome, macropain) activator subunit 1 (PA28 alpha) (PSME1)* and the *proteasome (prosome, macropain) activator subunit 2 (PA28 beta)* (*PSME2*) genes are located. These genes encode proteasome activators PA28α and β subunits. These are two subunits of PA28 which is an activator of the proteosome which plays an important role in antigen presentation mediated by the major histocompatibility complex class I (Dubiel *et al.* 1992). Wang *et al.* (2004) reported evidence that a polymorphism in the *PSME1* gene is associated with weaning weight in pigs. At these QTL on SSC4 and SSC7, no individual additive or dominance effects were identified, therefore these QTL mainly express their effects through AA interactions with each other. A further epistatic effect (AA) was identified between QTL on SSC8 and SSC6 for chemical body composition measured at 90 kg body weight. There are no reports of QTL around the same genomic location of SSC8. In the same marker interval as this QTL, the *microsomal triglyceride transfer protein large subunit (MTP)* gene is located (Estelle *et al.* 2005). *MTP* is involved in the transfer of lipids during lipoprotein assembly in the liver and intestine (Hussain 2000) and *MTP* expression has been shown to be influenced by fatty acids in pigs (Lu *et al.* 2002). Mohrmann *et al.* (2006a) reported QTL for chemical body composition at 30 kg body weight in a slightly different location of SSC6 than the QTL identified in this study. QTL have also been reported around this location of SSC6 for loin and ham percentage in the carcass, and intramuscular fat content (cited in Table 4.6). At both of these QTL no significant additive or dominance effects were identified, indicating that these QTL

mainly express their effects through an AA interaction with each other. An interaction was identified in the present study between two genomic locations of SSC2 for chemical body composition at 120 kg body weight. These QTL were not identified from previous analysis of this data, although there are reports of QTL for lean and fat tissue around both locations of SSC2 (cited in Table 4.6). For chemical body composition measured at the final weight (140 kg body weight), an interaction was identified between the telomeric end of the q arm of SSC4 and SSC14. In this location of SSC4 a QTL has been reported for carcass weight (cited in Table 4.6). This QTL lies in the same interval as the QTL for chemical body composition at 90 kg body weight as well as the candidate gene *TGFBR3*. On SSC14, QTL have been reported around this location for number of muscle fibres and loin weight (cited in Table 4.6). A candidate gene around this location includes the *stearoyl-CoA desaturase* gene (Ren *et al.* 2004). This gene has been implicated in adiposity in mice (Cohen *et al.* 2002; Ntambi *et al.* 2002) and therefore may be a potential candidate for fatness traits.

4.4.2 Growth traits

QTL were previously identified in this population for PAR and DG for all growth periods (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapters 2 and 3). In the present study epistatic QTL were found to influence PAR and DG for all growth periods (30-60, 60-90, 90-120 and 120-140 kg body weight). PAR and DG are regulated by the same epistatic QTL pairs at each growth stage but epistatic QTL pairs differed between growth stages. The late stage of the growth period in pigs is associated with the deposition of fat tissue, which is costly to the pig producer as it is associated with higher feeding costs and a lower market value of the final product. Interestingly, epistatic QTL pairs were only found to influence the accretion of this tissue in the latest growth stage (120-140 kg). Apart from previous analyses of these data, there are no reports of QTL for accretion rates of protein or lipid. In the same location of SSC7 as QTL were identified for DG and PAR for early growth, a large number of QTL have been reported

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for daily gain as well as carcass traits including lean and fat tissue (cited in Table 4.6). Candidate genes in this location include the *PSME1* and *PSME2* genes. At the QTL on SSC14 for DG and PAR from 30 to 60 kg, QTL have been reported around this location for fat cuts percentage, number of muscle fibres and loin weight (cited in Table 4.6). At the telomeric end of the p arm of SSC2, where QTL were identified for DG and PAR for 60 to 90 kg body weight, QTL have been reported for a number of carcass characteristics, as well as lean and fat tissue (cited in Table 4.6). In this location, an imprinted QTL has been mapped to the *insulin-like growth factor 2 (IGF2)* locus with large effects on muscle mass and fat deposition (Nezer *et al.* 1999). Van Laere *et al.* (2003) showed that this was caused by a nucleotide substitution in intron 3 of *IGF2*. This region of SSC2 interacted with SSC8 in this study. No significant additive or dominance effects were identified at either QTL, indicating that they only express their effects through AA and DA interactions with each other. A further QTL was identified for PAR from 60 to 90 kg on SSC8 in a slightly different location. Around this location QTL have been reported for ham weight and belly weight (cited in Table 4.6). In the present study, this region of SSC8 interacted with a region of SSC14 where there are no reports of QTL in the literature. Again these QTL showed no significant individual QTL effects and therefore they mainly exert their effects through AA and AD interactions. For the growth period from 90 to 120 kg, a QTL was identified on SSC1 for DG and PAR with significant dominance effects. This QTL was already identified from previous analysis for both PAR and DG and in the same location for loin weight, however showed significant additive effects (Mohrmann *et al.* 2006a). Around the same genomic location, QTL have been reported for a number of lean and fat tissue characteristics, diameter of muscle fibres and average daily gain (cited in Table 4.6). This region interacted with a region of SSC2 where QTL have been reported for lean tissue and fatness (cited in Table 4.6). For the final growth stage (120-140 kg body weight) an interaction was identified between QTL on SSC6 and SSC13. On SSC6, the QTL was identified around the same genomic location as the *RYR1* gene. A large number of QTL have been reported in this location for lean and fat tissue characteristics (cited in Table 4.6). At the QTL on SSC13, QTL have been reported for ham weight and backfat (cited

in Table 4.6). The only QTL for LAR identified in this study was for the final growth stage (120-140 kg body weight) and was between QTL on two genomic locations of SSC7 (27 and 51 cM) and between SSC7 (18 cM) and SSC10. Around the QTL on SSC7 at 27 cM, QTL have been reported for abdominal fat and backfat (cited in Table 4.6). On SSC7 around 51 cM, QTL have been reported for backfat, carcass length, shoulder meat weight and body weight (cited in Table 4.6). These QTL were not identified from previous analysis, and no individual QTL effects were identified in the present study, indicating that these QTL mainly express their effects through AA and DA interactions with each other. At the QTL at 18 cM, QTL have been reported for carcass length and external fat on shoulder (cited in Table 4.6). Around this location of SSC7 lies the *Colipase* gene. Colipase prevents pancreatic lipase activity being inhibited by surface active agents (Brown and Archibald 2002). This QTL had no significant additive or dominance effects in the present study and therefore mainly expressed its effects through interaction with SSC10. Around the QTL on SSC10, QTL have been reported for a number of lean and fat tissue as well as growth traits (cited in Table 4.6). The results of the present study indicate that the genomic regulation of chemical body composition and growth is a complex process which is affected by numerous QTL and gene interactions, which are turned on and off at different stages of growth.

At present, no other studies have investigated the importance of epistasis to the genomic control of growth in pigs. There is however evidence in the literature for epistatic QTL pairs influencing growth in other species. Carlborg *et al.* (2003) investigated the contribution of epistasis to growth in a F_2 intercross between Red Jungle Fowl and White Leghorn chickens. Epistasis was found to be particularly important for early growth, where the foundation for growth is established by the development of internal organs. Carlborg *et al.* (2003) reported that epistasis was not as important for later growth, which involves the main deposition of body tissues. Following on from this study, Carlborg *et al.* (2004) carried out analysis of a cross between a White Leghorn line and a commercial broiler sire line. From this study they found that epistasis was an important contributor to the genetic variance of growth, with the largest effects on body

weight at 6 weeks of age and growth between 3 and 6 weeks of age. They also indicate evidence for a discrete set of interacting loci involved in earlier growth, supporting that the genetic regulation of early and late growth in the chicken differs. The results of the present study differs by indicating that epistasis is not only important for early growth, but for the entire growth period. Our results do however agree that the genomic regulation of growth and body compositions differs at different stages of growth.

There is a lot of evidence in the literature indicating that epistasis has a strong influence on growth and obesity in laboratory animals (e.g. Routman and Cheverud 1997; Brockmann *et al.* 2000; Cheverud *et al.* 2001; Brockmann *et al.* 2004; Ishikawa and Namikawa 2004; Yi *et al.* 2004a; Carlborg *et al.* 2005; Ishikawa *et al.* 2005; Yi *et al.* 2006). In contrast to the results of Carlborg *et al.* (2003; 2004) in chickens, Yi *et al.* (2006) reported that epistasis had a more pronounced effect for body weight at later stages of growth in mice. In contrast, Ishikawa *et al.* (2005) found that epistatic effects were more pronounced in the early stages of growth in mice. Carrying on from the studies of Carlborg *et al.* (2003; 2004) in chickens, Le Rouzic *et al.* (2008) demonstrated that the effects of many genes are dependent on genetic interactions with other loci. They found that when comparing the epistatic QTL pairs, the loci overlapped to a large extent with those previously identified in a one-dimensional scan. In particular, the two loci with the most pronounced effects in the one-dimensional scan also showed the most interactions with other loci. In the present study, however, this was not the case.

No data were available in the present study for chemical body composition or growth prior to 30 kg body weight. It would be interesting to know if epistasis is more important during the immediate post-natal period.

Table 4.6. Reports of similar QTL in the literature around similar locations as the QTL identified in the present study for chemical body composition and growth traits

Trait	SSC (position)	Marker interval	QTL reported in the same location in other studies
Chemical body composition (protein and lipid content)			
120 kg body weight	2 (43)	*SW240 – SW1026*	Lee *et al.* (2003a)
120 kg body weight	2 (63)	*SW1026 – SW1370*	Varona *et al.* (2002); Lee *et al.* (2003a); Wimmers *et al.* (2006)
140 kg body weight	4 (121)	*MP77 – SW856*	Malek *et al.* (2001a)
90 kg body weight	4 (130.1)	*SW856*	Malek *et al.* (2001a); Knott *et al.* (2002)
90 kg body weight	6 (32)	*SW2406 – SW1841*	de Koning *et al.* (2000); Milan *et al.* (2002)
60 kg body weight	6 (87)	*SW122 – S0228*	Rohrer (2000); Grindflek *et al.* (2001); Varona *et al.* (2002); Yue *et al.* (2003a); Edwards *et al.* (2008)
90 kg body weight	7 (100)	*SWR2036 – SW632*	Milan *et al.* (2002); Nezer *et al.* (2002); Sanchez *et al.* (2006)
60 kg body weight	7 (140)	*SW2537 – SW764*	-
90 kg body weight	8 (105,106)	*SW1551*	-
140 kg body weight	14 (91-95)	*SW1557 – SWC27*	Milan *et al.* (2002); Wimmers *et al.* (2006)
Growth traits			
DG and PAR 90-120 kg	1 (119)	*SW1828 – SW1301*	Beeckmann *et al.* (2003a); Geldermann *et al.* (2003); Kim *et al.* (2006); Wimmers *et al.* (2006)
DG and PAR 60-90 kg	2 (2, 4)	*SWR2516 – SW2623*	Milan *et al.* (2002); Kim *et al.* (2005); Sanchez *et al.* (2006); van Wijk *et al.* (2006)
DG and PAR 90-120 kg	2 (46)	*SW240 – SW1026*	Lee *et al.* (2003a)
DG and PAR 120-140 kg	6 (84)	*SW122 – S0228*	Rohrer (2000); Grindflek *et al.* (2001); Varona *et al.* (2002); Yue *et al.* (2003a); Edwards *et al.* (2006)
LAR 120-140 kg	7 (18)	*SWR1343 – SW2155*	Yue *et al.* (2003b)
LAR 120-140 kg	7 (27)	*SWR1343 – SW2155*	Yue *et al.* (2003b)
LAR 120-140 kg	7 (51)	*SW1369 – SW1856*	Rattink *et al.* (2000); Bidanel *et al.* (2001); Yue *et al.* (2003b); Sanchez *et al.* (2006)
DG and PAR 30-60 kg	7 (79)	*SWR2036 – SW632*	Malek *et al.* (2001b); Milan *et al.*(2002); Nezer *et al.*(2002); Geldermann *et al.* (2003); Yue *et al.* (2003b); Ponsuksili *et al.* (2005); Kim *et al.* (2006); Sanchez *et al.* (2006); Edwards *et al.* (2008)
PAR 60-90 kg	8 (67)	*S0086 – SW374*	Milan *et al.* (2002)
DG and PAR 60-90 kg	8 (93, 94)	*SW374 – SW1551*	
LAR 120-140 kg	10 (9)	*SWR136 – SW1894*	Dragos-Wendrich *et al.* (2003c); Rohrer *et al.* (2005); Kim *et al.* (2006)
DG and PAR 120-140 kg	13 (27)	*SWR1941 – SW1407*	Malek *et al.* (2001b); van Wijk *et al.* (2006)
PAR 60-90 kg	14 (12.4)	*SW857 – S0089*	-
DG and PAR 30-60 kg	14 (88, 89)	*SW1557 – SWC27*	Nezer *et al.* (2002); Dragos-Wendrich *et al.*, (2003a)

Definition of symbols: DG, daily gain; PAR, protein accretion rate; LAR, lipid accretion rate.

4.4.3 Daily feed intake and food conversion ratio

Understanding the genomic regulation of food intake and feed efficiency is of particular interest in pig production, particularly in commercial pig breeds where intense selection for increased lean content and reduced backfat thickness has had negative effects on food intake capacity, thus reducing the potential for maximal protein deposition. Therefore, it is of great economic benefit to understand the genomic regulation of these traits in order to optimise food intake based on maximal protein deposition. Previous analysis of the data of the present study by Mohrmann *et al.* (2006a) and Duthie *et al.* (2008; Chapter 2) have already provided some information about the genomic regulation of feed intake and feed efficiency. Regions of the genome were identified where Pietrain alleles were associated with reduced feed intake. Furthermore, regions were identified where Pietrain alleles were associated with increased feed intake (cryptic QTL). This may provide the opportunity to increase feed intake in this commercial breed. The results of the present study indicate that epistasis contributes to the genomic regulation of feed intake and feed efficiency throughout the entire growth period from 60 to 140 kg body weight. There are no reports in the literature for epistatic QTL influencing feed intake and feed efficiency. For the growth stage from 60 to 90 kg, AA and DA effects were identified between SSC7 and SSC13 for FCR. No QTL have been reported in this location of SSC7. The QTL on SSC13 was previously identified by Mohrmann *et al.* (2006a) in the same location as for PAR for the same growth period. In this location of SSC13, Houston *et al.* (2005) reported a suggestive QTL for DFI. A further interaction was identified between QTL on SSC8 and SSC7 for FCR from 60 to 90 kg, in a location where no QTL have been reported for FCR. Epistatic QTL were identified in this study for DFI from 60 to 90 kg, on SSC10 and SSC13. There are no reports in the literature for QTL for DFI in these locations. For a later growth period from 90 to 120 kg, two interactions were identified between SSC2 and SSC14. Houston *et al.* (2005) reported QTL for DFI around the same location as the QTL for FCR identified in the present study on SSC2. Houston *et al.* (2005) also reported suggestive QTL for feeding rate and FCR around the QTL identified on SSC14. Further QTL were identified for DFI from

120 to 140 kg on SSC2 and SSC13 around the same locations as the suggestive QTL reported by Houston *et al.* (2005) for the same trait.

Estelle *et al.* (2008) identified a number of epistatic QTL pairs for muscle fibre traits in pigs and reported that the interactions formed a network of connected epistatic QTL pairs. Carlborg *et al.* (2006) also reported a similar network system in chickens. The present study has shown that the genomic regulation of growth and chemical body composition is a complex process involving many QTL throughout the genome and interactions between QTL. Information about epistatic QTL is fundamental to obtaining a fuller understanding of the genomic networks influencing biological systems. In addition to information about the effect of individual QTL or genes, an understanding of the effect of interactions is important to build up a fuller understanding of the genomic networks which influence variation in biological systems (Carlborg and Haley 2004).

In the present study epistatic QTL were analysed for on a trait by trait basis, however, the genomic control of growth and body composition is probably more complex, and interactions probably exist between traits. Investigating interactions between traits was not the objective of the present study.

Epistasis has been found to be an important source of genetic variation of quantitative traits in crops, laboratory animals as well as livestock species (e.g. Carlborg *et al.* 2003; Carlborg *et al.* 2005; Xu and Jia 2007). Epistatic QTL in pigs have been reported so far for reproduction traits (Bidanel 1993; Rodriguez *et al.* 2005; Noguera *et al.* 2006), coat colour (Hirooka *et al.* 2002), meat quality traits (meat colour and intramuscular fat content) (Ovilo *et al.* 2002; Szyda *et al.* 2006) and muscle fibre traits (Estelle *et al.* 2008) and in the present study for growth, feed intake, food conversion ratio and chemical body composition. Therefore, it may be of interest to exploit epistatic QTL within selection strategies. Particularly, the AA effect was found to be the most prevalent for the traits analysed in this study. These AA genetic effects have been shown

to be heritable (Goodnight 1988). Jannink (2003) studied the AA effects in response to selection and found that epistatic gene action may condition greater and more long-term response to selection than additive gene action. Furthermore, to optimise the use of QTL in breeding, e.g. in marker assisted selection, it is important to account for epistasis within QTL mapping, to prevent QTL remaining undetected, and to prevent the estimated effects of QTL being biased, leading to invalid interpretations of the importance of many QTL (Carlborg 2006).

Chapter 5

Epistatic quantitative trait loci analysis of carcass characteristics in pigs reveals genomic interactions of QTL including the locations of *IGF-2* or *MC4R*

Abstract

The present study focussed on the identification of epistatic QTL pairs for body composition traits (carcass cuts, lean tissue and fat tissue weights) measured at slaughter weight (140 kg body weight) in a three generation full-sib population developed by crossing Pietrain sires with a crossbred dam line. For the QTL analysis, 386 animals were genotyped for 88 molecular markers covering chromosomes SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14. In total, 24 significant epistatic QTL pairs were identified with the additive-by-additive genetic interaction being the most prevalent. Epistatic QTL were identified across all chromosomes except for SSC13 and epistatic QTL pairs accounted for between 5.8% and 10.2% of the phenotypic variance. Eight epistatic QTL pairs were between QTL which resided on the same chromosome and 16 between QTL which resided on different chromosomes. SSC1, SSC8, SSC9, SSC2, SSC6 and SSC4 harboured the highest number of epistatic QTL. The epistatic QTL pairs with the highest effects were for entire loin weight between two locations on SSC7, and for carcass length, between two genomic locations on SSC1, explaining 10.2% and 9.5% of the phenotypic variance, respectively. Epistatic associations were identified between regions of the genome which contain the *IGF2* or *MC4R* genes with QTL residing in other genomic locations. QTL in the region of the *MCR4* gene and on SSC7 showed significant positive dominance effects for entire belly weight, which were offset by negative dominance-by-dominance interactions between these QTL. In contrast, the QTL in the region of the *IGF2* showed significant negative dominance effects for entire ham weight, which were largely overcompensated by positive additive-by-dominance genetic effects with a QTL on SSC9. The study shows that epistasis is of great important for the genomic regulation of body composition of pigs and contributes substantially to the variation in complex traits.

5.1 Introduction

Numerous quantitative trait loci (QTL) have been reported for carcass characteristics in pigs (e.g. Geldermann *et al.* 2003; Karlskov-Mortensen *et al.* 2006; Liu *et al.* 2007). These studies have focused on identifying the individual QTL effects (additive, dominance and imprinting), without considering interactions between loci (epistasis). When epistasis is ignored, some QTL may remain undetected, and the effects of the identified QTL can be severely biased (Carlborg 2006). Furthermore, the inclusion of epistasis provides a better understanding of the genomic control of economically important traits.

Evidence exists for epistatic QTL in pigs, for reproductive traits (Bidanel 1993; Rodriguez *et al.* 2005; Noguera *et al.* 2006), coat colour (Hirooka *et al.* 2002), meat quality (Ovilo *et al.* 2002; Szyda *et al.* 2006) and muscle fibre traits (Estelle *et al.* 2008). Studies in chickens have shown that epistasis is involved in the genomic regulation of growth traits (Carlborg *et al.* 2003; Carlborg *et al.* 2004). Also, studies in mice have identified epistatic QTL for growth and obesity (e.g. Brockmann *et al.* 2000; Yi *et al.* 2004ab; Yi *et al.* 2006). Generally these studies suggest that different networks of interactions are involved in the genomic regulation of different groups of traits.

