Institut für Tierwissenschaften, Abt. Tierzucht und Tierhaltung
der Rheinischen Friedrich – Wilhelms – Universität Bonn

Detection and characterization of QTL in a porcinen Duroc-Pietrain resource population

I n a u g u r a l – D i s s e r t a t i o n
zur Erlangung des Grades

Doktor der Agrarwissenschaft
(Dr. agr.)
der
Hohen Landwirtschaftlichen Fakultät
der
Rheinischen Friedrich – Wilhelms – Universität
zu Bonn

vorgelegt im August 2005

von

Guisheng Liu

aus

Wuhan, China

D98

Referent:	Prof. Dr. K. Schellander
Korreferent:	Prof. Dr. H. Sauerwein
Tag der mündlichen Prüfung:	05 Oktober 2005

Diese Arbeit wurde mit finanzieller Unterstützung vom KAAD durchgeführt.

Dedicated to my beloved parents, who used to concern about me and encourage me during my study but both passed away during the final phase of my study. I missed them at the last moment; I also have not taken care of them during their illness.

Detection und Characterization für QTL

in einer porcinen Duroc-Pietrain Resourcenpopulation

In der vorliegenden Arbeit wurde ein Genomescan zur Detektion von QTL in der F_2-Generation einer porcinen Ressourcenpopulation aus einer reziproken Kreuzung der Rassen Duroc und Pietrain durchgeführt. 1085 F_2 Nachkommen in 38 Vollgeschwister-Familien wurden mit diesen drei Generationen produziert. Die Tiere wurden an 73 informativen Mikrosatellitenmarkern genotypisiert, die 79.5 % der 18 *sus scrofa*-Autosomen nach USDA/MARC.2 Karte abdeckten. Genetische Karten wurden mit der Crimap-software konstruiert. Weibliche Karten, männliche Karten und Kombinierte Karten hatten ein Größe von 2142.8 cM, 1660 cM, beziehungsweise 1821.6 cM. Least-Square-Regression-Intervall-Kartierung wurde zur Detektion von QTL zwischen den molekularen Markern und 32 phänotypischen Merkmalen durchgeführt. Die Merkmale umfassten Körpergewicht, Wachstumsparameter, Schlachtkörperzusammensetzung und Fleischqualität. Signifikante Schwellenwerte wurden durch Permutationstests ermittelt. Insgesamt wurden 71 QTL auf nahezu allen Autosomen mit Ausnahme von SSC10 mit einem Ein-QTL-Modell identifiziert. Von diesen QTL waren 52 mit einem 5 % chromosomenweiten Signifikanzniveau, sehn QTL auf SSC1, SSC2, SSC6, SSC8, SSC9, SSC16 und SSC17 waren mit 5 % genomweiter Signifikanz, neun QTL auf SSC1 und SSC7 waren mit 1 % genomweiter Signifikanz. Für den pH-Wert des Fleisches, der post-mortem im *m. long. dorsi* und *m. semimembranosus* gemessen wurde, überstiegen zwei QTL den genomweiten 1 % Schwellenwert in derselben Region auf SSC1, mit einem Konfidenzintervall von 20 ~ 42 cM. Unsere Ergebnisse erscheinen wertvoll und interessant für weitere Untersuchungen, wie Feinkartierung dieser Region mit dem Ziel der Identifizierung positioneller Kandidatengene zur Verbesserung der Fleischqualität. Weiterhin zeigten unsere Resultate, dass die statistische Aussagekraft der QTL-Kartierung durch multiple QTL-Analysen gesteigert werden könnte. Identische Resultate wurden durch Co-Faktor- und Zwei-QTL-Modell-Analysen ermittelt. Dreizehn imprinted QTL für Fett- und Magerfleischanteilmerkmale wurde auf SSC2, SSC5, SSC6 and SSC11 gefunden. Bei Verwendung der multiplen QTL-Analyse mit Imprinting wurden zwei QTL auf SSC2 gleichzeitig Segregation gefunden: einer zeigte Imprintingexpression, hauptsächlichen paternale Expression in der proximalen Region; der andere Mendelische Expression in der distalen Region.

Detection and characterization of QTL
in a porcine Duroc-Pietrain resource population

In the present thesis, genome scans were performed for QTL detection in a F2 resource population which was reciprocally crossed between Duroc and Pietrain. 1085 F_2 progeny within 38 full-sib families were produced from these three generations of commercial pigs. These animals were characterized for 73 informative microsatellites that covered 79.5 % of 18 *sus scrofa* autosomes according to USDA/MARC.2 map. Genetic maps were constructed by Crimap software. Female maps, male maps and sex average maps were 2142.8 cM, 1660 cM and 1821.6 cM Kosambi, respectively. Least square regression interval mapping was conducted for QTL detection between these molecular markers and 32 phenotypic traits. These traits included body weight, growth traits, body composition and meat quality. Significant thresholds were determined by permutation test. In total, seventy-one QTL were identified on almost all autosomes except SSC10 by the one-QTL model. Among those QTL, fifty-two QTL were detected at the 5 % chromosome-wide significant level, ten QTL on SSC1, SSC2, SSC6, SSC8, SSC16 and SSC17 were significant at the 5 % genome-wide level, nine QTL on SSC1 and SSC7 were significant at the 1 % genome-wide level. For meat pH value which was measured post-mortem in *m. long. dorsi* and in *m. semimembranosus*, respectively, two QTL were exceeding the 1 % genome-wide threshold in the same region on SSC1, confidence interval was 20 ~ 42 cM. Our results are valuable and interesting for further work such as fine mapping in this region, then identification of positional candidate gene, in order to improve the meat quality. Furthermore, our findings demonstrated that the statistical power of QTL mapping could be increased considerably by multiple QTL analyses. Identical results were obtained by cofactor analyses and by the two-QTL model analyses. Thirteen suggestive imprinted QTL for fat traits and leanness traits were obtained on SSC2, SSC5, SSC6 and SSC11. By using two-QTL model with imprinting, also by using cofactor analyses, two QTL were found simultaneously segregation on SSC2: one indicated imprinting expression, mainly paternal expression in the proximal region; another indicated Mendelian expression in the distal region.

Contents Pages

List of abbreviations

A	Adenine
AFLP	Amplified fragment length polymorphism
BAC	Bacterial artificial chromosome
bp	Base pairs
cDNA	Complementary DNA
cM	CentiMorgan
CI	Confidence interval
DMSO	Dimethyl sulfoxide
DNA	Desoxyribonucleic acid
dNTP	Deoxynuceotide triphosphate
DuPi	Duroc-Pietrain resource population
EDTA	Ethylenediaminetetraacetic acid
EST	Expressed sequence tag
kb	Kilobase pairs
LD	Linkage disequilibium
LOD	Log of the odds
LS	Least squares
mg	Milligram
ml	Milliliter
ML	Maximum likelihood
MAS	Marker assisted selection
MQM	Multiple QTL mapping
mRNA	Messenger RNA
MS	Microsatellite
ng	Nanogram
OD	Optical density
PCR	Polymerase chain reaction
QTL	Quantitative trait locus
QTN	Quantitative trait nucleotide
RAPD	Random amplified polymorphic DNA

RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
rpm	Rotations per minute
SAS	Statistical Analysis System software
SD	Standard Deviation
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
TBE	Tris-Boric acid-EDTA buffer
TE	Tris-EDTA buffer
µg	Microgram
µl	Microliter
µM	Micromol

List of tables Page

List of figures Page

List of appendixes Page

1 Introduction

Genetic theory and practice have evolved enormously over the past two decades. Quantitative genetics has now been jointed with molecular genetics creating new methods and insights into understanding biological processes. Today more widely than ever before, animal breeders are using knowledge and techniques from the different fields of molecular biology for manipulation and improvement of their livestock.

Update an important aspect of quality of life in Human being is the availability of healthy high quality food. Domestication of the pig occurred some 9,000-11,000 years ago (Reed et al. 1984) and it has been a tremendously important food source in several civilizations. Approximately one billion pigs are now raised worldwide and pork is the dominant meat source representing 40 % of all the red meat eaten. Natural and artificial selection have been the main force for the genetic modification of the domestic swine. Modern biological discoveries and technological improvement in management practices have revolutionized pork production.

The pork industry is diversifying into multiple pork chains. These chains have specific attributes relative to the consumer base they serve. Many chains have specifications regarding carcass lean and meat quality. There is concern that the quality merit of pork filling these chains may be eroding. This is fueled by preliminary results from the 2003 National Pork Quality Audit in USA that revealed that the frequency of pale, soft and exudative (PSE) pork in the USA has increased from 10.2 % in 1996 to 15.5 % (Bates et al. 2003). This increased frequency in PSE pork may be due in part to unfavorable correlated change accumulated as lean yield improved in U.S. pork. Selection for rapid lean growth rate in swine frequently results in production of animals that yield inferior quality meat. Genetic correlations of carcass leanness to ultimate pH (-0.13), reflectance (0.16) and drip loss (0.05) (Sellier 1998) indicate lowered meat quality with increased carcass leanness. Additionally, Wood (1985) reported increased occurrence of less juicy pork products with leaner pigs.

This unfavorable correlated change in meat quality can be overcome by inclusion of meat quality attributes and their related associations with lean growth in the selection objective of terminal as well as maternal lines and breeds. However, collection of meat quality data requires animal harvest and is expensive, thus limiting the utility of this

option. In addition, geographic locations of nucleus herds to slaughter plants may prohibit regular collection of meat quality data. An alternative can be selection for markers or major genes that have a significant and favorable association with meat quality traits under selection consideration.

The aim of this work was:

Whole genomes scan in a Duroc-Pietrain F2 resource population to dissect genome region which is underlying body weight, growth traits, body composition and meat quality traits.

Literature review

In some studies, individual genes with direct and measurable effects on quantitative traits (so called major genes) have been detected. A handful of such genes exist, including the Boorola gene (Davis et al. 1982), which raises litter size in sheep, and the double muscling gene in cattle, which increases lean meat yield (Grobet et al. 1997). However, the majority of those genes affecting quantitative traits does not have directly measurable effects on the traits and thus can not be detected by segregation analysis. A quantitative trait has a continuous distribution and examples of traits that belong to this group are body weight and milk yield. These traits are also referred to as complex, multifactorial or polygenic traits because they are influenced by several genes as well as environmental factors. Due to advances in molecular genetic and statistical methodology, it has become possible to map individual genetic factors with smaller effects on the quantitative traits, known as **Q**uantitative **T**rait **L**oci (QTL). Genes that affect quantitative trait variation in a population are called QTL.

QTL mapping is basically a genome-wide inference of the relationship between phenotypic values of quantitative traits and genotypes of QTL. This relationship includes the effects of QTL, the number of QTL and genomic positions of QTL. This relationship is also called the genetic architecture of quantitative traits. Depending on the data and the nature of molecular markers used for mapping analysis, what is usually identified as a QTL is a segment of chromosome that affects a quantitative trait, not necessarily a single locus. A very important study in quantitative genetics is to localize QTL on a genetic linkage map and further through more detailed genetic studies to characterize QTL, which may include the identification of DNA sequence polymorphisms that cause the quantitative trait variation.

QTL mapping shares the basic principle with qualitative gene mapping: testing association between marker genotypes and quantitative phenotypes. The QTL may contribute to different extent to the phenotypic trait. The methods are also used to infer the mode of inheritance, which gives a better understanding of the genes responsible for quantitative traits. Identifications of QTL are important for our understanding of genetic nature of quantitative trait variation within a population and between populations or species. Biologically, it is important to know how many genes are involved for a quantitative trait within and between populations.

One of the applications for this knowledge is Marker Assisted Selection (MAS), where knowledge about the QTL genotype can help animal breeders to further increase the genetic progress of the domestic animals, particularly for traits with low heritability or that can only be measured in one sex. Second application of this knowledge is positional cloning of candidate genes. Therefore, it is very important to study QTL, it is also the first step toward to functional genetic analysis of quantitative traits.

2.1 The pig genome

The pig genome is of similar size (3 x 10^9 bp), complexity and chromosomal organization (2n = 38, including meta- and acrocentric chromosomes) as the human genome. Comparative genetic maps have indicated that the porcine and human genomes are more similarly organized than compared to the mouse. The mean length of conserved syntenic segments between human and pig is approximately twice as long as the average length of conserved syntenic segments between human and mouse (Ellergren et al. 1994, Rettenberger et al. 1995). Furthermore, the organizational similarities between the human and porcine genomes reflect similarities at the nucleotide level. In more than 600 comparisons of non-coding DNAs aligned by orthologous exonic sequences on human chromosome 7, pig (cow, cat and dog) sequences consistently grouped closer to human and non-human primate sequences than did rodent (mouse and rat) sequences (Green 2002). The numbers of conserved homologous blocks mapped within porcine chromosomes are reported to be 145 with respect to the mouse and 149 to the human genome (http://www.informatics.jax.org, January 2005). Polymorphic loci and homologies between species provide the basis for analyzing genes causing variability of quantitative traits.

Currently, moderate to high-resolution genetic linkage maps containing highly polymorphic loci (Type II) have been produced using independent mapping populations (Rohrer et al. 1996). Additionally, physical mapping methods such as somatic cell hybrid analysis, in situ hybridization and ZOO-FISH have been employed to enrich the Type I marker map and to perform comparative analysis with map-rich species such as the human and mouse. To date, more than 5,000 mapped loci are catalogued for the pig genome (http://www.thearkdb.org). Recently, whole-genome radiation hybrid (WG-RH) panels have been generated for swine (Hawken et al. 1999) resulting in rapid

increase in the number of expressed sequences being mapped facilitating comparative mapping with other species (Rink et al. 2002). The swine genomics community has also acquired access to resources such as bacterial artificial chromosome (BAC) libraries providing approximately 35X coverage of the swine genome. These BAC resources have facilitated the production of higher resolution physical maps in specific chromosomal regions and support the construction of sequence-ready mapping resources for the porcine genome. This includes the creation of a pig-human comparative map and the initial construction of a whole genome BAC contig. Finally, large scale sequencing of expressed sequence tags (ESTs) in conjunction with genomic sequencing has permitted the identification of single nucleotide polymorphisms (SNPs) that can be used to finely map traits (e.g. disease resistance). Thus, the tools and informations are being developed to permit application of genomics to improving the health and performance of pigs.

Most recently, the most significant opportunity comes from the recent decision by the NIH to add the pig to the list of animals for complete genome sequencing (http://www.genome.gov/10002154, and: www.swinegenomics.com). This scientific recognition provides the basis for creating an international consortium to secure funding to complete this initiative. When finished, this sequence will permit rapid identification of genes and targeting chromosomal regions for rapid SNP assays to create new screening tools as well as for the development of new drugs and medicines that promote animal health and performance.

2.2 Genetic markers and genetic maps

Sax (1923) first used pattern and pigment markers in beans to analyze genes affecting seed size by investigating the segregation ratio of F2 progeny of different crosses. For the subsequent 70 years, analyses continued to use visible phenotypic markers and protein variants. However, along with recent revolutionary advances in molecular genetics, several types of markers based on DNA sequence polymorphism have been developed, for instance, Restriction Fragment Length Polymorphisms (RFLPs, Botstein et al. 1980), Simple Sequence Length Polymorphisms or Simple Sequence Repeats (SSLPs or SSRs Jeffreys et al. 1985, Weber and May 1989, also named microsatellites), Amplified Fragment Length Polymorphisms (AFLPs, Vos et al. 1995), Single

Nucleotide Polymorphisms (SNPs, Landegren et al. 1988). Microsatellites or SSLPs (Ellergren 2004) are the most widely used DNA markers to conduct a genome scanning. They are highly informative, highly abundant and approximately randomly distributed across the whole genome. Moreover, it is easy to genotype using automated methods based on PCR (Dodgson et al. 1997).

As the genomes of several organisms have been sequenced, SNPs are now becoming the standard molecular markers for a wide range of biological studies including genome scanning. SNPs are the most frequent type of DNA variation. They occur once per 1000-2000 base pairs in the human genome and approximately 3 million SNPs are already recorded in the human SNP database (e.g. dbSNP). Nucleotide diversity indexes are reported to be 1/1331 bp in humans (Sachidanandam et al. 2001), 1/443 bp in cattle (Heaton et al. 2001), 1/515 bp in mice (Lindblad-Toh et al. 2000). Recently, Fahrenkrug et al. (2002) reported porcine SNP densities that translate in an index of 1/609 bp.

The access to large numbers of DNA markers has made it possible to develop comprehensive genetic maps encompassing all regions of genome in various organisms (Donis-Keller et al. 1987, Marklund et al. 1996, Groenen et al. 1998).

Linkage mapping in pig was first reported by Andresen and Baker (1964) for loci of the C and J blood groups. Since then, the number of markers described for the porcine map has increased rapidly from 28 loci in 1984 (Echard 1984) to approximately 4081 loci of which 2,493 markers are in the database and 1,588 are designated as genes (http://www.thearkdb.org, March 2005). Rapid advances in molecular genetics have led to the development of dense genetic maps. Significant contributions to porcine linkage mapping came from the USDA-MARC projects (Rohrer et al. 1994 and 1996), the European PiGMaP consortium (Archibald et al. 1995), the Nordic Map consortium (Marklund et al. 1996) and the Japanese programme NIAI (Mikawa et al. 1999). Genetic markers used for linkage mapping in pig have been mainly microsatellite loci, but include also monogenic morphological trait variants, polymorphic proteins or enzymes, erythrocyte antigens, restriction fragment length polymorphisms (RFLPs) and single nucleotide polymorphisms (SNPs). The USDA-MARC.2 map indicates a total porcine map length of approximately 23 Morgans. Detection and localization of QTL on the genetic map is based on co-segregation between alleles at marker loci and alleles at the QTL. The genetic maps have been used in many gene and QTL mapping studies,

which have identified and localized a large number of QTL for various traits in pigs (Hu et al. 2005).

Table 2.1: Comparison of different DNA-marker systems

	RFLP	RAPD	SSR	AFLP	SNP
Principle	restriction, Southern blotting, hybridization	DNA amplification with random primers	PCR of simple sequence repeats	restriction, ligation of adapters, selective PCR	detection of single base substitution
Type of polymorphisms	single base changes, insertions, deletions	single base changes, insertions, deletions	changes in number of repeats	single base changes, insertions, deletions	single base changes
Level of polymorphisms	high	medium	very high	medium	low
inheritance	co-dominant	dominant	co-dominant	dominant	co-dominant
Number of loci analyzed per assay	1~2	5~10	1	100~150	1~10,000
DNA required per assay	2-10 µg	20 ng	50 ng	0.5-1.0 µg	20ng
Development costs	high	low	high	medium	high
Repeatability	very high	low	very high	high	very high
Usage in labour	intensive	easy	easy	initially difficult	easy

It is important to select markers having sufficient information to maximize the probability to detect the co-segregation between markers and QTL, especially in outbred

pedigree. The informativeness of a marker is commonly evaluated for its polymorphism information content (PIC) representing the probability for a marker to be informative in a family segregation analysis (Botstein et al. 1980). Markers with a PIC above 0.7 are generally considered as highly informative genetic markers (Hearne et al. 1992). Another parameter similar to PIC is heterozygosity.

2.3 Strategies for QTL mapping

QTL mapping can be also divided into single-marker analysis and interval mapping. Interval mapping can be further divided into single QTL mapping and multiple QTL mapping, according to estimating methods of regression parameters that can be divided into maximum likelihood interval mapping and least square regression interval mapping. Moreover, QTL mapping can be done one-dimensional search, two-dimensional search and multiple dimensional searches simultaneously. Here it will be described the construction of resource populations and models for QTL mapping, then the methods of QTL mapping will be outlined individually.

2.3.1. Construction of resource populations

The first step in QTL mapping is establishment of a mapping resource population, which maximizes the chance to have such genes and traits segregating. Crosses between inbred lines are highly efficient for detecting QTL. The crossed lines have a high degree of homozygosity at marker loci and QTL, and their resulting offspring will have high linkage disequilibrium between alleles of all linked loci. Crosses between outbred lines are common in species, where inbred lines do not exist in farm animals. The major disadvantage with outbred line crosses is that the degree of homozygosity at marker loci is lower than in inbred lines and genotypes are unknown for the QTL.

Two different strategies have been successfully used for QTL mapping in swine. Firstly a number of QTL have been identified using intercrosses between divergent populations, e.g., wild boar vs. European domestic pig; Chinese Meishan vs. European domestic pig; or Iberian vs. European white domestic pig. Secondly, used linecross between commercial breeds. Both strategies are based on the fact that a given QTL shows higher segregation in a cross between two lines, which has been fixed or nearly

fixed for different alleles at trait loci under strong directional selection for different purposes. However, recent research results demonstrated that several of the major QTL for growth and fatness traits previously mapped in experimental crosses appear to be segregating within commercial populations (Evans et al. 2003, Nagamine et al. 2003, Vidal et al. 2005). These studies open important perspectives for the use of commercial populations in QTL mapping.

Several types of populations can be derived from a cross between divergent lines, including F2, single- or double- backcross and recombinant inbred. A F2 is more powerful than an individual backcross for detecting QTL of additive effect, and can also be used to estimate the degree of dominance for detected QTL. In general, several traits are considered in each study and the level and direction of dominance will depend upon the trait. The F2 or a combination of the two backcrosses can be used to detect four types of interaction between two loci: additive by additive, additive by dominance, dominance by additive and dominance by dominance. The single backcross can only be used to detect the additive by additive interaction effect. Thus, the F2 makes a more thorough investigation of epistasis possible, but a larger population size is needed to obtain the same power to detect epistasis. Varona et al. (2002) used simulations to evaluate the power to detect epistasis in outbred F2 line crosses. Their studies indicate that the power to detect an interaction effect of size 1-5 % of the phenotypic variance ranges 50 ~ 80 % in populations of 200-400 individuals.

2.3.2 Genetic modeling of QTL mapping

As an example in F2 population, we denote the alleles at the QTL to be Q and q, then the possible genotypes are QQ, Qq and qq. A QTL can be modeled by an allele substitution effect. The additive model assumes a linear relationship for the three QTL genotype classes, where the heterozygote individuals (Qq) have an intermediate phenotype to the homozygote (QQ and qq). The additive effect is then the effect obtained by replacing the low effect allele (q) for the high effect allele (Q). Sometimes the additive effect is also expressed as the positive and negative deviation of the respective homozygote from the mean of both homozygote: $a= \{p(QQ)-p(qq)\}/2$. In many cases the heterozygote phenotypes deviates from the mean of the two homozygotes. The situation, where the heterozygote phenotype is closer to either one of

the homozygote, is called dominance, and the allele that mainly influences the phenotype is called dominant. A dominance effect can be modeled by including a parameter for the deviation of the heterozygous phenotype from the mean of the two homozygote: d = p(Qq) - { p(QQ) + p(qq) } / 2. Both the additive and dominance effects are commonly used in QTL mapping. For some loci in mammals, only one of the two alleles is expressed. The expression is determined by the parental origin of the allele and this phenomenon is known as genetic imprinting. Imprinting can be modeled by treating the heterozygote obtaining maternal and paternal alleles separately: imprinting= p{Q(from sire)q}-p{Q(from dam)q}. Genetic models including imprinting have recently been used for QTL mapping in livestock (Knott et al. 1998, Nezer et al. 1999, Jeon et al. 1999, de Koning et al. 2000, 2002, Rattink et al. 2000, Thomsen et al. 2004).

2.3.3 Single-marker analysis and interval mapping

When the collection of genotypic and phenotypic data is completed, statistical analysis can be performed to identify the positions of QTL that underlie phenotypic differences between two breeds and estimate their effects. The general procedure of QTL mapping is: marker genotypes are used to estimate probabilities of the breed-origin of each gamete at each position through the genome. These conditional probabilities of QTL are calculated to estimate coefficients of additive and dominance components for a putative QTL at each position. Then, regression of phenotypic values on these coefficients is performed to calculate F-ratios (or other test statistic) testing the existence of a QTL at a given position in the genome. The F-ratios are plotted against map position along the entire genome. The most likely location of QTL in a linkage group corresponds to the position with the highest F-ratio.

Regression of phenotype on genotype at a marker location is called a single marker analysis and can be used to estimate the effects of QTL linked to the marker. In a genome scan composed of multiple single marker tests, the best estimated location for a QTL is taken as the genomic location of the marker with the highest statistical support for an association of the marker genotype with an effect on the phenotype. The major disadvantage with this method is that it is not possible to distinguish whether the

detected effect is due to tight linkage between the marker and a QTL with a small effect or loose linkage to a QTL with a large effect.

To overcome the disadvantage of the single marker test, Lander and Botstein (1989) introduced the concept of interval mapping to disentangle the estimates of the location and genetic effect of a QTL. Interval mapping uses marker brackets instead of individual markers in the analysis and this makes it possible to get independent estimates of location and effect of the QTL. Since then, this method has become the basis for most QTL mapping methods. Within the concept of interval mapping, various methods have been proposed for estimation of location and genetic effects, and for significant test.

2.3.4 Maximum likelihood and least square

Methods based on maximum likelihood (ML) estimation of location and genetic effects are widely used in QTL mapping. A QTL mapping procedure based on ordinary least squares regression (LS) was introduced (Haley and Knott 1992, Martinez and Curnow 1992). The basic principle is to estimate the probabilities of unknown QTL genotypes in the marker intervals, and from these calculate regression coefficients to be used for estimation of QTL location and effect.

There are several differences between LS and ML. ML analyses typically have assumed that only two alleles are segregating at the QTL (although this would not be necessary), this assumption may make ML approach less robust than LS for livestock populations under selection (Knott and Haley 1996). In LS there is no direct estimator for QTL parameters (other than location). In ML, the distribution of a phenotype is a mixture of normal distributions with different means corresponding to the QTL genotypes, while in LS the phenotype has a single (normal) distribution with mean equal to a weighted average of QTL genotypic means, the weights being the probabilities of QTL genotypes (or alleles) computed prior to the analysis and conditional on the observed marker information. For a single marker, QTL position and effect cannot be separately estimated with LS, but these are estimable with ML. In ML, no normality of phenotype for reasons other than QTL segregation could falsely suggest the presence of a QTL. If the QTL is identical to a marker, LS and ML are identical (Hoeschele et al. 1997). LS and ML methods give equal parameter estimates when the QTL genotypes are exactly

known. When there is uncertainty in the genotypes, which is always the case when the QTL are located in between markers, both methods give different results. The advantage of ML is that they use the full information from the marker-trait distribution and is thus expected to be powerful. Disadvantages are a high computational demand, difficulties to modify the basic model and the need to construct specific analysis programs to perform the analyses. Construction of the maximum likelihood equations is rather straightforward, but obtaining the maximum likelihood estimates is much more difficult (Lynch and Walsh 1998). This fact becomes increasingly important when many QTL are included in the model and multidimensional searches are performed. Today true multidimensional global QTL searches using ML methods are not feasible.

Kao (2000) has, analytically and numerically by simulation, investigated the differences between QTL mapping based on maximum likelihood and linear regression. His study indicates that the maximum likelihood based methods can be more accurate, precise and powerful at the cost of an increased computational demand. The properties of the methods in real data where there are likely to be violations of model assumptions, such as unequal variances within QTL genotype classes, segregation distortion and unusual inheritance patterns, were not evaluated. It is therefore difficult to assess the properties of the methods in the analysis of experimental data sets. Simulation and theoretical studies in Martinez and Curnow (1992) and Haley and Knott (1992) indicate that the differences are very small when the QTL are well separated, but that the LS method using one-QTL models gives biased results when two or more QTL are linked. This methodology has been proved to be a good approximation to the maximum likelihood based methods. On the other hand, in practice multiple QTL models are more easily studied using LS because of the lower computational demand, hence, it also simplifies modifications to the basic model and can be performed in standard statistical computer packages. So explicitly including more QTL in the model is a possible remedy. Haley et al. (1994) later extended the method to analyses of crosses between outbred lines. A small bias in the estimation of QTL location and effect parameters using LS is of little practical importance if the main objective is detection of important regions for further experimental studies. LS methods make it feasible to try several different multiple QTL models (Ljungberg 2004).

2.3.5 Permutation test in QTL mapping

Since QTL mapping involves multiple statistical tests throughout the genome, the selection of a significant threshold is a key issue of the procedure. Correction for multiple testing is necessary, since the use of a nominal significant threshold will lead to an elevated type I error (large number of falsely detected QTL). Various methods have been suggested to deal with the multiple comparisons (e.g. Lander and Botstein 1989, Kruglyak and Lander 1995, Benjamini and Hochberg 1995, Southey and Fernando 1998). Empirical estimation of overall significant thresholds can be done in a wide range of population designs by resampling techniques, such as permutation testing (Churchill and Doerge 1994). Here the observed trait values are randomly shuffled over individuals (genotypes) generating a sample with the original marker information, but with trait values randomly assigned over genotypes. The test statistic is then computed in the new sample, and the procedure is repeated many times, generating an empirical distribution of the test under the hypothesis of no marker-trait associations. By keeping the marker information for each individual together, the approach account for differences in marker densities, missing genotypes and segregation distortion. That means it can be applied for a wide range of population designs. The major drawback with this method is a 1,000 to 10,000 fold increase in computational demand, which in some cases causes severe restrictions for the use of the method in practice.

2.3.6 Multiple QTL mapping (MQM)

Interval mapping, as described by Lander and Botstein (1989), was designed to map single QTL, and does not consider other, linked or unlinked, QTL affecting the trait. This decreases the power and resolution of the procedure when more than one QTL affects the trait. To overcome this, several authors (e.g. Jansen 1992, 1993, Jansen and Stam 1994, Zeng 1993, 1994) have proposed extensions of interval mapping to mapping of multiple QTL. The basic concept of these methods is to include markers, or previously detected QTL, as cofactors in the model when interval mapping is used to search further for additional QTL, since both markers or putative QTL are factors (in statistical sense), they are dealt with in exactly the same way. The effects of linked QTL can be reduced by including markers linked to the interval of interest, whereas including

unlinked markers can partly account for the segregation variance generated by unlinked QTL. This method is developed for inbred line cross experiments.

For the outbred line cross model, Knott et al. (1998) and Brockmann et al. (1998) describe this strategy extendedly which cofactors are first selected for the individual chromosomes, and subsequently selected across chromosomes by backward elimination. De Koning et al. (2001a) developed this cofactor strategy in detail for simultaneous analysis of multiple chromosomes to increase the power and the precision of QTL mapping in out-bred populations. Following this time, cofactor strategy (also named MQM) were implemented widely by Brockmann et al. (2000, 2001, 2004), Olsen et al. (2002), Viitala et al. (2003), Carlborg et al (2003, 2004), Bennewitz et al. (2004), Holmberg and Andersson-Eklund (2004), Schulman et al. (2004), Zhang et al. (2004), Szyda et al. (2005).

