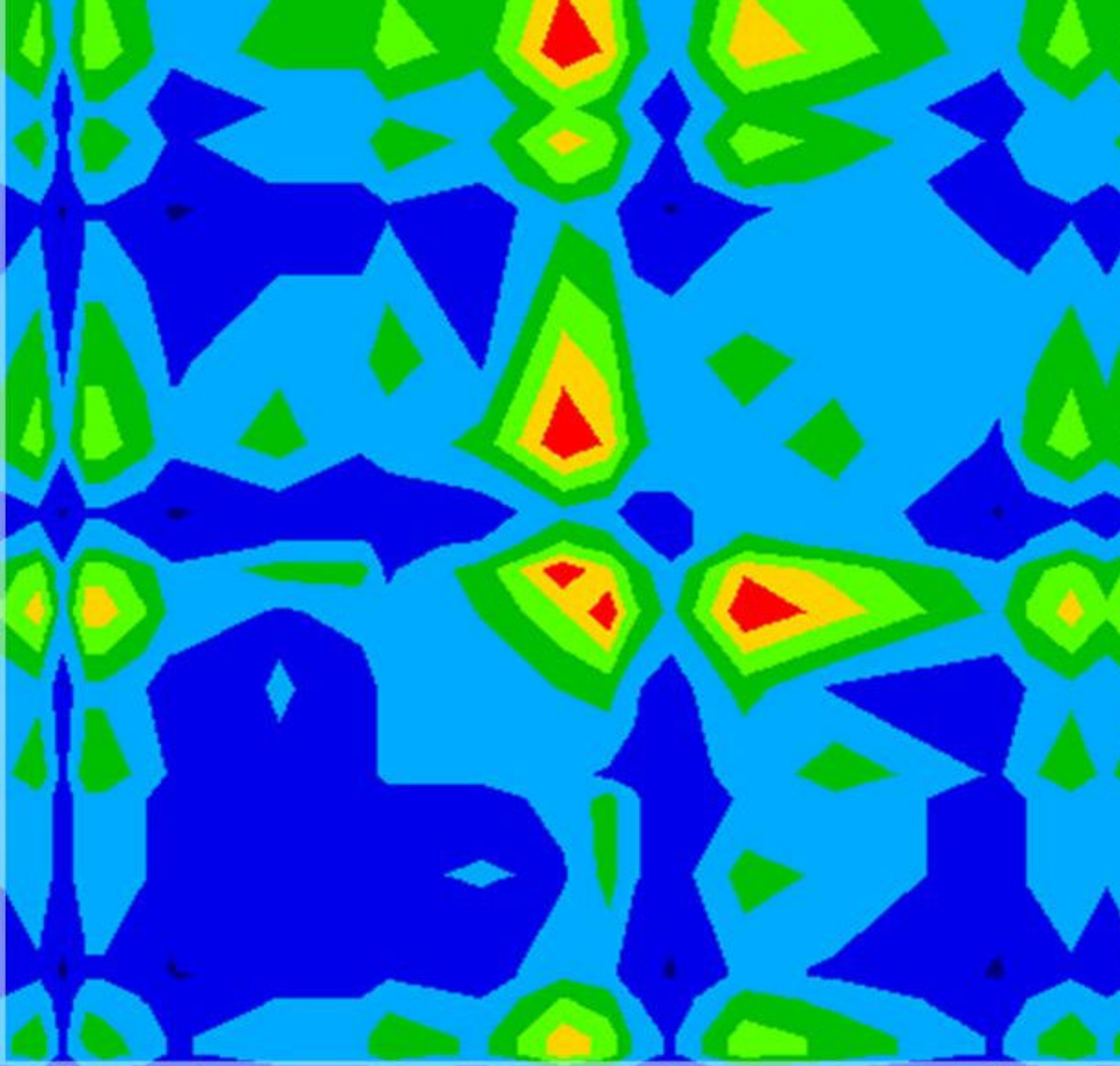

The Genetics of Pork Quality

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Promotor: **Prof. dr. M.A.M. Groenen**
Persoonlijk hoogleraar bij de leerstoelgroep Fokkerij en Genetica, Wageningen Universiteit

Co-promotor: **Dr. ir. H. Bovenhuis**
Universitair docent bij de leerstoelgroep Fokkerij en Genetica, Wageningen Universiteit

Promotiecommissie:
Prof. dr. F.A. van Eeuwijk
Wageningen Universiteit
Prof. dr. R.G.F. Visser
Wageningen Universiteit
Dr. ir. J.W. Merks,
Institute for Pig Genetics B.V., Beuningen
Prof. dr. L.B. Schook
University of Illinois at Urbana-Champaign, USA

The Genetics of Pork Quality

Henricus Johannes van Wijk

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The Genetics of Pork Quality

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H.J. van Wijk
Institute for Pig Genetics B.V., Beuningen, The Netherlands, and
Animal Breeding and Genetics Group, Wageningen University, Wageningen, The Netherlands