Body composition of pigs may be controlled by a complex set of interactions; however, there is currently a lack of knowledge for epistatic QTL involved in the genomic regulation of lean and fat tissue of pigs. This is most likely because of the computational demand associated with this type of analysis, rather than epistasis not being important for the genomic regulation of these traits.

In the present study, epistatic QTL pairs were investigated for a number of carcass cuts as well as lean and fat tissue traits in a commercial pig population, developed by crossing Pietrain sires with a crossbred dam line.

5.2 Materials and methods

5.2.1 Design and data

The QTL mapping experiment of this study was based on data from a resource family of a three generation full-sib design. The resource family was created by mating seven Pietrain grandsires, which were unrelated, to 16 grandams of a crossbred dam line (Leicoma × (Landrace × Large White)). The Pietrain sires were all heterozygous at the *ryanodine receptor 1 (RYR1)* locus. Eight boars and 40 sows of the F_1 generation were mated to produce two litters of the F_2 generation comprising of 315 pigs of 49 families. Animals of the F_2 generation were either housed individually or in groups of up to 15 pigs of mixed sex in straw-bedded pens. Individual housed pigs (48 gilts and 46 barrows) were fed manually and feed consumption was recorded from these animals weekly. Group housed animals (117 gilts and 10 barrows) were supplied food by an electronic feeding station (ACEMA 48), which recorded feed consumption at every visit. All animals were provided with one of three pelleted diets containing 13.8 MJ ME/kg and 1.2% lysine, 13.8 MJ ME/kg and 1.1% lysine, or 13.4 MJ ME/kg and 1.0% lysine for weight ranges 30-60, 60-90 and 90-140 kg body weight, respectively. All animals were provided with *ad libitum* access to diets, which were formulated above requirement in order to reach maximal protein deposition. For a more detailed description of the management of this project see the studies of Landgraf *et al.* (2006ab) and Mohrmann *et al.* (2006ab).

5.2.2 Carcass composition

Phenotypic data of body composition were collected from pigs slaughtered in a commercial abattoir at 140 kg body weight. Using the AutoFOM device, measurements of valuable carcass cuts were obtained. This device adopts an automatic ultrasound scanning technique to produce a three-dimensional image of the carcass (Brondum *et al.* 1998). Using the AutoFOM device, measurements were obtained for average fat thickness, belly weight, lean content, lean content of the belly as well as weights of entire and trimmed shoulder, loin and ham without bones. Thereafter, the right carcass side of each pig was dissected into primal carcass cuts neck, shoulder, loin, ham and belly weights. Neck, shoulder, loin and ham cuts were further dissected into lean and fat tissue. Moreover, weights of jowl, thick rib, flank, front as well as hind hock, tail and claw were recorded. From the cold left carcass side, further measurements were obtained including carcass length, sidefat thickness; at the $13^{th}/14^{th}$ rib interface loin eye area, fat area, and thinnest fat measure (fat degree B); fat content and area of the belly. Protein content of loin and intramuscular fat content was measured in the *musculus longissimus thoracis et lumborum* using near-infrared reflectance spectroscopy. Additional information about the dissection of carcasses is presented in the study of Landgraf *et al.* (2006b). Table 5.1 outlines mean values and standard deviations of traits analysed in the present study.

Table 5.1 Means and standard deviations (SD) of carcass characteristics measured on pigs of the F_2 generation

Trait	Mean	SD	Number of Records
AutoFOM traits			
AF average fat thickness (mm)	22.295	4.989	313
AF entire shoulder weight (kg)	6.176	0.406	313
AF shoulder lean meat weight (kg)	4.577	0.408	313
AF entire loin weight (kg)	6.265	0.396	313
AF loin lean meat weight (kg)	3.764	0.352	313
AF entire ham weight (kg)	13.573	0.814	313
AF ham lean meat weight (kg)	9.511	1.052	313
AF entire belly weight (kg)	9.168	0.548	313
AF lean content (%)	50.509	6.403	313
AF lean content of belly (%)	43.741	7.891	313
Carcass characteristics – dissected carcass cuts			
Entire neck weight (kg)	5.316	0.505	306
Neck weight without external fat (kg)	4.160	0.430	306
External neck fat weight (kg)	1.156	0.285	306
Entire shoulder weight (kg)	8.452	0.564	307
Shoulder weight without external fat (kg)	5.910	0.584	307
External shoulder fat weight (kg)	1.403	0.261	307
Entire loin weight (kg)	9.163	0.730	308
Loin weight without external fat (kg)	6.650	0.624	308
External loin fat weight (kg)	2.513	0.645	308
Entire ham weight (kg)	16.908	0.997	310
Ham weight without external fat (kg)	11.568	1.087	310
External ham fat weight (kg)	2.566	0.493	310
Belly weight (kg)	6.461	0.655	308
Jowl weight (kg)	1.914	0.284	306
Thick rib (kg)	1.441	0.217	307
Flank weight (kg)	1.789	0.407	308
Front hock weight (kg)	1.139	0.189	307
Hind hock weight (kg)	1.430	0.141	310
Tail weight (kg)	0.429	0.134	310
Hind claw (kg)	0.914	0.122	310
Carcass characteristics – standard performance test			
Carcass length (cm)	107.947	49.296	310
Sidefat thickness[1] (cm)	3.847	0.866	315
Thinnest fat measure[1] (cm)	1.725	0.552	314
Loin eye area *M.l.t.l.*[1,2] (cm^2)	54.160	6.767	314
Fat area *M.l.t.l.*[1,2] (cm^2)	24.514	5.884	314
Fat content of belly (%)	53.508	8.272	306
Fat area of belly (cm^2)	23.789	6.782	306
Intramuscular fat content (%)	1.343	0.542	313
Protein content of loin (%)	24.215	2.066	313

[1]collected at the 13th/14th rib interface.

[2]measured on *musculus longissimus thoracis et lumborum*.

5.2.3 Genotypic data

From the F_0, F_1 and F_2 animals, blood samples were collected from the *vena jugularis* and their genomic DNA was isolated. Chromosomes SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14 were chosen for genotyping due to their likely associations with carcass cuts as well as lean and fat tissue. All pigs were genotyped for 88 informative microsatellite markers of which 10, 9, 9, 9, 10, 8, 9, 9, 8 and 7 genomic markers were located on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14, respectively. Based on the published USDA linkage map, markers and their distances were selected (http://www.marc.usda.gov; Rohrer *et al.* 1996). This linkage map provided all information relating to their position and alleles, outlined in Table 5.2. The average distance between markers was 16.0, 16.5, 16.3, 20.6, 17.3 18.4, 17.3, 16.0, 18.0 and 17.4 cM and the largest gaps between markers were 27.7, 25.2, 26.5, 28.7, 26.2, 23.1, 21.7, 20.8, 24.0 and 23.6 cM on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14, respectively.

Table 5.2 Markers used in the present QTL mapping project, their relative map position using USDA pig map, number of different alleles, polymorphic information content in the F_2 generation (PIC) and heterozygosity in F_1 generation (H)

Marker	SSC	Position (cM)	H	Number of alleles	PIC
SW1514	1	0.0	0.79	8	0.75
SW1515	1	16.4	0.67	8	0.68
SW1332	1	29.2	0.63	4	0.37
SW1851	1	44.6	0.73	4	0.53
SW1430	1	58.5	0.81	6	0.76
SWR982	1	86.2	0.88	7	0.77
SW1311	1	100.8	0.58	6	0.62
SW1828	1	118.5	0.90	7	0.69
SW1301	1	140.5	0.83	5	0.67
SW2512	1	144.0	0.77	6	0.55
SWR2516	2	0.0	0.67	5	0.48
SW2623	2	9.8	0.68	5	0.63
SWR783	2	23.7	0.51	3	0.30
SW240	2	42.0	0.84	7	0.78
SW1026	2	60.6	0.47	6	0.55
SW1370	2	74.8	0.91	8	0.69
SWR2157	2	89.2	0.78	8	0.68
SWR345	2	114.4	0.87	8	0.75
S0036	2	132.1	0.85	7	0.80
SW2404	4	0.0	0.91	10	0.81
SW489	4	8.0	0.66	5	0.53
S0301	4	27.1	0.72	6	0.56
S0001	4	41.8	0.66	6	0.65
SW839	4	62.3	0.44	4	0.45
S0214	4	79.3	0.80	6	0.74
SW445	4	105.8	0.91	10	0.77
MP77	4	120.0	0.87	8	0.74
SW856	4	130.1	0.98	14	0.84
MP35	6	0.0	0.70	6	0.59
SW2406	6	21.4	0.74	8	0.61
SW1841	6	41.5	0.98	15	0.88
S0087	6	62.8	0.75	5	0.59
SW122	6	83.3	0.85	7	0.69
S0228	6	105.2	0.69	6	0.68
SW1881	6	121.1	0.96	8	0.76
SW322	6	149.8	0.79	8	0.72
SW2052	6	164.6	0.79	9	0.78
SW2564	7	0.0	0.69	5	0.49
SWR1343	7	12.2	0.83	4	0.53
SW2155	7	32.9	0.67	4	0.48
SW1369	7	48.2	0.77	8	0.68
SW1856	7	61.5	0.69	5	0.48
SWR2036	7	78.2	0.81	9	0.77
SW632	7	104.4	0.77	6	0.67
SWR773	7	117.3	0.56	3	0.46
SW2537	7	139.5	0.69	7	0.63
SW764	7	156.0	0.76	5	0.65

Table 5.2 continued

Marker	SSC	Position (cM)	H	Number of alleles	PIC
SW2410	8	-1.3	0.42	4	0.44
SW905	8	20.8	0.71	6	0.71
SWR1101	8	38.3	0.88	12	0.75
SW444	8	52.5	0.85	7	0.76
S0086	8	62.2	0.69	6	0.56
SW374	8	82.8	0.88	5	0.63
SW1551	8	105.9	0.75	6	0.66
S0178	8	127.7	0.54	7	0.68
SW983	9	4.0	0.81	6	0.61
SW21	9	15.1	0.65	5	0.50
SW911	9	36.8	0.75	7	0.68
SW2401	9	57.1	0.71	6	0.68
SW2571	9	73.3	0.46	6	0.61
S0019	9	86.4	0.75	6	0.62
SW2093	9	103.6	0.90	6	0.77
SW174	9	122.9	0.81	3	0.51
SW1349	9	142.5	0.81	7	0.75
SW830	10	0.0	0.67	7	0.64
SWR136	10	7.6	0.77	6	0.72
SW1894	10	23.2	0.65	4	0.50
SW2195	10	44.0	0.48	3	0.42
SW173	10	56.1	0.35	4	0.39
SW1041	10	67.5	0.46	3	0.41
SW2043	10	87.7	0.56	5	0.72
SW1626	10	108.0	0.79	11	0.68
SW2067	10	128.0	0.81	7	0.69
S0282	13	0.0	0.90	8	0.77
SWR1941	13	14.1	0.87	7	0.71
SW1407	13	27.2	0.88	11	0.83
SW864	13	43.1	0.63	5	0.64
S0068	13	62.2	0.78	9	0.72
SW398	13	79.3	0.69	6	0.66
SW2440	13	102.2	0.96	6	0.79
S0291	13	126.2	0.83	8	0.79
SW857	14	7.4	0.87	9	0.74
S0089	14	14.0	0.67	7	0.71
SW245	14	32.0	0.77	7	0.71
SW342	14	53.2	0.79	7	0.71
SW1081	14	72.1	0.87	6	0.65
SW1557	14	87.9	0.64	4	0.49
SWC27	14	111.5	0.45	8	0.41

5.2.4 Statistical analysis

Because of the computational demand of a genomic scan for epistatic QTL, the analysis was performed in two stages, following Estelle *et al.* (2008). In the first stage, a 5 cM scan was carried out across all genomic positions in order to pre-select potential candidate regions with epistatic effects with the model:

$$y_i = sex_i + MHS_i + batch_i + \beta slwt_i + C_{aa}I_{aa} + C_{ad}I_{ad} + C_{da}I_{da} + C_{dd}I_{dd} + e_i, \qquad [1]$$

where y_i is the i-th individual phenotype. Fixed effects and covariates were fitted in the model depending on their significance for the trait. For all traits *sex*, *RYR1* genotype (*MHS*) and *batch* were included in the model and slaughter weight (*slwt*) was considered as covariate β. I_{aa}, I_{ad}, I_{dd} and I_{dd} are the additive × additive (AA), additive × dominance (AD), dominance × additive (DA) and dominance × dominance (DD) epistatic effects, respectively. These four epistatic effects were estimated, following the Cockerham's decomposition (Cockerham 1954), by regressing on a linear combination of the individual QTL origin probabilities:

$C_{aa} = P_1(QQ)P_2(QQ) - P_1(QQ)P_2(qq) - P_1(qq)P_2(QQ) + P_1(qq)P_2(qq),$
$C_{ad} = P_1(QQ)P_2(Qq) - P_1(qq)P_2(Qq),$
$C_{da} = P_1(Qq)P_2(QQ) - P_1(Qq)P_2(qq),$
$C_{dd} = P_1(Qq)P_2(Qq),$

where P_1 and P_2 refers to the probability of QTL at location 1 and 2, respectively, and $P(QQ)$ is the probability of being homozygous of the grandpaternal sire line (Pietrain) $P(qq)$ is the probability of being homozygous of the grandmaternal dam line and $P(Qq)$ is the probability of being heterozygous (Varona *et al.* 2002). This model [1] was tested against a null model where no epistatic effects were estimated:

$$y_i = sex_i + MHS_i + batch_i + \beta slwt_i + e_i, \tag{2}$$

Interacting QTL pairs with P-values < 0.001 were selected for further analyses.

In the second stage, a complete epistatic model including the individual QTL effects was applied using a 1 cM scan around the pre-selected positions obtained in the first stage. This model included, besides all environmental effects, the individual additive and dominance genetic effects as well as epistatic genetic effects:

$$y_i = sex_i + MHS_i + batch_i + \beta slwt_i + C_{a1}a_1 + C_{a2}a_2 + C_{d1}d_1 + C_{d2}d_2 + C_{aa}I_{aa} + C_{ad}I_{ad} + C_{da}I_{da} + C_{dd}I_{dd} + e_i,$$

$$\tag{3}$$

where a denotes the individual additive genetic effect and C_a represents the difference in probabilities of being homozygous for alleles of the grandpaternal sire line (QQ) and being homozygous for alleles from the grandmaternal dam line (qq). A positive additive genetic value indicates that the allele originating from the grandpaternal sire line (Pietrain) showed a higher effect than the allele from the grandmaternal dam line and *vice versa*. The effect d represents the individual dominance genetic effects and C_d gives the probability of being heterozygous. The dominance effect is defined as deviation of heterozygous animals from the mean of both types of homozygous animals. A positive dominance value indicates an increase in the trait of interest as a result of a heterozygous genotype and *vice versa*. This model [3] was tested against a null model that contained only the individual QTL effects:

$$y_i = sex_i + MHS_i + batch_i + \beta slwt_i + C_{a1}a_1 + C_{a2}a_2 + C_{d1}d_1 + C_{d2}d_2 + e_i, \tag{4}$$

Epistatic interactions were reported as significant if they had a nominal P-value < 0.001. All analyses were performed with QxPak software (Perez-Enciso and Misztal 2004).

This program uses mixed models and the maximum likelihood method to estimate the QTL location and effects. The analysis of QxPak proceeds in two main stages. In the first stage the probabilities of alleles being identical-by-descent are calculated using a Monte Carlo Markov Chain algorithm. In the second stage, the mixed model equations are built and the QTL estimates are obtained using a maximum likelihood approach via the expectation-maximisation algorithm. At each putative position the likelihood ratio is computed and the estimates for the parameters are those where the likelihood is highest. In this analysis the significance is tested with a likelihood ratio test which consists of computing minus twice the difference in log-likelihoods between the alternative and the null models (Perez-Enciso and Misztal 2004).

5.3 Results and discussion

In total, 24 significant epistatic QTL pairs were identified. Of these, ten epistatic QTL pairs were identified for entire carcass characteristics (lean and fat) (Table 5.3), seven for lean tissue characteristics (Table 5.4) and seven for fat tissue characteristics (Table 5.5). Epistatic interactions were identified between QTL on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, and SSC14. No epistatic QTL were identified on or with SSC13. Epistatic QTL pairs explained between 6.2% and 10.2% of the phenotypic variance for entire carcass characteristics (lean + fat), between 5.9% and 8.4% for lean tissue characteristics, and between 5.8% and 6.8% for fat tissue characteristics. Eight of the significant epistatic QTL pairs were between QTL which resided on the same chromosome, on SSC1, SSC2, SSC4, SSC6, SSC7 and SSC8. All types of epistatic effect were identified (AA, AD, DA and DD) in this study, with the AA interaction the most prevalent. The epistatic QTL pairs with the highest effect were for entire loin weight between two locations on SSC7, and for carcass length between two genomic locations on SSC1. These QTL explained a large proportion of the phenotypic variance, at 10.2% and 9.5%, respectively.

Table 5.3 Evidence of epistatic interactions for entire carcass characteristics (lean + fat) measured after dissection and by the AutoFom device (AF)

Trait	LR	P value	Q0 chr (pos)[1]	Q1 chr (pos)[1]	% var[2]	Q0_a ± SE[3]	Q0_d ± SE[3]	Q1_a ± SE[3]	Q1_d ± SE[3]	Q01_aa ± SE[4]	Q01_ad ± SE[4]	Q01_da ± SE[4]	Q01_dd ± SE[4]
Carcass length (cm)	30.88	3.2E-06	1 (45)	1 (59)	9.5	-25.821 ± 22.207	**87.477 ± 27.611**	32.301 ± 22.341	18.625 ± 25.921	16.776 ± 21.645	33.960 ± 26.596	**-103.698 ± 28.612**	**-90.181 ± 31.885**
AF entire belly weight (kg)	21.68	2.3E-04	1 (88)	7 (148)	6.7	-0.086 ± 0.075	**0.309 ± 0.118**	0.033 ± 0.070	**0.663 ± 0.130**	0.061 ± 0.069	-0.122 ± 0.130	-0.021 ± 0.108	**-1.040 ± 0.212**
Hind hock weight (kg)	22.52	1.6E-04	1 (35)	8 (107)	6.5	-0.035 ± 0.028	**0.126 ± 0.053**	0.036 ± 0.028	0.066 ± 0.052	**0.120 ± 0.025**	0.049 ± 0.050	-0.038 ± 0.048	**-0.190 ± 0.091**
Hind claw (kg)	22.33	1.7E-04	1 (63)	9 (23)	7.1	0.031 ± 0.023	0.073 ± 0.038	0.010 ± 0.021	0.072 ± 0.042	**-0.074 ± 0.021**	-0.061 ± 0.040	-0.055 ± 0.035	**-0.196 ± 0.068**
Entire ham weight (kg)	23.51	1.0E-04	2 (10)	9 (66)	7.3	-0.213 ± 0.124	**-0.408 ± 0.194**	-0.177 ± 0.119	-0.369 ± 0.231	**-0.242 ± 0.118**	**0.881 ± 0.225**	**0.436 ± 0.187**	0.529 ± 0.345
Belly weight (kg)	27.93	1.3E-05	4 (130.1)	4 (31)	8.7	0.043 ± 0.085	**-0.282 ± 0.127**	-0.140 ± 0.084	-0.187 ± 0.143	**-0.410 ± 0.083**	-0.170 ± 0.142	0.170 ± 0.123	**0.433 ± 0.209**
Entire neck weight (kg)	21.50	2.5E-04	6 (71)	6 (86)	6.8	**-0.876 ± 0.380**	0.015 ± 0.457	**0.844 ± 0.389**	-0.191 ± 0.441	-0.450 ± 0.328	**1.067 ± 0.473**	**-1.145 ± 0.503**	-0.322 ± 0.565
Entire loin weight (kg)	33.11	1.1E-06	7 (77)	7 (86)	10.2	1.227 ± 1.537	2.197 ± 1.142	-1.451 ± 1.509	2.571 ± 1.353	**2.954 ± 0.953**	-0.524 ± 1.990	1.282 ± 1.779	-1.764 ± 1.467
Flank weight (kg)	19.90	5.2E-04	7 (88)	10 (23)	6.2	0.056 ± 0.067	0.086 ± 0.124	0.037 ± 0.069	0.121 ± 0.113	**0.249 ± 0.066**	-0.167 ± 0.106	-0.227 ± 0.118	-0.308 ± 0.197
AF entire shoulder weight (kg)	24.97	5.1E-05	8 (21)	8 (37)	7.7	**0.849 ± 0.273**	-0.491 ± 0.340	**-0.729 ± 0.254**	-0.699 ± 0.397	**-0.684 ± 0.225**	**-0.991 ± 0.415**	0.665 ± 0.343	0.484 ± 0.504

Definition of symbols: LR, likelihood ratio; chr, chromosome.
Values in bold represent significant additive, dominance or epistatic effects.
[1]Positions of the QTL in cM.
[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no epistatic effects - residual variance of model with epistatic effects)/residual variance of model with no epistatic effects.
[3]Estimated additive (a) and dominance (d) effects and their standard errors (SE) of the individual QTL.
[4]Estimated additive x additive (aa), additive x dominance (ad), dominance x additive (da) and dominance x dominance (dd) effects and their standard errors (SE).