Generally there are three strategies for cofactor selection: forward selection, backward elimination and stepwise regression. In the forward selection approach, at each stage the best new cofactor satisfying the selection criterion is added until no further candidates remain. This approach is often used in QTL analysis. The backward elimination procedure starts with a multiple regression model, using a full set of cofactors (all putative QTL/markers) evenly spread over the genome. The unimportant or least important cofactors are dropped one by one until all remaining cofactors essentially meet the selection criterion. This is a satisfactory approach, especially if we wish to see all the variables in the model in order “not to miss any QTL”. The full model gives an unbiased estimate of the maximum amount of variance explainable by (non-interacting) cofactors (QTL). In order to exclude redundant cofactors, the selection criterion should be stringent, but not so stringent that important cofactors (those flanking the QTL) are thereby excluded. One of the disadvantages of the backward elimination procedure is that for a cofactor “once out” means “always out”. Backward elimination followed by a stepwise procedure, including new cofactors and dropping old ones, may help to overcome this at the cost of more computation. Alternatively, replacing important cofactors by neighbours, not present in the initial set of cofactor, can also help in fine-tuning the model.

These methods generally increase the power of detecting additional QTL and improve the precision in the estimates of QTL location. However, this method assumes that there are no interactions between the QTL included in the model (Jansen 2003).

Above, the major approaches to QTL mapping have been described. Several other approaches are also available, including Bayesian methods and the use of a genetic algorithm, nevertheless, are continuing to develop. These new methods may become important in the future, but are beyond the scope of this elementary description of statistical methods for QTL mapping.

2.3.7 One-dimensional search and bi-dimensional search

Methods that consider multiple QTL simultaneously have three advantages: greater power to detect QTL, greater ability to separate linked QTL, and the ability to estimate interactions between QTL. These more complex methods may facilitate the identification of additional QTL and assist in elucidating the complex genetic architecture underlying many quantitative traits.
Model selection is the principal problem in multiple QTL methods. The main concern is the formation of appropriate criteria for comparing models. The simplest multiple QTL method, multiple regression, should be used more widely, although, like analysis of variance, it suffers in the presence of appreciable missing marker genotype data. A forward selection procedure using interval mapping (i.e., the calculation of conditional LOD curves) is appropriate in cases of QTL that act additively and makes proper allowance for missing genotype data. MQM (Multiple QTL mapping) is an improved method, although it is also computationally intensive, can in principle map multiple QTL and identify interactions between QTL. The important aspects of the model selection problem require much further study.

2.3.8 Fine mapping of QTL

2.3.8.1 Fine mapping using current recombinations

Initial genome-wide scan analysis typically assigns a QTL to a 10-20 cM region. To further reduce the region of likely QTL location using current recombinations, additional markers need to be placed in the initial region at 0.5-2 cM. Such an approach utilizes recombinant chromosomes from a heterozygous parent and has been termed the chromosome dissection method (Thoday 1961, Soller and Andersson 1998).

2.3.8.2 Fine mapping using historical recombinations

Positional cloning or candidate positional cloning requires the assignment of a gene to a region of 0.3 cM or less (Falconer and Mackay 1996), which will often not be feasible with chromosome dissection methods due to the limited number of current recombinations in livestock or human populations. Positional cloning of monogenic diseases has been successful after assignment of the gene to such a small region by methods utilizing historical recombinations or linkage disequilibrium (LD). There are different types of LD and LD is influenced by multiple factors such as selection, admixture, genetic drift, mutation, migration, co-ancestry and population expansion (Xiong and Guo 1997). LD fine mapping methods assume that LD is primarily due to the introduction of a variant on an ancestral haplotype via mutation (or migration), which is partially preserved in descendants of the current generation.
Until now methodologies for fine mapping, especially combining linkage and LD mapping seems a promising approach and will be fully developed in the near future, e.g., extended to include dependency among markers in the haplotype, marker mutation, multiple origins of QTL alleles, population history and multiple QTL. They will be carefully evaluated and compared via simulated and real data to determine their usefulness for fine mapping of QTL (Hoeschele 2003, Olsen et al. 2004, 2005).

2.3.9 Power of QTL mapping

The accuracy of QTL mapping depends on a number of factors: the heritability of the trait, the number of genes involved, the interactions of the genes, the distribution of the genes over the genome, the statistical distribution of the random non-genetic factors, the type of segregating population studied, the effective size of the population, the genome size, and the number of marker loci employed, as well as their distribution over the genome.
The power of detecting a QTL is limited in outbreds by the degree of informativeness of the markers and the QTL. For instance, when the frequency of a detectable QTL allele is very low (≤ 0.1) or very high (≥ 0.9), a pedigree is likely to contain only families which are not segregating for this allele, hence the QTL will not be detected. On the other hand, with the additive variance at a biallelic QTL being $2p(1-p)a^2$ (p is allele

frequency, *a* is substitution effect), the same amount of variance is explained with *p* near 0.5 and a smaller *a*-value; or *p* low or high and a large *a*-value, this may improve the detection of a QTL due to the large *a*-value (Jansen 2003).

The complexity of statistical methods for QTL mapping in outbred population depends on the structure of the population. For analysis of individual large families, or for joint analysis of a small number of such families and ignoring genetic ties among families, simpler methods used for analysis of line crosses can be adapted quite easily, e.g., choosing only informative markers and allowing for offspring with uncertain marker allelic inheritance. In contrast, multiple generational pedigrees, potentially with substantial amounts of missing data, require much more sophisticated methods of analysis.

2.4 Update QTL mapping results in swine

2.4.1 QTL database

Hu et al. (2005) have made a pig QTL database in the internet: http://www.animalgenome.org/QTLdb/, which contains 791 QTL representing 219 different traits from 73 publications during the past 10 years. The database and its peripheral tools make it possible to compare, confirm and locate on pig chromosomes the most feasible location for a gene responsible for a quantitative trait important to pig production.

2.4.2 General overview

Successful QTL mapping experiments can be categorized into 2 different types as described in section 2.3.1. First, a number of QTL has been identified using intercrosses between divergent populations, using indigenous (e.g., Chinese Meishan, Iberian) or rustic breeds (e.g. wild boars) crossed with commercial breeds or populations. This type crosses have a high degree of homozygosity at marker loci and QTL, and their resulting offspring will have high linkage disequilibrium between alleles of all linked loci. These studies have reported QTL for backfat thickness and carcass merit, e.g., the first QTL mapping in pigs using microsatellite markers and including most chromosomes was

reported by Andersson et al. (1994) for growth, fat deposition and small intestine length. Following that, several QTL studies were published (Bidanel et al. 2001, de Koning et al. 1999, 2000, 2001a, 2001b, 2001c, Gelderman et al. 1996, 2003, Jeon et al. 1999, Milan et al. 2002, Ovilo et al. 2002, Pazek et al. 1999, Rohrer et al. 1998a, b, Varona et al. 2002).

Second, studies that have used commercially viable breeds for resource population development have also reported putative QTL for carcass merit and meat quality traits, albeit fewer in number, e.g. Grindflek et al. (2001) reported a cross between Norwegian Landrace/Duroc and Norwegian Landrace/Yorkshire; Malek et al. (2001a, b) and Thomsen et al. (2004) studied a cross between Berkshire and Yorkshire; Nezer et al. (1999, 2002) used a cross between Large White and Pietrain. These results indicate that favorable QTL are segregating for carcass merit and meat quality traits and may be exploited with commercial breeding schemes.

Furthermore, recent research results (Evans et al. 2003, Nagamine et al. 2003, and Vidal et al. 2005) demonstrated that several of the major QTL for growth and fatness traits previously mapped in experimental crosses appear to be also segregating within commercial populations. These studies opened important perspectives for the use of commercial populations in QTL mapping and encourage for the application of marker-assisted selection procedures in pigs. They demonstrated that a considerable amount of phenotypic variance observed in commercial populations can be explained by segregation at major QTL that have not yet reached fixation through the long process of artificial selection. This finding might be explained by the fact that purebred populations are usually selected according to diverse criteria, a feature that diminishes the probability of allele fixation for the QTL that influence a single trait. Moreover, selection criteria have changed through time, country (or work group, farm) and introgression of foreign material in supposedly "pure" breeds is the rule rather than the exception. The existence of pleiotropic genes with alleles that are favourably correlated to some traits and unfavourably with others might also explain the maintenance of genetic diversity in selected pig populations.

This also implies that resources already available can be used to set up large-scale studies for the comparative analysis and fine mapping of genomic regions containing genes responsible for QTL of interest. Commercial populations of livestock species may in fact provide unique opportunities for the molecular characterization of QTL. This

opportunity exists because large amounts of phenotypic data are collected routinely for breeding purposes in farm animals and it is possible to study extensively in multi-generation pedigrees.

2.4.3 QTL with gametic imprinting effects

Parental genomes undergo modifications during gametogenesis. In human and mouse, most imprinted genes are arranged in chromosomal clusters, their linked organization suggests coordinated mechanisms controlling imprinting and gene expression (Morison et al. 1998, Constancia et al. 1998). The result is that some genes inherited from one parent are not completely expressed, or not at all. It is generally viewed that imprinting is involved in fetal growth and brain development (Tilghman 1999). This phenomenon of genomic imprinting has been shown to influence several genes and traits in animals (including humans, Morison et al. 2001) as well as plants (Alleman and Doctor 2000) and insects (Lloyd et al. 1999).

Genome scans can also be used to search for imprinted QTL. The imprinted QTL provided that the parental origin of alleles can be traced back from the F_2 to the F_1 parents (Knott et al. 1998). This prerequisite excludes F_2 crosses between inbred lines because the F_1 parents are all heterozygous for the same marker alleles. Knott et al. (1998) were the first to search for imprinted QTL in a genome scan. They inferred imprinting when effects differed significantly from Mendelian expression. Jeon et al. (1999) and Nezer et al. (1999) found paternal expression for muscularity in the IGF2 region of chromosome 2 in pigs. De Koning et al. (2000, 2001b, c) modified the approach of Knott et al. (1998) and reported a large number of imprinted QTL for growth and meat quality traits in pigs. De Koning et al. (2002) demonstrated that successful detection and inference on the mode of QTL expression puts greater demands on statistical tests and their interpretation. More recently, Thomsen et al. (2004) have further developed tests for parent-of-origin effects and to implement them to a cross between Berkshire and Yorkshire.

2.4.4 Results of multiple QTL mapping

Reports of linked QTL are very scarce in pigs. Until now, there are only a few available example results:

- Knott et al. (1998) reported suggestive evidence of linked QTL on SSC5 affecting growth rate, abdominal fat and small intestine length in Wild Boar × Large White crosses
- De Koning et al. (2000) reported two imprinted linked QTL with different parental expression affecting intramuscular fat content on SSC6
- Quintanilla et al. (2002) also reported significant linked QTL concerning growth traits on SSC1, SSC3, SSC7 in a Meishan × Large White crosses
- Ovilo et al. (2002) reported that significant linked QTL for meat quality traits appear at the same locations by the one QTL analysis with a similar level of significance, in an Iberian × Landrace intercross. There were 4 pairs of epistatic (interaction) QTL for meat quality. Varona et al. (2002) reported a pair epistatic QTL (genomewide significant) on SSC2 and SSC17 affecting live weight in the same population

Carlborg et al. (2003, 2004) who developed a multiple QTL mapping software and performed by themselves, have estimated the relative contribution of additive, dominance and epistatic effects to growth in chicken. They found, the contribution of epistasis was more pronounced prior to 46 days of age, whereas additive genetic effects explained the major portion of the genetic variance later in life. Some of the detected loci affected either early or late growth but not both. Very few loci affected the entire growth process, which points out that early and late growth, at least to some extent, have different genetic regulation.

2.5 Map-based cloning and from QTL to QTN

Once a QTL has been identified for the trait of interest, the next step is to find the genes causing the phenotype. The region harboring the QTL can be large and span some tens of cM. Narrowing down the region as much as possible is an important step towards the identification of the causative gene or genes. Additional crosses can be set up, either by intercrossing individuals from the same generation or backcrossing to either of the

parental lines to generate new generations and recombination between the QTL and surrounding markers. The QTL genotype of parental animals can be determined using progeny testing in such crosses. The results of such experiments make it possible to exclude the QTL from some parts of the region. When the region is small enough there may be only a few genes to investigate.

The genes underlying QTL have been characterized in a few instances. Two prominent examples in livestock are: identification of the *G* to *A* substitution in a silencer element controlling IGF2 transcription has a major effect on skeletal and cardiac muscle mass in pigs (Braunschweig et al. 2004, Jungerius et al. 2005); another, a nonconservative lysine to alanine substitution in the bovine DGAT1 gene has a major effect on milk yield and composition (Grisart et al. 2004).

These two successful examples were achieved with the limited resources that are available in the field of livestock genomics. These inferences are quite remarkable and demonstrated the value of livestock populations for the molecular dissection of complex traits. The detection of the QTN (quantitative trait nucleotide) underlying the SSC2 QTL illustrates this vividly. The *Q* to *q* substitution effect corresponds to a difference of 2–3 % in muscle mass, which would have been virtually impossible to detect in a phenotype-driven mutagenesis screen. Yet this mutation accounts for 25 % of the phenotypic difference in the F2 generation, its identification revealed a novel cis-acting regulatory element in IGF2, a gene that has been extensively studied using standard molecular biology. This procedure from QTL mapping to subsequent QTN identification has the potential to make a significant contribution to narrow the "phenotype gap", i.e., the lack of functional information from mutation-induced phenotypes for most mammalian genes.

Therefore, a more systematic use of livestock populations could very significantly contribute to a fundamental understanding of the molecular architecture of complex inherited traits. It will contribute to the identification of biochemical pathways affecting phenotypes that are not only of importance for agriculture but have relevance to human health as well.

2.6 Marker assisted selection (MAS)

The information about the co-segregation between traits and marker loci can not only be used for characterization of the genes influencing the trait, but also for possible implementation of these loci directly in breeding programs; this is potentially of great economic value. Discussion of the possible uses of molecular marker information to accomplish this sort of goal goes back at least to Neimann-Sorenson and Robertson (1961), but has been given renewed impetus in recent years by rapid advances in molecular biology. There is a number of ways in which marker information might be used for these purposes, but only one area will be considered here.

Suppose we have a population in which one or, more usually, many QTL are segregating. We wish to increase the value of some trait in subsequent generations by selecting certain individuals from the population to form the next generation. Traditionally this has been done solely on the basis of phenotypic information of individuals and their relatives, but incorporating information on the marker genotypes of individuals in marker assisted selection (MAS) has the potential to improve the rate of progress. In this case, different approaches are required for inbred and outbred populations. For these research fields, a number of additional complications must be faced when trying to implement MAS schemes, especially in outbred population. Moreover, the development of theory for MAS remains an active area. Perhaps the most promising current approach is the implementation of Bayesian approaches via Markov Chain Monte Carlo (MCMC), just like described by Whittaker (2003).

3. Materials and methods

3.1 Materials

3.1.1 Resource population

For this study, a F_2 resource population based on the cross of Duroc and Pietrain pig (DUPI resource population) was used to map loci affecting a number of economically important traits using microsatellite markers.

The Pietrain breed, originating from the village of Pietrain in Belgium, is characterized by its exceptional muscularity and leanness. Pietrain boars are therefore used for their carcass improving ability in terminal crosses all over the world. However, Pietrain animals have relatively poor growth features (such as daily gain) and modest mothering characteristics and milk production. Moreover, a large proportion of the animals suffer from malignant hyperthermia, associate with porcine stress (PSS) and pale soft exsudative meat (PSE) syndromes. In many respects, the Duroc, a breed of pigs with its origin in the eastern United States and in the Corn Belt, has complementary features. Duroc produce lower grade, fattier carcasses, but grow faster, are prolific and good rearers and are resistant to stress. Crosses between this two breeds therefore offer the possibility to identify the allelic variants responsible for their differences. This opportunity is particularly relevant since the corresponding variation is being exploited in the present breeding programs.

Initially, the F_1 generation was produced by mating of four Duroc boars to eight Pietrain sows and two Pietrain boars mating to five Duroc sows separately. The F_1 animals were reciprocally assigned to produce the F_2 animals, therefore, 13 ‘DuPi’ F_1 females were assigned to two ‘PiDu’ F_1 boars and 14 ‘PiDu’ F_1 females were assigned to three ‘DuPi’ F_1 boars.

All pigs were kept at the experimental research farm ‘Frankenforst’ of the University of Bonn and exposed to uniform environmental conditions. Piglets were weaned at 28 days of age and placed in collective pens in the post-weaning unit until 10 weeks of age. Male piglets were castrated. All animals were individually weighed at birth, at weaning, at the beginning and at the end of the testing, respectively. The F_2 pigs were given an *ad libitum* diet containing 16 % crude protein, 1 % lysine, 0.6 % (methionine + cystine),

0.6 % threonine and 13 MJ metabolizable energy during the whole testing period from 10 to 22 weeks of age, slaughtered approximately at 85 kg of slaughter weight, the average age at slaughter was 177.6 ± 15.6 days. A total of 1085 F_2 pigs from 38 full-sib families were generated between May 2000 and October 2003. The 19 founder animals were tested and were found to be free of the mutation at the ryanodine receptor locus which is responsible for halothane susceptibility.

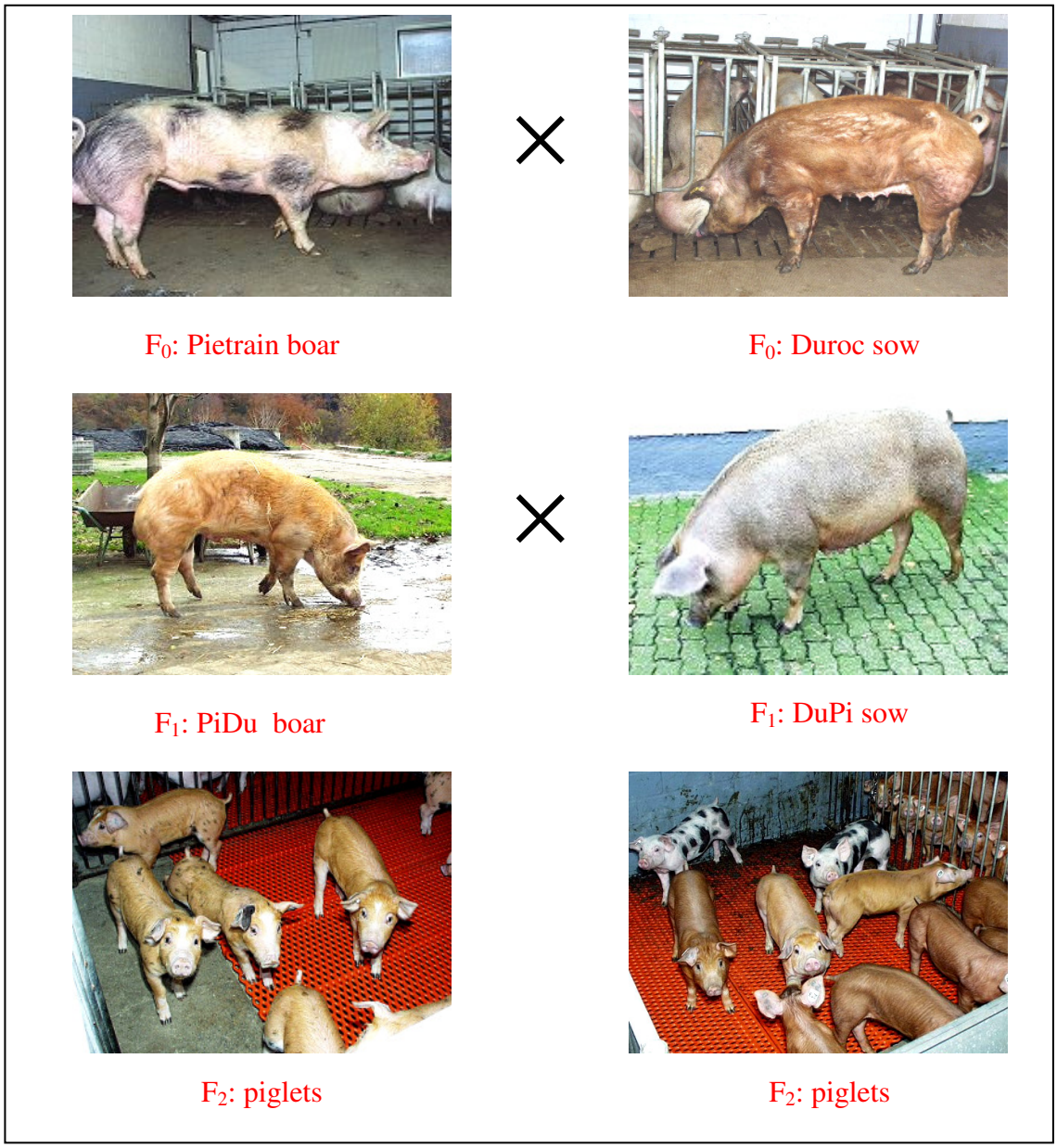

Figure 3.1 Structure of the Duroc-Pietrain resource population.

The sibship pedigrees of the three generations of F_2 Duroc-Pietrain resource population was illustrated in figure 3.1 and figure 3.2.

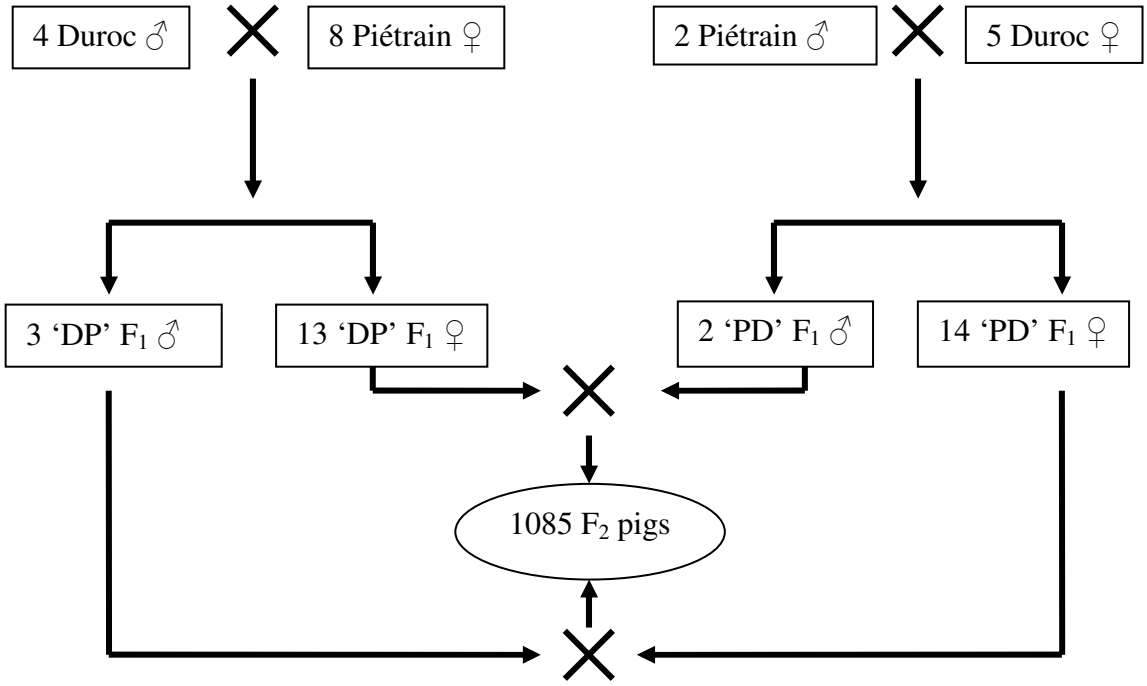

Figure 3.2 Pedigree structure of the Duroc-Pietrain resource population.

Table 3.1: Equipments used

Automated sequencer	LI-COR	4200	MWG (Ebersberg)
Thermocycler	MJ Research	PTC100	Biozym, Hess Oldendorf
Thermocycler	BIO RAD	iCycler	BIO RAD (München)
Centrifuge	HERMLE	Z233MK	HERMLE (Wehingen)
Thermoshaker	Gerhardt	-	Gerhardt (Bonn)
Incubator	Memmert	BB16	Memmert (Schwabach)
UV Transilluminator	Uniequip	Uvi-tec	Uniequip (Martinsried)
Spectrophotometer(UV)	DU®-62	PM2K	Unterschleissheim-Lohhof
WasserReinigungsAnlagen	Millipore	Milli Q	Millipore (Eschborn)
pH-Star. Opto-Star, LF-Star, Scan-Star			Mattaeus, Poettmess, Germany
Ez-Driploss			Germany
Instron-4310			Germany

3.1.2 Laboratory materials

The following materials were used for molecular genetic analysis in laboratory. They include equipments, software, chemicals, solutions, buffers and primers.

3.1.2.1 Equipments

Equipments used in this study are given in table 3.1.

3.1.2.2 Software used

Software used in this study are in table 3.2.

Table 3.2: Software used

Image Analysis program (Version 4.10)	LI-COR Biotechnology, USA
OneDscan	Scanalytics Inc., Billerica, MA
SAS Version 8.2	SAS Institute Inc., Cary, NC, USA
Crimap Version 2.4	Green et al. 1990
GDA 1.1	Lewis and Zaykin. 2002
QTL Express	Seaton et al. 2002

3.1.2.3 Chemicals

Biomol (Hamburg): Phenol

Biozym Diagnostik (Hessisch-Oldendorf): Sequagel XR sequencing gel (National Diagnostics) and SequiTherm Excel^{TM}II DNA sequencing kit (Epicentre Technologies)

GeneCraft (Münster): Taq polymerase

MWG Biotech (Ebersberg): Oligonucleotide primers.

Roth (Karlsruhe): Acetic acid, Ampicillin, Ammonium peroxydisulphate (APS), Boric acid, Bromophenol blue, Calcium chloride, Chlorofrom, Dimethyl sulfoxide (DMSO), dNTP, Ethylenediaminetetraacetic acid (EDTA),

Ethanol, Ethidium bromide, Formadehyde, Formamide, Glycerin, Hydrochloric acid, Hydrogen peroxide (30 %), Isopropyl β-D-thiogalactoside (IPTG), N,N´-dimethylformamide, Nitric acid, Peptone, Proteinase K, Sodium dodecyl sulphate (SDS), Silver nitrate, Sodium carbonate, Sodium chloride, Sodium hydroxide, N, N, N´, N´-Tetramethylethylene-diamine (TEMED), Tris, 5-bromo-4-chloro-3-indolyl-β-D-galactopyra-noside (X-gal), Xylencyanol and Yeast extract.

Serva Electrophoresis GmbH (Heidelberg): Acrylamide (molecular biology grade) and Bisacrylamide.

Sigma-Aldrich Chemie GmbH (Taufkirchen): 2, 2´Azino bis (3-ethylbenzthiazo-line-6-sulphonic acid) diammonium salt (ABTS), Agarose, Blue dextran, Calcium chloride, Diethyl barbituric acid, Ethylene glycol-bis (2-amino-ethylether)-N,N,N´,N´-tetraacetic acid (EGTA), Gelatin, Fetal calf serum, Gutamin, Histopaque-1077, Isopropanol, Magnesium chloride, Penicillin, Phytohemagglutinin (PHA), RPMI-1640 medium, Streptomycin G, Sodium barbituric acid, Sodium barbiturate, *Taq* polymerase, VectoretteII system.

3.1.2.4 Solutions and buffers

All solutions used in this investigation (table 3.3) were prepared with deionized and demineralised (Millipore) water and pH was adjusted with sodium hydroxide or hydrochloric acid.

3.1.2.5 Primer sequences used

In this study, all primer sequences were derived from published porcine sequences which were in the databank:

http://iowa.thearkdb.org/anubis?singlespecies=pig
http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9823

Table 3.3: Solutions and buffers used in this study

Name	Composition of consistion	Volume or weight
APS solution (10 %):	Ammoniumpersulfat	5 g
	Water (added to)	50 ml
Polyacrylamide gels:	Sequagel XR	20 ml
	Sequagelbuffer	5 ml
	APS (10 %)	250 µl
	DMSO	200 µl
Blue dextran buffer:	Blue dextran	50 mg
	ddH_2O	950 µl
	EDTA (0.5M) (186.1 mg/ml)	50 µl
	Formamide added to	5 mL
SDS solution (10 %):	Sodium dodecylsulfat	10 g
	Water	100 ml
Sequence loading buffer:	Formamide	83 % (v/v)
	EDTA pH 8.0	4 mM
	Blue dextran	10 mg / ml
Silane solution:	Silane	3 µl
	Ethanol 95 %	1 ml
10× TBE-buffer	Tris	108 g
	Boric acid	55 g
	EDTA (0.5M) (186.1mg/ml)	40 ml
	Water added to	1000 ml
1× TE-buffer	Tris (1M)	10 ml
	EDTA(0.5M) (186.1mg/ml)	2 ml
	Water added to	1000 ml
Digestion buffer	NaCl	100 mM
	Tris-HCl	50 mM
	EDTA	1 mM
Phenol-Chloroform	Phenol	500 ml
	Chloroform	480 ml

3.1.2.6 Websites used in this study

http://www.genome.iastate.edu/pig

http://www.animalgenome.org/QTLdb/

www.swinegenomics.com

http://linkage.rockefeller.edu/soft/list.html

http://www.marc.usda.gov/genome/genome.html

3.2. Methods

3.2.1 General molecular genetic strategy

The figure 3.3 illustrated the strategy of this whole study.