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Abstract: This thesis describes the genetics of carcass composition and pork quality traits. A large population of commercial finishers was extensively phenotyped for growth, carcass composition and meat quality traits. Genetic parameters were estimated based on those measurements. The population was genotyped using 73 microsatellite markers covering approximately 50 percent of the genome. The covered genome regions were pre-selected based on published QTL for carcass composition and meat quality traits that were mainly obtained from divergent crosses. Significant evidence for QTL was obtained. Although with chromosome-wise significance values of 5% the obtained evidence was not very strong despite the large population size. Additional half-sib families and markers were typed to validate and further investigate the map position of QTL identified in regions on chromosomes 2, 4, 11, 13 and 14. A variance component (VC) analysis method using linkage and linkage disequilibrium was applied to further characterize and refine the map position of the QTL. The results were compared with results obtained by the classic regression analysis method. The VC analysis results reveal the considerable contribution of the dam haplotypes to the variance of meat quality traits. An accurate positioning of the QTL however was not yet possible with the marker density so far. Therefore, new microsatellites were developed by *in silico* analysis of BAC-end sequences (BES) of BACs on the porcine physical map and genomic shotgun sequences. This resulted in the identification of thousands of new markers covering the porcine genome with over 200 new markers in the region of interest on SSC4. The ~200 markers were tested and resulted in ~60 markers that were informative and used in an effort to further fine mapping of the QTL on SSC4.

Table of Contents

Chapter 1

General introduction

The first paragraph of this introduction is derived from the following paper: Heuven H.C.M., H.J. van Wijk, and J.A.M. van Arendonk. 2003. Combining traditional breeding and genomics to improve pork quality. Outlook in Agriculture Vol.32 no.4: 235-239.

General introduction

Traditionally pig breeding programmes mainly focused on production efficiency with breeding goal traits like growth, back fat thickness, feed efficiency or reproductive performance. Selection efforts reduced the cost of production successfully (Ollivier, 1998). More recently, consumer preferences for food products refocused industry emphasis towards improving of pork quality (Martinez and Zering, 2004). This led to an expanded breeding objective *i.e.* including meat quality. Consequently, the genetics of pork quality has become subject of increased research during the last decade (Cameron, 1993; Hovenier, 1993a, 1993b, Sellier, 1998; De Vries, 1998; Verbeke et al., 1999; Knap et al., 2002; Rosenvold and Andersen, 2003). Meat quality is influenced by a large number of factors including muscle characteristics (fiber size and type, fat and connective tissue), production and environmental conditions (growth rate, nutrition, age, (pre-) slaughter conditions and post-mortem aging) and the genetics of the animals (breed and growth potential). Genetic variation in pork quality is illustrated by the detection of a few major genes influencing meat quality which are being utilized in pig breeding schemes. Most notably is the hal mutation in the ryanodine receptor (RYR1 or halothane gene) responsible for the PSE phenotype (Fujii et al., 1991) and the RN gene responsible for the “acid meat” condition (Le Roy et al., 1990). Meat quality traits are considered as quantitative traits *i.e.* traits with a multifactorial background that are influenced by environmental factors and are under polygenic control. Genomic regions harboring genes affecting these traits are termed quantitative trait loci (QTL). Selection for meat quality traits is hampered by the need for extensive and expensive measurements on slaughtered relatives. It is expected that knowledge of the underlying genes will greatly contribute to selection response through marker-assisted selection (MAS). The key challenge is the identification of the functional mutation that is responsible for the effect on the quantitative trait. A first success in pigs with regard to the identification of a functional mutation underlying a QTL was obtained recently. It has been shown that a QTL with major effect on muscle growth and fat deposition is caused by a mutation in intron 3 of the IGF2 gene (Van Laere et al., 2003).

A large number of studies reported the identification of QTL in pigs for a variety of traits (reviewed by Bidanel and Rothschild, 2002; Geldermann et al., 2003). Most of the studies were conducted on crosses between breeds and focused on the major production traits like fatness and

growth. QTL identified in crosses between breeds do not necessarily segregate within commercial breeds and therefore segregation needs to be verified first in commercial breeds to allow implementation of MAS on a within-breed basis. Further, QTL affecting production traits are of less interest for breeding than e.g. QTL affecting meat quality because production traits can effectively be improved by 'classical' breeding. Therefore, prior to application of MAS in commercial breeding considerable efforts need to be dedicated to verify the segregation of previously detected QTL and to possibly identify new QTL for traits which are of particular interest for MAS. These are traits with low heritability, sex-limited or traits for which phenotypic observations are hard to obtain, e.g. because measurements are expensive or phenotypes such as disease resistance and meat quality cannot be obtained on selection candidates. This thesis describes the detection and subsequent fine mapping of QTL for carcass composition and meat quality traits in a commercial pig line. The detection of QTL was directed towards targeted genome regions known for harboring carcass composition and meat quality QTL based on literature.