Table 5.4 Evidence of epistatic interactions for lean tissue characteristics measured after dissection and by the AutoFom device (AF)

Trait	LR	P value	Q0 chr (pos)[1]	Q1 chr (pos)[1]	% var[2]	Q0_a ± SE[3]	Q0_d ± SE[3]	Q1_a ± SE[3]	Q1_d ± SE[3]	Q01_aa ± SE[4]	Q01_ad ± SE[4]	Q01_da ± SE[4]	Q01_dd ± SE[4]
Protein content of loin (%)	21.64	2.4E-04	2 (93)	2 (117)	6.7	**1.160 ± 0.323**	**1.208 ± 0.405**	**-1.225 ± 0.323**	**1.204 ± 0.402**	**1.378 ± 0.317**	**-1.142 ± 0.406**	**1.162 ± 0.408**	**-1.049 ± 0.504**
AF lean content of belly (%)	21.87	2.1E-04	2 (9)	8 (55)	6.7	1.973 ± 1.168	**5.305 ± 1.773**	0.130 ± 1.057	**4.762 ± 2.012**	**-3.022 ± 1.058**	-1.582 ± 1.990	-3.089 ± 1.631	**-10.239 ± 2.954**
Loin eye area M.l.t.l.[5,6] (cm^2)	27.71	1.4E-05	2 (22)	9 (136)	8.4	-2.565 ± 1.318	-4.516 ± 2.300	-1.856 ± 1.255	-3.497 ± 2.459	**4.275 ± 1.170**	**6.658 ± 2.314**	**5.782 ± 2.110**	**11.189 ± 4.045**
Protein content of loin (%)	19.69	5.7E-04	4 (121)	7 (1)	6.1	-0.149 ± 0.089	0.067 ± 0.128	0.142 ± 0.090	0.127 ± 0.127	-0.140 ± 0.089	**0.522 ± 0.127**	-0.145 ± 0.128	-0.036 ± 0.186
Loin weight without external fat (kg)	22.05	2.0E-04	4 (89)	14 (66)	6.9	0.088 ± 0.113	-0.358 ± 0.196	**-0.226 ± 0.105**	-0.022 ± 0.202	**0.328 ± 0.102**	0.034 ± 0.196	**0.505 ± 0.178**	0.508 ± 0.334
Loin weight without external fat (kg)	18.71	9.0E-04	6 (28)	8 (60)	5.9	0.094 ± 0.116	0.061 ± 0.213	-0.086 ± 0.108	0.335 ± 0.223	**0.409 ± 0.101**	-0.188 ± 0.207	0.271 ± 0.190	-0.468 ± 0.379
Neck weight without external fat (kg)	19.64	5.9E-04	6 (145)	9 (58)	6.3	-0.099 ± 0.070	**0.323 ± 0.131**	0.130 ± 0.071	**0.431 ± 0.121**	**0.158 ± 0.065**	**0.249 ± 0.112**	-0.047 ± 0.124	**-0.776 ± 0.225**

Definition of symbols: LR, likelihood ratio; chr, chromosome.

Values in bold represent significant additive, dominance or epistatic effects.

[1]Positions of the QTL in cM.

[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no epistatic effects - residual variance of model with epistatic effects)/residual variance of model with no epistatic effects.

[3]Estimated additive (a) and dominance (d) effects and their standard errors (SE) of the individual QTL.

[4]Estimated additive x additive (aa), additive x dominance (ad), dominance x additive (da) and dominance x dominance (dd) effects and their standard errors (SE).

[5]collected at the 13th/14th rib interface.

[6]measured on *musculus longissimus thoracis et lumborum*.

Table 5.5 Evidence of epistatic interactions for fat tissue characteristics measured after dissection and by the AutoFom device (AF)

Trait	LR	P value	Q0 chr (pos)[1]	Q1 chr (pos)[1]	% var[2]	Q0_a ± SE[3]	Q0_d ± SE[3]	Q1_a ± SE[3]	Q1_d ± SE[3]	Q01_aa ± SE[4]	Q01_ad ± SE[4]	Q01_da ± SE[4]	Q01_dd ± SE[4]
External ham fat weight (kg)	21.79	2.2E-04	1 (48)	1 (118)	6.8	-0.080 ± 0.071	0.168 ± 0.108	0.107 ± 0.072	-0.073 ± 0.101	-0.131 ± 0.072	-0.092 ± 0.100	**-0.367 ± 0.106**	-0.185 ± 0.150
Intramuscular fat content (%)	20.82	3.4E-04	1 (126)	4 (94)	6.4	**0.257 ± 0.090**	0.087 ± 0.151	0.102 ± 0.088	0.246 ± 0.154	-0.036 ± 0.085	**-0.721 ± 0.150**	-0.171 ± 0.142	-0.288 ± 0.258
AF average fat thickness (mm)	19.56	6.1E-04	1 (142)	6 (119)	6.1	**-1.656 ± 0.672**	-0.419 ± 0.988	0.217 ± 0.652	1.858 ± 1.159	**2.293 ± 0.651**	**4.014 ± 1.153**	-1.053 ± 0.956	0.248 ± 1.700
External neck fat weight (kg)	19.61	6.0E-04	4 (1)	4 (120)	6.1	0.015 ± 0.033	-0.075 ± 0.048	-0.050 ± 0.033	-0.056 ± 0.049	**-0.139 ± 0.033**	-0.031 ± 0.048	0.005 ± 0.048	0.130 ± 0.070
Fat content of belly (%)	19.54	6.2E-04	4 (106)	6 (12)	6.2	1.027 ± 1.069	0.734 ± 1.572	-0.478 ± 1.007	-0.788 ± 1.858	**-4.737 ± 1.020**	-2.345 ± 1.885	-0.796 ± 1.478	-0.003 ± 2.669
Thinnest fat measure[5] (cm)	18.70	9.0E-04	6 (42)	8 (56)	5.8	-0.131 ± 0.088	**-0.462 ± 0.145**	-0.121 ± 0.079	**-0.369 ± 0.155**	**-0.234 ± 0.078**	0.112 ± 0.153	0.166 ± 0.134	**0.789 ± 0.248**
External loin fat weight (kg)	18.90	8.2E-04	6 (150)	9 (57)	6.0	0.130 ± 0.099	-0.115 ± 0.174	-0.078 ± 0.097	**-0.336 ± 0.165**	**-0.235 ± 0.093**	**-0.325 ± 0.159**	-0.023 ± 0.165	**0.901 ± 0.299**

Definition of symbols: LR, likelihood ratio; chr, chromosome.

Values in bold represent significant additive, dominance or epistatic effects.

[1]Positions of the QTL in cM.

[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no epistatic effects - residual variance of model with epistatic effects)/residual variance of model with no epistatic effects.

[3]Estimated additive (a) and dominance (d) effects and their standard errors (SE) of the individual QTL.

[4]Estimated additive x additive (aa), additive x dominance (ad), dominance x additive (da) and dominance x dominance (dd) effects and their standard errors (SE)

[5]collected at the 13th/14th rib interface.

5.3.1 Entire carcass characteristics

Weights of important carcass cuts are economically important for the market value of the carcass. In the present study, ten epistatic interactions were identified for entire carcass cuts.

Large DA and DD interactions were identified between two close genomic locations on SSC1 for carcass length. These QTL were not identified in previous individual QTL analyses. Around the location of these QTL there are numerous reports of QTL for entire carcass cuts, lean and fat tissue, as well as growth (cited in Table 5.6).

A further DD interaction was identified between QTL on SSC1 and SSC7 for entire belly weight measured by the AutoFom device. The QTL on SSC1 was previously identified in individual QTL mapping analyses by Mohrmann *et al*. (2006a) and in both studies this QTL showed a significant dominance effect. Around this location of SSC1, numerous QTL have been reported for lean tissue and fat tissue (cited in Table 5.6). This QTL is in the vicinity of the *melanocortin-4 receptor* locus (*MC4R*) which is located close to *SWR982* at 86 cM. This locus is important for controlling energy balance and body weight, and hence is a candidate gene for traits associated with feed intake and energy-homeostasis related traits (Meidtner *et al.* 2006). There are many reports of an association of *MC4R* with growth and fatness (Kim *et al.* 2000; Park *et al.* 2002; Houston *et al.* 2004; Meidtner *et al.* 2006). Meidtner *et al.* (2006) reported that *MC4R* could be a useful marker to increase growth of the slow-growing Pietrain breed by increasing feed intake. No QTL were identified in previous analysis on SSC7, which was surprising because there is strong evidence for QTL on SSC7 in the literature (e.g. Milan *et al.* 2002; Nezer *et al.* 2002; Yue *et al.* 2003b; Kim *et al.* 2005; Sanchez *et al.* 2006). Therefore, the QTL identified on SSC7 has only expressed its effects through DD interactions with SSC1.

A QTL on SSC1 showed an interaction with a QTL on SSC8 for hind hock weight. Neither of these QTL were identified in previous analyses of the present data, which may be expected because the negative interaction effect is almost as high as the sum of the individual QTL effects. Around the same location of SSC1 a QTL has been reported for growth rate (cited in Table 5.6). However there are no reports in the literature confirming the QTL on SSC8.

A further location of SSC1 showed an interaction with SSC9 for weight of hind claw. These QTL were not identified in previous analyses. These QTL showed no significant additive or dominance effects and only expressed their effects through novel interactions between additive as well as dominance effects. The QTL on SSC1 for hind claw was around the same location as that for carcass length close to *SW1430*. Around this location of SSC1 the *insulin-like growth factor-1 receptor* (*IGF1R*) is located. *Insulin-like growth factor 1* (*IGF-1*) plays an important role in regeneration, metabolism and proliferation in a variety of cell types (Schweiger *et al.* 2005). In association with *IGF-1*, *IGF1R* regulates growth and differentiation of a variety of cells and controls body weight, not only after birth but also during the pre-natal stage (Kopecny *et al.* 2002). Many QTL for carcass traits have been identified around this location (cited in Table 5.6) and therefore this is a candidate gene for growth and body composition.

Several epistatic effects were identified between the telomeric end of the p arm of SSC2 and 66 cM of SSC9 for entire ham weight. In the present study the QTL on SSC2 and SSC9 showed substantial interactions between additive and dominance effects which more than offset the negative effects associated with dominance and AA genetic effects. From previous analysis of these data, numerous QTL were identified around this location of SSC2 where Pietrain alleles were associated with increased lean tissue and reduced fatness (Duthie *et al.* 2008; Chapter 2). Whereas the QTL on SSC2 was affected by individual dominance effects, the QTL on SSC9 showed no significant individual QTL effects in the present study. However, from previous analysis, QTL were identified in this genomic location for entire shoulder weight and shoulder weight without external

fat (Duthie *et al.* 2008; Chapter 2). There are reports of QTL around this location of SSC2 for carcass traits, lean tissue and fat tissue and around the region of SSC9 for body weight (cited in Table 5.6). In the region of the QTL on SSC2, a paternally expressed QTL which affects growth and fat deposition has been mapped to the *insulin-like growth factor 2 (IGF2)* locus (Jeon *et al.* 1999; Nezer *et al.* 1999). Van Laere *et al.* (2003) showed that this QTL is caused by a nucleotide substitution in intron 3 of this gene.

Interactions between additive or dominance effects were identified between two locations of SSC4, on the p arm (31 cM) and the telomeric end of the q arm (130.1 cM), for belly weight. The QTL on the p arm was not identified in previous individual QTL analysis of the data, whereas a QTL was identified for lean content at 33 cM, for which Pietrain alleles were associated with decreased lean tissue (Duthie *et al.* 2008; Chapter 2). QTL have been reported around 31 cM for numerous carcass traits as well as lean and fat tissue, however only a single QTL has been reported at the telomeric end of the q arm for daily gain (cited in Table 5.6). The QTL at 31 cM is located between *S0301* and *S0001*. Within this marker bracket lies the *F-BOX protein 32* (*FBXO32*) gene (Yu *et al.* 2005). Expression of this gene has been found to increase in myotubules during muscle atrophy, whereas mice deficient in *FBXO32* were resistant to atrophy (Bodine *et al.* 2001). This gene could be an important gene for muscle mass development (Glass 2003). Moreover, between these markers is the *exostoses (multiple) 1* gene which is located in the same position as *S0301* (27.1) (Cepica *et al.* 2002). This is a candidate gene for growth related traits.

For entire neck weight, AD and DA interactions were identified between two close genomic locations of SSC6 (71 and 86 cM). There are numerous reports in the literature for QTL associated with carcass traits, lean and fat tissue in these locations (cited in Table 5.6). No QTL were detected near 71 cM from previous individual QTL analyses of this data, but Mohrmann *et al.* (2006a) reported a large number of QTL around the QTL at 86 cM for several carcass cuts (lean + fat), fat tissue, lean tissue characteristics and chemical body composition, at which Pietrain alleles were associated with decreased

fat tissue and increased lean tissue. The significant additive effect identified at the QTL (86 cM) in the present study indicated that Pietrain alleles were associated with increased neck weight. This QTL is in the same genomic location as the *RYR1* locus (Rohrer *et al.* 1996). A mutation at this locus is associated with malignant hyperthermia syndrome (Fujii *et al.* 1991). This locus is significantly associated with production traits in pigs (Kadarmideen 2008).

A novel epistatic AA QTL pair was identified on two locations of SSC7 for entire loin weight. No individual QTL effects were identified at either of these QTL outlining why they were not identified from previous individual QTL analyses. There are reports of QTL around these locations for numerous carcass characteristics (cited in Table 5.6). The QTL at 86 cM is located between *SWR2036* and *SW632*. Between these markers the *proteasome (prosome, macropain) activator subunit 1 (PA28 alpha) (PSME1)* and the *proteasome (prosome, macropain) activator subunit 2 (PA28 beta)* (*PSME2)* genes are located. *PSME1* and *PSME2* encode proteasome activators PA28α and β subunits which are two subunits of PA28 which is an activator of the proteosome and plays an important role in antigen presentation mediated by the major histocompatibility complex class I (Dubiel *et al.* 1992). Wang *et al.* (2004) reported that a polymorphism in the *PSME1* gene is associated with weaning weight. Therefore this may be a candidate gene for production traits in this location of SSC7.

An AA interaction was identified between SSC7 and SSC10 for flank weight. Again, at these QTL no individual QTL effects were identified. The QTL on SSC7 was located around the same region as for entire loin weight in the present study. Around this location of SSC7 there are reports of QTL for leanness, fatness and growth, whereas around this location of SSC10 a QTL has been reported for backfat (cited in Table 5.6).

For entire shoulder weight, AA and AD interactions were identified between two close genomic locations of SSC8 (21 and 37 cM). Duthie *et al.* (2008; Chapter 2) identified QTL at 37 cM for protein content of the loin, at which Pietrain alleles were associated

with less protein content. In this study, Pietrain alleles were associated with less shoulder weight at this QTL. The QTL at 21 cM was not identified before and therefore only exhibits effects through the interactions. QTL have been reported around these locations for numerous carcass traits, daily gain as well as lean tissue (cited in Table 5.6).

5.3.2 Lean tissue characteristics

One of the main goals of commercial pig production has been to increase lean tissue. A large number of studies have investigated QTL for lean tissue (e.g. Rohrer and Keele 1998b; Malek *et al.* 2001b; Geldermann *et al.* 2003) from individual QTL analysis. In the present study seven epistatic QTL pairs were identified for lean tissue characteristics.

For protein content of the loin tissue all fitted interactions as well as all individual QTL effects were significant between two genomic locations on SSC2 (93 and 117). A QTL was previously identified at 92 cM for shoulder weight without external fat (Duthie *et al.* 2008; Chapter 2). Around this location (93 cM), QTL have been reported for daily gain and backfat (cited in Table 5.6).

At the telomeric end of the p arm of SSC2, additive as well as dominance interactions were detected with SSC8 for lean content of the belly. The QTL on SSC2 is again around the same location as the *IGF2* gene, and numerous QTL were previously identified for lean and fat tissue QTL around this location of SSC2 (Duthie *et al.* 2008; Chapter 2). The dominance effects of the QTL on SSC8, however, were not detected in previous individual QTL mapping analyses of the data.

A slightly different location of SSC2 (22 cM) showed further interactions of all fitted combinations with SSC9 for loin eye area. No individual QTL effects were identified at these QTL, however QTL were reported in this resource family for lean tissue at the

same location on SSC2 (Duthie *et al.* 2008; Chapter 2). Interestingly, all interactions were positive, and may thus be an explanation for heterosis of these crosses in lean content. Around this location of SSC2, QTL have been reported for lean tissue as well as backfat and on SSC9 for fatness, daily gain and body weight (cited in Table 5.6).

In previous individual QTL mapping of the present resource family, no QTL were identified on SSC7 and only a few QTL were identified on SSC4. In the present study AD interactions were identified between these chromosomes for protein content of the loin. QTL have been reported around this location of SSC4 for carcass weight, body weight and liver weight (cited in Table 5.6). *Transforming growth factor, beta receptor III (TGFBR3)* is located around this location of SSC4. *Transforming growth factor- beta* is encoded by several genes including *TGFBR3* and is involved in tissue development and repair processes (Johnson *et al.* 1995).

Moreover, SSC4 showed positive interaction effects with SSC14 for loin weight without external fat. These positive interaction effects were almost four times as high as the negative additive genetic effects of the QTL on SSC14. These negative additive genetic effects at the QTL on SSC14 indicated that Pietrain alleles were associated with less lean meat of the loin. The QTL on SSC14 was at the same genomic location of SSC14 as the reported QTL for ham lean meat weight (Duthie *et al.* 2008; Chapter 2), where Pietrain alleles were also associated with decreased lean tissue weight, interpreted as a cryptic allele. Around both of these QTL, there are reports in the literature for QTL associated with numerous carcass characteristics, including lean and fat tissue (cited in Table 5.6). Around this location of SSC4 lies the *myocyte enhancer factor 2D* gene which is a member of the *myocyte enhancer binding factor 2* gene family (Wagenknecht *et al.* 2003). This gene is thought to be involved in myogenesis (Breitbart *et al.* 1993). The *myelin protein zero* gene is also located around this QTL (Wagenknecht *et al.* 2005), in the same location as QTL for carcass traits (lean and fat mass) (Cepica *et al.* 2003a). The *lamin A/C* locus is also identified in this region. This gene encodes lamins A and C (Wagenknecht *et al.* 2006). Sullivan *et al.* (1999) showed that mice lacking

lamins A have severely retarded postnatal growth and premature death and developed cardiac and skeletal myopathy. QTL for carcass traits were identified around this region of SSC4 (Cepica *et al.* 2003a) and therefore this is a candidate gene for muscle development and growth. Furthermore, *thioredoxin-interacting protein* gene is located in this region. This locus plays a crucial role in cell proliferation and growth (Yu *et al.* 2007). Yu *et al.* (2007) found significant effects of this gene on a number of important growth traits including carcass weight as well as daily gain in pigs. By comparison of two groups (slow and fast growth), they found that the expression of this gene was significantly lower in the fast growth group. Their results suggest that this gene influences growth.

A further interaction between additive genetic effects was identified between QTL on SSC6 and SSC8 for loin weight without external fat. No individual QTL effects were identified at these QTL, and these were not identified in previous analysis. Around the location of the QTL on SSC6, QTL have been identified for loin and ham percentage in the carcass and intramuscular fat content, and in the region of SSC8 QTL have been reported for a number of weights of carcass cuts and daily gain (cited in Table 5.6).