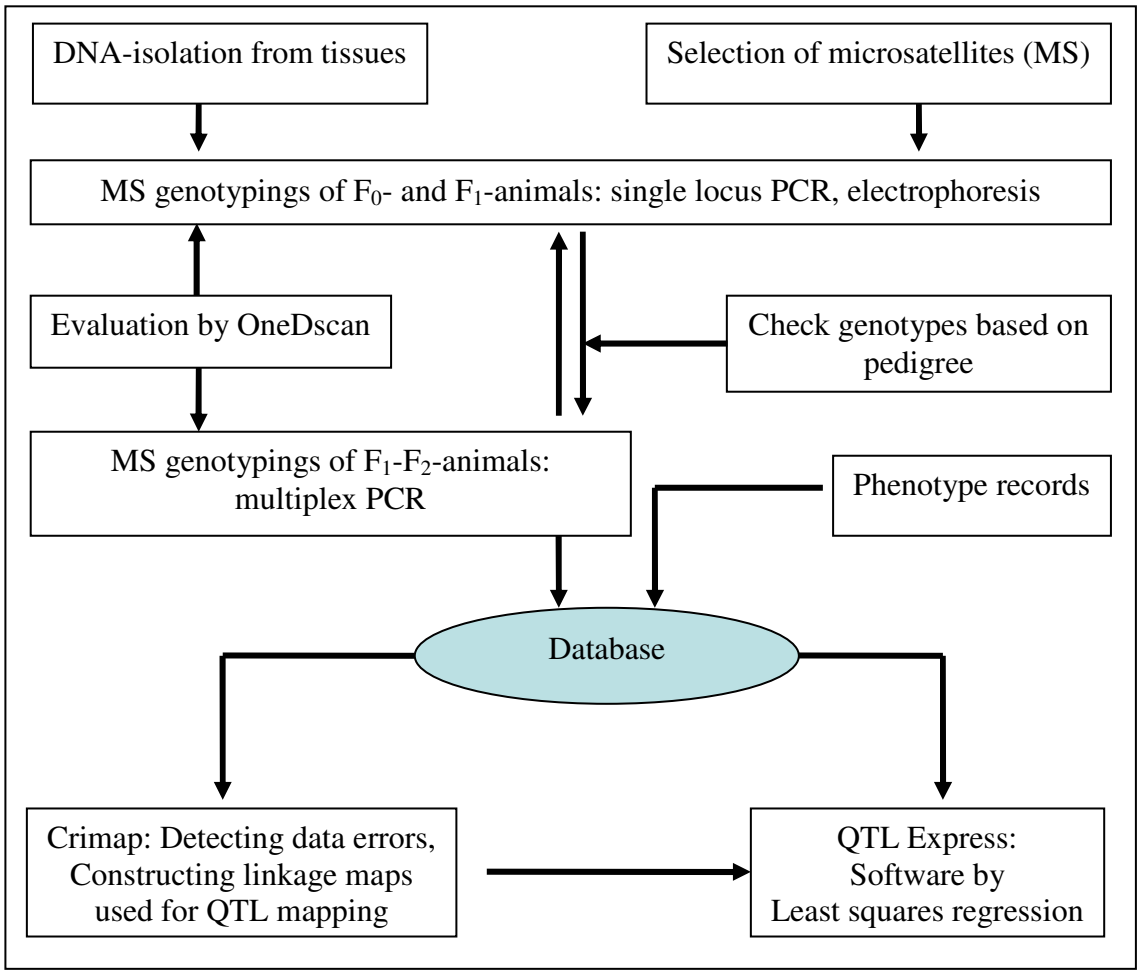

Figure 3.3: Schematic description of the whole study strategy

3.2.2 DNA isolation

In order to extract genomic DNA, approximate 0.2 g of sample tissue, which were tails or ear from piglets at birthday and stored at -20 °C, was cut into small pieces and put into a 2.2 ml sterilized microcentrifuge tubes. After sample was thawed, 700 µl digestion buffer, 70 µl SDS and 18 µl proteinase K (50µg/ml) were added. The mixture was incubated with shaking at 37 °C overnight. At the second day, 700 µl phenol-chloroform (1:1) was added, mixed for 15 sec and then centrifuged at 10000 rpm for 10 min at room temperature. The upper phase was transferred in a new tube. 700 µl of chloroform was added and centrifuged at 10000 rpm for 10 min at room temperature in order to remove the possible carryover of phenol. The upper phase was again transferred in a new 2.2 ml sterilized microcentrifuge tube. Then 700 µl of isopropanol and 70 µl 3M sodium acetate were added. After mixing and shaking vigorously, the DNA pellet was visible in the solution. After centrifuging at 4000 rpm for 4 minutes at 2 to 8 °C, the supernatant was removed; 100µl ethanol 70 % was added to wash and removed immediately. The DNA pellet was dried by opening the tube at room temperature for 30 to 60 min. 500 µl 1 × TE was added in order to dissolve the DNA pellet and kept at room temperature until the next day but with shaking the tubes several times in between. After dissolution of DNA (at least overnight), 10 µl was taken and diluted by the addition of 990 µl of 1×TE buffer. The mixture was swirled gently and the absorbance of this DNA dilution was measured at 260 and 280 nm wavelength in Spectrophotometer UV/visible light (DU®-62). The concentration of the DNA was calculated with the formula:

$$\text{Concentration of DNA (µg/ml)} = OD_{260} \times 50 \times DF$$

DF = dilution factor = 100

Meanwhile, the DNA quality was checked according to the formula: Ratio = OD_{260nm}: OD_{280nm} (the ratio is normally smaller than 2) and by agarose electrophoresis 2 % gels with 0.8 µg/ml ethidium bromide. Finally, good quality DNA was diluted to 50 ng/µl with 1×TE buffer and stored at the temperature of 4 °C for PCR.

3.2.3 Selection of microsatellites

In this study, 73 informative microsatellites were selected from 18 *sus scrofa* autosomes from database, mainly referencing to USDA/MARC.2 map covering 79.50 % of 18 autosomes(Appendix 1) based on three criteria: maximal information content; distribution over the whole chromosome from proximal to distal as possible; avoid choosing microsatellites with null allele.

3.2.4 Genotyping of F_0 and F_1 samples

The PCR of single marker was carried out for the F_0 and F_1 samples together in order to correctly identify their genotypes (table 3.4). Those genotypes were used to identify the genotypes of their F_2 animals according to the pedigree information.
Polymerase Chain Reaction (PCR) was carried out in 12.5 µl reaction volume containing: 1.5µl genomic DNA (50ng/µl), 200.00 µM dNTPs, 0.20 picomol of both forward and reverse primer, 0.5 U Taq-polymerase with supplied $MgCl_2$-free buffer (BioTherm) and 1.25 µl buffer with 50 mM $MgCl_2$ and the rest was added to ddH_2O to 12.5 µl reaction volume.

3.2.5 Multiplex PCR to genotype F_1 and F_2 samples

The aim of the multiplex-PCR for F_2 is rapidly genotyping and reduction of the cost. For optimizing multiplex PCR set, following steps were taken into account:

- In order to avoid overlapping the alleles of microsatellites, the distance between microsatellites should be more than 30 bp, so that each allele of microsatellites in the same multiplex PCR set could clearly be read after electrophoresis;
- Each microsatellite integrated into one multiplex set should have the same annealing temperature;
- The primer sequences of microsatellites should have no complementary sequence longer than 4 bp, especially at the 3'-end of primers to avoid primer dimmer;

- Then carry out PCR to test the candidate complex set;
- Primer concentration needs to be optimized, since high concentration of primer will inhibit amplification efficiency of other microsatellites in a multiplex set. Primer concentration often needed several times to test each other;
- PCR by-products need to be minimized. Sometimes, by-product could be avoided by control primer concentration;
- When the several microsatellites were highly compatible, they were used to amplify the F_2 samples. By the implementation of the multiplex PCR, it need to several times to modify or re-integrate the multiplex set. From my experience, the concentration of genomic DNA samples should be uniform. Polymerase was also critical factor for multiplex PCR.

Throughout those steps, there were 28 fine multiplex PCR sets from 73 microsatellites used for genotyping the whole F_2 animal samples. There were 2-4 microsatellites in each complex set (appendix 1).

3.2.6 Electrophoresis of PCR products

PCR products (table 3.4) were run in a Li-COR 4200 Automated Sequencer. Initially, polyacrymide gel was made for electrophoresis. Both plates were cleaned twice with water and ethanol (75 %) and dried with paper. 100 µl of binding silane were applied onto the area of one glass plate, where the comb is inserted. These plates and spacers were assembled and fixed as sandwich with rails. Gel solution containing Sequa Gel XR (SQG-XR-842 MWG, National Diagnostics) 15ml, Sequa Gel Complete Buffer Reagent (National Diagnostics) 3.75 ml, DMSO (Carl Roth GmbH) 200 µl and APS (10 % w/v) 150 µl, was filled in between the two plates. A comb was inserted on the gel sandwich and placed in a horizontal position allowing polymerization for half to one hour. After that, PCR products were diluted 1:10 (or 1:15) with dextranblue-buffer and loaded on 6 % polyacrymide gel. At the same time, standard size markers (it was mix solution of 75, 100, 105, 120, 145, 175, 200, 204, 230, 255, 300, 320 bp) were loaded at both side lanes (sometimes also at the middle lane). Electrophoresis was performed in 1× TBE-buffer at 50 °C, 50 W, 40 mA and maximal 1500 V. The gel image data was analysed by using Image Analysis program, version 4.10 (LI-COR Biotechnology).

Table 3.4: Single and multiplex PCR programs

Normal PCR for single or multiplex microsatellites				Touch down PCR for single or multiplex microsatellites			
Function	°C	Time (min)	Cycles	Function	°C	Time (min)	Cycles
Initial denatu-ration	95	3	1	Initial denatu-ration	95	3	1
Denatu-ration	94	0.5	32~42	Denatu-ration	94	0.5	Cycles equal to touch down number
Annealing	48 ~ 62	0.5 ~ 1		Annea-ling	(62°C - 1°C) /cycle to 48~60°C	0.5 ~ 1	
Extension	72	1 ~ 1.5		Extension	72	1 ~ 1.5	
End extension	72	10	1	Denatu-ration	94	0.5	28~40 (Appen-dix 1)
Keep	4	∞		Annea-ling	48~62 correspon-ding to each mutiplex set	0.5 ~ 1	
				Extension	72	1 ~ 1.5	
				End extension	72	10	1
				Keep	4	∞	

Table 3.5: Definition and abbreviation of traits studied

Trait group	Definition and abbreviation
Meat quality	pH1ko: pH-value 45 minutes post-mortem in *m. long. Dorsi* between 13^{th}/14^{th} rib
	Ph24ko: pH-value 24 h post-mortem in *m. long. Dorsi* between 13^{th}/14^{th} rib
	pH1si: pH-value 45 minutes post-mortem in *m. semimembranosus*
	pH24si.: pH-value 24 h post-mortem in *m. semimembranosus*
	LF1ko (mS/cm): conductivity 45 minutes post-mortem in *m. long. dorsi* between 13^{th}/14^{th} rib
	LF24ko (mS/cm): conductivity 24 h post-mortem in *m. long. dorsi* between 13^{th}/14^{th} rib
	LF1si (mS/cm): conductivity 45 minutes post-mortem in *m. semimembranosus*
	LF24si (mS/cm): conductivity 24 h post-mortem in *m. semimembranosus*
	Meat colour (Mcolor) (%): 24 h postmortem in *m. long. dorsi* between 13^{th}/14^{th} rib, 5 repeated measurements at different points
	Drip loss (Dloss): see text
	Cooking loss (Closs): see text
	Thaw loss (Thloss): see text
	Shear force (Shforce): see text
Muscling	LEA: Loin eye area on *m. long. Dorsi* at 13^{th}/14^{th} rib (cm^2)
	Estimated leanness content of belly (EBLC): = 65.942 + 0.145*(loin eye area) - 0.479*(fat area) – 1.867*(side BFT) – 1.819*BFT-lo
	Estimated leanness content of carcass (ECLC): 59.704 – 1.744*(BFT-lo) – 0.147*(fat area) + 0.222*(loin eye area) – 1.175*(BFT-10) – 0.809*(BFT-sh) – 0.378*(side BFT) – 1.801*(F1314).

Table 3.5: Definition and abbreviation of traits studied (cont.)

Trait group	Definition and abbreviation
Body weight and growth rate	Birth weight (BWT) (g)
	Weaning weight (WWT) (kg)
	Test start weight (TSW) (kg)
	Carcass weight: carcass weight with kidneys after 24h slaughtered (kg)
	Average daily gain 1 (ADG1) (g/day): from birth to weaning
	Average daily gain 2 (ADG2) (g/day): from weaning to test start
	Average daily gain 3 (ADG3) (g/day): from test start to slaughter
	Average daily gain 4 (ADG4) (g/day): from birth to slaughter
Fat deposition	F1314: Back fat depth on *m. long. Dorsi* at $13^{th}/14^{th}$ rib (cm)
	BFT-sh: Shoulder fat depth (cm): depth of fat and skin on muscle at thickest point, average of 3 measurements
	BFT-10: Fat depth at 10^{th} rib (cm): depth of fat and skin on muscle at thinnest point, average of 3 measurements
	BFT-lo: Loin fat depth (cm): depth of fat and skin on muscle at thinnest point, average of 3 measurements
	Average back fat depth (BFT-av) (cm): mean value of shoulder fat depth, fat depth at 10^{th} rib, and loin fat depth
Other traits	Dressing yield (%): chilled carcass weight relative to live weight at slaughter
	FFV: fat area in relation to loin eye area at $13^{th}/14^{th}$ rib
	Carcass length (CL) (cm): length of carcass from second cervical vertebra to pelvis

Abbreviation of traits was in the parenteses.

3.2.7 Evaluation of allele fragments

The alleles of microsatellite fragments were identified with the software 'ONE-DSCAN'. This program calibrates the length of microsatellite fragments in relation to

known fragment standard marker (75 bp; 100 bp; 105 bp; 120 bp; 145 bp; 175 bp; 200 bp; 204 bp; 230 bp; 255 bp; 300 bp; 325 bp). The software 'ONE-DSCAN' has own manipulations. In general there were 3 steps: Identification of the region of bands which will be evaluated; Identification of samples and standard markers; Identification of fragment length.

3.2.8 Definition and measurement of quantitative traits

The phenotypic data of F_2 animals were collected according to the German performance test directives (ZDS, 2003). The definition and abbreviations of traits, the numbers of records, means and standard deviation were shown in table 3.5. Several meat quality traits categorised in meat pH-value, meat conductivity and meat color groups were measured by Star-series equipment (Rudolf Matthaeus Company, Germany). Muscle pH was measured at 45 minutes (pH1) and 24 hours postmortem (pH24), respectively; both in *m. long. dorsi* at 13^{th} ~14^{th} rip and in the *m. semimembranosus* muscles, respectively. Meat conductivity is measured by using LF-Star at the same places as those for measuring pH. Muscle color was measuring at 24 hours postmortem using Opto-Star. Drip loss was scored based on a bag-method that used a size-standardized sample that was collected at 48 hours post-mortem from the *m. long. dorsi*. The sample was weighed, suspended in a plastic bag, held at 4 °C for 24 hours, and re-weighed at the end of the holding time (Honikel et al. 1986; Kauffman et al. 1986a, b). Drip loss was calculated as a percentage of loss weight based on the beginning weight of a sample. To obtain cooking loss, a loin cube was taken from the longissimus, weighed, placed in a polyethylene bag and incubated in water at 75°C for 50 minutes. The bag was then immersed in flowing water at room temperature for 30 minutes and the solid portion in it was reweighed. Cooking loss was obtained as the difference of the sample weights before and after the treatment. The method of measuring thawing loss is similar to that for cooking loss. Shear force was measured by the Instron-4310 equipment.

3.2.9 Statistical analyses

3.2.9.1 Heterozygosity

Heterozygosity is a widely used measure of the allelic diversity or informativeness of a genetic marker. The informativeness of a genetic marker increases as heterozygosity increases. The heterozygosity of a genetic marker is estimated by

$$H = 1 - \sum_{i=1}^{k} p_i^2$$

where p_i is the frequency of the i^{th} allele and k is the number of alleles (Nei 1987; Ott and Goldstein 1992).

3.2.9.2 Polymorphism information content

Alternative measure of the informativeness of a genetic marker in outbred species is the polymorphic information content (Botstein et al. 1980). The PIC of a genetic marker is estimated by

$$PIC = 1 - \sum_{i=1}^{k} p_i^2 - \sum_{i=1}^{k} \sum_{j=i+1}^{k} p_i^2 p_j^2$$

where p_i is the frequency of the i^{th} allele and k is the number of alleles (Botstein et al.1980, Ott and Goldstein 1992).
Regarding a codominant genetic marker, PIC was developed for ascertaining the allele transmitted by an affected heterozygous parent carrying a dominant disease allele (Ott and Goldstein 1992). PIC estimates the probability that the co-dominant marker genotype of an offspring can be used to deduce which of two marker alleles were transmitted by a parent carrying a dominant disease allele. The term polymorphic information content is alternatively and frequently used for Heterozygosity and possibly other measures of marker informativeness.

3.2.9.3 Linkage analysis and genetic map construction

The data obtained from genotyping were used to construct linkage maps. The data were firstly checked for any genotyping errors by using Pedcheck (Version 1.1) (O'Connell 1998). Then multipoint linkage analyses were carried out for males, females and sex average with the 2.4 version of the Crimap software (2.4 version) (Green 1990).

Various Crimap options were used to determine marker orders and marker distances within linkage groups. Recombination units were converted into map distances using the Haldane mapping function for QTL mapping.

3.2.9.4 QTL analyses

QTL analyses were performed using the regression approach implemented in "QTL Express" (Seaton et al. 2002. http://latte.cap.ed.ac.uk/) which is designed for the analyses of three generation pedigrees derived from a cross between outbred lines. This approach assumes that the founder populations are fixed for alternative QTL alleles in the F2 population. These two alleles will be denoted Q for the Duroc allele and q for the Pietrain allele. Under this assumption, the probability (p) of a F_2 individual being one of four possible QTL genotypes [p(QQ), p(Qq), p(qQ) or p(qq)], conditional on the marker genotypes at any putative location in the genome, were computed as described by Haley et al. (1994). These probabilities were then used in a least squares framework to investigate the genetic model underlying the trait of interest. Sex average distances were used in all analysis, since Knott et al. (1998) showed that using sex-specific maps had limited effects on the results. The different hypotheses (linked QTLs, genomic imprinting) were tested by computing at every cM of the whole genome, the reduction in sum of squares (F-ratio test) caused by adding the new component/s to a no-QTL and to one QTL models, as described below. By this procedure, the additive and dominance (and imprinting if it exists) coefficients and the F-ratio values were calculated. The proportions of F_2 phenotypic variance that was explained by QTL effects was calculated as reduction of the residual mean square within the F_2 generation.

Models of QTL analysis

Single Mendelian QTL model

One QTL regression model was used:

$$y_{ijk} = \mu + s_j + f_k + \beta\, \mathrm{cov}_{ijk} + c_{ai}a + c_{di}d + \varepsilon_{ijk} \qquad \text{[model 1]}$$

Where:

y_{ijk} is the phenotype of the i^{th} F_2 offspring;

μ is the overall mean;

s_j is the j^{th} fixed sex effect, j = 1,2;

f_k is the k^{th} fixed contemporary group effect, here is full-sib family (k = 1 ~ 38);

β is the regression coefficient on the covariate;

cov_{ijk} is a covariate that varied according to the trait analysed:

- Total number born in a litter and sow parity number, both as covariates for BWT;
- BWT, the number of pigs weaned and age at weaning as covariates for WWT;
- WWT and the age at beginning of test, both as covariates for test start weight ;
- BWT and the number of pigs weaned, both as covariates for ADG_1;
- WWT as covariate for ADG_2;
- Weight at test start as covariate for ADG_3;
- BWT as covariate for ADG_4;
- Test start weight and days at test, both as covariates for food consumption and food conversion ratio, respectively;
- Carcass weight as covariate for backfat traits, loin eye area, meat traits, meat quality traits and all of other fat traits.

c_{ai} is the additive coefficient of the i^{th} individual at a putative QTL location in the genome;

c_{di} is the dominant coefficient of the i^{th} individual at a putative QTL location in the genome;

a, d are the additive and dominant effects of a putative QTL, respectively;

ε_{ijk} is the residual error

Two Mendelian QTL model

To distinguish between the presence of one QTL with a large effect and two linked QTL with smaller effects, a two-dimensional QTL search at 1-cM grid was carried out for those linkage groups, where significant evidence for one QTL was detected by one QTL model, also by the cofactor analysis. The presence of two QTL in the same linkage

group was tested by adding additive and dominance effects for a second QTL in the model:

$$y_{ijk} = \mu + s_j + f_k + \beta\, \mathrm{cov}_{ijk} + c_{ai1}a_1 + c_{di1}d_1 + c_{ai2}a_2 + c_{di2}d_2 + \varepsilon_{ijk} \qquad \text{[model 2]}$$

Where: y_{ijk}, μ, s_j, f_k, β, cov_{ijk} and ε_{ijk} have the same meaning as in model [1]. Which a_1, a_2, d_1, d_2 are, respectively, additive and dominance effects for QTL_1 and QTL_2, c_{ai1}, c_{ai2}, c_{di1}, c_{di2} are the corresponding coefficients. Coefficients c_{a1}, c_{d1}, c_{a2} and c_{d2} were calculated conditional upon the markers, as follows:

$$c_{a1} = p_1\,(QQ) - p_1\,(qq)$$
$$c_{d1} = p_1\,(Qq)$$
$$c_{a2} = p_2\,(QQ) - p_2\,(qq)$$
$$c_{d2} = p_2\,(Qq)$$

Where: p_1 and p_2 are the probabilities for configurations QQ, Qq and qq in location 1 and location 2. A 1-cM grid search was performed in QTL Express by fitting model [2] to estimate the effects of two QTL at separate positions within the same linkage group simultaneously, examining all possible pairs of locations, to test whether the two-QTL model explained significantly more variation than the best QTL from the one-QTL analysis. Two F-statistics were computed. The first F-value was obtained by contrasting model [2] with a no QTL model with 4 df in the numerator (F_{4df}). When F-ratio (F_{4df}) reached the suggestive threshold, a second F-value was calculated by contrasting model [2] with QTL model [1] with 2 df in the numerator (F_{2df}). The presence of two QTL on the linkage group was concluded only when both F-statistics reached a significant threshold

One QTL model with imprinting

The presence of imprinting effects (*i*) was tested by considering the paternal or maternal origin of grandparental (Duroc or Pietrain) alleles, including the difference between the two classes of heterozygotes in the model as suggested by Knott et al. (1998):

$$y_{ijk} = \mu + s_j + f_k + \beta\, \mathrm{cov}_{ijk} + c_{ai}a + c_{di}d + c_{ii}i + \varepsilon_{ijk} \qquad \text{[model 3]}$$

Where y_{ijk}, μ, s_j, f_k, β, cov_{ijk} , a, d, c_{ai}, $c_d i$ and ε_{ijk} have the same meaning as in model [1]. i is the imprinting effect, and c_{ii} = p(Q(from sire)q) − p(qQ(from dam)) is the corresponding coefficient. Model [3] was first contrasted to a no QTL model with 3 degrees of freedom in the numerator (F_{3df}). When significant, model [3] was contrasted to the best one QTL model (model [1]) with 1 degree of freedom in the numerator (F_{1df}), in order to test the significance of the imprinting effects.

Two QTL model with imprinting

In this study we explore further two-dimensional QTL with parent-of-origin effect searching at 1-cM grid. This analysis was carried out for those linkage groups where significant evidence for a single QTL was detected by one QTL model. The presence of two QTL in the same linkage group was tested by adding additive, dominance and imprinting effects for a second QTL in the model:

$$y_{ijk} = \mu + s_j + f_k + \beta\,\text{cov}_{ijk} + c_{ai1}a_1 + c_{di1}d_1 + c_{ii1}i_1 + c_{ai2}a_2 + c_{di2}d_2 + c_{ii2}i_2 + \varepsilon_{ijk}$$

[model 4]

Where y_{ijk}, μ, s_j, f_k, β, cov_{ijk} and ε_{ijk} have the same meaning as in model [1], whereas that a_1, a_2, d_1, d_2, c_{ai1}, c_{ai2}, c_{di1}, c_{di2} have the same meaning as model [2]. The i_1 and i_2 are imprinting effects for QTL_1 and QTL_2, coefficients c_{i1} and c_{i2} were calculated conditional upon the markers, as follows:

$$c_{i1} = p_1\,(\text{Q(from sire)q}) - p_1\,(\text{qQ(from dam)})$$
$$c_{i2} = p_2\,(\text{Q(from sire)q}) - p_2\,(\text{qQ(from dam)})$$

Where p_1 and p_2 are the probabilities for configurations Q(from sire)q and qQ(from dam) in locations 1 and 2. A 1-cM grid searching was performed by fitting model [4] to estimate the effects of two QTL at separate positions within the same linkage group simultaneously, examining all possible pairs of locations, to test whether the two-QTL model explained significantly more variation than the best QTL from the one-QTL model. Two F-statistics were computed. The first F-value with 6 degrees of freedom in the numerator (F_{6df}) was obtained by contrasting model [4] to a no QTL model. When F_{6df} reached the suggestive threshold, a second F-value with 3 degrees of freedom in the

numerator (F_{3df}) was calculated by contrasting model [4] to model [3]. The presence of two QTL on the linkage group was concluded only when both F-statistics reached a suggestive level of significance.

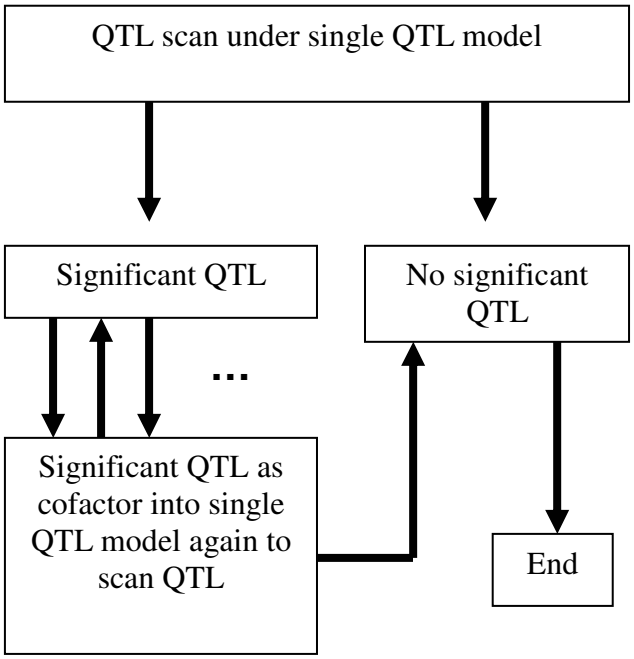

Figure 3.4: Cofactor (multiple) analysis procedure.

Multiple QTL mapping (MQM) analyses

A multiple (cofactor) analysis can partly account for the variance generated by other segregating QTL and substantially increases power to detect a QTL and precision of estimating the QTL position (Lynch and Walsh 1998).

The use of markers as cofactors in outbred populations may not be possible since markers are not uniformly informative in crosses of inbred lines (Spielman et al. 1996). The approach of fitting postulated QTL as cofactors on the same and other chromosomes may overcome this, so here will be performed a cofactor approach using postulated QTL, mainly on the same chromosome.

As described in the literature review, there are 3 approaches for the cofactor analyses: forward search; backward elimination and stepwise regression. Here we used mainly forward search with combination of stepwise regression.

The process was: when a significant QTL was identified (denote as QTL_1), then using this QTL_1 as cofactor (background) to search further alternative QTL, we denote here QTL_2, this procedure will be done until no new significant QTL and estimated locations of identified QTL are stable (denoted QTL_3, QTL_4 …) are found. Cofactors were dropped from the analysis if the corresponding significant level was lower than the threshold calculated at every round for each linkage group.
This procedure was described and implemented by de Koning et al. (2001a). After finishing the final round, we performed further analyses by using new QTL_2 as cofactors to re-detect the first QTL_1, since QTL_1 was the net result from accepting the effects of several QTL, therefore we need to detect again (figure 3.4).

Significant thresholds and confidence interval

Detection of QTL was based on a F statistic that was computed from sums of squares explained by the additive and dominance (also inclusive imprinting if it exists) coefficients for the QTL. Significant thresholds were determined empirically by data permutation test as described by Churchill and Doerge (1994). In this study three levels of significant threshold were set: 5 % chromosome-wide threshold, signed * ; 5 % genomewide threshold, signed ** ; 1 % genomewide threshold, signed *** . A total of 10,000 permutations were performed for each chromosome × trait combination. We did not observed distinct difference of estimated thresholds between traits, but differed more by chromosome as in Malek et al. (2001a). Because computational requirements prevented permutation test to be conducted, significant thresholds were derived based on seven representative traits: average BFT, BFT at shoulder, BFT at 13^{th} ~ 14^{th} rib, fat area, loin eye area, pH24ko and pH24si. Average thresholds across these 7 traits were used for significant testing for all traits. Average 5 % chromosome-wide thresholds ranged from 4.03 to 5.39 by different chromosomes. Thresholds for chromosome-wide significant at the 5 % level correspond approximately to suggestive significant at the genome-wise level (Lander and Kruglyak, 1995). A list of average thresholds by chromosome was as following: (SSC1) 4.72, (SSC2) 4.61, (SSC3) 4.80, (SSC4) 4.71, (SSC5) 4.73, (SSC6) 4.91, (SSC7) 5.01, (SSC8) 4.62, (SSC9) 4.59, (SSC10) 4.76, (SSC11) 4.57, (SSC12) 4.45, (SSC13) 4.64, (SSC14) 4.48, (SSC15) 4.77, (SSC16) 4.59, (SSC17) 4.46, (SSC18) 4.46.

Genome-wise significant thresholds also differed slightly by trait. Average genome-wise thresholds across these seven traits were 7.96 (**) and 9.72 (***) for the 5 % and 1 % levels, which were used for significant testing for all traits. Genome-wise threshold values were similar to those obtained by Malek et al. (2001a) who analyzed also in a commercial line cross.

Table 3.6: Significant threshold levels used in this study

SSC[a]	F_{1df}			F_{2df}			F_{3df}		F_{4df}		
	5 % chr	5 % gen	1 % gen	5 % chr	5 % gen	1 % gen	5 % chr[b]	5 % gen	5 % chr	5 % gen	1 % gen
2	6.62	12.7	16.0	4.61	7.96	9.72	-	6.18	-	-	-
5	6.83	12.7	16.0	4.73	7.96	9.72	-	6.18	-	-	-
6	7.15	12.7	16.0	4,91	7,96	9.72	-	6.18	-	-	-
9	-	-	-	4.59	7.96	9.72	-	-	3.32	5.22	6.19
11	6.55	12.7	16.0	4.57	7.96	9.72	-	6.18	-	-	-
16	-	-	-	4.59	7.96	9.72	-	-	3.32	5.22	6.19

Table 3.6: Significant threshold levels used in this study (continued).

SSC[a]	F_{6df}		
	5 % chr	5 % gen	1 % gen
2	2.82	4.19	4.88
5	-	-	-
6	-	-	-
9	-	-	-
11	-	-	-
16	-	-	-

[a] SSC meant *sus scrofa* chromosome. F_{1df} meant F statistic with 1 df in nominator. Significant at the 5 % chromosomewide level (5 % chr), the 5 % genomewide level (5 % gen) and the 1 % genomewide level (1 % gen). [b] The threshold of 5 % chromosomewide level (5 % chr) varied by chromosome, see table 4.9 ~ table 4.13. F value with one (F_{1df}), four (F_{2df}) and six (F_{6df}) degrees of freedom in the numerator were obtained by approximate approach (see text). The empty frames in the table mean no use of significant levels in this study.

Since the parent-of-origin effects were identified only on SSC2, SSC5, SSC6 and SSC11, also only affected for fat traits and leanness traits, therefore, the test statistic of 5 % chromosome-wise thresholds for one-QTL model with imprinting (model [3], F_{3df}) was individually obtained per trait and per chromosome, by 1,000 permutations. Detail results see table 4.8, table 4.9, table 4.11 and table 4.12. An approximate significant threshold in bi-dimensional scans was obtained as described by Knott et al. (1998): the

F ratio with two degrees of freedom in the numerator obtained from the null hypothesis was converted into a probability of the F ratio under a standard F distribution. Subsequently, the F ratio that would give this probability under F ratio with one, three, four and six degrees of freedom in the numerator were also obtained from the standard F distribution. The significant threshold levels used in this study were showed in table 3.6.

As advised by de Koning et al. (2001a), we estimated individually thresholds by permutation test for specific cofactor analyses; detail results see table 4.7 and table 4.9.

As shown by Mangin et al. (1994), the method "drop-off" (Lander and Botstein 1989) tends to give underestimated confidence interval (CI). Therefore, confidence intervals were obtained using the chi-square drop approximation (Mangin et al. 1994). The 95 % threshold was $\chi^2_{2.95} = 3.85$. Thus, the 95 % confidence interval limits were obtained at the chromosome locations where the F-statistics decreased 3.85/2 = 1.92 units starting in both directions from the position corresponding to the maximum F statistic. This method performs reasonably well for QTL with large effect but is not valid for QTL with small effect (Mangin et al. 1994). Therefore, in this study no CI was given for chromosome-wise significant QTL, but given CI for the QTL which reached the 5 % and 1 % genome-wise significant threshold.