Meat quality

As was pointed out by e.g. Cameron (1993), meat quality is a composite concept and therefore hard to measure in a simple and unique manner. The term "quality" serves the meat as product as well as the way the meat is produced. Consumers, retailers, processing industry and slaughterhouses hold different perceptions on the definition of meat quality. The different perceptions can roughly be summarized as follows: eating (sensory) quality, nutritional quality, technological quality, hygienic quality and ethical quality (Sellier, 1998). Measurement of each of the different meat quality perceptions involves different characteristics or traits. Table 1 lists technological and sensory quality characteristics together with frequently considered carcass characteristics. In this thesis the emphasis is on technological quality traits which are relatively easy to measure and of relevance for the slaughterhouses such as pH, colour (redness, yellowness, and reflectance), water-holding capacity (WHC), marbling and firmness.

Table 1. Carcass composition and pork quality traits (extended table of Sellier, 1998). Asterisks indicate the principal traits for different links in the chain.

Perception	Traits	Attributes of quality	Producer	Slaughter	Processor	Consumer
	Killing out percentage	Carcass composition	*	*		
	Carcass length		*	*	*	
	Percentage lean meat		*	*	*	
	Back fat thickness		*	*	*	
	Loin muscle area/ depth/ weight		*	*	*	
	Ham weights		*	*	*	
Sensory quality	Post mortem proteolysis	Fresh meat		*	*	*
	Texture				*	*
	Flavor				*	*
	Tenderness					*
	Juiciness					*
	Appearance				*	*
	Intramuscular fat				*	
Technological quality	Color (Minolta & JCS)	Processed pork products			*	*
	Marbling				*	*
	Firmness				*	
	pH (initial & ultimate)					
	Water-holding capacity					*
	Cooking loss				*	
	Conductivity					
	Muscle glycogen content					
	Muscle fiber characteristics					
	Seasoning loss (dry cured ham)				*	
	Processing yield				*	
Nutritional quality	Protein				*	
	Lipid				*	
	Vitamins					
	Minerals					
	Digestibility					

The importance of pork traits is strongly determined by their role in valorisation of the carcass. Muscle mass is the major determinant but the quality of meat is related to muscle growth and of growing importance for the consumer. Structural changes due to protein degradation are influenced by muscle type and development, temperature and pH, and they affect WHC and color. The technological meat quality characteristics pH, colour and WHC are of most importance for the processing industry.

The **pH** of pork is influenced by many factors, such as breed, gender, physical activity, feeding regime, stress during the pre-slaughter period and environmental factors around slaughter. The different factors affect post-mortem metabolism and therewith meat pH. Due to lack of oxygen in the post-mortem muscle, ATP is regenerated by an anaerobic glycolytic breakdown of glycogen which causes accumulation of lactic acid in the muscle. Lactic acid causes acidification and hence decreased pH in the muscle. Two pH measurements are commonly used, initial pH (pHi) measured at 45 min. post mortem and ultimate pH (pHu) measured at 24 hours post-mortem. A normal pH decreases from pH 7.2 in the living muscle to a pHu range of 5.1 - 5.5.
Acute stress prior to slaughter may lead to a rapid pH decrease immediately post-mortem resulting in pale, soft and exudative (PSE) condition of meat due to changes in biochemical and physical processes (Offer and Knight, 1988). An ultimate pH that is higher than normal may result in dark, firm and dry (DFD) meat. DFD meat is often the result in animals experiencing long-term stress or exercise before slaughter that used all their glycogen reserve (Tarrant, 1989). In general, pH influences the enzymatic activity in the meat and the electric charges of the muscle proteins. There is a strong relationship between pH, water-holding capacity, and color. Meat with high pH tends to be darker and fails to oxygenate (bloom).

Water-holding capacity (WHC) is the ability of meat to retain moisture during storage or processing. The water content in the muscle is ~75%. Most water is held in between the myofilaments actin and myosin of the myofibrils which amounts to 80% of the muscle cell (Offer and Knight, 1988). The WHC of meat is influenced by pH due to electrostatic charges of meat proteins. As a result of post-mortem pH changes in the muscle, electrostatic charges of the myofilament proteins alter, influencing the WHC. WHC in meat is at a minimum at the iso-

electric point (pI) of the proteins. WHC decreases with decreasing pH until the pI of the myofilaments is reached in the pH range of 5.1 to 5.5. At higher pH electrostatic charges attract water leading to less expressed juice and lower cooking loss. Drip loss (exudate after 24 hours refrigerating) or purge loss (exudate after several days of refrigerating) are common WHC measurements.