SSC6 showed further epistatic effects with SSC9 for neck weight without external fat. At both individual QTL, heterozygous animals were associated with increased lean weight. Mohrmann *et al.* (2006a) reported, for the same resource family, QTL around this location of SSC6 for lean and fat tissue showing dominance effects, whereas the QTL on SSC9 was not previously identified. The negative DD effects may be the reason for not detecting the QTL on SSC9 in an individual QTL mapping approach. QTL have been reported around this location of SSC6 for carcass length and loin eye area, and on SSC9 for lean weight and loin eye area (cited in Table 5.6). The QTL on SSC9 was situated close to *SW2401*. A candidate gene which is situated close to this marker includes *succinate dehydrogenase complex, subunit D (SDHD)*, one of the subunits of succinate dehydrogenase complex. Guimaraes *et al.* (2007) outlined that this gene is a candidate for production traits, because of its role in the SDHD complex in the process

of aerobic respiration. They reported that expression levels of this gene were associated with growth and meat quality traits in pigs. Furthermore, Zhu *et al.* (2005) reported an association of this gene with loin muscle area.

5.3.3 Fat tissue characteristics

Selection for reduced fatness has been an important goal within pig breeding over the last 50 years. Fat tissue has negative associations with consumer acceptability and the economic value of the carcass and is associated with waste and environmental impact. In the present study, epistatic interactions for seven traits associated with fatness were identified.

Epistatic DA genetic effects were identified in two genomic locations of SSC1 for external ham fat weight (48 and 118 cM). This QTL for external ham fat weight at 48 cM along with that for carcass length were identified close to *SW185* on SSC1. In a previous individual QTL analysis of the data, Mohrmann *et al.* (2006a) reported QTL at 119 cM of SSC1 for entire loin weight and external loin fat weight, attributed to dominance effects. The QTL on SSC1 for external ham fat weight at 118 cM is close to *SW1828.* At both of these QTL a large number of QTL have been reported for carcass traits, lean tissue, fat tissue and daily gain (cited in Table 5.6).

SSC1 also showed an AD interaction with SSC4 for intramuscular fat content. Significant additive effects at the QTL on SSC1 indicated that the alleles from the Pietrain breed were associated with higher intramuscular fat content. However, this positive additive genetic effect were offset by an almost three times higher negative interaction effect with SSC4. The QTL on SSC1 and SSC4 were not identified in previous individual QTL mapping of the data. However, numerous QTL have been identified around both of these QTL for carcass characteristics, lean and fat tissue, as well as body weight (cited in Table 5.6).

Furthermore, SSC1 showed interactions with SSC6 for average fat thickness measured by the AutoFom device. The QTL on SSC6 was previously reported by Mohrmann *et al.* (2006a). However, they estimated a significant individual dominance effect, whereas in the present study it is shown that it is more likely due to an interaction between additive and dominance effects. Around this location of SSC1 there are a large number of reports for fat tissue, along with lean tissue and growth and around the location of SSC6 for fatness, leanness and growth (cited in Table 5.6).

An AA genetic interaction was identified between the two telomeric ends of SSC4 for external neck fat weight. No individual QTL effects were identified at these QTL, and they were not identified in previous analysis of the data. At the telomeric end of the p arm, there are reports of QTL for fat tissue, as well as body weight and belly weight. At the telomeric end of the q arm however, there are no reports of QTL for fatness, but for carcass weight, body weight and liver weight (cited in Table 5.6).

A different location of SSC4 showed AA genetic interactions with SSC6 for fat content of the belly. At these QTL no individual QTL effects were identified and they were not identified in previous analysis. Around this location of SSC4 no QTL have been reported for fat tissue, but there are reports of QTL for lean tissue. In the region of the QTL on SSC6 there is only one report of QTL for ham weight (cited in Table 5.6).

For thinnest fat measure, additive or dominance interactions were identified between SSC6 and SSC8. In addition, significant dominance effects were identified at both QTL, indicating that heterozygous animals were associated with thinner fat at both QTL. An interaction was previously described in this study, for loin weight without external fat between SSC6 and SSC8. The QTL on SSC8 were both identified between *SW444* and *S0086*. The QTL on SSC6 were not identified in the same marker bracket. At the location of the QTL on SSC6, Mohrmann *et al.* (2006a) found significant dominance effects influencing chemical body composition (protein and lipid content) measured at

30 kg body weight. At these QTL heterozygous animals were associated with less lipid and protein content of the empty body and less protein content of the fat-free substance. There are no reports of QTL in the literature for similar traits around either QTL.

For external loin fat weight interactions were identified between similar genomic locations of SSC6 and SSC9 as that of neck weight without external fat. At the QTL on SSC9, heterozygous animals are associated with less fat weight of this carcass cut and increased lean. The QTL on SSC6 has been previously reported by Mohrmann *et al.* (2006a) for this resource family for many fat tissue characteristics. Furthermore, there are reports in the literature for fatness QTL around both QTL (cited in Table 5.6).

Table 5.6 Reports of QTL in the literature around similar locations as the QTL identified in the present study

Trait	SSC (position)[3]	Marker interval	Other studies confirming the QTL[4]
Entire carcass characteristics (lean + fat)			
Hind hock weight (kg)	1 (35)	*SW1332 – SW1851*	de Koning *et al.* (2001a)
Carcass length (cm)	1 (45)	*SW1851 – SW1430*	Malek *et al.* (2001a); Beeckmann *et al.* (2003a); Geldermann *et al.* (2003)
Carcass length (cm)	1(59)	*SW1430 - SWR982*	Beeckmann *et al.* (2003a)
Hind claw (kg)	1 (63)	*SW1430 - SWR982*	Beeckmann *et al.* (2003a)
AF entire belly weight (kg)	1 (88)	*SWR982 - SW1311*	Nezer *et al.* (2002); Beeckmann *et al.* (2003a); Karlskov-Mortensen *et al.* (2006)
Entire ham weight (kg)	2 (10)	*SW2623 – SWR783*	de Koning *et al.* (2001a); Milan *et al.* (2002); Geldermann *et al.* (2003); Lee *et al.* (2003a)
Belly weight (kg)	4 (31)	*S0301 – S0001*	Cepica *et al.* (2003a); Geldermann *et al.* (2003); Kim *et al.* (2006)
Belly weight (kg)	4 (130.1)	*SW856*	Knott *et al.* (2002)
Entire neck weight (kg)	6 (71)	*S0087 – SW122*	Yue *et al.* (2003a)
Entire neck weight (kg)	6 (86)	*SW122 – S0228*	Rohrer (2000); Grindflek *et al.* (2001); Varona *et al.* (2002); Yue *et al.* (2003a); Edwards *et al.* (2006)
Entire loin weight (kg)	7 (77)	*SW1856 – SWR2036*	Malek *et al.* (2001a); Milan *et al.* (2002); Geldermann *et al.* (2003); Yue *et al.* (2003b)
Entire loin weight (kg)	7 (86)	*SWR2036 – SW632*	Nezer *et al.* (2002); Kim *et al.* (2005); Ponsuksili *et al.* (2005); Edwards *et al.* (2008)
Flank weight (kg)	7 (88)	*SWR2036 – SW632*	Nezer *et al.* (2002); Kim *et al.* (2005); Ponsuksili *et al.* (2005); Edwards *et al.* (2008)
AF entire belly weight (kg)	7 (148)	*SW2537 – SW764*	-
AF entire shoulder weight (kg)	8 (21)	*SW905 – SWR1101*	Quintanilla *et al.* (2002); Sato *et al.* (2003)
AF entire shoulder weight (kg)	8 (37)	*SW905 – SWR1101*	Beeckmann *et al.* (2003c)
Hind hock weight (kg)	8 (107)	*SW1551 – S0178*	-
Hind claw (kg)	9 (23)	*SW21 – SW911*	-
Entire ham weight (kg)	9 (66)	*SW2401 – SW2571*	Cepica *et al.* (2003c)
Flank weight (kg)	10 (23)	*SWR136 – SW1894*	Quintanilla *et al.* (2002)
Lean tissue characteristics			
AF lean content of belly (%)	2 (9)	*SWR2516 – SW2623*	de Koning *et al.* (2001a); Milan *et al.* (2002); Geldermann *et al.* (2003); Lee *et al..* (2003a)
Loin eye area *M.l.t.l.*[1,2] (cm²)	2 (22)	*SW2623 – SWR783*	Lee *et al.* (2003a)
Protein content of loin (%)	2 (93)	*SWR2157 – SWR345*	Malek *et al.* (2001a); Lee *et al.* (2003a)
Protein content of loin (%)	2 (117)	*SWR345 – S0036*	-
Loin weight without external fat (kg)	4 (89)	*S0214 – SW445*	Perez-Enciso *et al.* (2000); Varona *et al.* (2002); Cepica *et al.* (2003a); Geldermann *et al.* (2003)
Protein content of loin (%)	4 (121)	*MP77 – SW856*	Malek *et al.* (2001a); Cepica *et al.* (2003a)
Loin weight without external fat (kg)	6 (28)	*SW2406 – SW1841*	de Koning *et al.* (2000); Milan *et al.* (2002)
Neck weight without external fat (kg)	6 (145)	*SW1881 – SW322*	Malek *et al.* (2001a); Edwards *et al.* (2008)
Protein content of loin (%)	7(1)	*SW2564 – SWR1343*	-
AF lean content of belly (%)	8 (55)	*SW444 – S0086*	-
Loin weight without external fat (kg)	8 (60)	*SW444 – S0086*	Casas-Carrillo *et al.* (1997); Milan *et al.* (2002); Kim *et al.* (2005)
Neck weight without external fat (kg)	9 (58)	*SW2401 – SW2571*	Rohrer *et al.* (2005)
Loin eye area *M.l.t.l.*[1,2] (cm²)	9 (136)	*SW174 – SW1349*	Cepica *et al.* (2003c); Kim *et al.* (2006)
Loin weight without external fat (kg)	14 (66)	*SW342 – SW1081*	Dragos-Wendrich *et al.* (2003a); Geldermann *et al.* (2003); van Wijk *et al.* (2006)

Table 5.6 continued

Trait	SSC (position)[3]	Marker interval	Other studies confirming the QTL[4]
Fat tissue characteristics			
External ham fat weight (kg)	1 (48)	*SW1851 – SW1430*	Malek *et al.* (2001a); Beeckmann *et al.* (2003a); Geldermann *et al.* (2003)
External ham fat weight (kg)	1 (118)	*SW1311 – SW1828*	Beeckmann *et al.* (2003a); Geldermann *et al.* (2003); Kim *et al.* (2006)
Intramuscular fat content (%)	1 (126)	*SW1828 – SW1301*	Rohrer and Keele (1998ab); Rohrer (2000); Beeckmann *et al.* (2003a); Edwards *et al.* (2008)
AF average fat thickness (mm)	1 (142)	*SW1301 – SW2512*	Rohrer and Keele (1998a); Bidanel *et al.* (2001); Quintanilla *et al.* (2002); Beeckmann *et al.* (2003a); Sanchez *et al.* (2006)
External neck fat weight (kg)	4 (1)	*SW2404 – SW489*	Marklund *et al.* (1999); Milan *et al.* (2002)
Intramuscular fat content (%)	4 (94)	*S0214 – SW445*	Perez-Enciso *et al.* (2000); Varona *et al.* (2002); Cepica *et al.* (2003a); Geldermann *et al.* (2003)
Fat content of belly (%)	4 (106)	*SW445 – MP77*	Cepica *et al.* (2003a); Geldermann *et al.* (2003)
External neck fat weight (kg)	4 (120)	*MP77*	Malek et *al.* (2001a); Cepica *et al.* (2003a)
Fat content of belly (%)	6 (12)	*MP35 – SW2406*	van Wijk *et al.* (2006)
Thinnest fat measure[1] (cm)	6 (42)	*SW1841 – S0087*	-
AF average fat thickness (mm)	6 (119)	*S0228 – SW1881*	Varona *et al.* (2002); Sato *et al.* (2003); Kim *et al.* (2006); Edwards *et al.* (2008)
External loin fat weight (kg)	6 (150)	*SW322 – SW2052*	Kim *et al.* (2005)
Thinnest fat measure[1] (cm)	8 (56)	*SW444 – S0086*	-
External loin fat weight (kg)	9 (57)	*SW911 – SW2401*	Rohrer *et al.* (2005)

[1]collected at the 13th/14th rib interface.

[2]measured on *musculus longissimus thoracis et lumborum*.

[3]Positions of the QTL in cM.

[4]References of other studies reporting QTL for similar traits in similar regions of the genome.

There are numerous reports of QTL in the literature for carcass characteristics, lean tissue and fat tissue in pigs in many genomic locations throughout the genome (e.g. Rohrer and Keele 1998ab; Bidanel *et al.* 2001; Milan *et al.* 2002; Geldermann *et al.* 2003; Sanchez *et al.* 2006; Liu *et al.* 2007). Previous analysis of the phenotypic data from the commercial population (Pietrain sires x crossbred dam line) of the present study identified numerous QTL for entire carcass characteristics, as well as lean and fat tissue characteristics (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapter 2). However, in these studies the role of epistasis in the genomic regulation of body composition has not been considered. To date, there is limited evidence for epistatic QTL across all species of livestock. This is most likely because tools and methodologies have not been available for this type of research, and because of the computational demand associated with the analysis.

In pigs, epistatic QTL have been reported so far, for reproductive traits (Bidanel 1993; Rodriguez *et al.* 2005; Noguera *et al.* 2006), coat colour (Hirooka *et al.* 2002), meat quality traits (meat colour and intramuscular fat content) (Ovilo *et al.* 2002; Szyda *et al.* 2006) and muscle fibre traits (Estelle *et al.* 2008). No epistatic QTL have been reported for body composition, such as entire carcass cuts, lean tissue and fat tissue characteristics. The present study is the first report to estimate epistatic interactions for carcass characteristics measured at slaughter weight in the pig.

Carlborg and Haley (2004) outlined the importance of a relatively large data set for the analysis of epistatic QTL. Small data sets will only detect epistatic QTL pairs with large effects. In the present study, a large number of epistatic QTL pairs were identified; this study is only the first step in understanding the contribution of epistasis to the genetic control of body composition in pigs. The present study has not covered the whole genome, therefore there are probably many more epistatic interactions involved in the genomic regulation of body composition.

Estelle *et al.* (2008) identified numerous significant epistatic QTL pairs for muscle fibre traits in a pig population of Iberian × Landrace F_2 cross, using a similar methodology as the present study. They identified all two-locus epistatic effect (AA, AD, DA, DD) but did not find that any particular epistatic effect was prevalent in their study. The interactions identified in the present study were at different genomic locations than those of Estelle *et al.* (2008). This may be because muscle fibre traits are under different genomic control, or due to breed differences as the study by Estelle *et al.* (2008) was based on an experimental cross between Iberian and Landrace pigs. They found that the epistatic interactions formed a network of connected pairs of epistatic QTL. They also indicated that this may be a common phenomenon as Carlborg *et al.* (2006) reported similar networks. Estelle *et al.* (2008) found that SSC10 and SSC11 behaved as hubs for this network. There is no clear evidence of this type of network in our study. However, SSC1, SSC8, SSC9, SSC2, SSC6 and SSC4 seemed particularly active with respect to epistasis. SSC10 did not seem as important in our study, with only one interaction being identified on SSC10, and SSC11 was not genotyped in the present study.

Information about the involvement of epistatic QTL in the genomic regulation of body composition is limited in livestock. There is, however, some evidence of the involvement of epistasis in the genomic regulation of growth in chickens, particularly early growth (Carlborg *et al.* 2003; Carlborg *et al.* 2004). Furthermore, there is a lot of evidence indicating an important role for epistatic interactions in the genomic control of growth and obesity of mice. Routman and Cheverud (1997) reported epistatic QTL for adult body weight. Brockmann *et al.* (2000) reported epistatic effects for serum concentrations of leptin, insulin and IGF-1, body weight, abdominal fat weight and muscle weight. They reported co-coordinated regulation of body and muscle weight by the interaction of two pairs of loci, one of which also influenced serum concentration of lipid. They indicated that these interactions may contribute to the high genetic correlation between body and muscle weight. Yi *et al.* (2004a) also found that epistasis played an important role controlling obesity in mice. They reported that different groups of traits were influenced by different interactions, such that a different genetic

architecture was identified for obesity traits and total cholesterol. They also found that the total phenotypic variance explained by epistatic interactions was higher than those explained by main effects. The epistatic QTL pairs identified in the present study also contributed to higher proportions of the phenotypic variance than QTL identified from single QTL analysis. The proportions of phenotypic variance explained in the present study by epistatic QTL pairs were approximately double that of the individual QTL identified from the individual QTL analysis (Duthie *et al.* 2008; Chapter 2) and therefore may be simply the combination of two QTL. In a further study of mice, Yi *et al.* (2004b) reported an epistatic effect between mouse chromosomes 7 and 3 for hepatic lipase activity. The QTL on chromosome 7 was detected in non epistatic analysis in the same location. The QTL on chromosome 3 had a weak main effect on hepatic lipase activity and was not detected in the non epistatic analysis, however chromosome 3 was found to interact strongly with chromosome 7. Further studies in mice reported epistatic QTL pairs for abdominal fat percentage, abdominal fat weight, body weight, kidney weight and spleen weight (Carlborg *et al.* 2005) and organ weights and limb length traits (Wolf *et al.* 2006). Yi *et al.* (2006) found that epistasis was more important for body weight in mice at older ages, than younger ages in contradiction to Ishikawa *et al.* (2005) who found that epistasis was more important for early growth than late stages of growth in mice. Yi *et al.* (2006) also found that epistasis influenced fatness and organ weights.

In the present study a large number of epistatic QTL pairs were identified which were involved in the regulation of many carcass traits, including lean and fat tissue weights in pigs. It is obvious from this study and from studies of poultry and mice that epistasis is important for the genomic regulation of growth and body composition. Information about epistatic interactions can add to our understanding of the genomic networks which form the fundamental basis of biological systems. In addition to knowledge about the individual QTL or genes which influence a biological system, information about the effect of interactions between genes will build on the understanding of the genomic networks which influence variation in biological systems (Carlborg and Haley 2004).

Future QTL analyses should therefore focus their attention on uncovering the role of epistasis in the genomic regulation of economically important traits.

Chapter 6

Quantitative trait loci for meat quality traits in pigs considering imprinting and epistatic effects

Abstract

The aim of the research was to gain a better understanding of the genomic regulation of meat quality by investigating individual QTL and pairs of epistatic QTL in a three generation full-sib population (Pietrain x crossbred dam line). In total, 386 animals were genotyped for 96 markers covering several chromosomes. Analysed traits included pH at 45 minutes (pH_{45}) and 24 hours (pH_{24}) post-mortem, reflectance value, conductivity and meat colour. Thirteen significant individual QTL were identified. The most significant QTL were detected on SSC1 and SSC9 for pH_{45} and pH_{24}, respectively, on SSC4 for meat colour and on SSC8 for conductivity, accounting for between 3.4% and 4.7% of the phenotypic variance. Nine significant epistatic QTL pairs were detected accounting for between 5.7% and 10.9% of the phenotypic variance. The epistatic QTL pairs showing the largest effects were for reflectance value between two locations of SSC4, and for pH_{45} between SSC10 and SSC13, explaining 9.5% and 10.9% of the phenotypic variance, respectively. Furthermore, 10 significant QTL with imprinting effects were identified. This study indicates that meat quality traits are influence by a large number of QTL, expressed partly by imprinting, as well as a complex network of QTL interactions.

6.1 Introduction

In the past 50 years, selection strategies in pigs have been mainly focussed on the genetic improvement of production traits such as growth rate, lean content, backfat thickness and feed efficiency (Roehe *et al.* 2003; Kanis *et al.* 2005; van Wijk *et al.* 2005). There has been considerable progress in the genetic improvement of pigs through artificial selection of superior animals without knowledge of the underlying genomic regulation of these traits (Andersson 2001; Georges 2001; Weller 2001; Dekkers and Hospital 2002). Intensive artificial selection has resulted in a substantial increase in loin muscle area and reduction in backfat thickness, an indication that these body composition traits have high genetic determination (Andersson 2001; Roehe *et al.* 2003). This selection for increased leanness has however partly been unfavourably associated with meat eating quality characteristics (Schwab *et al.* 2006). Understanding the genetic regulation of meat quality is therefore becoming more important (Karlsson *et al.* 1993; De Vries *et al.* 1994; Knapp *et al.* 1997; Oksbjerg *et al.* 2000; Kanis *et al.* 2005; Aaslyng *et al.* 2007).