4. Results and discussion

4.1 Microsatellite characterization

By the procedure of genotyping, 5 microsatellites had null alleles; 4 microsatellites showed no polymorphisms in this resource population; 40 samples were false; about 75 animal DNA samples were difficult to genotype. All of them were excluded from analyses. Finally, a total of 73 microsatellite genotypes of 1085 F_2 animals could be analysed, all together about 92,200 genotypes were made, inclusive F_0, F_1 and F_2.
As shown in appendix 2, across all 73 microsatellites, 360 different alleles were identified in the founder animals, of which 101 alleles (28.1 %) were found exclusively in Duroc and 137 alleles (38.1 %) exclusively in Pietrain. Although the comparison was influenced by different numbers of founder animals, the data indicated that the groups of founder animals used for propagation of the F_2 families were genetically diverse.
Average heterozygosity and information content was 0.750 and 0.572 (appendix 3). Information content for 14 markers was less than 0.5 on an individual basis.

4.2 Linkage maps

Seventy-three microsatellites (MSs) from 18 autosomes which covered 79.50 % of 18 *sus scrofa* chromosomes (SSC) according to USDA-MARC.2 map (Rohrer et al. 1996) have been used for QTL mapping. The female maps, male maps and sex averaged maps were 2142.8 cM, 1660 cM respectively 1821.6 cM Kosambi in lengths (table 4.1).

4.2.1 Marker order

The orders of 73 microsatellite loci were almost in accordance with the published USDA-MARC.2 map (Rohrer et al. 1996), except of S0226 on SSC2 and S0220 of SSC6 as those were not available in USDA-MARC.2 map. The order S0220-S0059-S0003 in this study agreed with PiGMaP.1 (Archibald et al. 1995). The marker S0226 is at the same position with SW14 in PiGMaP.1.5, in SSC2_refl1149 and in SSC2_refl1231, further, the order SW834-SW14 exists in USDA-MARC.2 map,

therefore, the order SW834-S0226 in our study is most likely correct. On the other hand, the order also was supported by high LOD scores in this population.

4.2.2 Differences in recombination rates between sex-specific maps

Maps which calculated from maternal meioses on average across all 18 autosomes were 1.3 times longer than the paternal chromosomes (table 4.1). However, female maps were shorter or the same length as the male maps on SSC1 and SSC13. These observations were consistent with the PiGMaP.1 (Archibald et al. 1995), Nordic.2 (Marklund et al. 1996) and NIAI (Mikawa et al. 1999) maps. Since the USDA-MARC.2 map has no sex map, it could compare sex map ratio with the present study.

Table 4.1: Sex-average, female and male linkage maps

SSC	MSs	Female map (K-cM)	Male map (K-cM)	Sex average map (K-cM)	Average maker interval per SSC (cM)	Contri-bution (R %)	Sex average map (H-cM)
1	SW1515	0.0	0.0	0.0	29.7	6.52	0.0
	SW1851	33.1	20.4	28.1			35.6
	S0155	18.4	92.8	34.8			46.0
	SW1301	58.5	54.1	55.7			81.9
	Sum	110.0	167.4	118.7			163.5
2	SW2443	0.0	0.0	0.0	20.4	4.48	0.0
	SW240	88.0	38.4	51.1			73.6
	SW834	16.4	25.6	21.5			26.0
	S0226	7.1	10.9	9.0			9.8
	Sum	111.4	74.9	81.6			109.4
3	SW72	0.0	0.0	0.0	21.1	4.62	0.0
	S0164	32.5	30.7	31.5			40.8
	SW2570	11.8	22.9	13.4			15.2
	S0002	42.2	24.6	39.3			53.4
	sum	86.5	78.2	84.2			109.4

Table 4.1: Sex-average, female and male linkage maps (cont.)

SSC	MSs	Female map (K-cM)	Male map (K-cM)	Sex average map (K-cM)	Average maker interval per SSC (cM)	Contri-bution (R %)	Sex average map (H-cM)
4	S0227	0.0	0.0	0.0	30.5	6.70	0.0
	S0001	43.6	41.9	42.7			59.1
	S0214	26.0	28.0	27.0			34.0
	S0097	64.7	26.5	52.3			75.8
	Sum	134.3	96.4	122.1			168.9
5	SW1482	0.0	0.0	0.0	28.3	6.22	0.0
	S0005	53.0	43.0	47.0			66.4
	IGF1	43.5	22.1	33.6			44.1
	SW967	33.4	35.8	32.7			42.7
	Sum	129.9	100.9	113.3			153.2
6	S0035	0.0	0.0	0.0	13.5	5.95	0.0
	S0087	62.3	63.2	62.5			94.3
	SW1067	13.4	4.5	8.3			9.0
	SW193	6.6	3.7	5.2			5.5
	S0300	2.4	1.2	1.4			1.4
	S0220	1.3	2.1	1.9			1.9
	S0059	21.7	15.5	18.6			22.8
	S0003	7.8	12.5	10.3			11.4
	Sum	115.4	102.5	108.3			146.3
7	S0025	0.0	0.0	0.0	19.3	6.35	0.0
	S0064	37.8	20.4	28.4			36.1
	S0102	26.0	27.4	26.6			33.4
	SW175	10.8	6.9	8.8			9.6
	S0115	25.2	24.4	25.0			31.0
	S0101	25.7	28.1	26.8			33.7
	Sum	125.6	107.2	115.6			143.8

Table 4.1: Sex-average, female and male linkage maps (cont.)

SSC	MS	Female map (K-cM)	Male map (K-cM)	Sex average map (K-cM)	Average maker interval per SSC (cM)	Contri-bution (R %)	Sex average map (H-cM)
8	SW2611	0.0	0.0	0.0	28.9	5.97	0.0
	S0086	65.4	77.9	70.0			108.3
	S0144	22.0	18.0	19.6			23.3
	SW61	19.2	18.8	19.3			22.9
	Sum	106.6	114.8	108.8			154.5
9	SW21	0.0	0.0	0.0	16.9	4.63	0.0
	SW911	23.0	22.0	22.3			27.1
	SW54	38.3	22.9	32.0			41.6
	S0109	14.1	1.8	10.0			11.0
	S0295	39.5	21.7	20.0			23.9
	Sum	114.8	68.4	84.3			103.6
10	SW830	0.0	0.0	0.0	52.3	8.61	0.0
	S0070	100.0	56.5	77.8			123.1
	SWR67	81.3	75.9	79.0			125.0
	Sum	181.3	132.4	156.8			248.1
11	SW2008	0.0	0.0	0.0	16.6	3.64	0.0
	S0071	68.2	12.8	31.3			40.5
	S0009	13.7	7.2	10.4			11.5
	SW703	27.1	21.0	24.6			30.4
	Sum	109.1	40.9	66.3			82.4
12	SW2490	0.0	0.0	0.0	38.2	6.29	0.0
	SW874	71.8	53.5	62.3			93.9
	SW605	62.2	39.1	52.3			75.8
	Sum	134.0	92.7	114.6			169.7

Contribution (R) was calculated by dividing the length of a linkage group by the total length (1821.6 cM Kosambi in 18 autosomes). K-cM meant in Kosambi cM; H-cM meant in Haldane cM.

Table 4.1: Sex-average, female and male linkage maps (cont.)

SSC	MSs	Female map (K-cM)	Male map (K-cM)	Sex average map (K-cM)	Average maker interval per SSC (cM)	Contri-bution (R %)	Sex average map (H-cM)
13	S0219	0.0	0.0	0.0	26.5	5.82	0.0
	SW344	51.6	23.5	41.5			57.0
	SW398	21.2	53.7	39.9			54.4
	S0289	25.1	24.4	24.6			30.4
	Sum	97.9	101.6	106.0			141.8
14	SW857	0.0	0.0	0.0	26.3	5.98	0.0
	S0007	44.5	70.1	52.6			76.3
	SWC27	83.6	53.4	56.4			83.1
	Sum	128.1	123.5	109.0			159.4
15	S0355	0.0	0.0	0.0	20.3	4.46	0.0
	SW1111	25.8	22.0	23.7			29.1
	SW936	38.0	34.0	35.6			47.3
	SW1119	23.0	21.2	22.0			26.7
	Sum	86.8	77.1	81.3			103.1
16	S0111	0.0	0.0	0.0	29.6	4.87	0.0
	S0026	91.0	45.0	56.5			83.3
	S0061	42.4	26.5	32.4			42.2
	Sum	133.4	71.5	88.8			125.5
17	SW335	0.0	0.0	0.0	34.3	5.64	0.0
	SW840	57.2	24.2	42.1			58.1
	SW2431	100.0	41.4	60.6			90.8
	Sum	157.2	65.6	102.8			148.9
18	SW1023	0.0	0.0	0.0	19.7	3.24	0.0
	SW787	36.8	13.1	22.3			27.1
	SWR414	43.7	31.0	36.7			49.1
	Sum	80.5	44.0	59.1			76.2

Table 4.2: Summary of the whole QTL results

Trait[a]	Numbers of significant QTL						Sum % vars[f]
	5 % chr[b]	5 % gen[b]	1 % gen[b]	imp[c]	mult[d]	Imp + mult[e]	
ADG_1	2						2.39
ADG_2	1						1.80
ADG_3	2						4.56
ADG_4		1					3.18
BWT	1						1.03
WWT	1						1.10
CL	2		1				7.55
Dressing	1						2.20
BFT-av	3		1				9.32
BFT-sh	3		1	1	1		9.87 (11.59)
BFT-10	2						4.62
BFT-lo	3	1					8.79
F1314	3		1	3		1	11.02
Side fat	3			2			6.76
Fat area	3	1		2			8.36
LEA	3	3			1		16.19 (17,76)
FFV	3	1	1	1		1	13.54
pH24ko	1		1				5.55
pH24si	1		1				6.96
LF24si	1						2.16
Dloss	2						6.89
Closs	1						3.40
Shforce	1						3.44
Mcolor	1						2.22
EBLC	3		1	2		1	10.46
ECLC	2	2	1	2			14.49
FCS	2						4.16
FCR	2						4.01

[a] The abbreviation of traits see table 3.5. [b] significant at the 5 % chromosomewide level (5 % chr.) which F statistic was different by chromosome, the 5 % genomewide level (5

% gen) (F > 7.96) and the 1 % genomewide level (1 % gen) (F > 9.72) by trait. [c] imprinting means the QTL numbers by model [3]. [d] multiple means trait numbers which obtained two QTL by multiple QTL analyses; [e] imprinting + multiple means trait numbers which two QTL obtained by multiple QTL analyses with imprinting effects; [f] Sum % variance = the sum of fraction phenotypic variance explained of all QTL by model [1]. In the parentheses are shown the phenotypic variance explained of QTL results by model [1] added that by model [2] (table 4.7). The portion of phenotypic variance explained of imprinted QTL did not show here; that could be seen the table 4.8, 4.9, 4.10, 4.11, 4.12 and table 4.13.

4.3 QTL results and discussion

4.3.1 General results of QTL mapping

In total, 71 QTL by model [1] were identified on almost all autosomes except SSC10. Some QTL on SSC10 were excluded as there was segregation distortion on this chromosome. Among those QTL, fifty-two QTL were significant at the 5 % chromosome-wise level, ten QTL on SSC1, SSC2, SSC6, SSC8, SSC16 and SSC17 were significant at the 5 % genomewide level, nine QTL on SSC1 and SSC7 were significant at the 1 % genomewide level. Those results were achieved by model [1].
Multiple QTL analyses, also with parent-in-origin effect, were performed in this study (table 4.2). Two QTL affecting loin eye area segregated on SSC9, meanwhile, two QTL affecting shoulder back fat thickness segregated on SSC16 (table 4.7). Thirteen suggestive imprinted QTL for different traits were obtained on SSC2, SSC5, SSC6 and SSC11. Two QTL segregated on SSC2 affecting three traits at the same locations (table 4.9), one QTL showed Mendelian expression; another indicated the imprinting expression. Several of those QTL found in this study have been identified in previous studies, which will be discussed on a trait-by-trait basis. In order to compare with the QTL database (Hu et al. 2005), the position of QTL was converted to cM Kosambi.

4.3.2 QTL for growth and body composition traits

4.3.2.1 Birth weight (BWT)

Evidence for a suggestive QTL affecting BWT (n=1057) was identified at 41.5 cM (F=5.19*, figure 4.1, table 4.3) on SSC12. It was overdominant (243.6), which means heterozygotes have biggest BWT. Interestingly, Knott et al. (1998) have also mapped a suggestive overdominance (-303.3) QTL for BWT on SSC12 in a cross between wild boar and Large White pigs.

Table 4.3: Results of QTL for ADG and body weight by model [1]

SSC	Traits[a]	F value[b]	Pos[c] (CI)	Add[d] (SE)	Dom[d] (SE)	% vars[e]
1	ADG_3	6.35*	62.9	21.01 (6.05)	8.30 (11.38)	2.10
1	ADG_4	9.30**	77.8 (50~92)	19.16 (4.57)	-14.54 (12.04)	3.18
4	ADG_1	5.40*	30.0	-5.90 (3.72)	23.63 (8.02)	1.24
4	ADG_2	5.07*	22.2	-10.32 (4.72)	28.61 (11.64)	1.80
4	ADG_4	4.91*	23.7	-8.03 (5.05)	34.33 (12.22)	1.71
9	WWT	4.77*	9.2	0.19 (0.09)	-0.33 (0.15)	1.10
9	ADG_1	4.98*	8.3	6.75 (3.06)	-10.91 (4.95)	1.15
12	BWT	5.19*	41.5	-13.12 (25.87)	243.65 (77.25)	1.03
18	ADG_3	6.41*	42.7	11.28 (6.36)	-35.93 (12.06)	2.29

[a] Abbreviation of traits see table 3.5. [b] F value with 2 df in nominator came from model [1] vs. model without QTL. * = 5 % suggestive chromosomewide significant level; ** = 5 % genomewide significant level; *** = 1 % genomewide significant level, respectively. [c] pos was the QTL position in Kosambi cM, the confidence interval (CI) was given in the parentheses only when it reached the 5 % and 1 % genomewide significant level, however, there was no CI given when it reached suggestive threshold (Mangin et al. 1994) . [d] Add (additive effects) = Duroc allele – Pietrain allele; [d] Dom (dominance effects) are relative to the mean of the two heterozygotes. Standard error (SE) is in the parentheses. [e] % var = the fraction of phenotypic variance explained by a QTL, as percentage of the residual variance in the F_2.

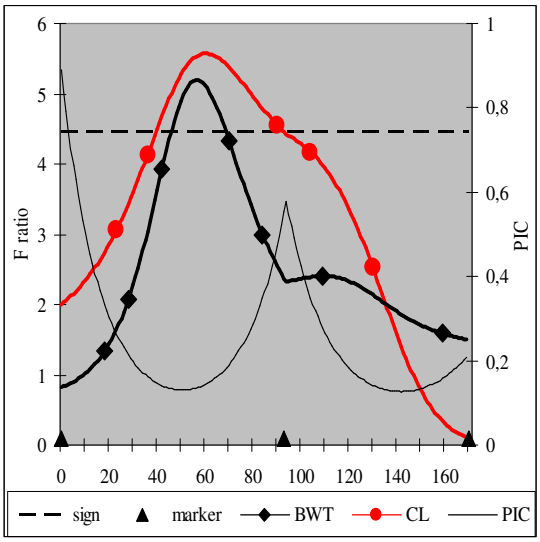

Figure 4.1: QTL on SSC12.

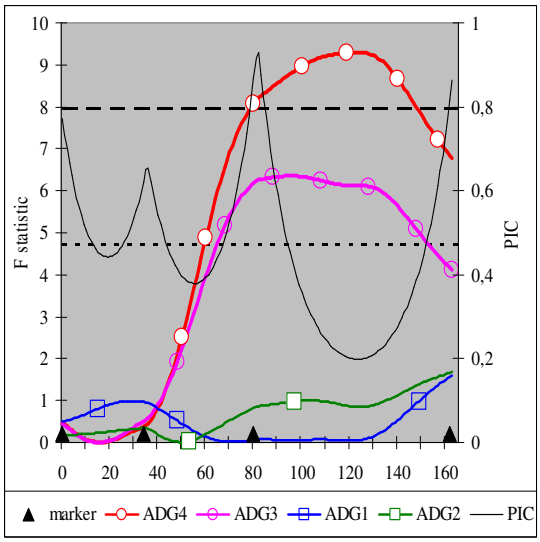

Figure 4.2: QTL for ADG on SSC1.

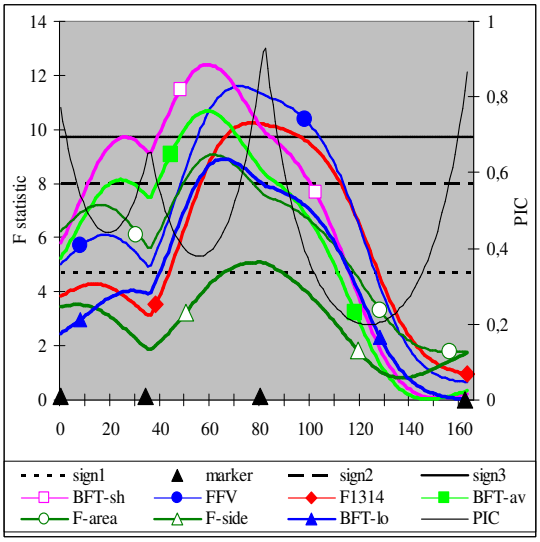

Figure 4.3: QTL for fat traits on SSC1.

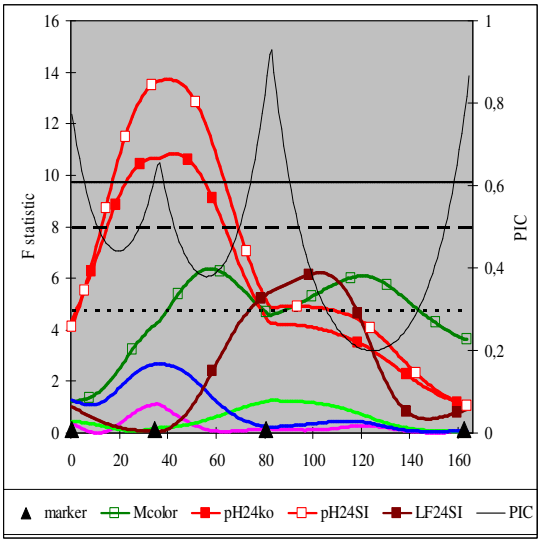

Figure 4.4: QTL for meat traits on SSC1.

Common legend: the Y axis on the left shows the F test statistic, three threshold levels: the short thick dashed line showed the suggestive significant level which the F value varied from 4.45 to 5.01 by chromosomes, see section 3.2.9.4; the longer thick dashed line indicated 5 % genomewide significant thresold (F = 7.96**); the thick solid line showed 1 % genomewide significant threshold (F = 9.72***). The black triangle on X axis presents the position of markers in Haldane cM. The thin solid curve shows the polymorphism information content (PIC) based on multiple markers, which value was according to Y axis on the right. Abbreviations of traits see in table 3.5.

For BWT, there were few QTL reported in previous studies (Knott et al. 1998, Paszek et al. 1999, Malek et al. 2001a) with no strong statistic support, both in divergent or in commercial cross populations. It is possible that BWT is mainly affected by maternal effects in the period of gestation, or affected by many genes which are interacting, thus QTL could not be detected by one-QTL model or by multiple no interaction QTL model (Carlborg et al. 2003, 2004). The overdominance effect also suggests interaction of loci.

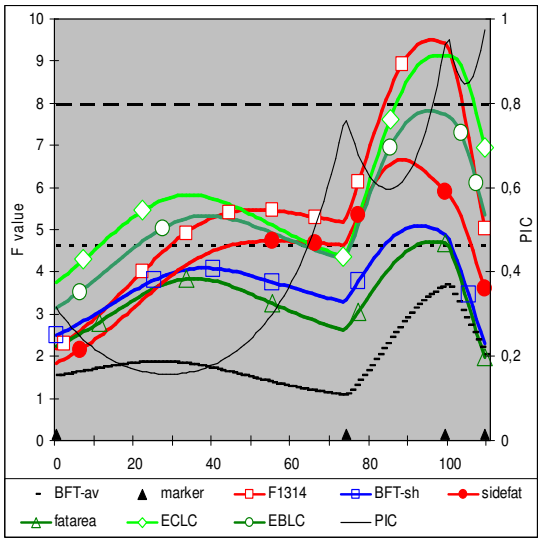

Figure 4.5: QTL for fat traits on SSC2.

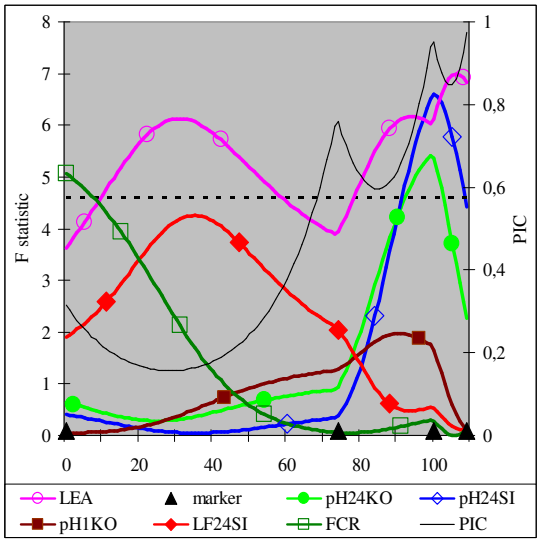

Figure 4.6: QTL for meat traits on SSC2

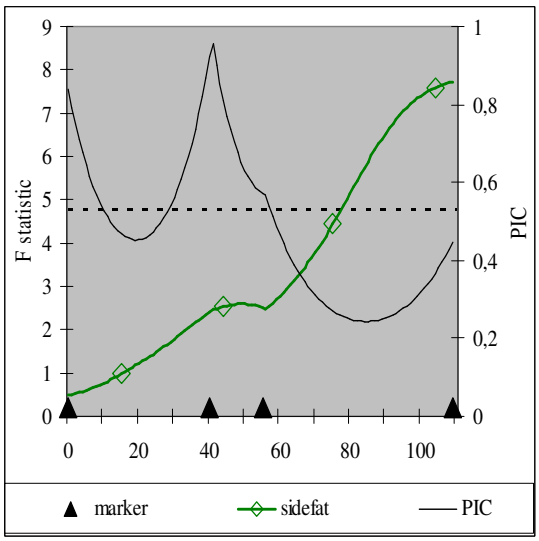

Figure 4.7: QTL for side fat on SSC3.

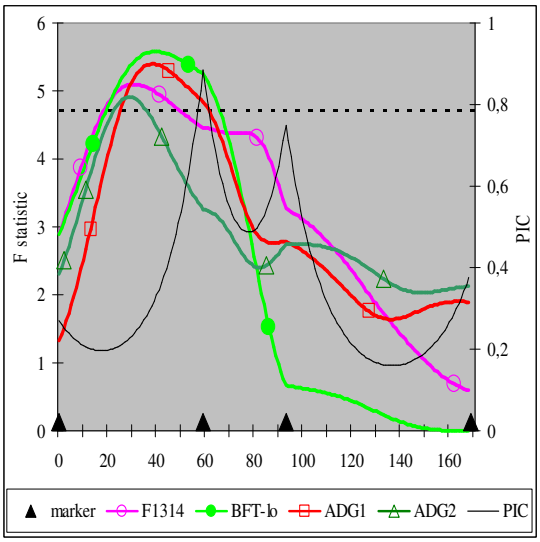

Figure 4.8: QTL on SSC4

Figure 4.5 ~ 4.8: common legends were the same as those of figure 4.1 ~ figure 4.4.

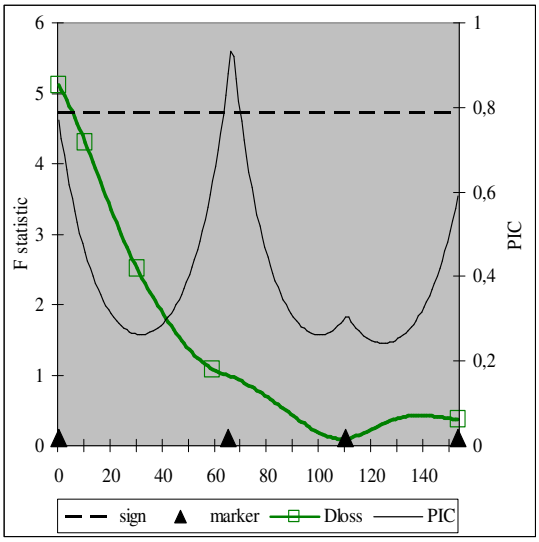

Figure 4.9: QTL for drip loss on SSC5.

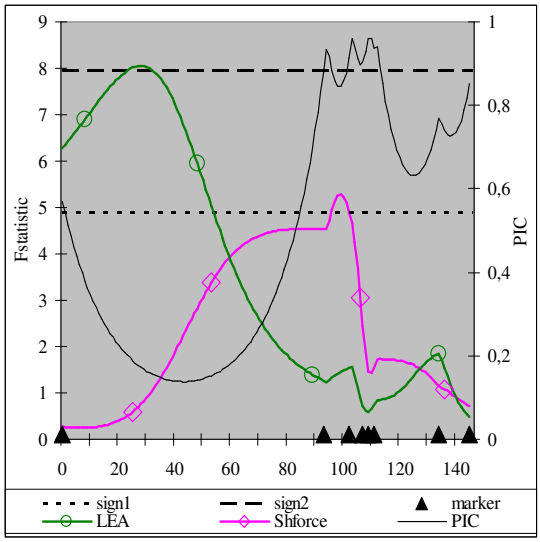

Figure 4.10: QTL on SSC6

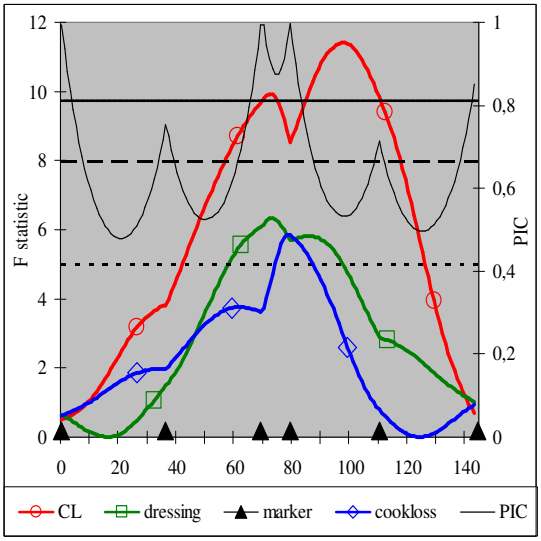

Figure 4.11: QTL on SSC7

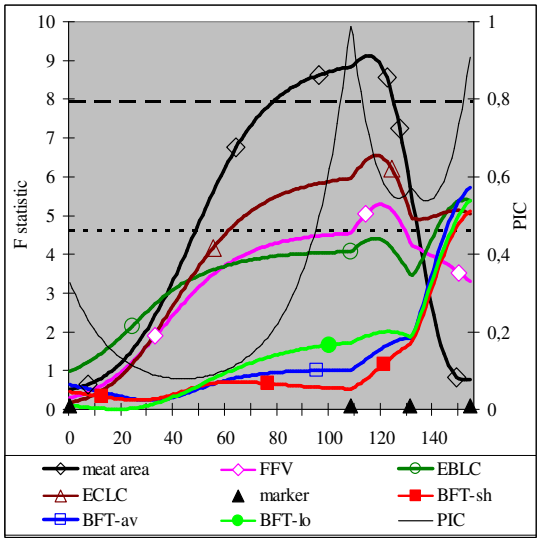

Figure 4.12: QTL on SSC8

Figure 4.9 ~ 4.12: common legends were the same as those of figure 4.3 ~ figure 4.4.

4.3.2.2 QTL for average daily gain (ADG)

QTL for ADG on SSC1

As shown in figure 4.2 and table 4.3, a QTL accounting for ADG_4 from birth to slaughter reached 5 % genomewide significant threshold at 77.8 cM on SSC1, CI: 50~92 cM. Duroc alleles produced pork with faster growth rate than Pietrain alleles.

Paszek et al. (1999) found a significant QTL (P<0.000027) affecting daily gain from birth to slaughter between markers SW373 and SW1301 of SSC1 in a Meishan × Yorkshire swine resource family, Rohrer (2000) also obtained a genomewide significant QTL for ADG in 8~18 weeks at 134 cM (marker interval: S0056~SW1301) in Meishan cross populations, the CI of both findings were confirmed by this study.

This QTL accounting for ADG_4 from birth to slaughter on SSC1 was mainly caused by ADG_3 (in test period), since ADG_1 from birth to weaning and ADG_2 from weaning to test start did not reach the significant level, and the major effect of this QTL was additive. This was in good agreement with the results from Carlborg et al. (2003, 2004), who have estimated the relative contribution of additive, dominance and epistasic effects to growth in chicken. They found that the contribution of epistasis was more pronounced prior to 46 days of age, whereas additive genetic effects explained the major portion of the genetic variance later in life. Some of the loci affected either early or late growth but not both. Very few loci affected the entire growth process, which pointed out that early and late growth, at least to some extent, have different genetic regulation. Additional analyses should be conducted to determine whether epistatic interactions exist between the detected loci in this population. We have also noticed that the region in this study has lowest information and a gap between markers S0155 ~ SW1301 is too large (55.7 cM Kosambi, see table 4.1, figure 4.2). More informative markers should be added in the interval between marker S0155~SW1301 to get the exact location and effect of the QTL for ADG.

QTL for ADG on Chromosome 4, 9, 18

Two suggestive QTL for ADG_1 were obtained on SSC4 (figure 4.8) and SSC9 (figure 4.13), one suggestive QTL for ADG_2 was on SSC4. Suggestive QTL for ADG_3 was on SSC18 (figure 4.19). QTL for ADG_3 on SSC18 and ADG_1 on SSC9 showed that heterozygotes had lower growth rate (table 4.3), heterozygotes had greatest growth rate for QTL on SSC4 than both homozygotes.