Color of the meat is determined by the physical properties (e.g. fiber types) of the muscles, as well as by the concentration and chemical state of the muscle pigment myoglobin, and to a lesser extent the blood pigment haemoglobin (MacDougall, 1982). Color may be assessed subjectively using a standard color chart like Japanese color scale or objectively using a Minolta chromameter. Objectively the internal or surface reflectance is measured as the light scattering properties of meat proteins, which are related to the physical structure of the muscle fibers. The method measures the lightness or luminescence (L* value (black=0 and white=100)), the redness (a* value (negative=green and positive=red)), and the yellowness (b* value (negative=blue and positive=yellow) (Van Heugten, 2000). The color may also be expressed in hue angle (color itself with 0°=red, 90°=yellow, 180°=green and 270°=blue) and chroma (intensity or purity or saturation) values. Increased chroma values indicate increased color intensity.
Meat color is influenced by the pH of the meat and the chilling process after slaughter. Residual enzyme activity will be high by high ultimate pH resulting in de-oxidation of the oxymyoglobin molecule and darker color (Lawrie, 1985).

Skeletal muscle growth and development are major determinants for muscle mass and meat quality. Therefore, understanding muscle growth and development is one of the important goals in animal and meat science. Muscles are based on contractile myofibrils organized in muscle fibers surrounded by the endomysium *i.e.* connective tissue. The muscle fibers are organized in bundles which are separated from each other by a connective tissue called perimysium. Muscle bundles surrounded by the epimysium form the muscle. Muscle fibers are the major component of a muscle. In the pig two types of muscle fibers are formed during the myogenesis; primary fibers (in the pig between days 25 to 50 of gestation) and secondary muscle fibers (day 50 to 90) which line up using the primary fibers as a scaffold. The primary fibers mature to red fibers whereas the secondary fibers mainly mature to white muscle fibers (Heyer, 2004). The different

fiber types show different morphological, contractile and metabolic characteristics. In pig muscles it has been shown that slow-twitch fibers (type 1 (slow-red)) have a higher aerobic capacity, a lower glycolytic capacity and contain a higher level of intracellular lipid and myoglobin than fast-twitch fibers (type 2 (fast-white)) (Morita et al., 1969; Klosowska and Klosowski, 1985; Essen-Gustavsson et al., 1994). Slow-twitch fibers have a higher demand for oxygen and are red due to higher concentrations of myoglobin. The fast-twitch fibers are further subdivided in fast 2a, 2x and 2b fibers. The frequency of fiber types varies between muscles. The *M. longissumus* of pigs consists of approximately 13% STO (slow twitch oxidative; red) fibers, 17% FTO (fast twitch oxidative (2a & 2x); intermediate) fibers, and about 70% FTG (fast twitch glycolytic (2b); white) fibers. Red oxidative and white glycolytic muscle fibers differ greatly in pre- and post-mortem metabolic and biochemical characteristics and many of these influence meat quality (Cassens et al., 1975; Kiessling et al., 1982; Klont et al., 1998). These include the rate and extent of decrease in pH postmortem, the concentration of lipids, (anti)oxidants such as haem and vitamin E, glycogen and glycolytic intermediates and the activities of various muscle specific enzymes, in particular the enzymes that regulate glycogen and glucose biosynthesis and protein metabolism. Type I muscle fibers have the lowest level of glycogen storage and metabolism followed by type 2b and type 2a.

The number and type of muscle fibers and their postnatal hypertrophy significantly determine muscle mass and meat quality after slaughter. Current research suggests that there is an optimum number of muscle fibers of moderate size that results in both high quantity and quality of meat (reviewed by Rehfeldt et al., 2004).

QTL mapping

Over the past 15 years, more than 1000 QTL have been reported in the pig (Hu et al., 2005, PigQTLdb: www.animalgenome.org/QTLdb/ or PigAce: https://acedb.asg.wur.nl/ (with QTL information available in the 'in-house' version of the database)). Until now, only a few QTL have been characterized at the gene level and implementation of MAS in commercial pig breeding is limited (Dekkers, 2004). There may be several reasons for this. Most of the QTL detection experiments were conducted on experimental crosses. It is not clear to what extend the detected QTL are polymorphic within commercial populations. Therefore it is necessary to perform QTL mapping within commercial populations. Another drawback is the low map

resolution of most of the experiments. For the purpose of MAS, Dekkers (2004) distinguished three types of markers: 1) direct markers; loci that code for a functional mutation, 2) linkage disequilibrium (LD) markers; loci that are in population-wide linkage disequilibrium with the functional mutation and 3) linkage equilibrium (LE) markers; loci that are in population-wide linkage equilibrium with the functional mutation in outbred populations. The markers used in a genome scan are not likely in population-wide linkage disequilibrium with the QTL and therefore analysis needs to be performed within families as well as selection. The use of those markers in other families requires that the linkage phase of the markers and the QTL must be established in each family. Therefore, the search for markers in population-wide linkage disequilibrium has become of much interest.