Meat quality is a complex trait with several criteria involved from technological to subjective meat eating quality characteristics. Technological aspects of meat quality refer to properties such as water holding capacity (e.g. drip loss during storage), intensity and homogeneity of colour, firmness, shelf-life, cooking loss and various processing yields (Sellier 1998; Otto *et al.* 2004). Consumer satisfaction with the product is influenced by traits associated with appearance, such as colour, leanness, amount of fat tissue and water holding capacity (Otto *et al.* 2006). Commonly used indicators of meat quality are pH at 45-60 minutes post-mortem and pH at 24 hours after slaughter (Sellier 1998). Intramuscular fat content is considered as having a favourable influence on tenderness and juiciness of the meat, however too high levels may be detrimental and negatively influence consumer acceptability.

The market price of the final product should include both carcass composition and meat quality (Otto *et al.* 2007a). As a result, breeding goals should include meat quality as well as production traits (van Wijk *et al.* 2005). A large number of genomic studies have been devoted to growth and body composition traits (e.g. Andersson *et al.* 1994; Rohrer and Keele 1998ab; Bidanel *et al.* 2001; de Koning *et al.* 2001a; Milan *et al.* 2002), however much less attention has been paid to meat quality traits. Recently, however, the interest in quantitative trait loci (QTL) associated with meat quality has increased (de Koning *et al.* 2001b; Grindflek *et al.* 2001; Paszek *et al.* 2001; Ovilo *et al.* 2002; Nii *et al.* 2005; Vidal *et al.* 2005).

The aim of this study was to gain further insight into the genomic regulation of meat quality. For this reason QTL analysis of meat quality traits was carried out across several autosomes as well as chromosome X in a F_2 pig population. The mode of inheritance was investigated as additive and/or dominance and the epigenetic effects of imprinting were tested. Furthermore a QTL scan for epistatic QTL pairs was carried out to examine the role of epistasis in the genomic regulation of meat quality.

6.2 Materials and methods

6.2.1 Design and data

The present study was based on data of a resource family of a three generation full-sib design. In the F_0 generation, seven unrelated Pietrain sires, which were all heterozygous at the *ryanodine receptor 1 (RYR1)* were mated to 16 sows of a crossbred dam line (Leicoma × (Landrace × Large White)). Eight boars and 40 sows of the F_1 generation were mated, whilst avoiding inbreeding, to produce the F_2 generation which comprised 49 families of 315 pigs across two litters. Animals of the F_2 generation were either housed individually or in groups of up to 15 pigs of mixed sex in straw-bedded pens. Individual housed animals comprised of 48 gilts and 46 barrows. These animals were fed manually with feed consumption recorded on a weekly basis. Group housed animals comprised 117 gilts and 104 barrows. These animals were supplied food by an electronic feeding station (ACEMA 48), which recorded feed consumption at each visit. Pigs were provided with one of three pelleted diets containing 13.8 MJ ME/kg and 1.2% lysine, 13.8 MJ ME/kg and 1.1% lysine, or 13.4 MJ ME/kg and 1.0% lysine for weight ranges 30-60, 60-90 and 90-140 kg body weight, respectively. Pigs were provided with *ad libitum* access to diets, formulated slightly above requirement, so they were able to reach maximal protein deposition. For more detail about the management of this project see the studies of Landgraf *et al.* (2006ab) Mohrmann *et al.* (2006ab).

6.2.2 Meat quality measurements

Means and standard deviations of traits analysed in the present study are presented in Table 6.1. Following slaughter at 140 kg body weight, reflectance was measured 45 min post-mortem (reflectance$_{45}$) simultaneously with the carcass grading information using the Fat-O-Meter device (FOM, SKF Technology, Herlev, Denmark) perpendicular to the *longissimus* muscle between the last 3rd and 4th rib. The pH was measured 45 minutes

post mortem (pH_{45} loin) on the intact carcass using a pH-STAR electrode (Matthäus, Nobitz-Klausa, Germany). The pH probe was inserted 4 cm deep into the *musculus longissimus dorsi* between the 13th and 14th thoracic vertebrae. Prior to the measurement, temperature was measured at the point the pH probe was placed and the pH was adjusted according to temperature. At 24 hours post-mortem, the carcass was cut between the 13th and 14th thoracic vertebrae and pH values (pH_{24} loin) were measured at the surface of *musculus longissimus dorsi*. After cleaning the cranial surface of the *musculus longissimus dorsi*, the colour of the muscle was measured by OPTO-STAR equipment at the same location (Matthäus, Nobitz-Klausa, Germany). The OPTO-STAR equipment measures the brightness of the meat sample whereby lower and higher values indicate paler and darker meat, respectively. At 24 hours post-mortem, the pH in the ham (pH_{24} ham) was measured 4 to 6 cm above the Symphisis pelvis in the *musculus semimembranosus* inserting the pH probe to a depth of 2 cm. Conductivity was taken 24 hours post mortem (conductivity$_{24}$) using LF-STAR (Matthäus, Nobitz-Klausa, Germany) inserted between the 14th and 15th thoracic vertebrae to a depth of 6 cm.

Table 6.1 Means and standard deviations (SD) of meat quality traits measured on pigs of the F_2 generation

Trait	Mean	SD
pH_{45} loin	6.242	0.399
pH_{24} ham	5.553	0.188
pH_{24} loin	5.448	0.132
Reflectance$_{45}$[1]	24.781	4.602
Conductivity$_{24}$	4.813	2.212
OPTO-STAR value	69.252	7.531

[1]measured by the Fat-O-Metre device.

6.2.3 Genotypic data

Blood samples were collected from all animals of the F_0, F_1 and F_2 generations from the *vena jugularis* and their DNA was isolated. Chromosomes chosen for genotyping were SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13, SSC14 and SSCX because of their likely associations with carcass characteristics and growth. All animals were genotyped for 96 informative microsatellite markers. Of these markers 10, 9, 9, 9, 10, 8, 9, 9, 8, 7 and 8 genomic markers were located on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13, SSC14 and SSCX, respectively. Markers and their distances were selected from the published USDA linkage map (http://www.marc.usda.gov; Rohrer *et al.* 1996) which provided all information relating to their positions and alleles (Table 6.2). Average distance between markers is 16.0, 16.5, 16.3, 21.0, 17.0, 18.4, 17.3, 16.0, 18.0, 17.4, and 18.3 cM and largest gaps between markers is 28.0, 25.2, 26.5, 29.0, 26.0, 23.1, 21.7, 20.8, 24.0, 23.6 and 22.4 cM on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13, SSC14 and SSCX, respectively.

Table 6.2 Markers used in the present QTL mapping project, their relative map position using USDA pig map, number of different alleles, polymorphic information content in the F_2 generation (PIC) and heterozygosity in F_1 generation (H)

Marker	SSC	Position (cM)	H	Number of alleles	PIC
SW1514	1	0.0	0.79	8	0.75
SW1515	1	16.4	0.67	8	0.68
SW1332	1	29.2	0.63	4	0.37
SW1851	1	44.6	0.73	4	0.53
SW1430	1	58.5	0.81	6	0.76
SWR982	1	86.2	0.88	7	0.77
SW1311	1	100.8	0.58	6	0.62
SW1828	1	118.5	0.90	7	0.69
SW1301	1	140.5	0.83	5	0.67
SW2512	1	144.0	0.77	6	0.55
SWR2516	2	0.0	0.67	5	0.48
SW2623	2	9.8	0.68	5	0.63
SWR783	2	23.7	0.51	3	0.30
SW240	2	42.0	0.84	7	0.78
SW1026	2	60.6	0.47	6	0.55
SW1370	2	74.8	0.91	8	0.69
SWR2157	2	89.2	0.78	8	0.68
SWR345	2	114.4	0.87	8	0.75
S0036	2	132.1	0.85	7	0.80
SW2404	4	0.0	0.91	10	0.81
SW489	4	8.0	0.66	5	0.53
S0301	4	27.1	0.72	6	0.56
S0001	4	41.8	0.66	6	0.65
SW839	4	62.3	0.44	4	0.45
S0214	4	79.3	0.80	6	0.74
SW445	4	105.8	0.91	10	0.77
MP77	4	120.0	0.87	8	0.74
SW856	4	130.1	0.98	14	0.84
MP35	6	0.0	0.70	6	0.59
SW2406	6	21.4	0.74	8	0.61
SW1841	6	41.5	0.98	15	0.88
S0087	6	62.8	0.75	5	0.59
SW122	6	83.3	0.85	7	0.69
S0228	6	105.2	0.69	6	0.68
SW1881	6	121.1	0.96	8	0.76
SW322	6	149.8	0.79	8	0.72
SW2052	6	164.6	0.79	9	0.78
SW2564	7	0.0	0.69	5	0.49
SWR1343	7	12.2	0.83	4	0.53
SW2155	7	32.9	0.67	4	0.48
SW1369	7	48.2	0.77	8	0.68
SW1856	7	61.5	0.69	5	0.48
SWR2036	7	78.2	0.81	9	0.77
SW632	7	104.4	0.77	6	0.67
SWR773	7	117.3	0.56	3	0.46
SW2537	7	139.5	0.69	7	0.63
SW764	7	156.0	0.76	5	0.65

Table 6.2 continued

Marker	SSC	Position (cM)	H	Number of alleles	PIC
SW2410	8	-1.3	0.42	4	0.44
SW905	8	20.8	0.71	6	0.71
SWR1101	8	38.3	0.88	12	0.75
SW444	8	52.5	0.85	7	0.76
S0086	8	62.2	0.69	6	0.56
SW374	8	82.8	0.88	5	0.63
SW1551	8	105.9	0.75	6	0.66
S0178	8	127.7	0.54	7	0.68
SW983	9	4.0	0.81	6	0.61
SW21	9	15.1	0.65	5	0.50
SW911	9	36.8	0.75	7	0.68
SW2401	9	57.1	0.71	6	0.68
SW2571	9	73.3	0.46	6	0.61
S0019	9	86.4	0.75	6	0.62
SW2093	9	103.6	0.90	6	0.77
SW174	9	122.9	0.81	3	0.51
SW1349	9	142.5	0.81	7	0.75
SW830	10	0.0	0.67	7	0.64
SWR136	10	7.6	0.77	6	0.72
SW1894	10	23.2	0.65	4	0.50
SW2195	10	44.0	0.48	3	0.42
SW173	10	56.1	0.35	4	0.39
SW1041	10	67.5	0.46	3	0.41
SW2043	10	87.7	0.56	5	0.72
SW1626	10	108.0	0.79	11	0.68
SW2067	10	128.0	0.81	7	0.69
S0282	13	0.0	0.90	8	0.77
SWR1941	13	14.1	0.87	7	0.71
SW1407	13	27.2	0.88	11	0.83
SW864	13	43.1	0.63	5	0.64
S0068	13	62.2	0.78	9	0.72
SW398	13	79.3	0.69	6	0.66
SW2440	13	102.2	0.96	6	0.79
S0291	13	126.2	0.83	8	0.79
SW857	14	7.4	0.87	9	0.74
S0089	14	14.0	0.67	7	0.71
SW245	14	32.0	0.77	7	0.71
SW342	14	53.2	0.79	7	0.71
SW1081	14	72.1	0.87	6	0.65
SW1557	14	87.9	0.64	4	0.49
SWC27	14	111.5	0.45	8	0.41
SW949	X	0.0	0.65	6	0.53
SW980	X	11.9	0.87	7	0.80
SW1903	X	33.0	0.87	5	0.70
SW2456	X	55.4	0.81	6	0.67
SW259	X	74.4	0.89	5	0.70
SW1943	X	87.4	0.70	5	0.70
SW707	X	107.9	0.49	4	0.59
SW2588	X	128.4	0.25	4	0.37

6.2.4 Statistical analysis

All QTL analyses for individual QTL and epistatic QTL were performed with QxPak version 3.0 (Perez-Enciso and Misztal 2004). This program used the maximum likelihood method for estimation of the position and effect of the QTL. The analysis of QxPak proceeds in two main stages. In the first stage the probabilities of alleles being identical-by-descent are calculated using a Monte Carlo Markov Chain algorithm. In the second stage, the mixed model equations are built and the QTL estimates are obtained using a maximum likelihood approach via the expectation-maximisation algorithm. At each putative position the likelihood ratio is computed and the estimates for the parameters are those where the likelihood is highest. In this analysis the significance is tested with a likelihood ratio test which consists of computing minus twice the difference in log-likelihoods between the alternative and the null models (Perez-Enciso and Misztal 2004).

The individual QTL genome scan was applied across all autosomes and the sex chromosome X, whereas the epistasis QTL genome scan was applied only to autosomes.

6.2.4.1 Individual QTL analysis

In the individual QTL analysis, only additive and dominance effects were estimated. In cases where the dominance effect was not significant, an additive only model was adopted. The individual QTL analysis of all traits was performed with the following model:

$$y_i = sex_i + MHS_i + batch_i + ht_i + sldat_i + \beta slwt_i + C_a a + C_d d + e_i, \qquad [1]$$

where y_i is the i-th individual phenotype. *Sex*, *RYR1* genotype (*MHS*), batch, housing type (*ht*: *individual or group housed*) and slaughter date (*sldat*) were fitted as fixed

effects in the model. Slaughter weight (*slwt*) was considered as a covariable *β*. The additive (*a*) and dominance (*d*) effects were estimated by consideration of the coefficients of C_a and C_d, respectively. The coefficient C_a was calculated for each individual and position as the probability of the individual being homozygous for alleles of the grandpaternal sire line (QQ) minus the probability of pigs being homozygous for alleles from the grandmaternal dam line (qq). The coefficient C_d is the probability of the individual being at the chromosomal position heterozygous (Qq). Moreover, traits were tested for QTL expressing paternal or maternal imprinting. In this analysis imprinting tested with models where only the allele of paternal origin is expressed (maternal imprinting) and only the allele of the maternal origin is expressed (paternal imprinting) i.e. setting the maternal or paternal coefficients to zero.

The QTL scans were performed every cM. QxPak provides the log likelihood ratios under the models tested and the associated nominal P-values, which were obtained by removing the QTL effect in the model [1]. A previous study by Perez-Enciso *et al.* (2000) showed that nominal P-values of 0.001 and 0.005 correspond to 1% and 5% chromosome-wide significance level, respectively. Therefore in the present study, nominal P-values of <0.0001, 0.001, 0.005 and 0.01 were treated as significant at the 0.1%, 1%, 5% and suggestive at the 10% chromosome-wide level, respectively.

6.2.4.2 Epistasis QTL analysis

Due to the substantial computational demand of a genomic scan for epistatic QTL, the analysis was performed in two stages, following Estelle *et al.* (2008). In the first stage, a 5 cM scan was carried out across all genomic positions in order to pre-select potential candidate regions of QTL expressing epistatic effects with the model:

$$y_i = sex_i + MHS_i + batch_i + ht_i + sldat_i + \beta slwt_i + C_{aa}I_{aa} + C_{ad}I_{ad} + C_{da}I_{da} + C_{dd}I_{dd} + e_i, \quad [2]$$

where I_{aa}, I_{ad}, I_{dd} and I_{dd} are the additive × additive (AA), additive × dominance (AD), dominance × additive (DA) and dominance × dominance (DD) epistatic effects, respectively. These four epistatic effects were estimated, based on Cockerham's decomposition (Cockerham 1954), by regressing on a linear combination of the individual QTL origin probabilities:

$C_{aa} = P_1(QQ)P_2(QQ) - P_1(QQ)P_2(qq) - P_1(qq)P_2(QQ) + P_1(qq)P_2(qq)$,
$C_{ad} = P_1(QQ)P_2(Qq) - P_1(qq)P_2(Qq)$,
$C_{da} = P_1(Qq)P_2(QQ) - P_1(Qq)P_2(qq)$,
$C_{dd} = P_1(Qq)P_2(Qq)$,

where P_1 and P_2 refers to the probability of QTL at location 1 and 2, respectively (Varona *et al.* 2002). This model [2] was tested against a null model where no epistatic effects were estimated. Interacting QTL pairs with P-values of < 0.001 were selected for further analyses.

In the second stage, the complete epistatic model [2] including the individual QTL effects was applied using a 1 cM scan around the pre-selected positions detected in the first stage. Again, this model was tested against a null model in which no epistatic effects were fitted. Epistatic interactions were reported as significant if they had a nominal P-value of < 0.001.

6.3 Results

6.3.1 Individual QTL Analysis

From the individual QTL analysis, 13 significant QTL were identified. The additive and dominance effects of these QTL are presented in Table 6.3.

Table 6.3 Evidence for quantitative trait loci (QTL) for meat quality characteristics using model with adjustment for *RYR1* genotypes.

SSC	Trait	LR	Pos[1]	% Var[2]	a ± SE[3]	d ± SE[4]
1	pH$_{45}$ loin	14.88**	45	4.7	**-0.098 ± 0.025**	-
2	pH$_{45}$ loin	11.65*	21	3.7	**-0.083 ± 0.031**	**0.115 ± 0.052**
2	pH$_{24}$ loin	10.62*	28	3.4	**0.037 ± 0.011**	**-**
4	pH$_{45}$ loin	7.60[a]	128	2.4	**-0.068 ± 0.024**	-
4	pH$_{24}$ ham	10.39[a]	58	3.2	0.010 ± 0.015	**-0.083 ± 0.026**
4	OPTO-STAR value	12.35**	119	3.9	**-2.083 ± 0.587**	-
6	pH$_{45}$ loin	11.38*	93	3.5	-0.058 ± 0.033	**0.171 ± 0.060**
7	pH$_{45}$ loin	9.11*	47	3.0	**-0.090 ± 0.030**	**5.912 ± 0.470**
7	pH$_{24}$ loin	9.62[a]	44	3.4	0.006 ± 0.011	**0.057 ± 0.020**
8	pH$_{24}$ ham	9.34[a]	1	2.6	-0.024 ± 0.012	**0.041 ± 0.018**
8	Conductivity$_{24}$	10.96**	107	3.4	**0.535 ± 0.160**	-
9	Reflectance$_{45}$	8.11*	138	2.6	**1.160 ± 0.404**	-
9	pH$_{45}$ loin	11.58*	142.5	3.7	-0.026 ± 0.031	**-0.186 ± 0.055**
9	pH$_{24}$ ham	12.00**	11	3.7	**-0.050 ± 0.014**	-
10	pH$_{24}$ ham	10.35[a]	21	3.2	0.011 ± 0.014	**-0.077 ± 0.024**
13	pH$_{45}$ loin	6.79[a]	43	2.2	**0.066 ± 0.025**	-
13	Conductivity$_{24}$	10.39[a]	62	3.3	-0.219 ± 0.138	0.680 ± 0.239
14	Reflectance$_{45}$	9.32*	40	3.0	**-1.247 ± 0.405**	-
14	pH$_{24}$ ham	7.34[a]	111.5	2.1	**0.052 ± 0.019**	-
X	pH$_{24}$ ham	9.05*	13	2.6	**-0.039 ± 0.013**	-
X	pH$_{24}$ loin	9.38[a]	33	3.4	0.011 ± 0.010	**0.045 ± 0.021**
X	OPTO-STAR value	10.92*	43	3.5	-0.317 ± 0.731	**4.851 ± 1.516**

Values in bold represent significant additive or dominance effects.

Definition of symbols: LR, likelihood ratio.

[1]Positions of the QTL in cM.

[2]Percentages of F$_2$ variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no QTL effect - residual variance of model with QTL effect)/residual variance of model with no QTL effect.

[3]Estimated additive (a) and dominance (d) effects and their standard errors (SE) of the individual QTL.

[a] implies suggestive at the 10% chromosome-wide level.

* and ** implies significance at the 5%, or 1% chromosome-wide levels, respectively.

6.3.1.1 Measurement of pH at 45 minutes post-mortem

Five significant QTL were identified for pH$_{45}$ loin. The QTL with the highest effect was identified on SSC1 close to *SW1851* explaining 4.7% of the phenotypic variance (Figure 6.1). A significant additive effect at this QTL indicates that Pietrain alleles are associated with lower pH$_{45}$ loin.