Table 4.4: Results of fat QTL by model [1]

SSC	Trait[a]	F value[b]	Position[c] (CI)	Add[d] (SE)	Dom[d] (SE)	% vars[e]
1	BFT-av	10.68***	42.7 (34~53)	0.09 (0.02)	-0.04 (0.04)	3.66
1	BFT-sh	12.40***	42.7 (33~52)	0.15 (0.03)	0.00 (0.06)	4.23
1	BFT-lo	8.91***	46.2 (33~54)	0.08 (0.02)	-0.07 (0.04)	3.07
1	F1314	10.24***	53 (38~75)	0.06 (0.01)	-0.04 (0.02)	3.52
1	Side fat	5.08*	54.1	0.10 (0.03)	-0.07 (0.05)	1.78
1	Fat area	9.06**	43.8 (32~64)	0.65 (0.16)	-0.53 (0.33)	3.12
2	F1314	9.51**	63.4 (58~67)	-0.06 (0.01)	0.03 (0.02)	3.27
2	BFT-sh	5.09*	61.2	-0.08 (0.03)	0.01 (0.04)	1.78
2	Side fat	6.66*	59	-0.13 (0.04)	-0.05 (0.06)	2.31
2	Fat area	4.72*	63.4	-0.40 (0.14)	0.20 (0.20)	1.65
3	Side fat	7.72*	70.4	-0.13 (0.04)	0.23 (0.10)	2.67
4	F1314	5.1*	24.3	-0.06 (0.02)	0.05 (0.05)	2.36
4	BFT-10	5.58*	31	-0.08 (0.02)	0.02 (0.05)	2.39
8	BFT-av	5.72*	93.7	0.05 (0.01)	-0.03 (0.02)	2.00
8	BFT-sh	5.10*	93.7	0.05 (0.02)	-0.07 (0.03)	1.78
8	BFT-lo	6.13*	93.7	0.06 (0.02)	-0.01 (0.02)	2.13
13	BFT-av	4.67*	20	-0.07 (0.03)	0.09 (0.07)	1.64
13	Fat area	5.55*	38.5	-0.57 (0.18)	0.36 (0.50)	1.94
15	BFT-10	6.41*	24.2	-0.03 (0.02)	-0.08 (0.02)	2.23
16	BFT-av	5.79*	62.4	0.04 (0.02)	0.10 (0.03)	2.02
16	BFT-sh	5.97*	63.4	0.07 (0.03)	0.15 (0.05)	2.08
16	BFT-lo	5.29*	62.4	0.03 (0.02)	0.11 (0.04)	1.85
17	BFT-lo	4.97*	38	-0.03 (0.04)	0.48 (0.15)	1.74
17	F1314	5.34*	29	0.00 (0.03)	0.34 (0.10)	1.87
17	Fat area	4.71*	28.4	0.15 (0.28)	3.07 (1.02)	1.65

[a, b, c, d, e] have the same meanings as in table 4.3.

Walling et al. (1998) found higher genomewide QTL for ADG from weaning to 25 kg at 69.6 cM on SSC4 with CI of 27.1~79.3 cM. De Koning et al. (2001b) reported a suggestive QTL for ADG from weaning to approximate 25 kg at 21 cM, CI: 4.1~27.1

cM. Knott et al. (2002) mapped a suggestive QTL at 27 cM for ADG from birth to 3 weeks (F ratio = 4.33). The CI of these findings was similar to the present study.

Malek et al. (2001a) have revealed a 5 % genomewide significant QTL for ADG on test at 99.3 cM on SSC4 (CI: 80.5-107.9 cM) in a Berkshire-Yorkshire F2 population. Those QTL were not confirmed by this study.

4.3.3 QTL for fat traits (table 4.4)

Some multiple QTL with/without imprinting effects were obtained on SSC2, SSC5, SSC9 and SSC16 which will be discussed later; here will be only described the results by one QTL model. The QTL for back fat traits jointly explained from 4.62 % to 11.02 % of the phenotypic variance in the F2 population (table 4.2, 4.4); however, some traits are obvious to be highly correlated. Our results indicated that Duroc alleles tended to be associated with fatter for the QTL on SSC1. In contrast, Pietrain alleles tended to increase back fat on SSC2, heterozygotes were slightly fatter on SSC16 and 17.

4.3.3.1 QTL for fat traits on SSC1 and on SSC2

A series of genomewide significant QTL affecting BFT at shoulder, BFT at loin, average BFT, fat area, ratio of fat area relative to meat area were obtained on SSC1 (Figure 4.3, table 4.4) almost in the same CI: 32~64 cM. They were similar to Geldmann et al. (2003) and Malek et al. (2001a), CI overlapped.
On SSC2, a genomewide significant QTL has been uncovered for fat depth at 13^{th} - 14^{th} rib at the distal region; at the same region several suggestive QTL mapped affecting fat area, affecting shoulder BFT and side fat thickness (figure 4.5). In a resource population based on Meishan and Dutch Large White and Landrace, QTL for backfat were shown in closer vicinity to our QTL (Rattink *et al.* 2000; de Koning *et al.* 2000, 2001c).

Table 4.5: Results of QTL for carcass composition by model [1]

SSC	Trait[a]	F value[b]	Pos[c] (CI)	Add[d] (SE)	Dom[d] (SE)	% vars[e]
1	FFV	11.60***	49.6 (38~74)	0.02 (0.00)	-0.01 (0.01)	4.50
1	ECLC	12.64***	50.8 (42~67)	-0.61 (0.13)	0.45 (0.22)	4.30
1	EBLC	11.45***	50.2 (40~68)	-0.68 (0.16)	0.56 (0.27)	3.92
2	LEA	6.98*	69.0	0.92 (0.25)	0.06 (0.36)	2.42
2	FFV	8.23**	62.9 (56~69)	-0.01 (0.00)	0.00 (0.01)	2.85
2	EBLC	7.82*	62.9	0.59 (0.15)	-0.06 (0.22)	2.71
2	ECLC	9.14**	64.0 (57~69)	0.52 (0.12)	-0.08 (0.18)	3.15
2	FCR	5.07*	0	-0.06 (0.02)	-0.05 (0.05)	1.99
6	LEA	8.05**	23.0 (0~36)	-0.69 (0.43)	-4.70 (1.26)	2.79
7	CL	11.41***	64.0 (57~71)	0.71 (0.15)	0.14 (0.27)	3.90
7	dressing	6.33*	50.8	-0.32 (0.09)	0.02 (0.14)	2.20
8	LEA	9.12**	74.0 (48~81)	-1.01 (0.26)	-0.81 (0.42)	3.14
8	FFV	5.28*	76.2	0.01 (0.00)	0.01 (0.01)	1.85
8	EBLC	5.41*	92.5	-0.44 (0.14)	0.24 (0.21)	1.89
8	ECLC	6.55*	75.0	-0.45 (0.13)	-0.30 (0.22)	2.28
9	LEA	7.87*	66.2 (54 ~ 68)	-1.30 (0.35)	0.78 (0.69)	2.72
9	FFV	5.07*	32.3	0.01 (0.00)	0.00 (0.01)	1.77
9	ECLC	5.56*	16.4	-0.40 (0.15)	0.45 (0.27)	1.94
12	CL	5.58*	43.2	-0.84 (0.25)	0.10 (0.73)	1.95
14	FCR	5.12*	10.8	0.05 (0.02)	-0.09 (0.05)	2.02
14	FCS	6.07*	25	0.06 (0.02)	-0.12 (0.06)	2.38
16	LEA	8.43**	56.3 (41~61)	1.14 (0.28)	0.10 (0.38)	2.91
17	LEA	6.35*	55.2	-1.45 (0.75)	-12.36 (4.33)	2.21
17	FFV	7.41*	31.0	0.01 (0.01)	0.10 (0.03)	2.57
17	EBLC	5.57*	31.7	-0.22 (0.32)	-3.90 (1.20)	1.94
17	ECLC	8.14**	52.0 (23~64)	-0.41 (0.35)	-7.16 (1.89)	2.82
18	CL	4.86*	49.0	-0.31 (0.14)	-0.51 (0.22)	1.70
18	FCS	4.61*	14	0.03 (0.02)	-0.06 (0.03)	1.82

[a, b, c, d, e] have the same meanings as in table 4.3.

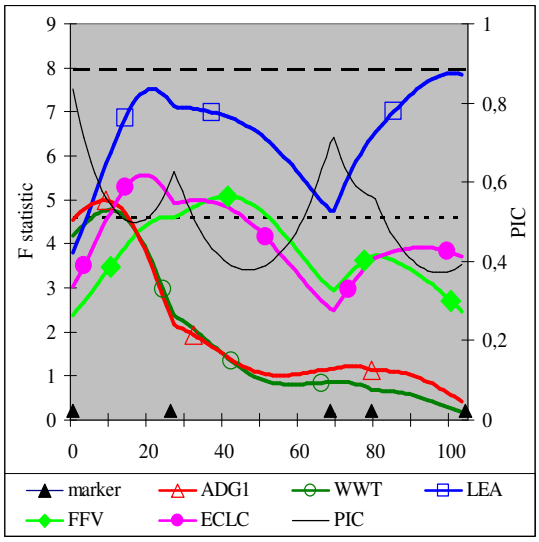

Figure 4.13: QTL on SSC9.

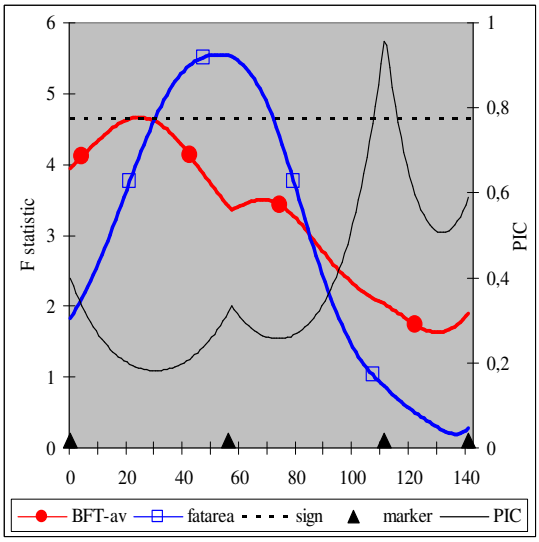

Figure 4.14: QTL on SSC13.

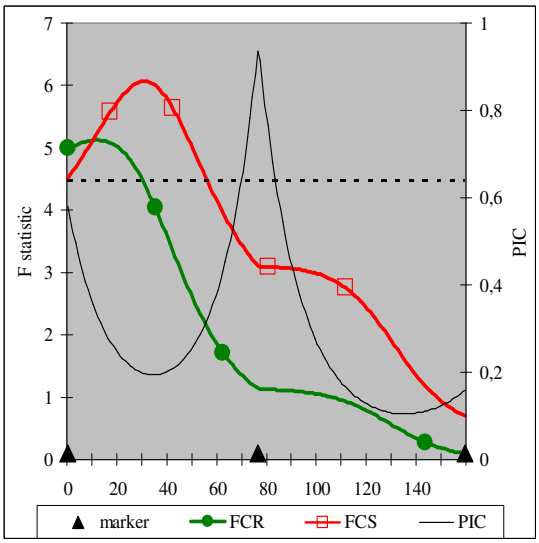

Figure 4.15: QTL on SSC14

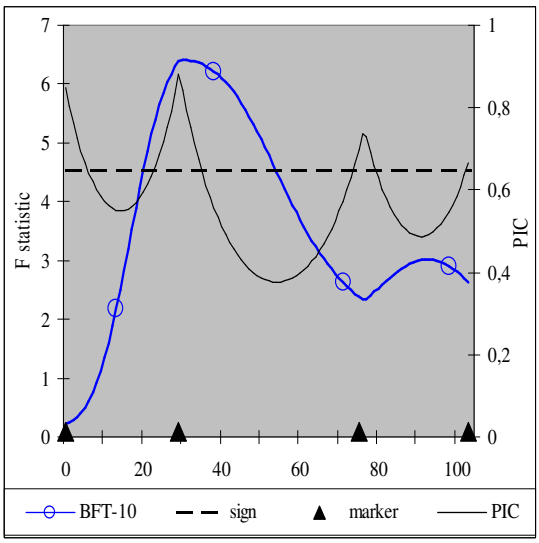

Figure 4.16: QTL for fat traits on SSC15

Figure 4.13 ~ 4.16: common legends were the same as those of figure 4.1 ~ figure 4.4.

4.3.3.2 QTL for fat traits on SSC3, 4, 8, 9, 15 and 17

A suggestive QTL responsible for side fat thickness was observed on SSC3 (figure 4.7), similar to Knott et al. (1998) who reported a suggestive QTL for BFT at 113 cM. Suggestive QTL for BFT at 10th rib was at 31 cM on SSC4 and for BFT at 13th-14th rib at 24.3 cM on SSC4 (figure 4.8). There were three new suggestive QTL at the end region on SSC8 for average BFT, shoulder BFT and loin BFT (figure 4.12), who's

location did not agree with Rohrer (1998a) and Bidanel et al. (2001). A suggestive QTL for ratio of fat area relative to meat area at 32.3 cM on SSC9 (figure 4.13) was near to the region of a QTL found by Rohrer (1998a). A suggestive QTL for fat area was found on SSC13 (figure 4.14), another for average BFT, which confirmed the results of Rohrer and Keele (1998a), Malek et al. (2001a) and Nezer et al. (2002). Yu et al. (1995) reported that PIT1 locus has significant association with average BFT. A suggestive QTL for BFT at 10^{th} rib was mapped on SSC15 that was consistent with Knott et al. (1998) and Thomsen et al. (2004) (figure 4.16). Three suggestive QTL were found on SSC16 affecting average BFT, shoulder BFT and loin BFT (figure 4.17). Suggestive QTL on SSC17 for fat area, for BFT at 13^{th}-14^{th} rib and for BFT at loin were identified; also a suggestive QTL was at 31 cM on SSC17 for ratio of fat area relative to meat area (figure 4.18), these QTL have been found for the first time.

4.3.4 QTL for loin eye area

We have detected seven QTL for loin eye area on SSC2, SSC6, SSC8, SSC9, SSC16 and SSC17; they jointly explained 16.19 % of the phenotypic variance in the F_2 population (table 4.5). Three QTL of them were significant at the 5 % genomewide level, Duroc alleles tended to produce pigs with the larger loin eye area on SSC2 and SSC16. However, Pietrain alleles had slightly larger loin eye area on SSC8 and SSC9. Heterozygotes had smallest loin eye area on SSC6 than both homozygotes.
The QTL on SSC2 (figure 4.6) and SSC8 (figure 4.12) confirmed results from Varona et al. (2002), who found QTL for loin eye area on SSC2 and 8 in the same region. The results on SSC6, 9, 16, 17 have been found for the first time in this population (figure 4.10, 4.13, 4.17 and 4.18, respectively).

4.3.5 QTL for carcass length

Our results revealed 1 % genomewide significant QTL for this trait on SSC7, with the Duroc alleles resulting in longer carcass length than the Pietrain (table 4.5, figure 4.11). On SSC7, Rohrer and Keele (1998b) and Geldermann et al. (2003) have got the similar confidence interval to the present study. However, our result was not in the same region reported by Nezer et al. (2002) and Sato et al. (2003). Other two suggestive QTL were

on SSC12 (figure 4.1) and SSC18 (figure 4.19). These effects totally accounted for 7.55 % of the F_2 variance in the F2 population (table 4.2).

4.3.6 QTL for meat pH value

The ultimate pH is the most commonly used trait to assess pork quality and usually is measured at 24 hours postmortem. Pork pH value is not a direct measure of quality, but it is correlated with the quality traits of color, drip loss and water-holding capacity. Muscle pH postmortem is also correlated with sensory panel traits such as tenderness and juiciness. A higher level of acidity within the muscle (lower pH) causes muscle proteins to denature and lose their ability to hold water. Therefore, meat with higher pH will tend to have more desirable characteristics such as darker color, less drip loss, more firmness and higher tenderness.

4.3.6.1 QTL for meat pH on SSC1

Two genomewide significant QTL ($P < 0.01$) were demonstrated for pH value at 24 hours post-mortem both in *m. long. dorsi* at 13^{th}~14^{th} rib and in *m. semimembranosus* (table 4.6, figure 4.4), respectively. They explained 4.66 % and 3.75 % of the phenotypic variance, respectively. Both QTL increased pH value with Duroc alleles. The confidence interval of both QTL were 20~42 cM (marker interval: SW1515~S0155), but do not match the region previously detected by Thomsen et al. (2004) and Beeckmann et al. (2003). Thomsen et al. (2004) have found a suggestive QTL for loin pH after 48 hours at 106 cM (marker interval: SW373~SW974) in a Berkshire-Yorkshire population. Beeckmann et al. (2003) have reported a suggestive QTL at 137.3 cM for pH value in 45 minutes post-mortem in *m. semimembranosus* in a family of Wild Boar crossed with Pietrain. Su et al. (2004) have mapped a suggestive QTL for pH value for *m. biceps femoris* at 3 cM in a Large White - Meishan resource family.

This two genomewide QTL provided valuable information for further work such as fine mapping in this region, then identification of positional candidate genes in order to perform further study or improve the meat quality.

Table 4.6: Results of QTL for meat quality by model [1]

SSC	Trait[a]	F value[b]	Pos[c] (CI)	Add[d] (SE)	Dom[d] (SE)	% vars[e]
1	pH24ko	10.97***	32.3 (20~42)	0.03 (0.01)	-0.01 (0.01)	3.75
1	pH24si	13.75***	30.4 (22~41)	0.05 (0.01)	0.00 (0.02)	4.66
1	LF24si	6.22*	67.0	0.43 (0.17)	-0.94 (0.37)	2.16
1	Mcolor	6.36*	41.5	1.35 (0.45)	-1.79 (0.93)	2.22
2	pH24ko	5.42*	65.0	-0.02 (0.01)	-0.01 (0.01)	1.80
2	pH24si	6.61*	65.5	-0.02 (0.01)	-0.02 (0.01)	2.30
5	Dloss	5.11*	0.0	0.18 (0.09)	-0.26 (0.09)	3.14
6	Shforce	5.28*	65.0	-1.11 (0.50)	1.78 (0.77)	3.44
7	Closs	5.85*	53.5	-0.39 (0.00)	0.66 (0.00)	3.40
18	Dloss	6.14*	48.0	-0.29 (0.09)	-0.10 (0.09)	3.75

All categories have the same meanings as in table 4.3.

4.3.6.2 QTL for meat pH on SSC2

On SSC2 (figure 4.6), there were two suggestive QTL for pH value at 24 hours post-mortem in m. *long. dorsi* at 13^{th} ~ 14^{th} rib and in *m. semimembranosus*, respectively (table 4.6, figure 4.15), which position was similar to Su et al. (2004), they have mapped a QTL affecting pH in *m. biceps femoris* at 67 cM (CI: 59.9~72.4 cM, marker interval: S0170~SW1883).

We were not able to confirm below results in previous studies. Malek et al. (2001b) have detected seven QTL for pH-related traits at the 5 suggestive QTL on SSC 5, 6, 14, and15, three of which were significant at the 5 % genomewide level on SSC 5 and SSC15, and one at the 1 % genomewide level on SSC 15 in a Berkshire-Yorkshire population. Geldermann et al. (1996, 2003) have demonstrated a QTL for pH on SSC6 near the HAL gene but using HAL-positive pigs, also a QTL on SSC X. De Koning et al. (2000, 2001c) also found QTL affecting pH on SSC4, 9, 11, 14, 18, and X, with a variety of modes of gene expression. Several studies reported QTL for this trait on SSC3 (Paszek et al. 1999; Ovilo et al. 2002; Su et al. 2004) and on SSC16 (Paszek et al. 1999).

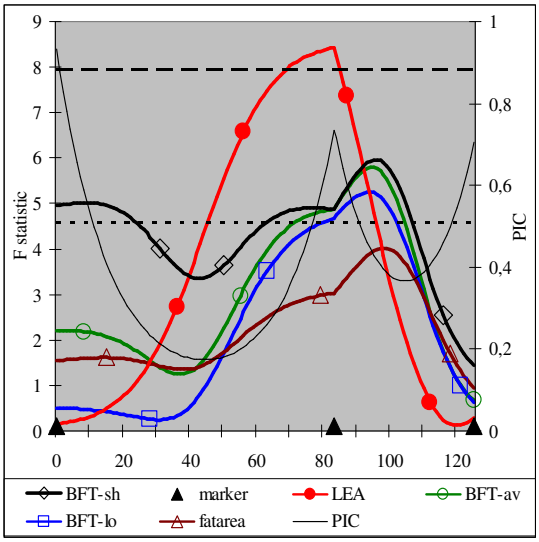

Figure 4.17: QTL on SSC16

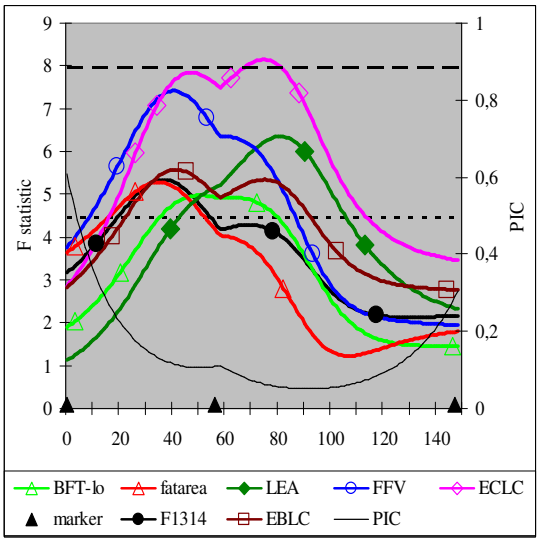

Figure 4.18: QTL on SSC17

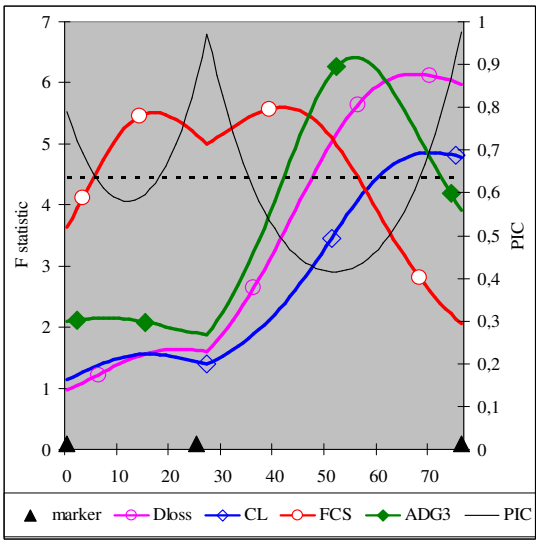

Figure 4.19: QTL on SSC18

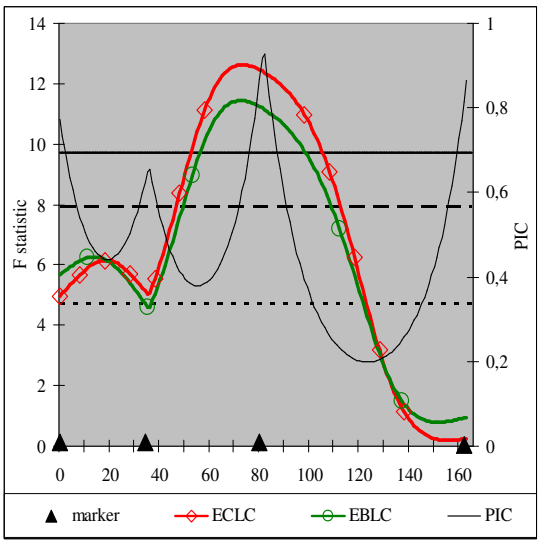

Figure 4.20: QTL for leanness content on SSC1

Figure 4.17 ~ 4.20: Common legends were the same as those of figure 4.1 ~ figure 4.4.

4.3.7 QTL for drip loss

Two suggestive QTL for drip loss were detected on SSC5 and SSC18 (table 4.6, figure 4.9 and 4.19), which jointly explained 6.89 % of the phenotypic variance in the F_2 population. Heterozygotes had less drip loss for QTL on SSC5; in contrast, Pietrain alleles got more drip loss on SSC18. Malek et al. (2001b) have obtained a suggestive

QTL for drip loss on SSC1, SSC2, SSC11 and SSC13, respectively; de Koning et al. (2000, 2001c) detected four QTL for drip loss, some with imprinted effects, on SSC 4, 6 (maternal), 14 (Mendelian), and 18 (paternal), those were not confirmed in this study.

4.3.8 QTL for meat conductivity, meat color, cooking loss and shear force

We have mapped a suggestive QTL affecting meat conductivity 24 h post-mortem in *m. semimembranosus* on SSC1 (figure 4.4), which explained 2.16 % of the phenotypic variance in the F_2 population. Heterozygotes showed less conductivity for this QTL. Yue et al. (2003a) have demonstrated two genomewide significant QTL for this trait on SSC6 near the HAL gene in a Meishan-Pietrain and a Pietrain-Wild Boar family, respectively. But they used HAL-positive pigs, which was not the case in this study.
A suggestive QTL affecting meat colour was on SSC1 in the present study (figure 4.4), which was located within the confidence interval reported by de Koning et al. (1999), but paternal expressed. Thomsen et al. (2004) have mapped a suggestive QTL on SSC1; its location was not the same like ours. A suggestive QTL for cooking loss was found on SSC7 near the QTL for the dressing trait (figure 4.11). A suggestive QTL for shear force is on SSC6 (figure 4.10).

4.3.9 QTL for estimated carcass leanness content and estimated belly leanness content

Five QTL for estimated carcass leanness content (ECLC) were detected on SSC1, 2, 8, 9 and 17 (table 4.2) with the QTL on SSC1, 2 and 7 reaching genome-wide significant. They jointly explained for 14.49 % of the phenotypic variance in the F_2 population. Pietrain showed more leanness for QTL on SSC1 (figure 4.20), SSC8 (figure 4.12), SSC9 (figure 4.13) and SSC17 (figure 4.18); in contrast, Duroc has more leanness for QTL on SSC2. These results were in good agreement with properties of both breeds. The same case happened by other traits such as meat quality, ADG and fat traits. We have also analysed multiple QTL inclusive parent-of-origin effects on SSC2 (section 4.3.12). Milan et al. (2002) reported genomewide significant QTL for estimated carcass leanness content on SSC1, 2, 7 and X in a Meishan and Large White F2 population, however, the definition was somewhat different with that in this study.

In this study, four QTL for estimated belly leanness content (EBLC) were detected on SSC1, 2, 8 and 17 with the QTL on SSC1 reaching genome-wide significant. This jointly explained for 10.46 % of the phenotypic variance in the F2 population (table 4.2). Pietrain showed more leanness for QTL on SSC1, 8 and SSC17; in contrast, Duroc had more leanness for QTL on SSC2.

4.3.10 QTL for dressing, food conversion ratio and food consumption

A suggestive QTL affected dressing (F = 6.33*) is at 50.8 cM on SSC7 (figure 4.11). Pietrain alleles have more dressing yield than Duroc alleles.
A suggestive QTL for food conversion ratio were detected at 0 cM on SSC2 (figure 4.6) and at 10.8 cM on SSC14 (figure 4.15). A suggestive QTL for food consumption were mapped at 25 cM on SSC14 (figure 4.15) and at 14 cM on SSC18 (figure 4.19).

4.3.11 Multiple QTL mapping

Cofactor analyses by model [1] and model [3] were performed and compared with the bidimensional search by model [2] or model [4]. In this section, also the following section 4.3.12 and section 4.3.13, the position was retained in cM Haldane in order to explain easily.

4.3.11.1 Multiple QTL mapping for shoulder BFT on SSC16

- Fitting the QTL at 98 cM (table 4.7, figure 4.20) as cofactor by model [1] to scan further, then another suggestive QTL was obtained at 0 cM (figure 4.22);
- Using of QTL at 0 cM as cofactor, a suggestive QTL was identified at 97 cM (figure 4.23);
- Fitting both QTL at 97 cM and 0 cM as cofactors together, no other suggestive QTL was detected;
- Since the position at 97 cM was not the same as the first result at 98 cM, we needed again use the QTL at 97 cM as cofactor to scan further, a suggestive QTL was still kept at the location of 0 cM (figure 4.24).

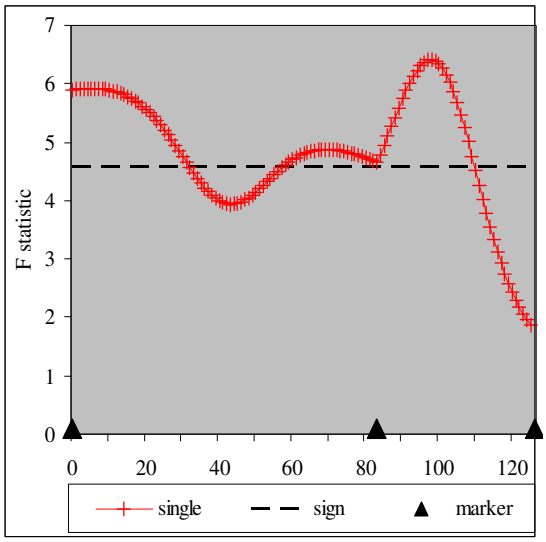

Figure 4.21: QTL for BFT-sh on SSC16.

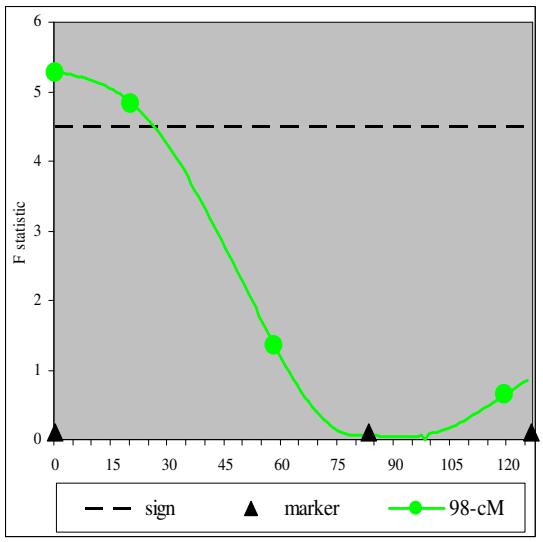

Figure 4.22: Cofactor at 98 cM.

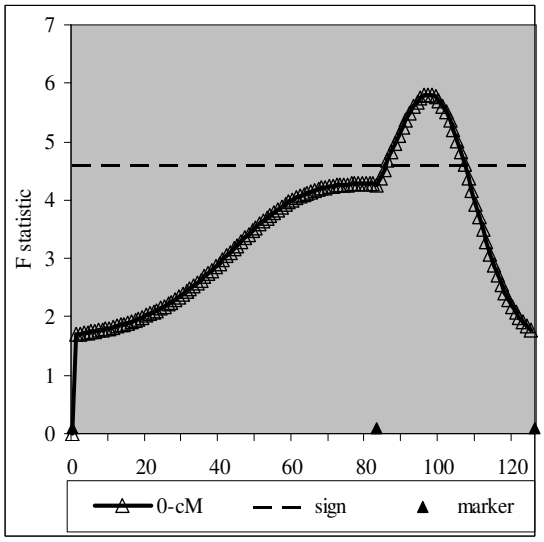

Figure 4.23: Cofactor at 0 cM.

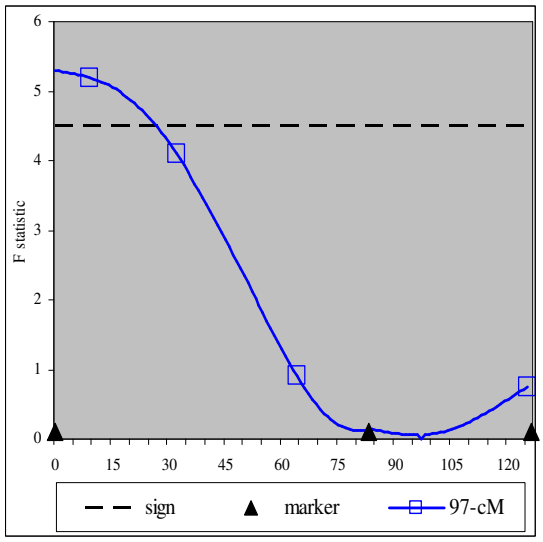

Figure 4.24: Cofactor at 97 cM.