Critically we may conclude that QTL mapping until to date has not been very successful in the identification of genes underlying complex traits. The usually applied linkage analysis approach for detecting QTL in genome scans has limited power and QTL are positioned inaccurate. However, in view of the time-span since it first became possible to map QTL in pigs, a serious advance has been made and several developments (from which some are restricted to crosses between inbred strains) have been published which may contribute to future success. These include genomic resources, animal resources, techniques and improved statistical methods (Flint et al., 2005). Whole genome scans for markers that are in population-wide linkage disequilibrium with QTL becomes feasible with the availability of dense marker maps combined with new analysis approaches. The availability of an increasing number of (complete) genome sequences is a source for an almost unlimited number of microsatellite and single nucleotide polymorphism (SNP) markers. With regard to new analysis methods, the variance component (VC) method combining linkage analysis (LA) and linkage disequilibrium (LD) between markers and QTL is gaining considerable attention (George et al., 2000; Meuwissen and Goddard, 2000, 2001). The classical linkage analysis, which is based on family data, and recent linkage disequilibrium analysis are complementary in gene mapping. In linkage analysis the marker intervals whose inheritances explain most of the phenotypic variance indicate the most likely position of the QTL (Meuwissen and Goddard, 2000). Positioning of the QTL is based on recombinants. The number of recombinants in the typed population is usually low and the mapping resolution therefore limited. Linkage disequilibrium exploits historical recombinations that occurred during the generations before the genotyped generation. Due to recombinations in

the subsequent generations following the base population, only markers closely linked to the QTL will be in linkage disequilibrium with the QTL alleles. Linkage disequilibrium mapping therefore may strongly contribute to the positioning of the QTL. A combined linkage and linkage disequilibrium mapping method was proposed by Meuwissen et al. (2002). The method optimally combines the recombinations present in the genotyped population and the preceding pedigree, and accounts for unknown background genes. The method also reduces the identification of false positive associations from LA or LD that may arise when applying the methods separately (Olsen et al., 2004). The improved method allows a more powerful and accurate mapping of QTL to regions of several centimorgan (cM) (provided sufficient markers) compared to the usually 20-40 cM regions of a typical genome scan. The use of QTL in breeding requires QTL mapped within the cM resolution. Provided that sufficient markers are available this seems to be in reach by the proposed combined linkage and linkage disequilibrium method. Fine mapping within the cM resolution may contribute considerably to the identification of quantitative trait genes. The average gene density in the pig is 10 genes per mega-base (Mb) or 12 genes per cM (3 billion base pairs, 2500 cM = 1.2Mb/cM, 30.000 genes). Fine mapping of QTL to such a resolution decreases the number of positional candidate genes considerably.

QTL in the pig

In the pig the first QTL study was carried out in 1994 (Andersson et al.). Since then quantitative trait loci have been identified for a large number of traits segregating within numerous breeds. Within the scope of the work described in this thesis a pig genome database (PigAce: https://acedb.asg.wur.nl/, Van Wijk et al., 2003) was set up from which the 'in house version' includes the results of the main QTL mapping studies that have been carried out in pigs. Recently, a specific porcine QTL database – PigQTLdb – became available (www.animalgenome.org/QTLdb/, Hu et al., 2005). With the start of the work described in this thesis there was a need for a conveniently arranged overview of the identified QTL. Such an overview could be used to increase the efficiency of future studies. It provides information about QTL-rich and QTL-poor genome regions with respect to different traits. Also, it may contribute to a more accurate positioning of QTL and the identification of the underlying genes which in turn will contribute to the implementation of QTL in MAS strategies. The main goal of setting

up a QTL database was to summarize the most important QTL mapping results in pigs in order to be able to select regions of interest for future research with regard to different traits of interest.

Prior to the start of a genome scan in the summer of 2002 a total of 69 papers reporting QTL in pigs were identified. From these papers significant QTL and their characteristics were imported into the PigAce database. This effort resulted in information on 360 QTL concerning 136 traits (Table 2), where all QTL out of the different publications were counted independently. There was not always consistency in the nomenclature of traits. Also, summarizing of results turned out to be difficult due to the different threshold definitions to conclude significance. Easy to measure traits, like growth and fatness, had been included in almost all experiments. Difficult (or expensive) to measure traits or female traits were investigated in a limited number of studies only and typically on smaller population sizes. Traits were grouped into main trait classes as growth, fatness, carcass, reproduction, and meat quality. The two main trait classes fatness and growth were cited most with close to 180 QTL or ~50 percent of the QTL.

Approximately 70 QTL were counted within the meat quality main trait class. QTL were reported on all pig autosomes except SSC16. Also no QTL were reported on SSCY but this sex-chromosome is often not included in QTL analyses. A frequency distribution of QTL throughout the genome is presented in Figure 1 with the number of QTL (mid point position) per 10 cM bins across the genome. The chromosomes SSC1 and SSC7 with both around 50 QTL contain the most QTL. Other chromosomes with a high number of QTL were SSC2, SSC4, SSC6 and SSC8. The selection of genome regions that were targeted in the genome scan as described in this thesis were based on the QTL database made. This also applies for the description of QTL per trait class as given below. To date many more QTL have been published. A total of 87 publications which have been processed into the publicly available PigQTL database resulted in 1147 QTL representing 235 traits (see also Table 2). This includes QTL identified with suggestive evidence that represent more than 50% of the QTL reported.