Figure 6.1
Likelihood ratio curve for evidence of quantitative trait loci for pH measured at 45 minutes post-mortem on SSC1. The horizontal line indicates the chromosome-wide significance level.

Further QTL, significant at the 5% chromosome-wide level, were identified on SSC2, SSC6, SSC7 and SSC9. The QTL identified on SSC2 was located close to *SWR783* at 21 cM and accounted for 3.7% of the phenotypic variance. At this QTL the grandpaternal Pietrain breed was associated with lower pH_{45} loin and a significant dominance effect indicated that heterozygous animals are associated with higher pH_{45} loin. On SSC6, between *SW122* and *S0228,* a QTL was identified at 93 cM explaining 3.5% of the phenotypic variance. A significant dominance effect at this QTL indicates heterozygous animals are associated with higher pH_{45} loin. A further QTL was identified on SSC7 close to *SW1369* at 47 cM, explaining 3.0% of the phenotypic variance. At this QTL Pietrain alleles are associated with reduced pH_{45} loin and heterozygous animals are associated with increased pH_{45} loin. The QTL on SSC9 was located at the telomeric end of the q arm and accounted for 3.7% of the phenotypic variance, with heterozygous animals associated with reduced pH_{45} loin.

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6.3.1.2 Measurement of pH at 24 hours post-mortem

Three significant QTL were identified for pH_{24}. The QTL with the highest significance, at the 1% chromosome-wide level, was identified at the telomeric end of the p arm of SSC9 for pH_{24} ham explaining 3.7% of the phenotypic variance (Figure 6.2). A significant additive effect at this QTL indicated that Pietrain alleles are associated with decreased pH_{24} ham.
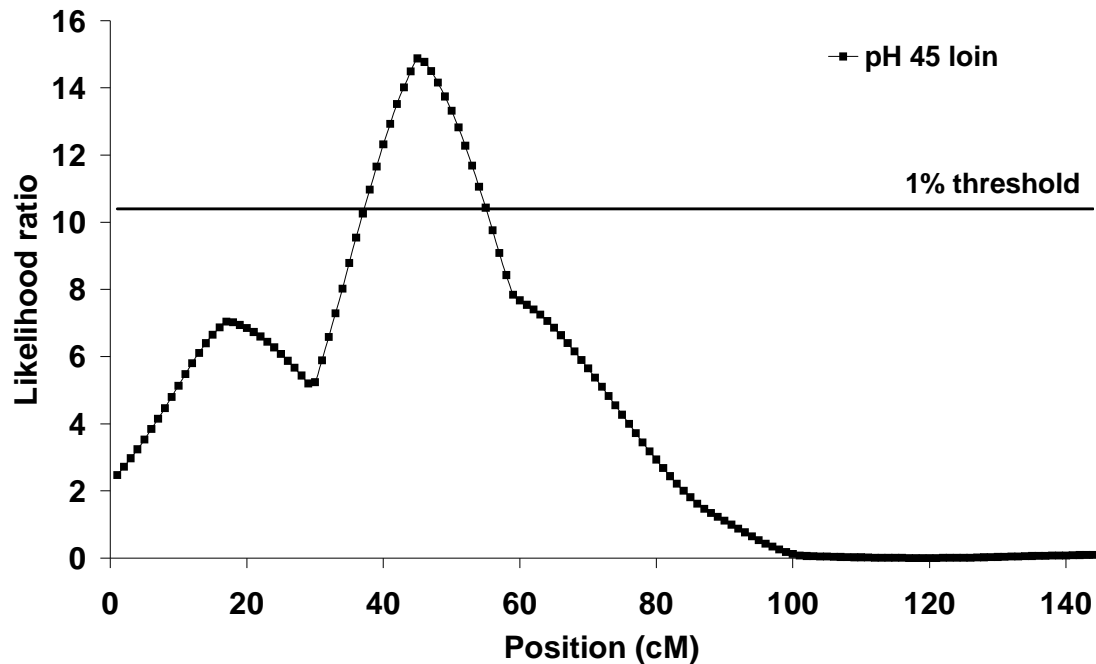

Figure 6.2
Likelihood ratio curves for evidence of quantitative trait loci for pH measured at 45 minutes and 24 hours post-mortem and reflectance value on SSC9. Horizontal lines indicate the chromosome-wide significance levels.

Further QTL, significant at the 5% chromosome-wide level were identified on SSC2 and SSCX. In the same region of SSC2 as the QTL for pH_{45} loin (Figure 6.3), between *SWR783* and *SW240*, a QTL significant at the 5% chromosome-wide level was identified for pH_{24} loin explaining 3.4% of the phenotypic variance. A significant additive effect at this QTL indicated that Pietrain alleles are associated with higher pH_{24} loin.
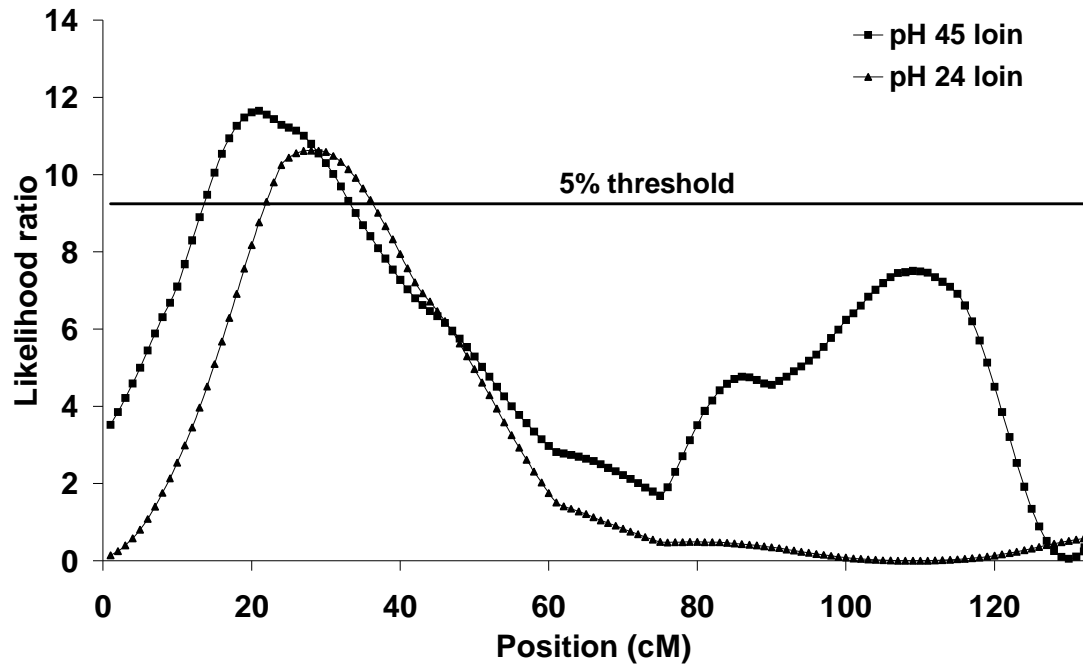
Figure 6.3
Likelihood ratio curves as evidence of significant QTL for pH measured at 45 minutes and 24 hours post-mortem in the loin cut on SSC2. The horizontal line indicates the chromosome-wide significance level.

At 13 cM of SSCX close to *SW980* a significant QTL was identified for pH_{24} ham. This QTL explained 2.6% of the phenotypic variance and a significant additive effect indicates that the grandpaternal Pietrain breed is associated with decreased pH_{24} ham.

6.3.1.3 Meat colour

For meat colour in the spectrum from pale to dark, two significant QTL were identified. The QTL with the highest effect, significant at the 1% chromosome-wide level, was identified on SSC4 at the telomeric end of the q arm for OPTO-STAR value, explaining 3.9% of the phenotypic variance (Figure 6.4). A significant additive effect indicates that Pietrain alleles are associated with reduced OPTO-STAR value indicating paler meat at this QTL.
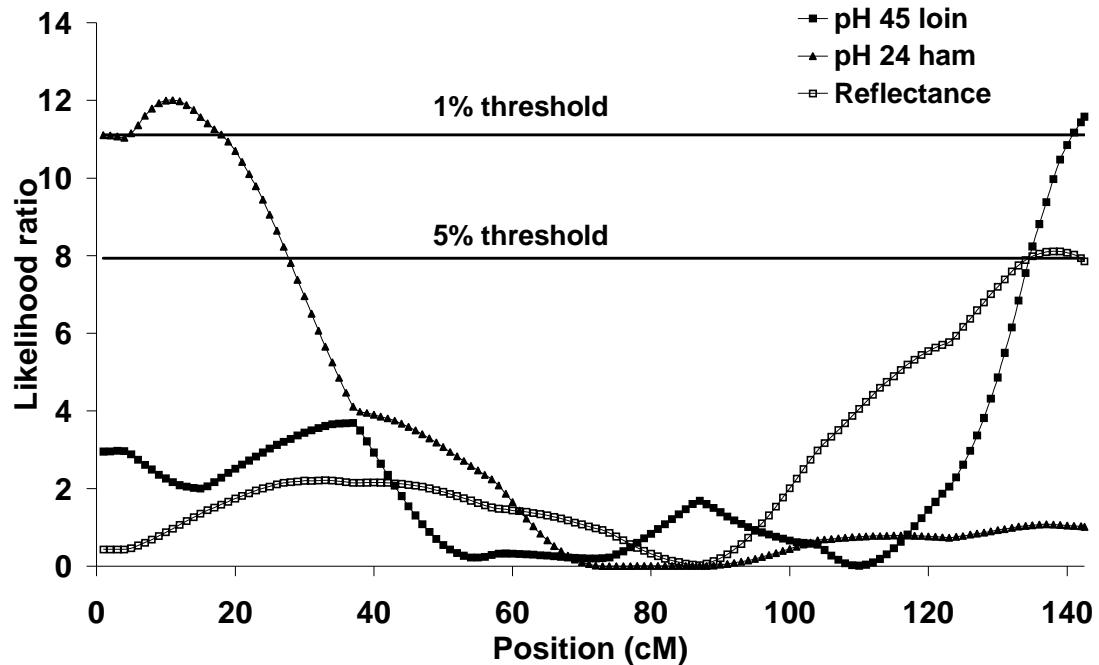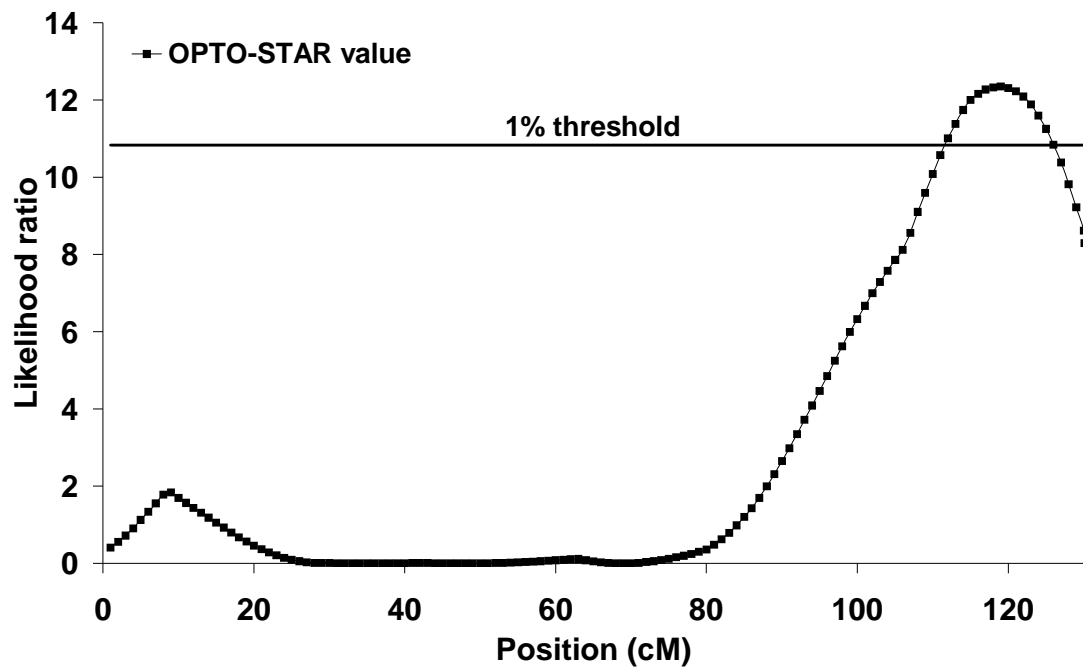

Figure 6.4
Likelihood ratio curve as evidence of significant QTL for meat colour (OPTO-STAR value) on SSC4. The horizontal line indicates the chromosome-wide significance level.

A further QTL for OPTO-STAR value was identified on SSCX, significant at the 5% chromosome-wide level. This QTL explained 3.5% of the phenotypic variance and a

significant dominance effect indicated that heterozygous animals are associated with higher OPTO-STAR value.

6.3.1.4 Conductivity 24 hours post-mortem

A single QTL was identified for conductivity$_{24}$ on SSC8 around no other QTL. This QTL explained 3.4% of the phenotypic variance and a significant additive effect indicated Pietrain alleles are associated with increased conductivity in the *musculus longissimus dorsi*.

6.3.1.5 Reflectance value (FOM)

A QTL for reflection value, significant at the 5% chromosome-wide level, was identified on SSC9 around a similar location as pH measured at 45 minutes post-mortem in the loin at the telomeric end of the q arm (Figure 6.2). This QTL was identified between *SW174* and *SW1349* and accounted for 2.6% of the phenotypic variance. A significant additive effect indicated that at this QTL the Pietrain breed was associated with higher reflection value. A further QTL for this trait was identified on SSC14 at 40 cM between *SW245* and *SW342*. This QTL was significant at the 5% chromosome-wide level and accounted for 3.0% of the phenotypic variance. A significant additive effect indicates that the grandpaternal Pietrain breed is associated with a lower reflectance value at this QTL.

6.3.1.6 Influence of adjustment for RYR1 genotypes

All results were adjusted for *RYR1* genotype (Tables 6.3, 6.5 and 6.6). However without adjustment, highly significant QTL at the 0.1% chromosome-wide level around the location of the *RYR1* gene for pH_{45} loin and $conductivity_{24}$ were revealed, with likelihood ratios of 42.07 and 36.25, respectively. These QTL explained high proportions of the phenotypic variance at 12.7% and 10.9%, respectively (see Table 6.4).

Table 6.4 Evidence for quantitative trait loci (QTL) for meat quality characteristics using model without adjustment for *RYR1* genotypes.

SSC	Trait	LR	Pos^1	% Var^2	$a \pm SE^3$	$d \pm SE^3$
1	pH_{45} loin	10.71*	45	3.4	**-0.110 ± 0.033**	-
2	pH_{24} loin	10.76*	30	3.4	**0.037 ± 0.011**	-
4	pH_{45} loin	13.55*	124	4.3	**-0.085 ± 0.033**	**0.129 ± 0.051**
4	$Conductivity_{24}$	10.61*	124	3.3	0.338 ± 0.179	**-0.723 ± 0.276**
4	OPTO-STAR value	14.30**	121	4.5	**-2.292 ± 0.599**	-
6	pH_{45} loin	42.07***	87	12.7	**-0.225 ± 0.036**	**0.155 ± 0.064**
6	$Reflectance_{45}$	7.11[a]	83	2.3	**1.035 ± 0.386**	-
6	$Conductivity_{24}$	36.25***	84	10.9	**1.182 ± 0.191**	-
6	OPTO-STAR value	10.53*	84	3.3	**-2.245 ± 0.686**	-
7	pH_{24} loin	10.78*	44	3.4	0.007 ± 0.011	**0.060 ± 0.020**
8	pH_{45} loin	9.46[a]	106	3.0	**-0.115 ± 0.043**	**-0.159 ± 0.077**
8	$Conductivity_{24}$	13.16**	106	4.1	**0.831 ± 0.227**	-
9	pH_{24} ham	10.68*	12	3.1	**-0.048 ± 0.015**	-
10	pH_{24} ham	10.55[a]	19	3.1	0.014 ± 0.015	**-0.078 ± 0.025**
14	$Reflectance_{45}$	6.77[a]	38	2.2	**-1.136 ± 0.434**	-
14	pH_{24} ham	7.13[a]	111	2.1	**0.052 ± 0.019**	-
X	OPTO-STAR value	12.25*	34	3.9	0.691 ± 0.672	**3.864 ± 1.422**
X	pH_{24} ham	8.99*	13	2.6	**-0.039 ± 0.013**	-

Values in bold represent significant additive or dominance effects.
Definition of symbols: LR, likelihood ratio.
[1]Positions of the QTL in cM.
[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no QTL effect - residual variance of model with QTL effect)/residual variance of model with no QTL effect.
[3]Estimated additive (a) and dominance (d) effects and their standard errors (SE) of the individual QTL.
[a] implies suggestive at the 10% chromosome-wide level.
* and ** implies significance at the 5%, or 1% chromosome-wide levels, respectively.

6.3.2 Imprinting

There is evidence for 10 significant QTL with imprinting effects across several autosomes (Table 6.5). On SSC1 a QTL with paternal imprinting was identified for reflectance$_{45}$, indicating that only the maternal allele is expressed at this QTL. A highly significant QTL at the 0.1% chromosome wide level was identified on SSC2 for pH$_{45}$ loin with maternal imprinting effects, indicating that only the paternal allele is expressed at this QTL. Maternal imprinting effects were identified on SSC7 for reflectance$_{45}$ at 20 cM. Paternal imprinting effects were also identified on two different genomic locations of SSC7 for pH$_{45}$ loin at 43 cM and for conductivity$_{24}$ at 153 cM. At the telomeric end of the p arm of SSC8, paternal imprinting effects were identified for meat colour, and in a second genomic location (53 cM) maternal imprinting effects were identified for reflectance$_{45}$. Maternal imprinting effects were identified at the telomeric end of the p arm of SSC10 for pH$_{24}$ loin. Further maternal imprinting effects were identified on SSC14 between 40 and 44 cM for reflectance$_{45}$ and pH$_{45}$ loin.

Table 6.5 Evidence for quantitative trait loci (QTL) with imprinting effects for meat quality characteristics with adjustment for *RYR1* genotypes.

SSC	Trait	LR	Pos[1]	% Var[2]	a ± SE[3]	Imprinting
1	Reflectance$_{45}$	8.90*	129	2.8	**-0.794 ± 0.264**	Paternal
2	pH$_{45}$ loin	15.94***	42	4.7	**-0.077 ± 0.019**	Maternal
7	Reflectance$_{45}$	10.11*	20	3.2	**-0.881 ± 0.275**	Maternal
7	pH$_{45}$ loin	12.87**	43	4.1	**-0.069 ± 0.019**	Paternal
7	Conductivity$_{24}$	8.43*	153	2.7	**0.292 ± 0.100**	Paternal
8	OPTO-STAR value	11.25**	3	3.6	**1.351 ± 0.399**	Paternal
8	Reflectance$_{45}$	11.66**	53	3.7	**0.765 ± 0.222**	Maternal
8	pH$_{45}$ loin	7.46[a]	55	2.4	**-0.062 ± 0.023**	Paternal
10	pH$_{24}$ loin	9.15*	1	3.4	**0.018 ± 0.006**	Maternal
14	Reflectance$_{45}$	10.51*	40	3.3	**-0.921 ± 0.282**	Maternal
14	pH$_{45}$ loin	8.01*	44	2.5	**0.050 ± 0.017**	Maternal
14	OPTO-STAR value	6.87[a]	15	2.2	**1.269 ± 0.481**	Maternal

Values in bold represent significant additive or dominance effects.
Definition of symbols: LR, likelihood ratio.
[1]Positions of the QTL in cM.
[2]Percentages of F$_2$ variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no QTL effect - residual variance of model with QTL effect)/residual variance of model with no QTL effect.
[3]Estimated additive (a) effects and their standard errors (SE) of the individual QTL.
[a] implies suggestive at the 10% chromosome-wide level.
* and ** implies significance at the 5%, or 1% chromosome-wide levels, respectively.