Figure 4.21 ~ 4.24: cofactor analyses for shoulder fat on SSC16. The common legends are: the dashed line presents the suggestive significant threshold; the black triangle on X axis presents the position of markers in Haldane cM. The red cross curve shows the initial result of QTL at 98 cM by model [1] (figure 4.21); the green round curve shows the QTL at 0 cM by cofactor at 98 cM (figure 4.22); the white triangle curve presents the suggestive QTL at 97 cM by cofactor at 0 cM (figure 4.23); the blue square curve shows the QTL at 0 cM by cofactor at 97 cM (figure 4.24).

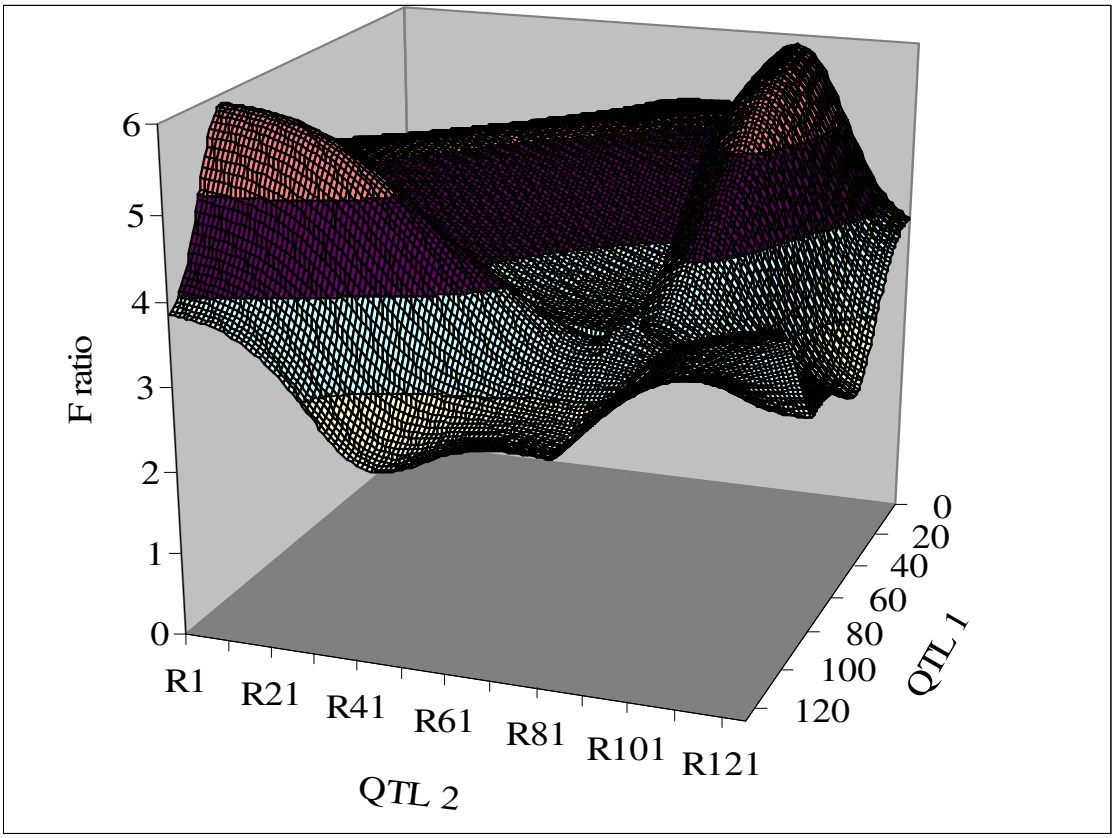

Figure 4.25: Bidimensional search results on SSC16 by model [2]. QTL_A at 97 cM; QTL_B at 0 cM, F_{4df} value = 5.9* (2 QTL vs 0 QTL); F_{2df} value = 5.29* (2 QTL vs 1 QTL) (table 4.7).

The bidimensional search was performed at 1-cM grid by model [2] (figure 4.25). The parameters by model [2] gave almost the same estimates in comparison with cofactor analyses. Only the dominance effect was small different (- 0.0853 vs. – 0.0849 from the table 4.7).

4.3.11.2 Multiple QTL mapping for LEA on SSC9

- When fitting the QTL at 101 cM (figure 4.26) as cofactor by model [1] to scan further, another suggestive QTL was obtained at 18 cM (figure 4.27);
- Using the QTL at 18 cM as cofactor, a suggestive QTL was located at 103 cM (figure 4.28);

- Fitting both QTL at 103 cM and 18 cM as cofactors, no further QTL was detected;
- As the position at 103 cM was not the same as result at 101 cM, we needed again use the QTL at 103 cM as cofactor to further scan; a suggestive QTL still remained at 18 cM (figure 4.29).

Table 4.7: Multiple QTL for LEA on SSC9 and shoulder BFT on SSC16

SSC	Trait[a]	QTL model[b]	F value[c]	Pos (CI)[d]	Add (SE)	Dom (SE)	% vars
9	LEA	Model [1]	7.87* (4.59)	101 (78~104)	-1.297 (0.352)	0.775 (0.692)	2.72
		Co. at 101 cM	4.55* (4.38)	18	-0.486 (0.312)	1.132 (0.542)	1.60
		Co. at 18 cM	5.05* (4.51)	103	-1.104 (0.363)	0.517 (0.686)	1.77
		Co. at 103 cM	4.60* (4.49)	18	-0.491 (0.311)	1.372 (0.542)	1.60
		Model [2]	F_{4df}=6.27*** F_{2df}=4.57*	QTL_1: 18 cM	-0.492 (0.312)	1.372 (0.540)	4.29
				QTL_2: 102 cM	-1.111 (0.365)	0.494 (0.692)	
16	BFT-sh	Model [1]	6.41* (4.59)	98	0.083 (0.034)	0.163 (0.060)	2.12
		Co. at 98 cM	5.28* (4.45)	0	0.049 (0.025)	-0.085 (0.034)	1.76
		Co. at 0 cM	5.80* (4.49)	97	0.070 (0.034)	0.162 (0.059)	1.92
		Co. at 97 cM	5.29* (4.45)	0	0.049 (0.025)	-0.085 (0.034)	1.76
		Model [2]	F_{4df}=5.9** F_{2df}=5.29*	QTL_1: 97 cM	0.070 (0.034)	0.162 (0.059)	3.84
				QTL_2: 0 cM	0.049 (0.025)	-0.085 (0.034)	

[a] the abbreviation of trait see the table 3.5. [b] the " Co. at 101 cM " means fitting the QTL at 101 cM as cofactor by model [1] to scan further. [c] F value with 4 df nominator came from model [2] vs. model without QTL; F value with 2 df nominator came from model [2] vs. model [1]. In the parenthesis was given significant threshold by 1,000 permutations which were advised by de Koning et al. (2001a). [d] position was in Haldane cM. Other categories have the same meanings as in table 4.3.

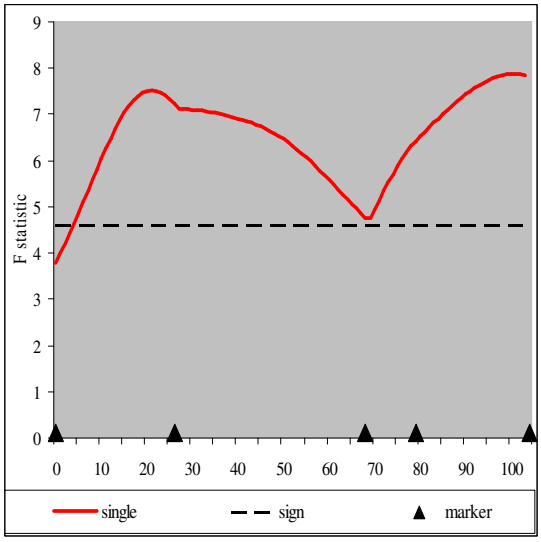

Figure 4.26: QTL for LEA on SSC9

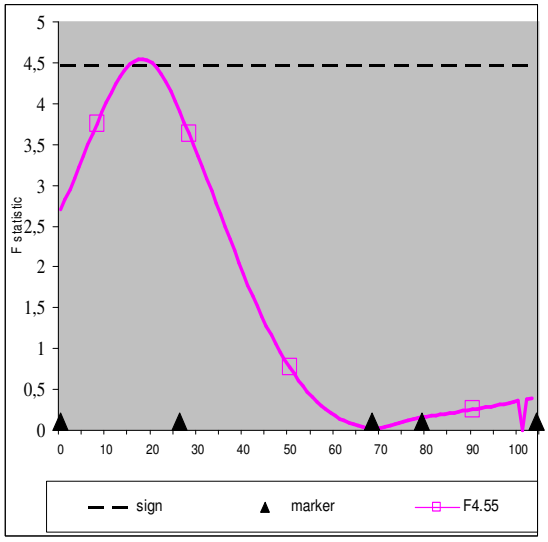

Figure 27: Cofactor at 101 cM.

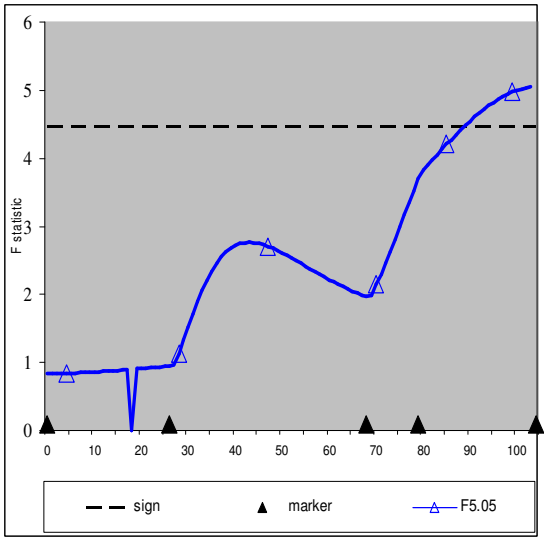

Figure 28: Cofactor at 18 cM.

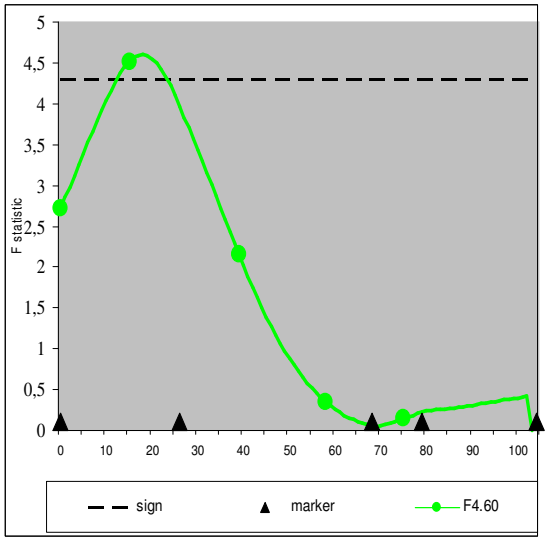

Figure 29: Cofactor at 103 CM

Figure 4.26 ~ figure 4.29: Cofactor analysis for loin eye area on SSC9. The common legends had the same meanings as that of figure 4.21 ~ 4.24. The red solid curve shows

the initial result of QTL at 101 cM by model [1] (figure 4.26); the red square curve shows the QTL at 18 cM by cofactor at 101 cM (figure 4.27); the blue triangle curve presents the suggestive QTL at 103 cM by cofactor at 18 cM (figure 4.28); the green round curve shows the QTL at 18 cM by cofactor at 103 cM (figure 4.29).

Table 4.8: Imprinted QTL for three fat traits on SSC2

Trait[a]	QTL model[b]	F value[c]	Pos[d]	Add[e] (SE)	Dom[e] (SE)	Impr[e] (SE)	% vars[f]
BFT-sh	Model [1]	5.09* (4.60)	92	-0.084 (0.026)	0.009 (0.042)	-	1.78
	Model [3]	3.80* (3.55)	10	-0.109 (0.039)	-0.024 (0.103)	-0.100 (0.043)	2.25
Side fat	Model [1]	6.66* (4.60)	88	-0.133 (0.037)	-0.051 (0.062)	-	2.31
	Model [3]	4.79* (3.60)	17	-0.158 (0.058)	-0.042 (0.167)	-0.184 (0.063)	2.50
Fat area	Model [1]	4.72* (4.60)	96	-0.396 (0.136)	0.204 (0.204)	-	1.65
	Model [3]	4.64* (3.70)	9	-0.569 (0.211)	0.539 (0.549)	-0.677 (0.233)	2.42

[a] The abbreviation of traits see table 3.5. [b] Models see section 3.2.9.4; [c] F value with 3 df in nominator came from model [3] vs. model without QTL. In the parenthesis was given significant threshold by 10,000 permutations. [d] Position was in Haldane cM. [e] Estimated imprinting effect is computed as the effects of paternal (Duroc) – maternal (Pietrain) alleles. Additive effect and dominance effect have the same meaning as before. Standard error (SE) is in the parentheses. [f] % variance = the fraction phenotypic variance explained by a QTL, as percentage of the residual variance in the F_2.

Bidimensional search was performed at 1-cM grid by model [2] (figure 4.30). The parameters by model [2] (table 4.7) gave almost the same results as in cofactor analyses, only the position of the second QTL had 1 cM difference (102 cM vs. 103 cM). Thomsen et al. (2004) mapped a suggestive QTL affecting loin eye area, its location was near that of our QTL_1.

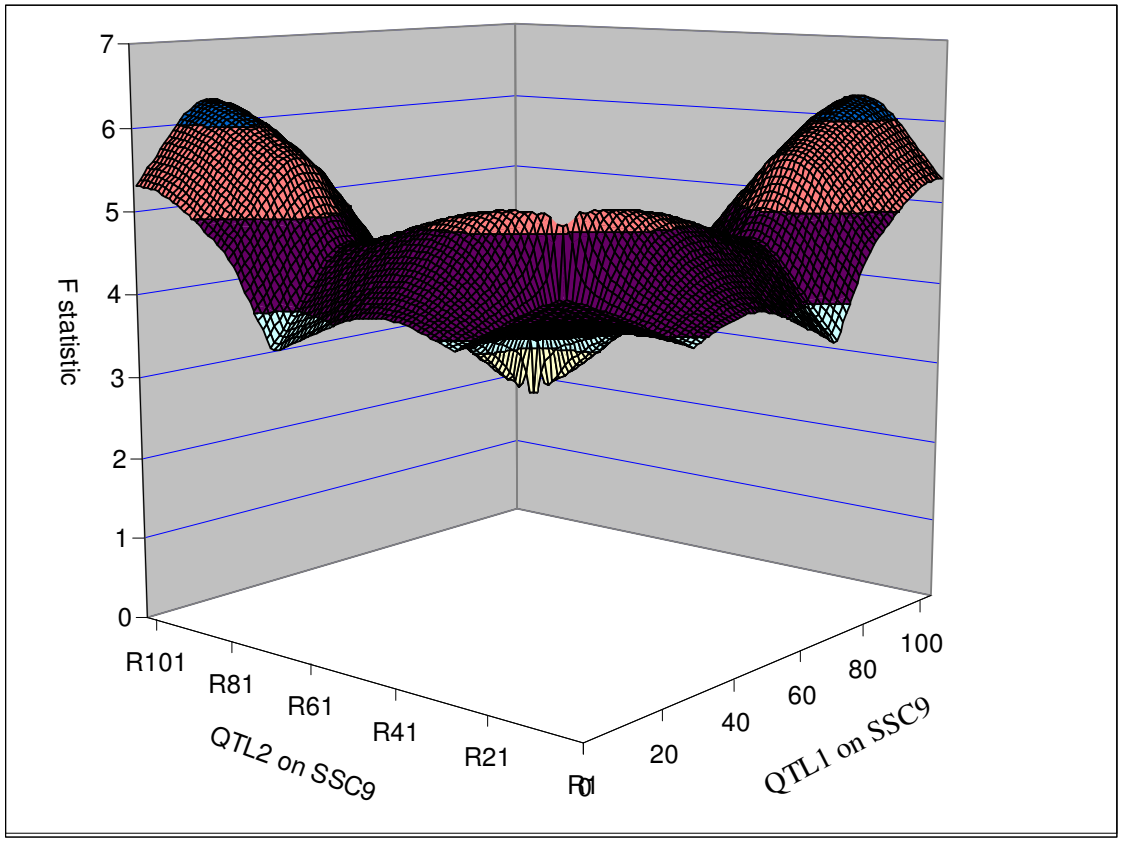

Figure 4.30: Bidimensional search results for LEA on SSC9 by model [2]. QTL_1 at 18 cM; QTL_2 at 102 cM, F_{4df} value = 6.27* (2 QTL vs 0 QTL; F_{2df} value = 4.57* (2 QTL vs 1 QTL) (table 4.7).

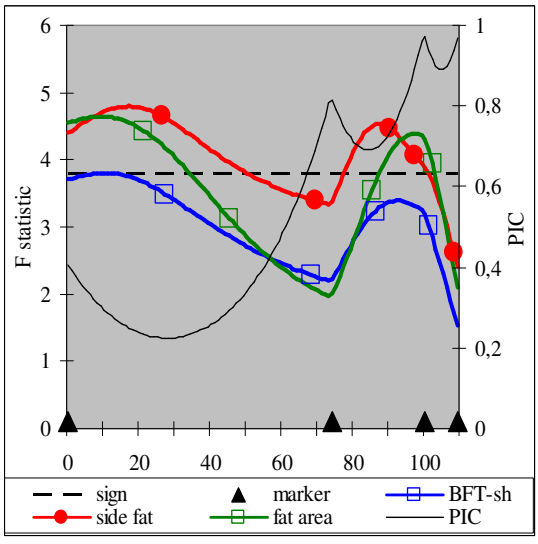

Figure 4.31: Imprinted QTL for three traits (side fat thickness, fat area, BFT at shoulder) on SSC2 by model [3] (table 4.8). The common legends had the same meanings as Figure 4.1 ~ 4.4.

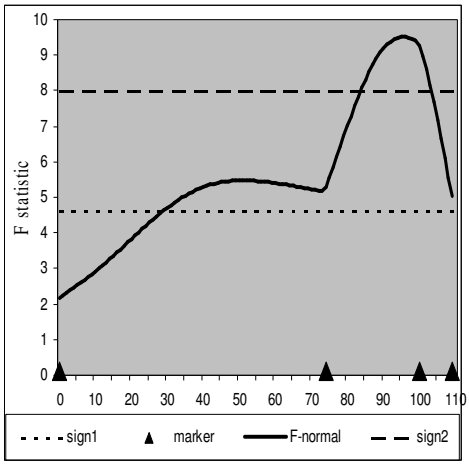

Figure 4.32: the peak of F plot curve (black) was at 96 cM by model [1]

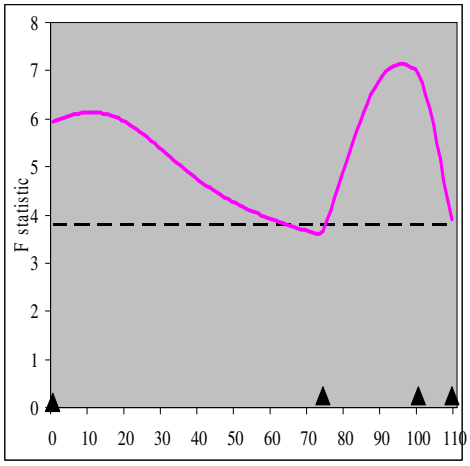

Figure 4.33: The peak of F plot curve (red) was at 95 cM by model [3].

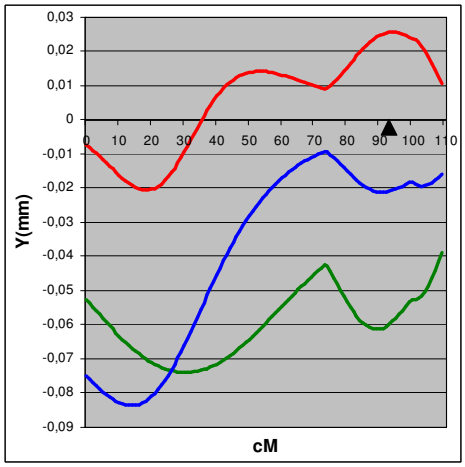

Figure 4.34: The distributions of imprinting, additive, dominance effects of trait F1314 on the whole SSC2, these were calculated by model [3]. The blue curve shows the imprinting effects; the green curve shows the additive effects; the red curve shows dominance effects. The black triangle shows the peak position of the QTL curve at 95 cM (table 4.9).

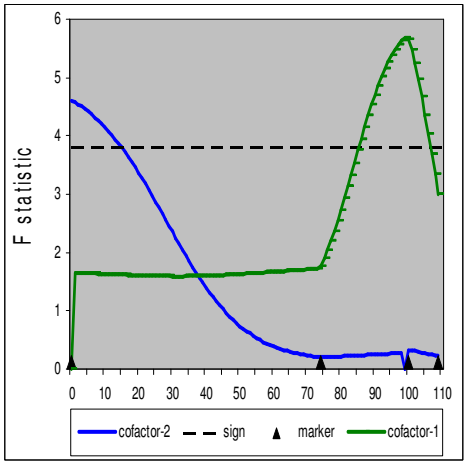

Figure 4.35: The results of cofactor analyses on SSC2. The blue curve shows the imprinted QTL at 0 cM by cofactor at 99 cM; the green curve shows the Mendelian QTL was at 99 cM by cofactor at 0 cM.

Figure 4.32 ~ 4.35 show the procedure of analyse imprinted QTL responsible for trait BFT at 13^{th} ~14^{th} rib (F1314). The common legends had the same meanings as figure 4.21 ~ 4.24.

Both examples from section 4.3.11.1 and section 4.3.11.2 provided a better explanation of the data than which by model [1]. This was in good agreement with Haley and Knott (1992), Martinez and Curnow (1992), Knott et al. (1997), Knott and Haley (1998).

4.3.12 Multiple QTL with imprinting on SSC2

As described in section 4.3.3.1 and section 4.3.9, a series of QTL in the distal of SSC2 has been detected for BFT at 13^{th} ~ 14^{th} rib, fat area, BFT at shoulder, side fat thickness, estimated carcass lean content and estimated belly leanness content. These QTL were derived by model [1], mean one Mendelian QTL model.

4.3.12.1 Simple case for three traits

When fitting model [3], suggestive imprinted QTL affecting three traits were directly obtained in the proximal region. These three traits were side fat, fat area and shoulder fat (figure 4.31 and table 4.8).

4.3.12.2 Expression of significant multiple QTL for other four traits

The trait BFT at 13^{th} ~14^{th} rib (F1314) had the other case than above three fat traits:

- By model [1], the peak of QTL curve was located at 96 cM (table 4.9, figure 4.32);
- Using model [3], the peak location of QTL curve was located at 95 cM (figure 4.33). However, the estimate effects of this location had no larger imprinting effects; the imprinting effect was obvious at approximal region (figure 4.34, table 4.9). The highest imprinting effects was at 14 cM, value was -0.0836;
- After cofactor analyses, a suggestive imprinted QTL was at 0 cM, meanwhile, another Mendelian expression QTL was at 99 cM (Figure 4.35, table 4.9).

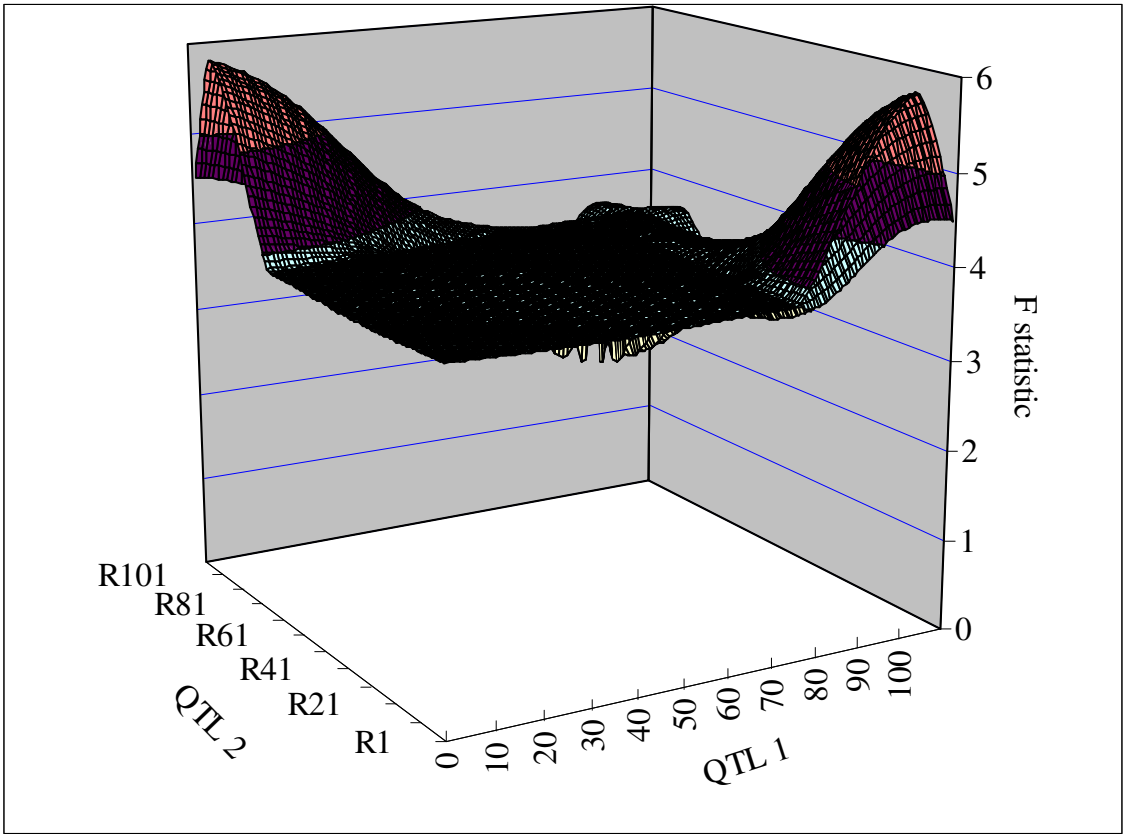

Figure 4.36: Two QTL for BFT at 13th ~14th rib on SSC2 by model [4].

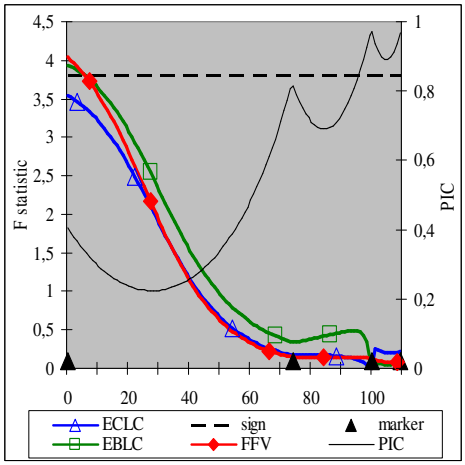

Figure 4.37: Imprinted QTL for EBLC, FFV and ECLC after cofactor analyses. Here did not show the Mendelian QTL as figure 4.35, the Mendelian QTL see table 4.9

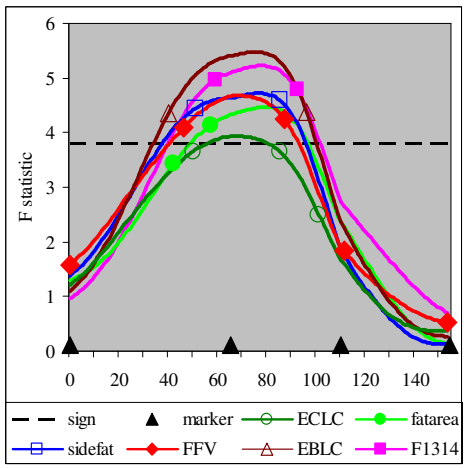

Figure 4.38: Imprinted QTL for six traits on SSC5

Table 4.9: Multiple QTL analyses for trait F1314, EBLC, FFV and ECLC on SSC2

Trait[a]	QTL model[b]	F value[c]	Pos[d]	Add[e] (SE)	Dom[e] (SE)	Imp[e] (SE)	% vars[f]
F1314	Model [1]	9.51** (4.61)	96	-0.057 (0.014)	0.025 (0.020)	-	3.27
	Model [3]	7.14** (3.67)	95	-0.058 (0.014)	0.026 (0.021)	-0.020 (0.013)	3.68
	Co. 95 cM by model [3]	4.20* (3.62)	0	-0.038 (0.019)	-0.006 (0.041)	-0.068 (0.021)	2.21
	Co. 0 cM by model [3]	5.69* (3.70)	99	-0.049 (0.013)	0.026 (0.018)	-0.012 (0.012)	2.97
	Co. 99 cM model [3]	4.61* (3.78)	0	-0.041 (0.019)	-0.005 (0.041)	-0.070 (0.020)	2.42
	Model [4]	F_{6df}=5.89*** F_{3df}=4.51*	QTL_1 : 99 cM	-0.049 (0.013)	0.026 (0.018)	-0.012 (0.012)	5.94
			QTL_2 : 0 cM	-0.041 (0.019)	-0.005 (0.041)	-0.070 (0.020)	
EBLC	Model [1]	7.82* (4.61)	95	0.588 (0.149)	-0.056 (0.227)	-	2.71
	Model [3]	5.45* (3.65)	94	0.600 (0.151)	-0.049 (0.235)	0.124 (0.146)	2.82
	Co. 94 cM by model [3]	3.64 (3.82)	0	0.479 (0.204)	0.004 (0.443)	0.620 (0.221)	ns
	Co. 0 cM by model [3]	4.15* (3.95)	100	0.485 (0.139)	-0.101 (0.193)	0.033 (0.134)	2.18
	Co. 100 cM by model [3]	3.94* (3.76)	0	0.517 (0.202)	0.006 (0.445)	0.630 (0.223)	2.07
	Model [4]	F_{6df}=4.65** F_{3df}=3.88*	QTL_1: 100 cM	0.485 (0.139)	-0.101 (0.193)	0.033 (0.134)	4.77
			QTL_2: 0 cM	0.517 (0.202)	0.006 (0.445)	0.630 (0.223)	

Table 4.9: Multiple QTL analyses for trait F1314, EBLC, FFV and ECLC on SSC2 (cont.)