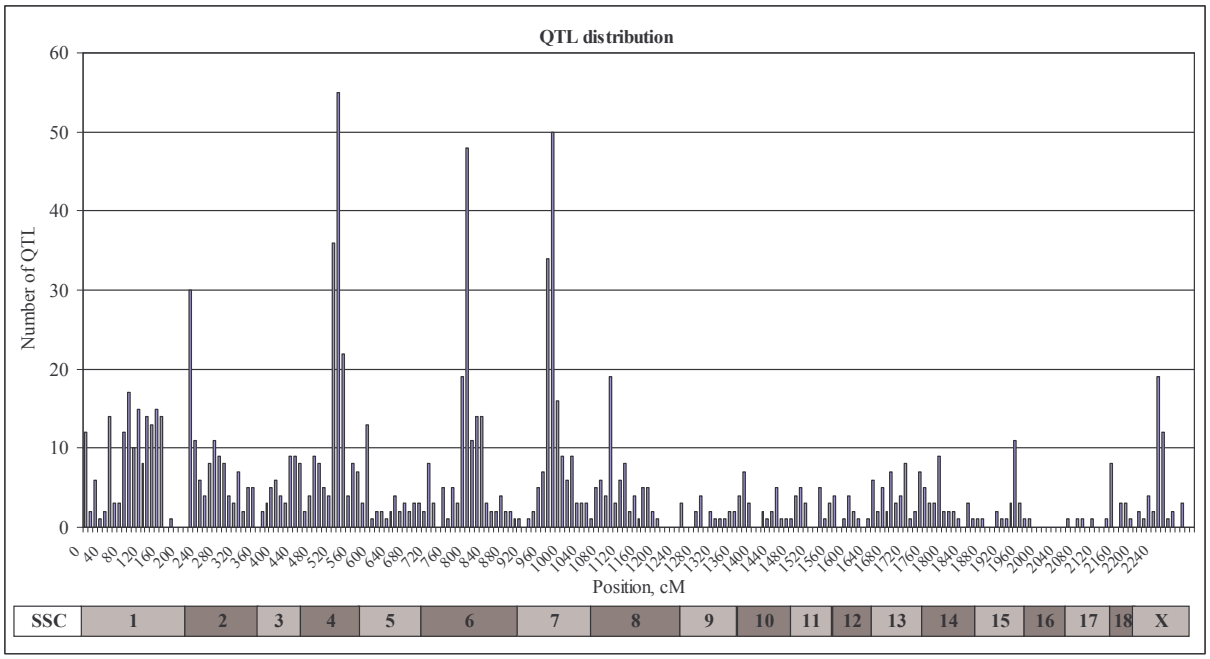

Figure 1. Distribution of QTL throughout the porcine genome with the number of QTL counted per 10 cM bins. The numbers in the gray bar indicates the different chromosomes.

Growth

Almost 90 QTL were reported for 25 growth traits. Life growth rate (LGR) and early growth rate (EGR) were the most frequently reported growth traits followed by test growth rate (TGR) and average daily gain (ADG). QTL affecting growth have been reported on 15pig autosomes. The chromosomes 1, 4 and 7 harbor most of the QTL affecting growth. QTL for any of the 25 recorded growth traits have not been reported on SSC11, SSC15 and SSC16.

Fatness

Back fat thickness (BFT) was the most frequently cited (fatness) trait. In addition, some publications used a subdivision where back fat thickness on first or last rib or lumbar were recorded as individual fatness traits. QTL affecting fatness have been reported on 12 of the pig autosomes and on the X chromosome. SSC1, SSC7 and SSCX are of most importance for fatness traits. SSC4 and SSC6 play a role as well. Striking is the strong correlation with the chromosomes harboring QTL for growth traits. The high number of records makes it possible to

confirm the independent observations. For example the BFT QTL on SSC7 is observed several times within an interval of 40 cM around the centromere of SSC7.

Table 2. Number of QTL per chromosome and trait class or trait type (further sub-division of the trait classes as considered in PigQTLDB) obtained from the QTL survey and PigQTLDB.