6.3.3 Epistasis

In total, 9 significant epistatic QTL pairs were identified (Table 6.6). Two epistatic QTL pairs were identified for pH_{45} loin, two for pH_{24}, one for reflectance$_{45}$, three for meat colour and one for conductivity$_{24}$. Epistatic interactions were identified between QTL on SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14. No epistatic QTL were identified on SSC1 and SSC2. Epistatic QTL pairs accounted for between 5.7% and 10.9% of the phenotypic variance. One of the significant epistatic QTL pairs was between QTL which resided on the same chromosome, on SSC4. All types of two-locus epistatic effects were identified, including AA, AD, DA and DD. The most significant epistatic QTL pairs (p value < 0.00001) were for reflectance$_{45}$ between two genomic locations of SSC4, and for pH_{45} loin between the telomeric end of the p arm of SSC10 and the telomeric end of the q arm of SSC13. These epistatic QTL pairs explained large proportions of the phenotypic variance, at 9.5% and 10.9%, respectively.

Table 6.6 Evidence of epistatic interactions for meat quality traits

Trait	LR	P value	Q0 chr (pos)[1]	Q1 chr (pos)[1]	% var[2]	Q0_a ± SE[3]	Q0_d ± SE[3]	Q1_a ± SE[3]	Q1_d ± SE[3]	Q01_aa ± SE[4]	Q01_ad ± SE[4]	Q01_da ± SE[4]	Q01_dd ± SE[4]
Reflectance$_{45}$	30.91	3.20E-06	4 (23)	4 (130.1)	9.5	0.873 ± 0.511	0.562 ± 0.867	**-1.866 ± 0.532**	-0.140 ± 0.741	-0.825 ± 0.514	**-2.083 ± 0.735**	**4.414 ± 0.893**	-0.475 ± 1.216
OPTO-STAR value	18.81	8.56E-04	4 (120)	6 (160)	5.9	**-2.533 ± 1.146**	**-3.617 ± 1.548**	**2.401 ± 1.118**	**-5.542 ± 1.963**	**3.122 ± 1.073**	0.766 ± 1.987	-3.052 ± 1.562	**6.908 ± 2.804**
OPTO-STAR value	19.80	5.50E-04	4 (8)	9 (76)	6.2	0.938 ± 1.026	2.296 ± 1.601	0.896 ± 0.953	3.190 ± 1.877	**3.944 ± 0.930**	0.506 ± 1.882	-0.425 ± 1.481	-4.534 ± 2.930
OPTO-STAR value	22.37	1.69E-04	4 (130.1)	14 (101)	7.0	**-4.778 ± 1.376**	**4.412 ± 2.099**	-0.432 ± 1.174	1.445 ± 2.605	**4.734 ± 1.183**	**6.676 ± 2.574**	0.954 ± 1.823	**-8.050 ± 3.895**
pH$_{24}$ loin	23.34	1.08E-04	6 (91)	9 (135)	7.0	0.045 ± 0.023	**0.100 ± 0.045**	0.024 ± 0.023	**0.122 ± 0.045**	0.036 ± 0.021	**-0.140 ± 0.041**	-0.052 ± 0.039	**-0.269 ± 0.080**
pH$_{24}$ ham	19.40	6.55E-04	7 (49)	9 (11)	5.7	-0.013 ± 0.026	**0.143 ± 0.043**	0.024 ± 0.027	0.038 ± 0.047	**0.081 ± 0.026**	0.038 ± 0.045	**-0.136 ± 0.042**	-0.130 ± 0.071
pH$_{45}$ loin	27.23	1.78E-05	7 (75)	14 (65)	8.4	0.108 ± 0.056	0.099 ± 0.097	**-0.118 ± 0.052**	**0.203 ± 0.099**	0.083 ± 0.051	**-0.251 ± 0.098**	**0.340 ± 0.088**	**-0.408 ± 0.164**
Conductivity$_{24}$	20.59	3.82E-04	8 (53)	10 (0)	6.4	0.073 ± 0.209	0.235 ± 0.365	0.005 ± 0.224	0.238 ± 0.307	**0.788 ± 0.201**	0.231 ± 0.284	-0.142 ± 0.365	-0.954 ± 0.500
pH$_{45}$ loin	35.52	3.63E-07	10 (2)	13 (122)	10.9	0.056 ± 0.041	**0.174 ± 0.058**	-0.016 ± 0.039	**0.238 ± 0.065**	**-0.185 ± 0.039**	0.015 ± 0.067	0.060 ± 0.055	**-0.325 ± 0.095**

Values in bold represent significant additive, dominance or epistatic effects.

Definition of symbols: LR, likelihood ratio; DG, daily gain; PAR, protein accretion rate; LAR, lipid accretion rate.

[1]Positions of the QTL in cM.

[2]Percentages of F_2 variance explained by the QTL calculated as the proportion of residual variances due to the QTL effect on the residual variances excluding the QTL effect: (residual variance of model with no epistatic effects - residual variance of model with epistatic effects)/residual variance of model with no epistatic effects.

[3]Estimated additive (a) and dominance (d) effects and their standard errors (SE) of the individual QTL.

[4]Estimated additive x additive (aa), additive x dominance (ad), dominance x additive (da) and dominance x dominance (dd) effects and their standard errors (SE).

6.4 Discussion

Intense selection of pigs for increased productivity, including increased leanness and reduced fatness, has been negatively associated with meat eating quality characteristics. In order to prevent a further decline in meat quality, it is important to gain an understanding of the genetic regulation of these traits and their associations with production traits. QTL analysis of physical and chemical body composition, daily gain, feed intake and feed efficiency in the same population of the present study have been previously reported (Mohrmann *et al.* 2006a; Duthie *et al.* 2008; Chapter 2).

Measurements of pH at 45 minutes and 24 hours post-mortem are commonly used indicators of meat quality. The rate of pH change post slaughter is one of the most important factors influencing meat quality. Abnormal rate of decline in pH is associated with three distinct abnormalities, PSE (pale, soft, exudative), dark, firm, dry, and acid meat (Sellier 1998; Klont 2005). Low pH is also associated with low water holding capacity and high drip loss (Otto *et al.* 2004; Otto *et al.* 2006). Ultimate pH also affects characteristics such as tenderness, juiciness and taste (Klont 2005).

A large number of QTL were identified in the present study for pH measured at different locations and times post slaughter. On SSC1, a QTL for pH_{45} loin was identified. There are no reports of QTL for pH around this location, however there are reports of QTL for pH in other locations of SSC1 (Ponsuksili *et al.* 2005). But, there are reports of QTL in the literature for carcass characteristics, including leanness and fatness around this location of SSC1 (e.g. Malek *et al.* 2001a; Beeckmann *et al.* 2003a; Geldermann *et al.* 2003).

QTL for pH_{45} and pH_{24} were identified on SSC2 at 21 and 28 cM, respectively. These were identified between *SW2623* and *SWR783* and between *SWR783* and *SW240*, respectively. No QTL have been reported in these locations of SSC2 for pH, however QTL have been reported in similar locations for drip loss and intramuscular fat content

(de Koning *et al.* 1999; van Wijk *et al.* 2006; Liu *et al.* 2007). In the same region as the QTL on SSC2 for pH, QTL have been reported in the literature for growth traits, lean and fat tissue characteristics (Rattink *et al.* 2000; Geldermann *et al.* 2003; Lee *et al.* 2003a) as well as feed intake (Houston *et al.* 2005).

A mutation at the *RYR1* gene, also known as the halothane gene, is associated with malignant hyperthermia syndrome (porcine stress syndrome) (Fujii *et al.* 1991). The stress susceptibility allele is associated with a fast rate of post-mortem decline in pH and pigs carrying this allele produce more PSE meat. The incidence of PSE meat is greater in breeds that exhibit extreme muscling (e.g. Pietrain) and has increased as a result of the intense selection for leanness (Clutter and Brascamp 1998). The *RYR1* gene is associated with improved leanness of carcasses and superior food conversion ratio (Clutter and Brascamp 1998), however this has been detrimental to meat quality, with a higher incidence of PSE meat (Clutter and Brascamp 1998; Garnier *et al.* 2003). In the present study, with no adjustment for the *RYR1* genotypes, QTL were detected in the vicinity of the *RYR1* gene, known to reside in the *S0087–SW122* marker interval at 63–83 cM of SSC6 (Rohrer *et al.* 1996), for meat colour, conductivity, reflectance value and pH, with likelihood ratios as high a 42.07 explaining proportions of the phenotypic variance up to 12.7% (Table 5). QTL have been reported in similar locations in the literature for pH, conductivity, marbling, meat colour and intramuscular fat content (Grindflek *et al.* 2001; Geldermann *et al.* 2003; Yue *et al.* 2003a; Edwards *et al.* 2008). From previous analysis of the data of the present study (Mohrmann *et al.* 2006a), QTL were identified for carcass cuts, lean and fat tissue as well as chemical body composition around this region of the *RYR1* gene. Pietrain alleles are associated with increased lean and decreased fat as well as decreased pH_{45}, indicating that selection for lean tissue has resulted in a decline in meat pH which is detrimental to meat quality. There is also published evidence for QTL for carcass cuts, lean and fat tissue in this region of SSC6 (Rohrer 2000; Varona *et al.* 2002; Geldermann *et al.* 2003; Yue *et al.* 2003a; Edwards *et al.* 2008).

On SSC7 a single significant QTL was identified for pH_{45} loin. Ovilo *et al.* (2002) reported a QTL for meat colour in a similar location. de Koning *et al.* (2001b) also reported a QTL for cooking loss (%) around this location of SSC7.

On SSC9, QTL were identified for pH_{24} ham. There are no reports of QTL for pH around this region of SSC9, however a QTL has been reported in this region for intramuscular fat content (Sato *et al.* 2003). In a second location of SSC9, at the telomeric end of the q arm, a significant QTL was identified for pH_{45} loin. Edwards *et al.* (2008) reported a suggestive QTL for meat colour in a slightly different location of this chromosome.

On SSCX, a QTL was identified for pH_{24} ham at 13 cM. At the telomeric end of the p arm of SSCX, QTL have been reported for conductivity (Cepica *et al.* 2003b), off flavour score (Rohrer *et al.* 2005) and moisture content (Edwards *et al.* 2008).

In the present study, at the QTL on SSC1, one on SSC2, SSC4, SSC9 and one QTL on SSCX, Pietrain alleles are associated with decreased pH, an indication that selection for leanness has negatively affected pH and thus meat quality. At one QTL on SSC2, Pietrain alleles are associated with higher pH at 24 hours post mortem. These QTL were identified in a region where no QTL were identified for body composition traits from previous analysis of this data, therefore providing the possibility of selecting for improved meat quality without affecting body composition traits.

Meat colour has an important impact on consumers' decisions and is therefore economically important. Most consumers prefer a reddish-pink colour in fresh pork. Meat cuts that are too dark, pale, or variable in colour may lower the consumer's perception of quality. QTL were identified in the present study for meat colour on SSC4 and SSCX. The grandpaternal Pietrain breed was associated with paler meat at the QTL on SSC4. There are reports of QTL for meat colour around this location of SSC4 by Ovilo *et al.* (2002) and Malek *et al.* (2001b). Reflectance value is another measure of

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meat colour. There are very limited QTL for reflectance value in the literature with only one reported on SSC2 (Jeon *et al.* 1999), probably because this trait has not been explored widely within QTL mapping studies. In the present study, QTL were identified for reflectance value on SSC9 and SSC14. At a similar location of SSC9, Edwards *et al.* (2008) reported a suggestive QTL for meat colour. In a similar location of SSC14, QTL have been reported for cooking loss (Malek *et al.* 2001b) and meat colour (de Koning *et al.* 2001b). Pietrain alleles are associated with increased reflectance values on SSC9 and reduced reflectance value on SSC14.

It has been recognised that optimal fat content in pigs may have been reached and further reductions in fatness is likely to negatively affect meat quality, therefore efforts to reduce backfat further by selection may not be desirable (Sellier 1998; Roehe *et al.* 2003). Intramuscular fat content is considered to have a positive influence on meat quality characteristics such as appearance, colour, tenderness, flavour and juiciness (de Koning *et al.* 1999; Roehe *et al.* 2003; Ciobanu *et al.* 2004). Pork with higher intramuscular fat content is expected to be more desirable with less variable eating quality than pork with lower intramuscular fat content. In order to preserve meat quality the possibility of reducing subcutaneous fat without reducing, or more desirably, with an increase in intramuscular fat content has been recognised (Roehe *et al.* 2003). Intramuscular fat content was not analysed in the present study, however a QTL was identified from previous analysis of the population of the present study by Duthie *et al.* (2008; Chapter 2) on SSC8. This QTL was identified close to a QTL for lipid accretion and in a region of no QTL for subcutaneous fat. This suggests there is potential to improve intramuscular fat without increasing subcutaneous fat.

There is evidence in this study for a number of imprinting effects throughout the genome which influence meat quality traits. Some of these were not identified using an additive and dominance only model. From previous analysis of the population in the present study, maternal imprinting was identified for fat tissue in the same location of SSC10 as maternal imprinting for pH was identified in the present study (Duthie *et al.* 2008;

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Chapter 2). de Koning *et al.* (2000) reported maternal imprinting on SSC2 for backfat thickness, i.e. paternal expression, in the same location as a QTL with paternal expression (i.e. maternal imprinting) was identified in the present study for pH_{45} loin. In a similar location of SSC10, where a QTL with maternal imprinting (paternal expression) for pH_{24} loin was identified in this study, Thomsen *et al.* (2004) reported a QTL with maternal expression for backfat and marbling score.

There is more evidence for imprinting effects in humans (see geneimprint, www.geneimprint.com) than has been reported in the pig. Investigation of the comparative regions of the human genome revealed imprinting effects which provide support for the imprinted QTL of the present study. A region of human chromosome 4 corresponds to the region of SSC8 where paternal imprinting effects (maternal expression) were identified for OPTO-STAR value. In this comparative region of the human genome there is evidence for genes also showing paternal imprinting effects including *Fibroblast growth factor receptor-like 1* and *KIAA1530* (Luedi *et al.* 2007). Further genes showing paternal imprinting effects have been reported in a region of human chromosome 9 including *FLJ46321 protein*, *LIM homeobox transcription factor 1, beta* and *Phosphohistidine phosphatase 1* (Luedi *et al.* 2007). This region of human chromosome 9 is comparative to the region of SSC1 where paternal imprinting was identified for reflectance value. The *Wilms tumor 1 gene* has been shown to have maternal imprinting effects (paternal expression) (Pal *et al.* 1990; Nordenskjöld *et al.* 1994) and resides in a location of human chromosome 11 which is comparative to the region of SSC2 where maternal imprinting effects were identified for pH_{45} of the loin tissue. Furthermore, paternal imprinting effects have been identified on genes which are located on human chromosome 19 (*Myeloid zinc finger 1*) (Luedi *et al.* 2007) and human chromosome 14 (*Delta-like 1 homolog*) (da Rocha *et al.* 2008). These regions are comparative to the region of SSC7 where a QTL with paternal imprinting was identified for conductivity in the present study.

In pigs, epistatic QTL have been reported so far for reproductive traits (Bidanel 1993; Rodriguez *et al.* 2005; Noguera *et al.* 2006), coat colour (Hirooka *et al.* 2002), meat quality traits (meat colour and intramuscular fat content) (Ovilo *et al.* 2002; Szyda *et al.* 2006) and muscle fibre traits (Estelle *et al.* 2008). From analysis of the data of the present study, a large number of epistatic interactions for carcass cuts, lean and fat tissue weights, chemical body composition and growth traits have been identified (Chapters 4 and 5). In the present study numerous epistatic QTL pairs for meat quality traits were identified. From previous analysis of the same population of the present study (Chapter 5), a similar interaction was identified for belly weight and external neck fat weight as was identified between two genomic locations of SSC4 for reflectance value in the present study. In a similar location to the epistatic QTL pair identified between SSC4 and SSC14 for meat colour, a similar interaction was previously identified for chemical body composition at 140 kg body weight (protein and lipid content) (Chapter 4). In the present study, an interaction was identified between SSC7 and SSC14 for pH_{45} loin. A similar interaction was previously identified for daily gain and protein accretion rate from 30-60 kg body weight (Chapter 4). Estelle *et al.* (2008) identified numerous significant epistatic QTL pairs for muscle fibre traits in a pig population of Iberian x Landrace F_2 cross, using a similar methodology as the present study. Estelle *et al.* (2008) suggest that the epistatic interactions they detected for muscle fibre traits formed a network of connected pairs of epistatic QTL. This may be a common phenomenon as Carlborg *et al.* (2006) reported similar networks. Estelle *et al.* (2008) found that SSC10 and SSC11 behaved as hubs for this network. The results of the present study indicate that SSC4 and SSC9 behaved as hubs for this activity. From the epistasis analysis in the present study, only two of the QTL were identified in the individual QTL analysis, on SSC4 for OPTO-STAR value, and on SSC9 for pH_{24} ham. These were two of the most significant QTL identified from the individual QTL analysis. Therefore there are numerous QTL which only express their effects through interaction with other loci, and these QTL cannot be detected from individual QTL analyses. It is therefore essential to consider epistasis within these QTL studies, to gain a fuller understanding of the genomic regulation of meat quality traits.

A large number of QTL were identified in the present study for meat quality traits. The positional associations of these QTL with QTL for body composition traits and their gene effects provide us with further insight into the genetic regulation of meat quality. Furthermore numerous epistatic QTL pairs influencing these traits were identified, indicating that the genomic control of meat quality is a complex process involving numerous QTL as well as a complex network of gene interactions. Knowledge about epistatic interactions is important for obtaining a thorough understanding of the genomic networks which form the basis of biological systems influencing meat quality traits (Carlborg and Haley 2004). Knowledge about the individual QTL or genes which influence variation in these systems is increasing. However, in order to build up a fuller understanding of the genomic networks which influence important biological systems, it is essential to uncover the role of epistasis and how the interaction between genes influences the variation in biological systems.

Chapter 7

General discussion

This Chapter will review the most significant findings of the previous Chapters and discuss their relevance to commercial pig production. Selection strategies based solely on phenotypic information of pigs have been a successful method of improving lean content and reducing fatness. This has been achieved with limited knowledge of the underlying genetic architecture of these economically important traits (Andersson 2001; Georges 2001; Weller 2001; Dekkers and Hospital 2002). As a consequence of this selection strategy, pig breeding has observed unfavourable changes in feed intake capacity as well as meat eating quality characteristics. Reduced feed intake capacity will limit the pigs' potential for maximal protein deposition and meat eating quality affects the commercial value of the market product. Therefore, it is essential to prevent further undesirable developments in these traits or even improve these traits. Furthermore, improving feed efficiency is becoming increasingly important within commercial pig production, particularly in light of growing feed costs. Consequently, the aim of this thesis was to investigate the genomic regulation of economically important traits in pig production, such as growth, body composition, feed intake, feed efficiency, carcass characteristics and meat quality.

The first objective was to gain insight into the genomic control of growth and body composition traits by using genomic scans for individual quantitative trait loci (QTL) across the autosomes. This work was reported in Chapter 2. A large number of QTL located throughout the genome were identified for physical body composition traits, including important carcass cuts (lean + fat tissue), lean tissue weights and fat tissue weights. These QTL were mostly confirmed by previous reports in the literature of QTL for similar traits. However, to the best of my knowledge there are currently no studies which have analysed phenotypic measurements taken from recent carcass grading techniques, such as the AutoFom device which is an automatic ultrasound scanning technique. This system achieves a faster and more accurate grading of the carcass (Brondum *et al.* 1998; Busk *et al.* 1999). In Chapter 2, a number of QTL were identified for measurements taken by the AutoFom device. These QTL are particularly interesting

as the information can be directly used to improve carcass quality defined by the market of interest.

In contrast to the large amount of information available in the literature for QTL associated with physical body composition, QTL for chemical body composition, such as protein and lipid content and change in the deposition of these components throughout growth, have only been reported in one study (Mohrmann *et al.* 2006a). Information about the deposition rates of protein and lipid tissue is important to accurately estimate the nutritional requirements of pigs, for the improvement of feed efficiency, to optimise the entire production system, to characterise the population of interest and within breeding to optimise feed intake capacity to allow maximal protein deposition. Even though this information is of great importance to pig production, studies have neglected the genomic control of chemical body composition, most likely because of the expense and difficulty associated with collecting these types of measurements in live animals. The use of the deuterium dilution technique, as a method of measuring chemical body composition based on total body water, has allowed for the collection of these data at different target weights in live animals in the resource population of this study (Landgraf *et al.* 2006a; Mohrmann *et al.* 2006b). From previous analysis of the data of this study across other chromosomes, Mohrmann *et al.* (2006a) firstly reported QTL for chemical body composition (protein and lipid contents) and deposition rates of these components based on the analysis of four chromosomes. Chapters 2 and 3 have added substantially to our understanding of the genomic regulation of chemical body composition traits. Different regions of the genome showed associations with protein and lipid contents as well as accretion rates of protein and lipid tissue. Chapters 2 and 3 also outlined that the genomic architecture underlying the regulation of chemical body composition and deposition differs between growth stages, such that different QTL seem to be switched on and off throughout growth.