Trait[a]	QTL model[b]	F value[c]	Pos[c]	Add (se)[e]	Dom (se)[e]	Imp (se)[e]	% vars[f]
FFV	Model [1]	8.23** (4.61)	95	-0.013 (0.003)	0.003 (0.005)	-	2.85
	Model [3]	6.02* (3.59)	95	-0.013 (0.003)	0.003 (0.005)	0.004 (0.003)	3.11
	Co. 95 cM by model [3]	3.96* (3.79)	0	-0.012 (0.004)	0.001 (0.010)	-0.013 (0.005)	2.08
	Co. 0 cM by model [3]	4.46* (3.86)	100	-0.011 (0.003)	0.003 (0.004)	0.002 (0.003)	2.34
	Co. 100 cM by model [3]	4.04* (3.75)	0	-0.013 (0.004)	0.000 (0.010)	-0.013 (0.005)	2.12
	Model [4]	F_{6df}=5.01*** F_{3df}=4.04*	QTL_1: 100 cM	-0.011 (0.003)	0.003 (0.004)	0.002 (0.003)	5.11
			QTL_2: 0 cM	-0.013 (0.004)	0.000 (0.010)	-0.013 (0.005)	
ECLC	Model [1]	9.14** (4.61)	97 cM	0.524 (0.123)	-0.080 (0.181)	-	3.15
	Model [3]	6.12** (3.84)	97 cM	0.525 (0.123)	-0.080 (0.181)	0.037 (0.119)	3.17
	Co. 97 cM by model [3]	3.33 (3.71)	0 cM	0.423 (0.173)	0.196 (0.379)	0.452 (0.188)	ns
	Co. 0 cM by model [3]	4.94* (3.75)	100 cM	0.452 (0.119)	-0.094 (0.165)	0.012 (0.115)	2.59
	Co. 100 cM by model [3]	3.55 (3.65)	0 cM	0.442 (0.172)	0.188 (0.380)	0.462 (0.190)	ns
	Model [4]	F_{6df}=4.85** F_{3df}=3.51	QTL_1: 100 cM	0.452 (0.119)	-0.094 (0.165)	-0.012 (0.115)	4.95
			QTL_2: 0 cM	0.442 (0.172)	0.188 (0.380)	0.462 (0.190)	

[b] "Co. 95 cM with imprinting" means QTL at 95 cM as cofactor by model [3] to scan further; also see text and see section 3.2.9.4; [c] In the parenthesis was given 5 % chromosomewide significant threshold by 1,000 permutations which advised by de Koning et al. (2001a). The F value with 6 df in nominator was by model [4] vs. model without QTL; the F value with 3 df in nominator is by model [4] vs. model [2]. [f] "ns" mean no significant. The other categories had the same meanings as in table 4.8.

Fitting the model [4], we found the identical results for trait F1314 on SSC2 when comparing with cofactor analyses under model [3] (table 4.9 figure 4.36), including the location and the estimates of effects. A suggestive QTL_1 affecting F1314 was at 99 cM, which expressed from Mendelian effects; another QTL_2 at 0 cM was mainly expressed from parent-of-origin effects.

Moreover, other three traits had the same case as BFT at 13^{th} -14^{th} rib. The three traits were ratio of fat area to meat area, estimated belly leanness content and estimated carcass leanness content (table 4.9, figure 4.37), but the QTL for the late trait (ECLC) was not reached the suggestive significant threshold.

After comparison of the results of those four traits (BFT at 13^{th} -14^{th} rib, fat area to meat area, estimated belly leanness content and estimated carcass leanness content (table 4.9), four conclusions could be drawn:

First, two QTL segregated on SSC2 and showed different expression: the QTL_1 was coming from mainly additive effects (Mendelian expression); QTL_2 was coming from parent-of-origin effects (imprinting expression);

Second, the locations of those 4 traits were the same: QTL_1 at 100 cM (besides the trait BFT at 13^{th} -14^{th} rib was at 99 cM); QTL_2 at 0 cM;

Third, the QTL_1 which account for F1314 and FFV were slightly favourable for Pietrain; in contrast, QTL_1 which accounted for leanness traits (ECLC and EBLC) major coming from Duroc alleles;

Fourth, the results by 2 QTL model were all identical with those by cofactor analyses, inclusive the estimates of position and effects. The identical results were also showed in the two examples of section 4.3.11. These identical results also demonstrated our thoughts in cofactor analyses (MQM) were likely correct. After identifying the 2^{nd} QTL (here we discuss the case of only two QTL segregating on a linkage group), we need to perform further cofactor analyses using the second QTL to re-identify the first QTL.

The final estimates of effects and position of a QTL could be derived by using another QTL as cofactor (see table 4.7, 4.9, 4.10), vice versa, $QTL_2 \leftrightarrow QTL_1$ reciprocally. Previous papers did not make the last step to re-detect the first QTL (e.g. de Koning et al. 2001a, Zhang et al. 2004, Szyda et al. 2005).

4.3.12.3 Further test statistic of imprinting effects

According to Knott et al. (1998), we have made a further test statistic which was imprinting model (model [3]) against Mendelian model (model [1]) so that test the parent-of-origin effects. The F values had 1 df in the nominator. The threshold was in table 3.6. When compared with standard F distribution for all traits, five traits showed obvious expression of parent-of-origin effects, besides the shoulder BFT (P = 0.021) and estimated carcass leanness content (P = 0.015, table 4.10).

Table 4.10: Test statistic for imprinting model versus Mendelian model on SSC2

Trait	Test model	F value (df = 1)	Imprinting	Paternal	Maternal
BFT-sh	Imprinting vs. Mendelian	5.36	-0.100	-0.209	-0.009
Side fat		8.46*	-0.184	-0.342	0.026
Fat area		8.47*	-0.677	-1.246	0.108
F1314		13.72**	-0.070	-0.111	0.029
FFV		7.92*	-0.013	-0.026	0.000
EBLC		8.45*	0.630	1.147	-0.113
ECLC		5.95	0.462	0.904	-0.020

F value with 1 df in the nominator came from model [3] vs. model [1]. The significant threshold levels were in table 3.6. The other categories had the same meanings as in table 4.8 and 4.9.

The imprinted QTL_2 affecting both fat traits and leanness traits were mainly paternal expression, but had opposite sign (table 4.10). These demonstrate which imprinted region has pleitropic effect with different regulation mechanism for fat trait meanwhile leanness trait. It was in good accordance with Jeon et al. (1999) and Nezer et al. (1999). They both found paternal expression for muscularity in the IGF2 region of chromosome 2 in pigs. Thomsen et al. (2004) obtained a numerous QTL with paternal expression for

backfat and loin muscle area in the distal region of chromosome 2, that were confirmed by our study.

We did not perform further significant analyses for paternal imprinting effects versus maternal imprinting effects like Thomsen et al. (2004), since the overall significant of these QTL did not seem so strong.

4.3.13 Imprinted QTL on SSC5

Table 4.11: Test statistic for imprinting model versus Mendelian model on SSC5

Trait	QTL model	F value (df = 3) [a]	Pos	Add (SE)	Dom (SE)	Imp (SE)	% vars	F value (df = 1) [b]
F1314	Model [3]	5.23* (3.77)	78	-0.02 (0.02)	-0.02 (0.03)	-0.06 (0.01)	2.72	14.17**
Side fat	Model [3]	4.72* (3.83)	76	-0.03 (0.038)	-0.08 (0.07)	-0.13 (0.04)	2.46	12.41*
Fat area	Model [3]	4.69* (3.88)	82	-0.03 (0.16)	-0.18 (0.30)	-0.61 (0.16)	2.34	13.21**
FFV	Model [3]	4.68* (3.93)	67	-0.002 (0.003)	-0.003 (0.004)	-0.01 (0.00)	2.44	13.12**
EBLC	Model [3]	5.48* (3.73)	75	0.08 (0.16)	0.252 (0.27)	0.63 (0.16)	2.85	15.58**
ECLC	Model [3]	3.94* (3.90)	67	0.10 (0.12)	0.05 (0.17)	0.38 (0.12)	2.07	10.98*

[a] F value with 3 df in the nominator came from model [3] vs. null QTL model. [b] F value with 1 df in the nominator came from model [3] vs. model [1]. The significant threshold levels were in table 3.6. The other categories had the same meaning as in table 4.8, 4.9 and table 4.11.

A series of suggestive imprinted QTL for fat traits and leanness content also appear on SSC5 at interval of 40~100 cM (marker interval: SW1482-IGF1) (table 4.12, figure 4.38). Similar to the results on SSC2, the imprinted QTL affecting both fat traits and leanness traits were mainly paternal expression; but that affecting fat traits had the opposite sign in comparison with which affecting leanness traits. These imprinted QTL

were not picked up by model [1] and showed higher significant effects when compared with model [1] (table 4.11).

4.3.14 Imprinted QTL on SSC6 and SSC11

Table 4.12: Test statistic for imprinting model versus Mendelian model on SSC6 and SSC11

Trait	QTL model	F value	Pos	Add (SE)	Dom (SE)	Imp (SE)	% vars	F value (df = 1)
F1314	Model [3]	3.87* (3.86)	145 (C6)	0.024 (0.013)	-0.002 (0.019)	-0.039 (0.013)	2.03	8.706*
F1314	Model [3]	4.43* (3.58)	71 (C11)	0.020 (0.016)	-0.023 (0.029)	-0.054 (0.016)	2.31	11.527*
EBLC	Model [3]	3.89* (3.88)	71 (C11)	-0.099 (0.174)	0.323 (0.312)	-0.547 (0.171)	2.04	10.241*
ECLC	Model [3]	3.90* (3.86)	138 (C6)	-0.069 (0.126)	0.240 (0.202)	0.407 (0.129)	2.04	9.884*

[a] Pos: position in Haldane cM. In the parentheses "C6"mean chromosome 6, "C11" mean chromosome 11. The other categories had the same meanings as table 4.12.

As shown in table 4.12, two suggestive imprinted QTL for fat thickness at 13^{th} ~ 14^{th} rib were detected on SSC6 and SSC11 and both showed maternal expression. One suggestive imprinted QTL for estimated belly lean content was identified at 71 cM on SSC11, meanwhile, a suggestive imprinted QTL for estimated carcass lean content was found at 138 cM on SSC6. When testing further the statistics for the parent-of-origin effects, the standard P value showed suggestive significant.

De Koning et al. (2000) have reported two imprinted linked QTL on SSC6 with different parental expressions affecting intramuscular fat content. The paternal expression QTL was near the region of the QTL affecting F1314 in the present study, however, our result showed maternal expression.

5 Summary and conclusion

We have detected a series of QTL segregating in the Duroc-Pitrain resource population. Some of the results obtained in this work were in agreement with previous findings and confirmed QTL regions which detected in other experimental crosses before. In most cases the effects of the putative QTL alleles met the specific features of the breeds, i.e. Pietrain alleles were associated with higher leanness content and lower fat content of the carcass, except for the fatness and carcass traits on SSC2.

We also uncovered some new QTL including the QTL for meat quality that were significant at genomewide level on SSC1. Two QTL for pH postmortem both in *m. long. dorsi* and in *m. semimembranosus* were exceeding the $P < 0.01$ genomewide critical value in the same location on SSC1. After the position has been refined, comparative mapping of this region will facilitate to get positional candidate genes and to determine whether the observed effect is due to a single gene or to a cluster of closely linked genes. No significant evidence was identified for drip loss on SSC1 in this study (almost reached the suggestive threshold, data not showed). A possible reason is that less F_2 animals for drip loss were available than for other meat quality traits (n = 342 vs. 599). The significant of this result might be improved by genotyping more F_2 animals and/or additional markers as demonstrated by Thomsen et al. (2004), or adding other new crosses with more informative meiosis such as increasing F_1 boars (Alfonso and Haley 1998, de Koning et al. 2002).

We have not observed strong statistic support for QTL affecting growth and fat deposition on SSC4, SSC6 and SSC7, which were amply reported in the exotic crosses by previous studies (Andersson et al. 1994; Wang et al. 1998; Pérez-Enciso et al. 2000; Bidanel et al. 2001). One possible explanation is that alleles are not fixed within the parental breed which causes a loss of power with regression methods (Andersson-Eklund et al. 1998), or the same alleles are fixed in both breeds.

QTL for fat traits were located at different region in comparison with those QTL for meat quality traits, which are in close agreement with other QTL data reported in divergent crosses (Andersson-Eklund et al. 1998; Ovilo et al. 2002; Varona et al. 2002, Evans et al. 2003) and in a purebred population (Vidal *et al.* 2005). On SSC1, eleven genome-wide significant QTL were detected for growth, fatness and leanness as well as meat quality traits. However, the confidence intervals of the QTL for these trait

complexes hardly overlap. Moreover, for fatness and leanness QTL reaching genome-wide significant were detected on SSC8, 16 and 17, where no QTL at all were found for growth and meat quality. This might be interpreted in the sense that meat quality, growth and carcass traits have a different genetic architecture though phenotypic correlation exists (Sellier et al. 1998; Huff-Lonergan et al. 2001).

In this Duroc-Pietrain population, the paternal QTL in the IGF2 region were in good agreement with previous studies, however, no evidence for parent-of-origin effects was obtained on growth performance or on meat quality. De Koning et al. (2000, 2001c) have reported a series of imprinted QTL affecting growth rate and meat quality such as color scores, pH value, drip loss, shear force and cooking loss in line-crossing of a Meishan and commercial Dutch F2 population. By further development of test approaches for detection of imprinted QTL, Thomsen et al. (2004) have found not only a series of higher genomewide QTL with paternal expression at the IGF2 loci, but also found two 5 % genomewide significant QTL with maternal expression for meat pH-value on different chromosomes. Moreover, our Duroc-Pietrain resource population also seemed not to be similar to Meishan and Large White population in France (Milan et al. 2002) which had no parent-of-origin effects at the IGF2 region. Milan et al. (2002) explained one possible reason could be that the imprinting effects are population (or haplotype) dependent.

As we know in human and mouse, most imprinted genes are arranged in chromosomal clusters, their linked organization suggests coordinated mechanisms controlling imprinting and gene expression (Morison and Reeve 1998, Constancia et al. 1998). In IGF2 cluster region there are not only imprinted gene expressions, but also biallelic expression. For instance, the human 11p15 imprinted domain which implicates the orthologous region in pigs is known to contain at least 9 imprinted transcripts, three of these are paternally expressed: LIT-1(KVLQT1-AS), IGF2, and IGF2-AS; six of these are maternally expressed; meanwhile contained at least 6 biallelic expression and 9 transcripts are not known or not precisely defined (Reik and Walter 2001). Comparison the results of human and mouse with the results in this study, also previous papers in pigs, could lead some futher topic of promising research.

References

Alfonso L and Haley CS (1998): Power of different F2 schemes for QTL detection in livestock. Anim Sci 66, 1-8

Alleman M and Doctor J (2000): Genomic imprinting in plants: observations and evolutionary implications. Plant Mol. Biol. 43,147-161

Andresen E and Baker LN (1964): the C blood group system in pigs and the detection and estimation of linkage between the C and J systems. Genetics 49, 379-386.

Andersson-Eklund L, Marklund L, Lundstrom K, Haley CS, Andersson K, Hansson I, Moller M, Andersson L (1998): Mapping quantitative trait loci for carcass and meat quality traits in a wild boar x Large White intercross. Journal of Animal Science 76, 694-700

Andersson L, Haley CS, Ellegren H, Knott SA, Johansson M, Andersson K, Andersson-Eklund L, Edfors-Lilja I, Fredholm M, Hansson I, Hakansson J, Lundstrom K (1994): Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science 263, 1771-1774

Archibald AL, Haley CS, Brown JF, Couperwhite S, McQueen HA, Nicholson D, W Coppieters W, Van de Weghe A, Stratil A, Wintero AK (1995): The PiGMaP consortium linkage map of the pig (*sus scrofa*). Mamm Genome 6, 157-175

Bates RO, Ernst CW, Doumit ME, Edwards DB, Raney NE (2003): Development of a Duroc x Pietrain swine resource population. Proc 28th National Swine Improvement Federation, Des Moines, IA, USA

Beeckmann P, Schröffel Jr J, Moser G, Bartenschlager H, Reiner G, Geldermann H (2003): Linkage and QTL mapping for *sus scrofa* chromosome 1. J Anim Breed Genet 120 (suppl1), 1-10

Benjamini Y and Hochberg Y (1995): Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B 57, 289-300

Bennewitz J, Reinsch N, Guiard V, Fritz S, Thomsen H, Looft C, Kühn C, Schwerin M, Weimann C, Erhardt G, Reinhardt F, Reents R, Boichard D, Kalm E (2004): Multiple quantitative trait loci mapping with cofactors and application of alternative variants of the false discovery rate in an enlarged granddaughter design. Genetics 168, 1019-1027

Bidanel JP, Milan D, Iannuccelli N, Amigues Y, Boscher MY, Bourgeois F, Caritez JC, Gruand J, Le Roy P, Lagant H, Quintanilla R, Renard C, Gellin J, Ollivier L, Chevalet C (2001): Detection of quantitative trait loci for growth and fatness in pigs. Genet Sel Evol 33, 289-309

Botstein D, White LR, Skolnick M, Davis RW (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32, 314-331

Braunschweig MH, Van Laere AS, Buys N, Andersson L, Andersson G (2004): IGF2 antisense transcript expression in porcine postnatal muscle is affected by a quantitative trait nucleotide in intron 3. Genomics 84, 1021-1029

Brockmann GA, Haley CS, Renne U, Knott SA, Schwerin M (1998): Quantitative trait loci affecting body weight and fatness from a mouse line selected for extreme high growth. Genetics 150, 369-381

Brockmann GA, Kratzsch J, Haley CS, Renne U, Schwerin M (2000): Single QTL effects, epistasis, and pleiotropy account for two-thirds of the phenotypic F2 variance of growth and obesity in DU6i × DBA/2 mice. Genome Res 10, 1941-1957

Brockmann GA, Haley CS, Wolf E, Karle S, Kratzsch J, Renne U, Schwerin M, Hoeflich A (2001): Genome-wide search for loci controlling serum IGF binding protein levels of mice. FASEB J 15, 978-987

Brockmann GA, Karatayli E, Haley CS, Renne U, Rottmann OJ, Karle S (2004): QTLs for pre and post weaning body weight and body composition in selected mice. Mamm Genome 15, 593-609

Carlborg Ö, Kerje S, Schutz K, Jacobsson L, Jensen P and Andersson L (2003): A global search reveals epistatic interaction between QTLs for early growth in the chicken. Genome Res 13, 413-421

Carlborg, Ö, Hocking PM, Burt DW and Haley CS (2004): Simultaneous mapping of epistatic QTL in chickens reveals clusters of QTL pairs with similar genetic effects on growth. Genet Res 83, 197-209

Churchill GA and Doerge RW (1994): Empirical threshold values for quantitative trait mapping. Genetics 138, 963-971

Constancia M, Pickard B, Kelsey G and Reik, W (1998): Imprinting Mechanisms. Genome Res 8, 881-900

Davis GH, Montgomery GW, Allison AJ, Kelly RW, Bray AR (1982): Segregation of a major gene influencing fecundity in progeny of Booroola sheep. New Zealand J Agric Res 25, 525-529

De Koning DJ, Janss LLG, Rattink AP, von Oers PAM, de Vries BJ, Groenen MAM, van der Poel JJ, de Groot PN, Brascamp EW, van Arendonk JAM (1999): Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs (*sus scrofa*). Genetics 152, 1679-1690

De Koning DJ, Rattink AP, Harlizius B, van Arendonk JAM, Brascamp EW, and Groenen MAM (2000): Genome-wide scan for body composition in pigs reveals important role of imprinting. Proc Natl Acad Sci USA 97, 7947-7950

De Koning DJ, Schulmant NF, Elo K, Moisio S, Kinos R, Vilkki J, Maki-Tanila A (2001a): Mapping of multiple quantitative trait loci by simple regression in half-sib designs. J Anim Sci 79, 616-622

De Koning DJ, Rattink AP, Harlizius B, Groenen MAM, van, E W Brascamp EW and Arendonk JAM (2001b): Detection and characterization of quantitative trait loci for growth and reproduction in pigs. Livest Prod Sci 72, 185-198

De Koning DJ, Harlizius B, Rattink AP, Groenen MAM, Brascamp EW, van Arendonk JAM (2001c): Detection and characterization of quantitative trait loci for meat quality traits in pigs. J Anim Sci 79, 2812-2819

De Koning, DJ, Bovenhuis H, van Arendonk JAM (2002): On the detection of imprinted quantitative trait loci in experimental crosses of outbred species. Genetics 161, 931-938

Dodgson JB, Cheng HH, Okimoto R (1997): DNA marker technology: a revolution in animal genetics. Poult Sci 76, 1108-1114

Donis-Keller H, Green P, Helms C, Cartinhour S, Weiffenbach B, Stephens K, Keith TP, Bowden DW, Smith DR, Lander ES (1987): A genetic linkage map of the human genome. Cell 51, 319-337

Echard G (1984): The gene map of the pig (*sus scrofa* domestica L) In: Genetic Maps, Vol3 Cold Spring Harbor Laboratory, New York, NY, 392-395

Ellegren H, Chowdharay BP, Johansson M (1994): a primary linkage map of the porcine genome reveals a low rate of recombination. Genetics 137, 1089-1100

Ellegren H (2004): Microsatellites: simple sequences with complex evolution. Nature Reviews Genetics 5, 435-445

Evans GJ, Giuffra E, Sánchez A, Kerje S, Dávalos G, Vidal O, Illán S, Noguera JL, Varona L, Velander I, Southwood OI, de Koning DJ, Haley CS, Plastow G, Andersson L (2003): Identification of quantitative trait loci for production traits in commercial pig populations. Genetics 164, 621-627

Falconer DS and Mackay TFC (1996): Introduction to quantitative genetics. Addison Wesley Longman, Harlow

Geldermann H, Muller E, Beeckmann P, Knorr C, Yue G, Moser G (1996): Mapping of quantative trait loci by means of marker genes in F2 generations of Wild boar, Pietrain and Meishan pigs. J Anim Breed Genet 113, 381-7

Geldermann H, Mueller E, Moser G, Reiner G, Bartenschlager H, Cepica S, Stratil A, Kuryl J, Moran C, Daoli R, Brunsch C (2003): Genom-wide linkage and QTL mapping in porcine F2 families generated from Pietrain, Meishan and Wild Boar crosses. J Anim Breed Genet 120, 363-393

Green E (2002): Comparative Sequencing of targeted regions in multiple vertebrates: reconnaissance for future genome explorations plenary lecture. Plant, Animal & Microbial Genome meeting, San Diego, Ca

Green P (1990): Document for CRI-MAP, version 24 Washington University School of Medicine, St Louis, MO, USA

Grindflek E, Szyda J, Liu Z, Lien S (2001): Detection of quantitative trait loci for meat quality in a commercial slaughter pig cross. Mamm Genome 12, 299-304

Grisart B, Farnir F, Karim L, Cambisano N, Kim J-J, Kvasz A, Mni M, Simon P, Frere J-M, Coppieters W, Georges M (2004): Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition. PNAS 101, 2398-2403

Grobet L, Martin LJ, Poncelet D, Pirottin D, Brouwers B, Riquet J, Schoeberlein A, Dunner S, Menissier F, Massabanda J, Fries R, Hanset R, Georges M (1997): A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nature Genetics 17, 71-74

Groenen MA, Crooijmans RP, Veenendaal A, Cheng H-H, Siwek M, van der Poel JJ (1998): A comprehensive microsatellite linkage map of the chicken genome. Genomics 49, 265-274

Haley CS, Knott SA (1992): A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69, 315-324

Haley CS, Knott SA, Elsen JM (1994): Mapping quantitative trait loci in crosses between outbred lines using least squares. Genetics 136, 1195-1207

Hawken RJ, Murtaugh J, Flickinger GH, Yerle M, Robic A, Milan D, Gellin J, Beattie CW, Schook LB, Alexander LJ (1999): A first generation porcine whole-genome radiation hybrid map. Mamm Genome 10, 824-830

Hearne CM, Ghosh S, Todd JA (1992): Microsatellites for linkage analysis of genetic traits. Trends Genet 8, 288-294

Heaton MP, Grosse WM, Kappes SM, Keele JW, Chitko-McKown CG, Cundiff LV, Braun A, Little DP, Laegreid WW (2001): Estimation of DNA sequence diversity in bovine cytokine genes. Mamm Genome 12(1), 32-37

Hoeschele I, Uimari P, Grignola FE, Zhang Q, Gage KM (1997): Advances in Statistical Methods to Map Quantitative Trait Loci in Outbred Populations. Genetics 147, 1445-1457

Hoeschele I (2003): Mapping quantitative trait loci in outbred pedigrees. In Handbook of Statistical Genetics (second edition) edited by Balding DJ, Bishop M and Cannings C. Wiley. 477-525

Holmberg M and Andersson-Eklund L (2004): Quantitative trait loci affecting health traits in Swedish dairy cattle. J Dairy Sci 87, 2653 - 2659

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

Hu ZL, Dracheva S, Jang W, Maglott D, Bastiaansen J, Reecy J, Rothschild M (2005): A quantitative trait loci resource and comparison tool for pigs: PigQTLDB. Abstract submitted to Plant and Animal Genome XIII, San Diego, CA, January 2005 http://wwwanimalgenomeorg/QTLdb/

Huff-Lonergan E, Baas TJ, Malek M, Dekkers JCM, Prusa K, Rothschild MF (2001): Correlations among selected pork quality traits. J Anim Sci 80, 617-627

Jansen RC (1992): A general mixture model for mapping quantitative trait loci by using molecular markers. Theor Appl Genet 85, 252-260

Jansen RC (1993): Interval mapping of multiple quantitative trait loci. Genetics 135, 205-211

Jansen RC and Stam P (1994): High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136, 1447-1455

Jansen RC (2003): Studying complex biological systems using multifactorial perturbation. Nature Rev Genet 4, 145-151

Jansen RC (2003): Quantitative trait loci in inbred lines. In Handbook of Statistical Genetics (second edition) edited by Balding DJ, Bishop M and Cannings C. Wiley. 445-476

Jeffreys AJ, Wilson V, Thein SL (1985): Hypervariable minisatellite' regions in human DNA. Nature 314, 67-73.

Jeon JT, Carlborg Ö, Törnsten A, Giuffra E, Amarger V (1999): Apaternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IFG2 locus. Nat Genet 21, 157-158

Jungerius B, van Laere AS, te Pas M, van Oost B, Andersson L, Groenen M (2005): The IGF2-intron 3-G3072A substitution explains a major imprinted QTL effect on backfat thickness in a Meishan x European white pig intercross. Genet Res 84, 95-101.

Kao C-H (2000): On the differences between maximum likelihood and regression interval mapping in the analysis of quantitative trait loci. Genetics 156, 855-865

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

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

Knott SA and Haley CS (1992): Maximum likelihood mapping of quantitative trait loci using full-sib families. Genetics 132, 1211-1222

Knott SA and Haley CS (1996): Methods for multiple-marker mapping of QTL in a halfsib population. Theor Appl Genet 93, 71-80

Knott SA, Neale DB, Sewell MM, Haley CS (1997): Multiple marker mapping of quantitative trait loci in an outbred pedigree of loblolly pine. Theor Appl Genet 94, 810-820

Knott SA and Haley CS (1998): Simple multiple marker sib-pair analysis for mapping quantitative trait loci. Heredity 81, 48-54

Knott SA, Marklund L, Haley CS, Andersson K, Davies W, Ellegren H, Fredholm M, Hansson I, Hoyheim B, Lundström K, MollerM, Andersson L (1998): Multiplemarker

mapping of quantitative trait loci in a cross between outbred Wild Boar and Large White pigs. Genetics 149, 1069-1080

Kruglyak L and Lander ES (1995): High-resolution genetic mapping of complex traits. Am J Hum Genet 56, 1212-1223

Landegren U, Kaiser R, Sanders J, Hood L (1988): A ligase-mediated gene detection technique. Science 241: 1077-1080.

Lander ES and Botstein D (1989): Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121, 185-199

Lander E and Kruglyak L (1995): Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11, 241-247

Lewis PO and Zaykin D (2002): Genetic Data Analysis: computer program for the analysis of allelic data, Version 1.1. Free program distributed by the authors over the Internet from http://lewis.eeb.uconn.edu/lewishome/software.html.

Lindblad-Toh K, Winchester E, Daly MJ, Wang DG, Hirschhorn JN, Laviolette JP, Ardlie K, Reich DE, Robinson E, Sklar P, Shah N, Thomas D, Fan JB, Gingeras T, Warrington J, Patil N, Hudson TJ, Lander ES (2000): Large-scale discovery and genotyping of single-nucleotide polymorphisms in the mouse. Nature Genet 24, 381-386

Ljungberg K, Holmgren S, Carlborg Ö (2004): Simultaneous search for multiple QTL using the global optimization algorithm DIRECT. Bioinformatics 20, 1887-1895

Lloyd VK, Sinclair DA, and Grigliatti TA (1999): Genomic imprinting and position-effect variegation in *Drosophila melanogaster.*. Genetics 151, 1503-1516

Lynch M and Walsh B (1998): Genetics and Analysis of Quantitative Traits. Sinauer Associates, Inc, Sunderland, MA. USA

Malek M, Dekkers JCM, Lee HK, Bass TJ and Rothschild MF (2001a): A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig I Growth and body composition. Mamm Genome 12, 630-636

Malek M, Dekkers JCM, Lee HK, Bass TJ, Prusa K, Huff-Lonergan E, and Rothschild MF (2001b): A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig II Meat and muscle composition. Mamm Genome 12, 637-645

Mangin B, Goffinet B, Rebai A (1994): Constructing confidence intervals for QTL location. Genetics 138, 1301-1308

Marklund L, Moller M, Hoyheim B, Davies W, Fredholm M, Juneja RK, Mariani P, Coppieters W, Ellegren H, Andersson L (1996): A comprehensive linkage map of the pig based on a Wild pig x Large White intercross. Anim Genet 27: 255-269

Martinez O and Curnow RN (1992): Estimating the locations and the sizes of the effects of quantitative trait loci using flanking markers. Theor Appl Genet 85, 480-488

Mikawa S, Akita T, Hisamatsu N, Inage Y, Ito Y, Kobayashi E, Kusumoto H, Matsumoto T, Mikami H, Minezawa M, Miyake M, Shimanuki S, Sugiyama C, Uchida Y, Wada Y, Yanai S, Yasue H (1999): A linkage map of 243 DNA markers in an intercross of Gottingen miniature and Meishan pigs. Anim Genet 30, 407-417

Milan D, Bidanel JP, Iannuccelli N, Riquet J, Amigues Y, Gruand J, Le Roy P, Renard C, Chevalet C (2002): Detection of quantitative trait loci for carcass composition traits in pigs. Genet Sel Evol 34, 705-728

Morison IM and Reeve AE (1998): A catalogue of imprinted genes and parent-of-origin effects in humans and animals. Hum Mol Genet 7, 1599-1609

Morison IM, Paton CJ, and Cleverley SD (2001): The imprinted gene and parent-of-origin effect database. Nucleic Acids Res 29, 275-276

Nagamine Y, Haley CS, Sewalem A, Visscher PM (2003): Quantitative trait loci variation for growth and obesity between and within lines of pigs (*sus scrofa*). Genetics 164, 629-635

Neimann-Sorenson A and Robertson A (1961): The association between blood groups and several production characteristics in the three Danish cattle breeds. Acta Agriculture Scandinavica 11, 163-196

Nei M (1987): Molecular evolutionary genetics. Columbia University Press, New York.