			Chromosome																					
		Trait class	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	X	Y	Total	%
QTL survey 2002		Growth	15	3	3	18	2	5	14	9	3	2	0	3	6	1	0	0	1	1	1	0	87	24.2
		Fatness	21	4	0	6	5	6	23	2	3	0	0	1	5	4	0	0	0	1	12	0	93	25.8
		Carcass	2	3	0	6	0	1	3	4	0	0	1	0	1	1	0	0	0	0	2	0	24	6.7
		Meat_quality	2	14	0	6	5	2	4	2	0	2	3	2	1	4	16	0	6	2	1	0	72	20.0
		Meatiness	7	1	2	1	0	1	4	2	0	0	2	0	0	3	0	0	0	0	3	0	26	7.2
		Muscle	0	1	0	2	0	7	3	0	0	0	0	0	0	0	0	0	0	0	0	0	13	3.6
		Reproduction	4	1	3	0	1	1	3	5	1	4	3	2	1	1	0	0	1	0	0	0	31	8.6
		Coat_color	0	2	1	0	1	3	0	7	0	0	0	0	0	0	0	0	0	0	0	0	14	3.9
		Total	51	29	9	39	14	26	54	31	7	8	9	8	14	14	16	0	8	4	19	0	360	100
PigQTLDB - autumn 2005	Trait type	Anatomy	49	49	17	66	6	42	44	21	2	5	5	6	8	5	0	0	0	0	14	-	339	29.6
		Behavioral	4	0	1	0	2	2	4	3	0	0	1	1	0	0	0	0	1	2	0	-	21	1.8
		Chemical	0	0	0	0	0	0	2	0	0	0	2	0	0	0	4	0	2	1	0	-	11	1.0
		Coat_color	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	-	2	0.2
		Conductivity	1	1	0	1	2	9	2	1	0	0	0	0	4	0	0	0	0	0	0	-	21	1.8
		Conformation	2	1	0	0	1	0	2	1	0	0	0	0	0	0	0	0	1	0	0	-	8	0.7
		Digestive_organ	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0	1	1	0	0	-	9	0.8
		Disease_resist.	0	0	0	0	2	4	0	0	1	0	0	0	0	0	0	0	0	0	0	-	7	0.6
		Endocrine	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	-	4	0.3
		Enzyme_activity	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	-	1	0.1
		Fat_composition	1	0	2	20	0	2	5	3	0	1	0	1	0	4	0	0	0	1	0	-	40	3.5
		Fatness	46	26	8	40	11	36	56	4	5	3	4	5	12	5	2	0	0	1	27	-	291	25.4
		Feed_conversion	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	-	4	0.3
		Feed_intake	3	0	0	1	1	0	0	0	1	0	1	0	1	0	0	0	0	0	1	-	9	0.8
		Flavor	0	5	0	1	0	0	0	0	0	0	0	1	0	1	2	0	1	0	1	-	12	1.0
		Growth	30	11	11	40	2	21	17	12	10	4	1	4	11	1	1	0	1	0	2	-	179	15.6
		Immune_capacity	1	1	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	-	8	0.7
		Litter_size	2	0	1	2	1	0	0	5	2	1	2	0	2	0	2	0	0	0	0	-	20	1.7
		Meat_color	1	5	2	6	3	2	5	2	0	0	0	1	2	4	5	0	3	1	0	-	42	3.7
		Odor	0	0	0	2	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	-	5	0.4
		pH	2	4	4	1	2	8	2	0	1	0	1	0	0	2	6	0	0	1	1	-	35	3.1
		Reprod._organ	5	2	2	0	1	1	2	8	0	4	1	2	0	0	0	0	0	0	0	-	28	2.4
		Reprod._traits	1	0	0	0	0	0	2	3	1	1	0	1	0	0	0	0	0	0	0	-	9	0.8
		Stiffening	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	-	3	0.3
		Texture	2	7	0	3	0	5	8	1	2	1	1	0	3	2	2	0	0	2	0	-	39	3.4
		Total	150	113	50	186	36	141	153	69	25	21	19	24	44	25	24	1	10	9	47	-	1147	100
	Trait class	Exterior	6	1	1	0	3	3	6	5	0	0	1	1	0	0	0	0	2	2	0	-	31	2.7
		Health	1	1	0	1	2	5	1	1	1	0	0	1	1	0	0	0	0	0	0	-	15	1.3
		Meat_Quality	102	97	33	140	24	110	125	32	10	10	13	14	29	23	21	0	6	7	43	-	839	73.1
		Production	33	12	12	43	5	22	17	14	11	4	2	5	12	2	1	1	2	0	3	-	201	17.5
		Reproduction	8	2	4	2	2	1	4	17	3	7	3	3	2	0	2	0	0	0	1	-	61	5.3
		Total	150	113	50	186	36	141	153	69	25	21	19	24	44	25	24	1	10	9	47	-	1147	100

Carcass composition

SSC2, SSC4, SSC7, and SSC8 contribute most to carcass characteristics. Together they harbor approximately two third of the carcass traits QTL reported on a total of 10 chromosomes. The centromeric part of SSC4 has the highest number of citations with regard to carcass traits and in particular carcass length. Carcass weight is influenced by SSC7 which is also associated with growth and fatness traits.