The market price of the final product is based on carcass quality, therefore the association between chemical and physical body composition is of great economic

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interest. Associations were identified between QTL for chemical body composition and deposition with QTL for physical body composition. For example, QTL for protein accretion (PAR) and lipid accretion (LAR) were identified in the same region as many QTL for lean and fat tissue where the Pietrain breed was associated with increased lean tissue and reduced fatness. The reason for these QTL for PAR and LAR is likely to be change in body composition. A further QTL for PAR was identified in a location of no other QTL than for daily gain, suggesting that growth rate *per se* is the likely reason for this QTL. QTL for LAR have been identified around different types of fat tissue, in one location around QTL for subcutaneous fat, and in another around only a QTL for intramuscular fat. Chapters 2 and 3 has provided further information about the genomic regulation of physical and chemical body composition and has outlined some important associations between these traits.

There is some indication in the literature that chromosome X is involved in the genomic regulation of growth and body composition of pigs (e.g. Knott *et al.* 1998; de Koning *et al.* 2001a; Cepica *et al.* 2003b; Geldermann *et al.* 2003). However, the methodologies and models applied in the majority of these studies were often not entirely appropriate for the analysis of this chromosome, which may have led to inaccurate estimates of QTL and some QTL remaining undetected. This is because accurate methodology for the analysis of chromosome X has previously not been available. However, Perez-Enciso *et al.* (2002) have developed methodologies which account more accurately for the unique features associated with chromosome X. This methodology has been implemented in the program QxPak (Perez-Enciso and Misztal 2004). The aim of Chapter 3 was to further elucidate the role of chromosome X in the genomic regulation of economically important traits. A particularly interesting finding was that the pseudoautosomal region, the region of chromosome X which is homologous to the Y chromosome, showed important associations with loin tissue characteristics. In this region the Pietrain breed was associated with higher weights of loin tissue characteristics. Within the pseudoautosomal region both maternal and paternal imprinting effects were identified for different traits. Paternal imprinting was identified for entire loin weight indicating

that only the maternal allele is expressed at this QTL. A QTL for neck weight without external fat showed maternal imprinting effects indicating that only the paternal allele is expressed at this QTL. This QTL for lean tissue of the neck was only identified when imprinting was accounted for and would otherwise have been missed with an additive and dominance only model. This outlines the importance of investigating imprinting. In the differential part of chromosome X, the region which is not homologous to the Y chromosome, a further QTL for LAR was identified. However, in this instance this was in a location of no QTL for fatness. There are many reports of QTL for fatness and leanness in this region of chromosome X (Milan *et al.* 2002; Perez-Enciso *et al.* 2002; Rohrer *et al.* 2005), thus it was particularly surprising that no QTL for these traits were identified in this study. This could be because lean tissue alleles are already fixed in these founder populations and that QTL reported in the literature for fat and lean tissue are not segregating in these commercial lines. This outlines the need to investigate QTL within commercial populations as the QTL identified in crosses of obese breeds such as the Meishan, Iberian or Wild Boar, may not be segregating in commercial populations, and therefore cannot be exploited within practical pig breeding programmes. The results of Chapter 3, however, confirm that chromosome X is involved in the genomic regulation of growth and body composition, however to a lesser extent than the autosomal chromosomes.

An undesirable consequence of selection for increased leanness has been a reduction in feed intake capacity of pigs, which limits the potential of pigs for maximal growth. In view of this, there is a requirement to improve feed intake capacity of pigs, without adverse effects on body composition. In Chapters 2 and 3, QTL were identified for daily feed intake (DFI). In general Pietrain alleles were associated with lower DFI, as expected, which is likely to be the result of long-term selection of this breed for increased leanness. In contrast, however, a QTL was identified on chromosome X, where the Pietrain breed was associated with increased feed intake at a late growth stage (120-140 kg body weight). This may provide an opportunity to improve feed intake capacity of this breed. This is important because growth of Pietrain is generally

restricted due to limited feed intake capacity. It is of particular interest to improve feed intake capacity at early stages of growth in the pig, when the pig is most efficient, and limit feed intake at later stages of growth to prevent extensive fat deposition which is associated with higher feed costs and lower commercial value of the market product.

One of the key goals within pig breeding programmes is to improve feed efficiency of pigs, particularly in light of increasing feed costs. In Chapter 2, a number of QTL for food conversion ratio (FCR) were identified. To date the reason for QTL for FCR have generally been unknown, however the results of Chapter 2 provide an interesting explanation for some of these QTL. On SSC2 the QTL for FCR was identified in a region of many QTL for body composition traits where the Pietrain breed was associated with increased lean tissue and reduced fatness. Therefore, indicating that in this location the reason for this QTL for FCR is change in body composition (increased leanness and reduced fatness). From previous analysis of these data, Mohrmann *et al.* (2006a) reported a QTL for FCR in a similar genomic location as QTL for PAR, indicating the reason for this particular QTL was lean growth *per se*. A further QTL for FCR was also identified in a region of no other QTL for growth and body composition, which may indicate that this QTL may be due to lower maintenance requirements of those animals.

An added complication to understanding the genomic control of economically important traits is the existence of interactions between QTL (epistasis). Epistasis has been largely ignored when trying to dissect the genetic regulation of economically important traits, even though quantitative geneticists have been aware of the likely contribution of epistasis for a long time. The main reason for this lack of attention is the unavailability of appropriate methodologies and software for this type of analysis. In order to further elucidate the genomic architecture underlying body composition, growth, feed intake and feed efficiency, Chapters 4 and 5 aimed to uncover the role of epistasis in the genomic regulation of these traits. Developments in methodology and available software allowed for the investigation of epistasis. There are no reports in the literature for epistatic QTL for the analysed traits in pigs and therefore the results of Chapters 4 and 5

are the first to report such effects. A large number of QTL were identified which were not identified from the individual QTL analysis of Chapter 2. The reason for this is because these QTL do not exhibit individual QTL effects (additive, dominance and imprinting), and mainly express their effects through interactions with other QTL. The epistatic QTL pairs identified in these Chapters also accounted for much higher proportions of the phenotypic variance than the QTL identified from the individual QTL analysis. In comparing the same QTL which were obtained from both individual QTL analysis and the epistasis analysis, in some cases slightly different estimates arose indicating that not accounting for epistasis may lead to inaccurate estimates of QTL. For example, the epistasis analysis of Chapter 4 identified QTL for daily gain and PAR with significant dominance effects. This was in contrast to results from previous individual QTL analysis which reported significant additive effects at these QTL (Mohrmann *et al.* 2006a). In turn this leads to wrong interpretations of the importance of QTL and lower response and economic gain when exploiting the QTL within breeding. Furthermore, interactions were identified between QTL of regions of the genome which are known to harbour genes, such as the *insulin-like growth factor 2 (IGF2)* or *melanocortin-4 receptor (MC4R)* genes, with QTL residing on other regions of the genome. The interaction between QTL in the region of the *MC4R* gene (SSC1) and SSC7 showed significant positive dominance effects for entire belly weight, which were offset by negative dominance-by-dominance interactions between these QTL. In contrast, the QTL in the region of the *IGF2* gene showed significant negative dominance effects for entire ham weight, which were largely overcompensated by positive additive-by-dominance effects with a QTL on SSC9. Although very little attention has been paid to epistatic QTL in the pig, there are a small number of reports for epistatic interactions influencing different groups of traits in the literature, such as reproductive traits (Bidanel 1993; Rodriguez *et al.* 2005; Noguera *et al.* 2006), coat colour (Hirooka *et al.* 2002), meat quality traits (meat colour and intramuscular fat content) (Ovilo *et al.* 2002; Szyda *et al.* 2006) and muscle fibre traits (Estelle *et al.* 2008). However, from a comparison of these studies it seems that different groups of traits are influenced by a different network of interactions. In different species of livestock evidence of epistatic QTL influencing

economically important traits is also limited. In cattle, evidence of epistatic interactions have been reported for meat tenderness between mutations at the *Calpain 1* gene and the *Calpastatin* gene (Barendse *et al.* 2007) and for fertility traits between genes involved in the POU1F1 pathyway (Khatib *et al.* 2009). At present there are no reports of epistatic QTL in sheep. In Chickens, epistasis has been shown to be particularly important for early growth in an intercross between Jungle Fowl and White Leghorn chickens (Carlborg *et al.* 2003). In a cross between a White Leghorn line and a commercial broiler line, epistasis has been found to be an important contributor to the genetic variance of growth, with the largest effects on body weight at 6 weeks of age and growth between 3 and 6 weeks of age (Carlborg *et al.* 2004). In contrast to livestock species, a large number of studies in mice indicate an important role of epistasis in the genomic regulation of growth and body composition. Routman and Cheverud (1997) identified a contribution of epistasis to the genomic control of adult body weight in mice. Brockmann *et al.* (2000) identified epistatic effects for serum concentrations of leptin, insulin and *Insulin-like growth factor 1*, body weight, abdominal fat weight and muscle weight in mice. In the same species, epistasis has also been reported to play an important role in controlling obesity (Yi *et al.* 2004a). Moreover, there are reports of epistatic QTL in mice for abdominal fat, body weight, kidney weight, spleen weight (Carlborg *et al.* 2005) as well as organ weights and limb length traits (Wolf *et al.* 2006). Yi *et al.* (2006) also reported that epistasis influenced fatness and organ weights in mice. Yi *et al.* (2006) reported that epistasis had a more pronounced effect for body weight at later stages of growth in mice, whereas Ishikawa *et al.* (2005) identified that epistasis was more important in early stages of growth in mice. Chapters 4 and 5 have confirmed the importance of epistasis in the genomic control of the considered traits, and outlined the importance of accounting for epistasis as not doing so leads to inaccurate estimates of QTL, and many QTL remaining undetected.

In addition to the traits discussed in this Chapter so far, meat quality traits are of great interest for pig breeding. The quality of the market product influences consumer satisfaction and subsequently the commercial value (Schwab *et al.* 2006). There has

been a reduction in the meat eating quality of pork, due to the intense selection for increased leanness. Measuring meat eating quality traits is expensive, difficult and can only be done after slaughter. Furthermore, the low heritability of these traits makes meat quality difficult to improve by selection (Gao *et al.* 2007). To date there is limited knowledge of the genes and interactions involved in the genomic regulation of meat quality. The first step in uncovering the underlying genetic architecture is to identify chromosomal regions influencing meat quality. Once the genetic regulation of meat quality traits is better understood, the information can be applied in breeding through marker assisted selection. Chapter 6 focussed on exploring the genomic regulation of a number of meat quality traits including pH measurements at different times after slaughter, meat colour measurements, reflectance values and conductivity. A large number of QTL were identified throughout the genome for meat quality traits as well as a complex network of QTL interactions. Similarly, as observed in Chapters 4 and 5, many QTL were not identified in the individual QTL analysis and only uncovered when epistasis was accounted for. This indicates that many QTL only exert their effects through interactions with other loci. Thus it is of great benefit to account for epistasis to gain a fuller and more accurate understanding of the genetic architecture underlying important traits. One of the main goals within breeding has been to reduce fat content. Intramuscular fat content is particularly important for meat quality (Sellier 1998; Roehe *et al.* 2003). Subcutaneous fat is however an undesirable characteristic which negatively influences the value of the commercial product. However, the opportunity for reducing subcutaneous fat, without reducing or even with an increase in intramuscular fat content has been recognised (Roehe *et al.* 2003). In Chapter 2 of this thesis, a QTL for lipid accretion was identified close to a QTL for intramuscular fat in a region of no QTL for subcutaneous fat. This suggests an opportunity to improve intramuscular fat without increasing subcutaneous fat.

A concern within the present study is multiple testing and the risk of false positive results, particularly with respect to the epistatic QTL analysis where a large number of tests were carried out. As research surrounding epistatic QTL analysis is in a preliminary

stage, an appropriate threshold has not been established. In order to minimise the risk of false positive results in the present study a more stringent threshold was applied to the epistatic QTL analysis than for the individual QTL analysis. Furthermore, it is important that the results of this study are verified by further research.

In animal breeding programs, the breeding goal is based on a number of traits in order to improve the genetic merit of a population (Dekkers 2004). Advances in molecular genetics provide tools which will provide opportunities to improve the genetic merit of livestock, through considerable changes to selection practices (Kennedy *et al.* 1990; Kappes 1999). Molecular genetics cannot replace traditional breeding strategies, but should be integrated in order to achieve the optimum improvement and the resulting economic benefits (Lande and Thompson 1990). The results of Chapters 2 and 3 have outlined that growth and body composition as well as feed intake and feed efficiency are influenced by several QTL located throughout the genome. As a result, selection strategies have to be developed to improve the overall genetic merit of these traits. Furthermore, it is essential that these strategies are flexible so as to adapt to different market demands. The use of molecular genetic information within breeding would achieve the most response and economic gain, if the underlying genetic architecture of economically important traits was completely clear and the number, position and effect of all genes was understood. However, at the present time this is far from reality as molecular and quantitative genetics can only partly explain the underlying genetic control of these traits. However, in conjunction with phenotypic information, breeding programs can be constructed which include genetic information (Dekkers and Hospital 2002). This will allow for a more accurate prediction of breeding values and therefore achieve a higher response and economic gain.

Information about most QTL reported in the literature is from crosses of lean with obese exotic breeds and cannot be used within commercial pig breeding. This is because the QTL identified in these crosses may not be segregating within the commercial populations which have been subjected to intense selection for improved growing-

finishing and carcass characteristics. The QTL identified in the present study can be directly exploited within pig breeding as they were identified within commercial populations.

Information about identified QTL can be used to improve the genetic improvement through marker assisted selection (Dekkers and Hospital 2002; Ovilo *et al.* 2002). Marker assisted selection can be used to optimise traits of economic importance by exploiting the linkage between QTL and genetic markers (Hayes and Goddard 2003). The large number of QTL mapping studies in livestock has been partly facilitated by the possible opportunities to exploit the identified QTL in marker assisted selection (Nezer *et al.* 2002). There is a lot of emphasis in the literature about the benefits that can be achieved using marker assisted selection, however the implementation of this technique has been limited and has not yet achieved the expected response (Dekkers 2004).

The general conclusion from investigations of the practical usefulness of marker assisted selection, is that it may be useful in achieving advances in genetic improvement, particularly in low heritability and sex-limited traits (Lande and Thompson 1990; Spelman and Bovenhuis 1998). Genetic improvement of meat quality traits are difficult using traditional selection strategies as these traits can only be measured on animals after slaughter, therefore, for selection purposes only information on relatives can be used (Otto *et al.* 2007b). Due to the difficulty in measuring meat quality traits and the fact that they mainly show low heritability, marker assisted selection may be particularly valuable for the improvement of meat quality traits (Dekkers and Hospital 2002; Gao *et al.* 2007; Otto *et al.* 2007b). In order to incorporate molecular genetic information within animal breeding will require a complete redesign of breeding strategies in order to make the most efficient use of the information (Dekkers and Hospital 2002).

The availability of markers associated with traits of economic interest in pig production is increasing steadily and will play an important role in selection strategies thereby increasing selection accuracy and thus improve genetic progress (Villanueva *et al.*

2002). The economics of using molecular genetics within animal breeding is the key determinant to its practical application (Dekkers and Hospital 2002). One of the most important questions is whether the benefits achieved from marker assisted selection are cost-effective.

There is also an opportunity to exploit information about epistasis within breeding. A large number of additive-by-additive effects were identified in Chapters 4, 5 and 6, thereby providing an opportunity to exploit this information within breeding. The additive-by-additive component provides the most opportunity as this component has been shown to be heritable (Goodnight 1988). This particular component has also been shown to generate greater and more long-term response to selection in comparison to additive gene action (Jannink 2003). Therefore, there is a potential benefit from understanding the role of epistasis, particularly the additive-by-additive component. Information about the use of the additive-by-additive component specifically within animal breeding is limited. Animal breeding focuses around the improvement of the additive genetic component, thereby ignoring non-additive effects, including epistasis. Based on the idea that interacting genes are often organised in physically linked gene clusters, Fury *et al.* (2006) investigated the use of a model which combines the additive-by-additive genetic effects and the additive genetic effect and reported that extra genetic gains can be achieved in the short-term but this is not sustainable in the long-term.

In conclusion, this thesis has provided a fuller understanding of the genetic regulation of growth and body composition of pigs. Chapter 2 outlined numerous QTL located throughout the genome and uncovered many "novel" QTL for chemical body composition and accretion rates of protein and lipid tissue. Furthermore, Chapter 2 provided insight into the biological reason underlying QTL for feed efficiency, which is of great economic benefit as improving feed efficiency is one of the most important traits in the breeding goal. Chapter 3 confirmed that chromosome X is involved in the genomic regulation of growth and body composition by applying accurate methodology for the analysis of this chromosome. Furthermore, Chapters 4 and 5 outlined the

complex nature of interactions involved in the genomic regulation of growth and body composition, and indicated the great benefits that can be achieved by accounting for these effects. Finally, Chapter 6 provided insight into the genomic architecture underlying meat quality traits. As these QTL were identified in a resource population of commercial breeds, they can be directly exploited within practical pig breeding programmes.

This study has provided substantial information about the genomic regulation of economically important traits in pig production. However, knowledge of the entire genomic regulation of these traits is still limited. Many of the QTL identified in this thesis have not been previously reported in the literature, particularly those for chemical body composition and deposition. Therefore it is important to confirm these QTL in other studies and to investigate whether they exist in other commercial breeds. This thesis also confirmed the importance of epistasis in the genomic regulation of growth, body composition and meat quality. However, epistatic QTL could only be investigated on an individual trait basis. The situation is probably much more complex and epistasis is likely to exist between traits. Unfortunately this could not be investigated in this thesis, as methodologies are unavailable at present. For the purpose of the research covered in this thesis, genotypic information was only available for markers on 11 of the 19 pig chromosomes. Therefore, there will be many more QTL which contribute to the genomic regulation of the traits considered in this study. The remaining chromosomes have not been genotyped in the present study because of restriction in the budget. It may be more cost-effective to genotype using the recently available single nucleotide polymorphisms (SNPs) marker chips.

Following on from the identification of QTL is the identification of the causative mutations underlying these QTL. The identification of these mutations is challenging in domestic animals due to the poor resolution of QTL studies. The confidence intervals surrounding QTL can be as high as 20 mega base pairs, a region which may contain up to hundreds of genes (Andersson 2008). A method of tackling this is genome-wide

association analysis using dense SNP marker chips. This method involves searching the genome for SNPs to be associated with the trait of interest. This method has become possible by the development of large collections of SNPs as well as effective methods for analysis (Andersson 2008). Therefore, further investigations with SNPs and genome-wide association is a promising method of investigating the underlying causative mutations of the QTL identified in the present study.

Utilizing QTL information in animal breeding is moving away from the approach of marker assisted selection and towards genomic selection, a strategy which was introduced by Meuwissen *et al.* (2001). In the simplest sense genomic selection is a genome-wide form of marker assisted selection (Meuwissen 2007). This approach utilises information about the association of large number of SNP markers located throughout the entire genome with phenotypic information. This has become feasible due to the availability of large numbers of SNP markers and the development of the porcine SNP chip. There are two main stages involved in genomic selection. In the first stage the effects of SNPs on traits of interest are estimated in a reference population. In this reference population genotypic information as well as phenotypic information is available for all animals. In the second stage these marker estimates are utilised to obtain the genetic value of animals which have only genotypic information available and no phenotypic information. As a result genomic selection can allow for breeding information to be obtained in a larger number of animals, therefore providing an opportunity to optimise genetic improvement by achieving a faster and more accurate rate of genetic gain (Goddard and Hayes 2007). This strategy has great potential for the genetic improvement of traits which are difficult to improve by traditional selection methods, including meat quality and feed efficiency.

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