Nezer C, Moreau L, Brouwers B, Coppieters W, Detilleux J, Hanset R, Karim L, Kvasz A, Leroy P, Michel G (1999): An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. Nat Genet 21, 155-156

Nezer C, Moreau L, Wagenaar D, Georges M (2002): Results of a whole genome scan targeting QTL for growth and carcass traits in a Pietrain x Large White intercross. Genet Sel Evol 34, 371-387

O'Connell, Weeks (1998): PedCheck (version 1.1): A program for detecting marker typing incompatibilities in pedigree data. American Journal of Human Genetics 63, 259-266

Olsen HG, Gomez-Raya L, Vage DI, Olsaker I, Klungland H, Svendsen M, Adnoy T, Sabry A, Klemetsdal G, Schulman N, Kramer W, Thaller G, Ronningen K, Lien S (2002): A genome scan for quantitative trait loci affecting milk production in Norwegian dairy cattle. J Dairy Sci 85, 3124-3130

Olsen HG, Lien S, Svendsen M, Nilsen H, Roseth A, Aasland Opsal M, Meuwissen THE (2004): Fine mapping of milk production QTL on BTA6 by combined linkage and linkage disequilibrium analysis. J Dairy Sci 87, 690-698

Olsen HG, Lien S, Gautier M, Nilsen H, Roseth A, Berg PR, Sundsaasen KK, Svendsen M, Meuwissen THE (2005): Mapping of a milk production quantitative trait locus to a 420-kb region on bovine chromosome 6. Genetics 169, 275-283

Otto SP and Goldstein DB (1992): Recombination and the evolution of diploidy. Genetics 131, 745-751

Óvilo C, Clop A, Noguera JL, Oliver MA, Barragán C, Rodríguez C, Silió L, Toro MA, Coll A, Folch JM, Sánchez A, Babot D, Varona L, Pérez-Enciso M (2002): Quantitative trait locus mapping for meat quality traits in an Iberian ×Landrace F2 pig population. J Anim Sci 80, 2801-2808

Paszek AA, Wilkie PJ, Flickinger GH, Rohrer GA, Alexander LJ, Beattie CW, Schook LB (1999): Interval mapping of growth in divergent swine cross. Mamm Genome 10, 117-122

Pérez-Enciso M, Clop A, Noguera JL, Óvilo C, Coll A, MFolch J, Babot D, Estany J, Oliver AM, Diaz I, Sánchez A (2000): A QTL on pig chromosome 4 affects fatty acid metabolism: Evidence from and Iberian by Landrace intercross. J Anim Sci 78, 2525-2531

Quintanilla R, Milan D, Bidanel JP (2002): A further look at quantitative trait loci affecting growth and fatness in a cross between Meishan and Large White pig populations. Genet Sel Evol 34, 193-210

Rattink AP, de Koning DJ, Faivre M, Harlizius B, van Arendonk JAM, Groenen MAM (2000): Fine mapping and imprinting analysis for fatness trait QTLs in pigs. Mamm Genome 11, 656-661

Reed CA (1984): The beginnings of animal domestication In: Mason IL(Ed) Evolution of Domesticated Animals. Longman Group Ltd. New York. 1-6

Reik W and Walter J (2001): Genomic imprinting: parental influence on the genome. Nature Rev Genet 1, 21-32

Rettenberger G, Klett C, Zechner U, Kunz J, Vogel W, Hameister H (1995): Visualization of the conservation of synteny between humans and pigs by heterologous chromosomal painting. Genomics 26, 372-378

Rink A, Santchi EM, Eyer KM, Roelofs B, Hess M, Godfrey M, Karajusuf EK, Yerle M, Milan D, Beattie CW (2002): A first-generation EST RH comparative map of the porcine and human genome. Mamm Genome 13, 578-587

Rohrer GA, Alexander LJ, Keele JW, Smith TP, Beattie CW (1994): A microsatellite linkage map of the porcine genome. Genetics 136, 231-45

Rohrer GA, Alexander LJ, Hu Z, Smith TPL, Keele JW, Beattie CW (1996): A comprehensive map of the porcine genome. Genome Res 6, 371-391

Rohrer GA and Keele JW (1998a): Identification of quantitative trait loci affecting carcass composition in swine: I Fat deposition traits. J Anim Sci 76, 2247-2254

Rohrer GA and Keele JW (1998b): Identification of quantitative trait loci affecting carcass composition in swine: II Muscling and wholesale product yield traits. J Anim Sci 76, 2255-2262

Rohrer GA (2000): Identification of quantitative trait loci affecting birth characters and accumulation of backfat and weight in a Meishan-White Composite resource population. J Anim Sci 78, 2547-2553

Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, Sherry S, Mullikin JC, Mortimore BJ,D Willey L, Hunt SE, Cole CG, Coggill PC, Rice CM, Ning Z, J Rogers J, Bentley DR, Kwok PY, Mardis ER, Yeh RT, Schultz B, Cook L, Davenport R, Dante M, Fulton L, Hillier L, Waterston RH, McPherson JD, Gilman B, Schaffner S , Van Etten WJ, Reich D, Higgins J, Daly MJ, Blumenstiel B, Baldwin J, N Stange-Thomann N, Zody MC, Linton L, Lander ES, Altshuler D, International SNP Map Working Group (2001): A map of human genome sequence variation containing 142 million single nucleotide polymorphisms. Nature 409, 928-933

Sato S, Oyamada Y, Atsuji K, Nade T, Shin-ichi Sato, Kobayashi E, Mitsuhashi T, Nirasawa K, Komatsuda A, Saito Y, Terai S, Hayashi T, Sugimoto Y (2003): Quantitative trait loci analysis for growth and carcass traits in a Meishan × Duroc F2 resource population. J Anim Sci 81, 2938-2949

Sax K (1923): The association of size differences with seed-coat pattern and pigmentation in Phaseolus vulgaris. Genetics 8, 552-560

Schulman NF, Viitala SM, de Koning DJ, Virta J, Maki-Tanila A, Vilkki JH (2004): Quantitative trait loci for health traits in Finnish Ayrshire cattle. J Dairy Sci 87, 443-449

Seaton G, Haley CS, Knott SA, Kearsey M, Visscher PM (2002): QTL Express: mapping quantitative trait loci in simple and complex pedigrees. Bioinformatics 18, 339-340

Sellier P (1998): Genetics of meat and carcass traits. In The Genetics of the Pig, Ed: Rothschild MF and Ruvinsky A, CABI, New York. USA. 463-510

Soller M and Andersson L (1998): Genomic approaches to the improvement of disease resistance in farm animals. Scientific and Technical Review 17, 329-345

Southey BR and Fernando RL (1998): Controlling the proportion of false positives among significant results in QTL detection. Proceedings of the 6th World Congress of Genetics Applied to Livestock Production Armidale, NSW, Australia 26, 341-244

Su, YH, Xiong YZ, Jiang SW, Zhang Q, Lei MG, Zheng R, Deng CY (2004): Mapping quantitative trait loci for meat quality traits in a Large White x Meishan cross. Acta Genetica Sinica 31, 132-136

Szyda J, Liu Z, Reinhardt F, Reents R (2005): Estimation of quantitative trait loci parameters for milk production traits in german Holstein dairy cattle population. J Dairy Sci 88, 356-367

Thoday JM (1961): Location of polygenes. Nature 191, 368-370

Thomsen H, Lee HK, Rothschild MF, Malek M, Dekkers JCM (2004): Characterization of quantitative trait loci for growth and meat quality in a cross between commercial breeds of swine. J Anim Sci 82, 2213-2228

Tilghman SM (1999): The sins of the fathers and mothers: Genomic imprinting in mammalian development. Cell 96, 185-193

Varona L, Óvilo C, Clop A, Noguera JL, Pérez-Enciso M, Coll A, Folch JM, Barragán C, Toro MA, Babot D, Sánchez A (2002): QTL mapping for growth and carcass traits in an Iberian by Landrace pig intercross: Additive, dominant and epistatic effects. Genet Res 80, 145-154

Vidal O, Noguera JL, Amills M, Varona L, Gil M, Jiménez N, Dávalos G, Folch JM, Sánchez A (2005): Identification of carcass and meat quality quantitative trait loci in a Landrace pig population selected for growth and leanness. J Anim Sci 83, 293-300

Viitala SM, Schulman NF, de Koning DJ, Elo K, Kinos R, Virta A, Virta J, Maki-Tanila A, Vilkki JH (2003): Quantitative trait loci affecting milk production traits in Finnish Ayrshire dairy cattle. J Dairy Sci, 86, 1828-1836

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frizjers A, Pot J, Peleman J and Kuiper M (1995) ALFP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414

Walling GA, Archibald AL, Cattermole JA, Downing AC, Finlayson HA, Nicholson D, Visscher PM, Walker CA, Haley CS (1998): Mapping of quantitative trait loci on porcine chromosome 4. Anim Genet 29, 415-24

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

Weber JL and May PE (1989): Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44, 388-396.

Whittaker JC (2003): Marker-assisted selection and introgression. In Handbook of Statistical Genetics (second edition) edited by Balding DJ, Bishop M and Cannings C. Wiley. 554-574

Wood JD (1985): Consequences of changes in carcass composition of meat quality. In Recent Advances in Animal Nutrition (1-Ed) by Haresign W and Cole DJA. Butterworth. London. 157-166

Xiong M and Guo SW (1997): Fine-Scale Genetic Mapping Based on Linkage Disequilibrium: Theory and Applications. Am J Hum Genet 60, 1513-1531

Yu TP, Tuggle CK, Schimtz CB, Rothschild MF (1995): Association of PT1 polymorphisms with growth and carcass traits in pigs. J Anim Sci 73, 1282-1288

Yue G, Stratil A, Kopecny M, Schröffelova D, Schröffel Jr J, Hojny J, Cepica S, Davoli R, Zambonelli P, Brunsch C, Sternstein I, Moser G, Bartenschlager H, Reiner G, Geldermann H (2003a): Linkage and QTL mapping for *sus scrofa* chromosome 6. J Anim Breed Genet 120 (suppl1), 45-55

ZDS, Zentral Verband der Deutschen Schweineproduktion e. V (2003): Richtlinie fuer die Stationspruefung auf Mastleistung, Schlachtkoerperwert und Fleischbeschaffenheit beim Schwein. December 10 2003, Bonn, Germany.

Zeng Z-B (1993): Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc Natl Acad Sci USA 90, 10972-10976

Zeng Z-B (1994): Precision mapping of quantitative trait loci. Genetics 136, 1457-1468

Zhang C, de Koning DJ, Hernández-Sánchez J, Haley CS, Williams JL, Wiener P (2004): Mapping of multiple quantitative trait loci affecting bovine spongiform encephalopathy. Genetics 167, 1863-18

Appendix 1: 28 Multiplex PCR sets from 73 Microsatellites

Set number	Marker	SSC[a]	IRD[b]	Min ~ Max[c]	Type of PCR[d]
1	S0086	8	700	154 ~ 184	54TD
	S0300	6	800	124 ~ 126	54TD
	IGF1	5	700	223 ~ 237	54TD
2	S0102	7	700	123 ~ 143	56TD
	SW344	13	700	150 ~ 174	56TD
	SW61	8	700	236 ~ 266	56TD
3	SW703	11	700	126 ~ 140	56*42
	SWC27	14	700	148 ~ 164	56*42
	S0097	4	700	181 ~ 246	56*42
4	SW1515	1	700	115 ~ 143	60*42
	SW1851	1	700	81 ~ 97	60*42
	SW1301	1	800	144 ~ 176	60*42
5	SW857	14	700	144 ~ 160	58TD
	SW830	10	700	176 ~ 204	58TD
	S0111	16	800	150 ~ 176	58TD
6	S0007	14	700	174 ~ 200	56*42
	SW787	18	800	150 ~ 166	56*42
7	SW240	2	700	94 ~ 114	56*42
	S0109	9	700	139 ~ 145	56*42
	SW2570	3	700	152 ~ 178	56*42
8	S0003	6	700	131 ~ 167	54TD
	S0001	4	700	177 ~ 200	54TD
	S0214	4	800	124 ~ 160	54TD
9	SW193	6	700	101 ~ 109	57TD
	S0035	6	700	176 ~ 186	57TD
10	SW2008	11	700	91 ~ 101	54TD
	SWR414	18	700	138 ~ 158	54TD

Appendix 1: 28 Multiplex PCR sets from 73 MSs (cont.)

set	Marker	SSC[a]	IRD[b]	Min ~ Max[c]	Type of PCR[d]
11	S0064	7	700	93 ~ 160	60TD
	S0002	3	700	189 ~ 209	60TD
	S0355	15	700	245 ~ 271	60TD
12	SW840	17	700	121 ~ 137	56*42
	S0071	11	700	168 ~ 200	56*42
	SW175	7	800	102 ~ 126	56*42
13	SW936	15	700	94 ~ 112	58*42
	SW398	13	700	166 ~ 188	58*42
	S0227	4	700	231 ~ 256	58*42
	S0009	11	800	122 ~ 133	58*42
14	S0144	8	700	208 ~ 218	54TD
	SW1067	6	800	144 ~ 175	54TD
15	SW21	9	700	123 ~ 139	54TD
	S0025	7	800	105 ~ 152	54TD
16	SW2431	17	700	151 ~ 169	57TD
	S0101	7	700	204 ~ 224	57TD
17	S0061	16	700	167 ~ 187	56*42
	S0295	9	700	228 ~ 256	56*42
	SW874	12	800	191 ~ 219	56*42
18	SW1482	5	700	98 ~ 136	56*42
	S0005	5	700	203 ~ 248	56*42
19	SW54	9	700	112 ~ 124	60TD
	SW1111	15	700	165 ~ 181	60TD
	SW2611	8	800	145 ~ 181	60TD
20	SW335	17	700	100 ~ 112	48TD
	S0059	6	700	126 ~ 152	48TD
	SW967	5	800	95 ~ 115	48TD

Appendix 1: 28 Multiplex PCR sets from 73 MSs (cont.)

set	Marker	SSC[a]	IRD[b]	Min ~ Max[c]	Type of PCR[d]
21	SW834	2	700	84 ~ 124	53*42
	S0226	2	700	181 ~ 205	53*42
	SW911	9	800	151 ~ 173	53*42
22	S0164	3	700	93 ~ 160	60*42
	S0220	6	800	143 ~ 158	60*42
	S0070	10	800	268 ~ 299	60*42
23	SW1023	18	800	94 ~ 117	58*42
	S0087	6	700	165 ~ 218	58*42
24	SW1119	15	700	144 ~ 160	58*42
	S0115	7	700	189 ~ 207	58*42
25	S0026	16	700	92 ~ 106	58*42
	SW2443	2	800	200 ~ 214	58*42
	S0155	1	700	150 ~ 166	58*42
26	SW2490	12	700	120 ~ 176	60TD
	S0289 A	13	800	126 ~ 178	60TD
27	SW72	3	700	101 ~ 113	56TD
	SWR67	10	800	125 ~ 147	56TD
28	SW605	12	700	109 ~ 134	54TD
	S0219	13	700	154 ~ 180	54TD

[a] *sus scrofa* chromosome. [b] IRD meant type labelled. [c] Min ~ Max meant the minimum and maximum length of a microsatellite fragment in this DuPi population. [d] 54TD meant from 62ºC to 54ºC each cycle minus 1°C touch down PCR, total cycles is 42 inclusive touch down cycles; 58*42 meant 58ºC annealing temperature 42 cycles PCR.

Appendix 2: Alleles of 73 microsatellitesin the founder generation of Duroc and Pietrain

Marker locus	Duroc			Pietrain			Sum
	Male(4)[1]	Female (5)	Excl. alleles	Male(2)	Female(8)	Excl. alleles	
SW1515	115, 133, 135	131, 133, 135	3	127, 133, 140	133, 137, 140	3	7
SW1851	94	86, 92, 94	1	92, 94, 98	92, 94, 98	1	4
S0155	154, 160	154, 160	1	156, 160	148, 156	2	4
SW1301	150, 162, 164, 172	164, 172	2	162, 166	160, 162, 164, 166	2	6
SW2443	204, 210	204, 210	1	210	206, 208, 210	2	4
SW240	94, 100, 110	94, 100, 110	1	94, 108, 110	92, 94, 96, 110, 112	4	7
SW834	96, 104, 112, 114, 120	104, 112, 114	3	114, 118, 128	104, 114, 118, 126, 128	3	8
S0226	181, 195	181, 195	2	189, 203, 205, 214	183, 203, 205	5	7
SW72	101, 111, 113, 117	103, 111, 113	1	101	101, 111, 113	0	5
S0164	214, 218, 220, 232	214, 300	4	244, 268, 288, 292	220, 252, 262, 288, 292	6	11
Sw2570	176, 178	160, 176	0	176, 178, 180	154, 160, 176,178, 180	2	5
S0002	202, 208, 210	204, 208, 210	2	189, 200, 210	189, 208, 210	2	6
S0227	228, 252	228, 252	0	228	228, 252	0	2
S0001	182, 188	178, 182, 188	1	180, 182, 186	180, 182, 186, 188	1	5
S0214	126, 128, 130, 134	126, 128, 136	3	124, 136	124, 128, 136	1	6
S0097	205, 234,	205, 234	0	234, 236, 238	205, 234, 236, 238, 240	3	5
SW1482	110, 114, 140	110, 114	1	110, 138, 140	106, 110, 140	2	5
S0005	237, 239, 247, 265	203, 239, 245, 247, 249, 265	6	233, 235, 241	203, 233, 235, 241	3	10
IGF1	228, 232, 236	228, 230, 232	2	228, 232	228, 232, 234	1	5

Appendix 2: Alleles of 73 microsatellitesin the founder generation of Duroc and Pietrain (cont.)

Marker locus	Duroc			Pietrain			Sum
	Male (4)[1]	Female (5)	Excl. alleles	Male (2)	Female (8)	Excl. alleles	
SW967	93, 97, 103	93, 97, 103	2	93, 115	93, 113, 115	2	5
S0035	174, 178	174, 178	1	178, 180	176, 178, 180, 182	3	5
S0087	180, 182	180, 210	2	165, 180	165, 180	1	4
SW1067	164, 170	164, 170	1	154, 172, 176	164, 168, 176	4	6
SW193	103	103	0	109	103, 109	1	2
S0300	124, 126	124, 126	0	124	124, 126, 132	1	3
S0220	147, 154	147, 154	1	145, 147, 150	145, 147, 152,	3	5
S0059	132, 150, 152	132, 150	1	150, 154	148, 150, 152	2	5
S0003	131, 145, 159	131, 145, 159	1	161, 163	131, 159, 161, 163	2	5
S0025	118	112, 118, 152	2	112	112, 114	1	4
S0064	102, 112, 114, 149	102, 112, 114, 149	2	92, 102, 112	92, 95, 102, 112	2	6
S0102	122, 134	122, 134, 136, 138, 140	3	130	124, 130, 136, 140	2	7
SW175	104, 128, 130	104, 130	1	104, 126, 128	104, 126, 128	1	4
S0115	191, 201, 205	191, 201, 205, 207, 209,	3	205, 207	193, 205, 207	1	6
S0101	208, 210	208, 210, 214	1	210, 212, 214	210, 212, 214	1	4
SW2611	175, 177, 179	171, 175, 177, 179	0	167, 177, 179	171, 175, 177, 179	1	5
S0086	154, 160, 164	154, 160	3	180	178, 180	2	5
S0144	212, 216, 218	216, 218	1	218	212, 218	0	3
SW61	236, 242, 248, 250, 256	236, 242, 248, 250, 256	4	238, 256, 264	238, 240, 256, 260	4	8

Appendix 2: Alleles of 73 MSs in the founder generation of Duroc and Pietrain (cont.)

Marker locus	Duroc			Pietrain			Sum
	Male (4)[1]	Female (5)	Excl. alleles	Male (2)	Female (8)	Excl. alleles	
SW21	126, 137	126, 137	0	126, 135, 137	126, 135, 137	1	3
SW911	159	153, 159	1	149, 159	155, 159, 165, 167	4	6
SW54	108, 124	108, 120, 124	1	115, 120	108, 115, 120	1	4
S0109	138, 142	138, 142	0	138, 142	138, 142	0	2
S0295	230, 232	230, 232	0	230, 232	226, 230, 232	1	3
SW830	180, 182	180	0	178, 180, 182	178, 180, 184	2	4
S0070	285	283, 285	2	267, 273, 281	264, 271, 273, 281	5	7
SWR67	125, 127	125, 127	0	125, 127	125, 127	0	2
SW2008	101, 103	87, 101, 103	1	101, 105	101, 103, 105	1	4
S0071	185, 190, 194	185, 190, 194	1	181, 183, 185, 190	181, 185, 190	2	5
S0009	126, 130	130	0	124, 130	124, 126, 130, 132	2	4
SW703	130, 140	130, 140	0	130, 134	130, 134, 140	1	3
SW2490	120, 158, 162, 166	120, 162, 166, 172	3	120, 156, 166	120, 148, 156	2	6
SW874	191, 197, 203, 205	191, 197	3	205, 209	205, 209	1	5
SW605	120, 132	120	0	120, 132	120, 122, 132	1	3
S0219	160, 172	160, 172	1	158, 160	158, 160	1	3
SW344	158, 172, 176	158, 170	2	158, 176	158, 162, 176, 183	2	6
SW398	165, 176	165, 176	1	178, 188, 190	176, 178, 188, 190	3	5
S0289	134, 140, 178	132, 134, 140	1	140, 178	134, 138, 140, 178	0	5
SW857	152, 156	152, 156	1	150, 152, 154	142, 150, 152, 154	3	5
S0007	158, 180, 196	158, 174, 180, 196	3	186, 188	180, 186, 188, 190, 198	4	8
SWC27	160, 164	160	1	160, 162	148, 160, 162	2	4

Appendix 2: Alleles of 73 microsatellites in the founder generation of Duroc and Pietrain (cont.)

Marker locus	Duroc			Pietrain			Sum
	Male(4)[1]	Female (5)	Excl. alleles	Male (2)	Female (8)	Excl. alleles	
S0355	243, 245	243	1	243, 247, 257, 270	243, 247, 257	3	5
SW1111	165, 173	165, 173, 175, 179	2	167, 173, 179	173, 177, 179, 185	3	7
SW936	*93, 102, 109*	*102, 109*	*2*	*95, 109*	95, 109, 115	*2*	5
SW1119	152, 156	156	0	148, 156	148, 150, 152, 156	2	4
S0111	152, 160, 162	152, 158, 162	2	154, 160	152, 154, 160, 180	2	6
S0026	98	94, 98, 104	1	96, 98, 102	94, 96, 98	2	5
S0061	167, 179, 181, 187	167, 179, 181, 187	1	167, 179, 181	167, 179, 181	0	4
SW335	100, 108, 110	100, 108	1	110	108, 110	0	3
SW840	128	128	0	128	120, 128	1	2
SW2431	159, 161	159, 161	1	161	155, 161	1	3
SW1023	93, 117, 132	93, 117, 132	1	115, 117,	93, 111, 117	2	5
SW787	152, 154, 156, 160	152, 154, 156, 158, 160	3	154, 156, 162	154, 162	1	6
SWR414	146, 148	146	1	148, 154	154, 158	2	4
sum			101			137	360
%			28.1%			38.1%	

[1]numbers in the parentheses are founder animals used to generate the F_1 animals. “excl. alleles” means: exclusive alleles in Duroc or Pietrain.

Appendix 3: Heterozygosity (He) and polymorphism information content (PIC) in F_1 animals

SSC	Locus	Alleles (F_1)	He		PIC		informative meioses	
				Average		Average		Average
1	S0155	4	0.906	0.852	0.625	0.594	2053	1830
	SW1301	6	0.938		0.692		2021	
	SW1515	7	0.750		0.530		1754	
	SW1851	4	0.813		0.530		1491	
2	SW2443	4	0.469	0.774	0.382	0.641	930	1697
	SW240	7	0.688		0.603		1657	
	SW834	7	0.938		0.811		2054	
	S0226	6	1.000		0.766		2146	
3	SW72	5	0.813	0.766	0.540	0.636	1932	1510
	S0164	10	1.000		0.792		2120	
	SW2570	5	0.531		0.626		626	
	S0002	5	0.719		0.585		1361	
4	S0001	4	0.813	0.665	0.569	0.517	1976	1423
	S0097	5	0.688		0.560		1077	
	S0214	6	0.813		0.694		1703	
	S0227	2	0.344		0.244		937	
5	SW1482	4	0.781	0.734	0.589	0.601	1905	1707
	S0005	9	1.000		0.849		2088	
	IGF1	5	0.625		0.530		1327	
	SW967	5	0.531		0.456		1508	
6	S0003	5	0.781	0.711	0.756	0.559	1711	1582
	S0035	5	0.750		0.593		1733	
	S0059	5	0.594		0.472		1182	
	S0087	4	0.813		0.505		1949	
	S0220	4	0.750		0.627		1603	
	S0300	3	0.594		0.509		1392	
	SW1067	6	0.938		0.713		2021	
	SW193	2	0.469		0.294		1067	
7	S0025	5	1.000	0.860	0.580	0.650	2154	1896
	S0064	6	0.750		0.718		1624	
	S0102	7	1.000		0.742		2162	
	SW175	4	0.938		0.679		2067	
	S0115	6	0.625		0.522		1430	
	S0101	4	0.844		0.658		1939	
8	S0086	4	1.000	0.735	0.579	0.581	2164	1602
	S0144	3	0.219		0.332		683	
	SW2611	4	0.750		0.567		1484	
	SW61	9	0.969		0.845		2079	

Appendix 3: Heterozygosity (He) and polymorphism information content (PIC) in F_1 animals (cont.)

SSC	Locus	Alleles (F_1)	He		PIC		informative meioses	
				Average		Average		Average
9	SW21	3	0.813	0.613	0.468	0.449	1947	1332
	SW911	6	0.688		0.551		1303	
	SW54	4	0.781		0.599		1543	
	S0109	2	0.438		0.323		562	
	S0295	3	0.344		0.304		1305	
10	S0070	7	1.000	0.812	0.716	0.541	2124	1667
	SWr67	2	0.656		0.375		1387	
	SW830	4	0.781		0.531		1490	
11	SW2008	4	0.813	0.844	0.599	0.626	1787	1724
	S0071	5	0.969		0.694		2147	
	S0009	4	0.719		0.629		1479	
	SW703	3	0.875		0.583		1485	
12	Sw2490	6	0.906	0.677	0.707	0.536	1998	1441
	SW874	4	0.875		0.689		1549	
	SW605	3	0.250		0.213		776	
13	S0219	3	0.719	0.672	0.423	0.510	1132	1369
	SW344	5	0.406		0.361		920	
	SW398	5	0.938		0.713		2076	
	S0289	6	0.625		0.544		1349	
14	S0007	9	1.000	0.688	0.825	0.571	2154	1426
	SW857	5	0.875		0.718		1542	
	SWC27	4	0.188		0.170		582	
15	S0355	4	0.906	0.797	0.560	0.589	1799	1781
	SW1111	7	0.969		0.784		2141	
	SW936	5	0.656		0.504		1656	
	SW1119	5	0.656		0.509		1530	
16	S0026	5	0.906	0.896	0.552	0.683	1921	1968
	S0061	4	0.813		0.647		1894	
	S0111	6	0.969		0.849		2091	
17	SW335	3	0.844	0.500	0.554	0.354	1566	978
	SW840	2	0.219		0.176		302	
	SW2431	3	0.438		0.333		1066	
18	SW1023	5	0.719	0.896	0.634	0.664	1731	2006
	SW787	5	1.000		0.707		2158	
	SWR414	4	0.969		0.651		2129	

Acknowledgments

I would like to express my sincere gratitude and greatest appreciation to my supervisor Mr. Prof. Dr. Karl Schellander, Animal Breeding and Hunsbadry Group from the Institute of Animal Science of University of Bonn, for offering me the opportunity to pursue a PhD degree, for his invaluable advices during the realization of this study. Prof. Schellander always encourages and supports me, especially in the difficult period of this study, gives me momentums to get further progress; pays great attention to my study and life in this Institute, solves my personal and technical problems worth of more mention; Prof. Schellander also has great patience for me. Here is lack of words to express my deepest thanks to Prof. Schellander.

I am also very grateful to Prof. Dr. Dr. agr. Helga Sauerwein, Physiology and Hygiene group from the Institute of Animal Science of University of Bonn, for her willingness to be a co-supervisor in this study.

There were larger difficulties at the beginning of my PhD study, not only language, but also laboratory work. Ms. Tina Kleinwächter guided me for the whole process of laboratory work. Without her help, I could not fulfill my PhD study.

About the work with this resource population, there were two difficulties, one was disappearance of the animal samples, needed to look for; another was that some critical samples were wrong, needed to identify which is correct. In these procedures of one year, I would to thank very much Dr. Heinrich Juenst, Dr. Klaus Wimmers, Mr. Ludger Buschen, Ms. Tina Kleinwächter and Ms. Türlay Türeyenler.

Regarding the DNA isolation from more than thouthand of tissue samples, my grateful thanks go to Ms. Steffi Heussner, Ms. Katrin Bauch, Ms. Nadine Leyer, Ms. Angelika Griep and Mr. Stefan Künne for a plenty of their collaboration.

For the data analyses, I would like to thank very very much Mr. Heinz-Josef Schreimachers. When I asked questions, he always quickly answered, gave me much patience. Without his help, I would be now unable to realize my PhD study.

I owe a heavy debt of gratitude and sincere thanks to PD Dr. Klaus Wimmers and PD. Dr. Siriluck Ponsuksili Wimmers, for their professional and technical advice.

My grateful thanks go to Dr. Ernst Tholen for his valuable advices, insightful comments and unlimited help for the difficulties of statistics analyses and correction of this manuscript.

By this opportunity, my grateful appreciation should be to Dr. DJ de Koning in Roslin Institute for his unforgettable advices and help, especially for analyses of imprinting effects in this thesis. Dr. Eduard Muráni had great contributions of this study. I also thank Dr. Wenhua Wei for his insightful comments.

I thank Dr. Danyel Jennen for support of this work after his coming into institute. My special thanks go to Dr. Dawit Tesfaye, Dr. Nares Trakooljul, Dr. Ali Kadanga, Mr. Heiko Buschbell, Mr. Patricio Ponce Barajas, Ms Elisabath Jonas, Ms. Anke Brings, Mr. Chirawath Phatsara and Mr. Lei Zhang as well as the other people, who are not mentioned here, for their valuable discussions and unforgettable help. I am very grateful to all the members of the administration, especially Mrs Bianca Peters, Mrs. Nicole Diel, Mrs Ulrike Schröter and Mr. Peter Müller.

I am very grateful to the Catholic Academic Exchange Service (KAAD, Germany) for the financial support during the first four years of my PhD study in Germany.

I deeply thank my parents who both passed away during the final phase of my study, all members of my family for their encouragement and inspiration in any time and support me to concentrate on my study in Germany. May their soul rest in peace.

In general, my special thanks to the supervision of Prof. Schellander, help of all people who have contributed in one way or the other for the realization of this study. So, if it could be said I have got any progress, it should been attributed to all people of above.

Curriculum vitae

Name:	Guisheng Liu
Date of birth:	18 February 1964
Place of birth:	Wuhan, Hubei Province, P. R. China
Sex:	male
Nationality:	P. R. China
Email	liuguish@yahoo.com

Education and Professional Experiences

1981 - 1985:	Bachelor in Animal Husbandry, Huazhong Agricultural University, 430070 Wuhan, China
1985 - 1988:	Master degree in Animal Genetics and Breeding, Huazhong Agricultural University, 430070 Wuhan, China
1988 - 1994:	Lecture in the Faculty of Animal Science and Veterinary Technology, Huazhong Agricultural University, 430070 Wuhan China
1994-1998:	Manager worked about animal food technology and technical service after sale, the administration in Wuhan Chiatai Co. (group), China
1998 - 1999:	Learned Germany course in Tongji Medicine University, Wuhan, China
1999 - 2000:	Advance Studies about Molecular Genetics, Molecular Biology and Advance English in Wuhan University, China
2000 - 2000:	Intensive course of Germany for six months, Bonn, Germany
2000 – Oct. 2005:	PhD studies in Molecular and quantitative Genetics. Institute of Animal Science, University Bonn