Reproduction

Approximately 30 QTL were recorded for reproduction traits. The number of nipples (NN) is simple to measure and is the most cited of the different reproduction traits recorded. SSC1, SSC8 and SSC10 contain most reproduction QTL. Other chromosomes that play a more modest role are SSC3, SSC7 and SSC11.

Meatiness or Muscle

Several tens of QTL were reported for certain specific muscle characteristics such as meatiness, muscle depth (MD), eye muscle area (MA), percentage white fibers (%WF) and intramuscular fat (IMF). The latter count for most of the muscle QTL reported. SSC6 is the predominant chromosome for IMF QTL. The independent observations are however scattered over the whole chromosome. Chromosomes SSC1 and SSC7 are of interest for QTL related to meatiness. The QTL on chromosome SSC1 are mainly localized at the telomeric end of the q-arm of the chromosome.

Meat quality

Special attention was given to meat quality traits to select genome regions for validation of segregation of meat quality QTL within a commercial crossbred as described in this thesis. In the 2002 survey over 70 QTL were recorded for approximately 30 different meat quality traits. The chromosomes SSC2 and SSC15 were identified as the most important chromosomes for quality traits. Chromosomes SSC4, SSC5, SSC7, SSC11, SSC14 and SSC17 were also of interest.

Typical quality traits are pH, color, marbling, firmness, drip loss, taste and smell. Some muscle traits, such as IMF and %WF, could be considered as quality traits as well but often were classified under muscle traits. The chromosomes SSC1, SSC6, SSC8, SSC10, SSC12, SSC13,

SSC18 and SSCX with one or a few meat quality trait QTL each were considered of minor interest with regard to the number of quality related QTL reported. Chromosome SSC6 however plays a major role in IMF. On four pig chromosomes (SSC3, SSC9, SSC16 and SSCY), no QTL related to any of the quality traits had been identified at the time of the survey. If resources are limited, these chromosomes seem to be of minor interest for validation or detection of meat quality traits QTL.

All together, nine chromosomes were identified as being of most interest with regard to meat quality traits. These are SSC2, SSC4, SSC5, SSC6, SSC7, SSC11, SSC14, SSC15 and SSC17. The meat quality QTL on these nine chromosomes were summarized in more detail:

- Chromosome SSC2 has a total length of approximately 140 cM. The quality related QTL are scattered throughout the whole chromosome with a concentration of QTL at the telomeric end of the q-arm of the chromosome. The IGF2 gene with a known mutation with effect on meatiness is located at the top of the p-arm of this chromosome (Van Leare et al., 2003).
- Chromosome SSC4 with a length of 130 cM harbors quality related QTL which mainly are located on the q-arm of the chromosome.
- SSC5 harbors quality related QTL which are all located on the second half of the chromosome.
- Chromosome SSC6 plays a major role in IMF and harbors several QTL for other quality related traits under which water-holding capacity, drip loss, pH and firmness. It is one of the longest pig chromosomes with a length of 165 cM. The halothane locus (RYR1 gene) with known effect on meat quality is located on SSC6 in the region of ~105-115 cM.
- SSC7 has a length of 155 cM. The QTL related to quality are scattered throughout the chromosome except for the tip of the p-arm.
- The quality related QTL on SSC11 are all reported on the tip of the p-arm of the chromosome.
- SSC14 has an estimated length of 110 cM with quality QTL scattered over the chromosome.
- On SSC15 QTL are reported for pH, color, tenderness (Tend), flavor, average glycogen (aGly) and average lactate (aLac). SSC15 is a relative short chromosome of ~100 cM. The reported quality related QTL are scattered over the chromosome from 40-100 cM. The RN gene which is located on this chromosome is known for its large effect on meat quality characteristics such as pH, water content and glycogen content (Le Roy et al., 1990). The RN gene is one of the few genes affecting meat quality QTL for which the functional mutation is known (Milan et al., 2000).

- SSC17 is a relatively short chromosome with quality related QTL reported on the telomeric end of the q-arm of the chromosome.

As mentioned earlier, the traits investigated differ widely. Performance traits like growth and fatness traits, for which phenotypic measurements are relatively easy and very frequently done, were subject of most of the published experiments. Traits which are more difficult or expensive to measure, like most meat quality traits were examined in a limited number of studies (see also the review of Bidanel and Rothschild, 2001). It is likely that the total number of QTL with effect on the most extensively studied traits (growth and fatness) is over-represented. Also, part of the studies recorded was carried out on a limited number of chromosomes (incomplete genome scans) and directed to (QTL-rich) genome regions based on previously published work. As a consequence it is very likely that the number of QTL on the most extensively studied chromosomes such as SSC 1, 2, 4, 6, 7, 8 and X is over-represented.
The comparison of results of different studies appears to be difficult. The main reason for this is the low consistency of trait nomenclature. The low accuracy of the estimated QTL positions also makes it difficult to compare studies, which is strengthened by the use of different marker maps. Positioning of the QTL on a reference map, which could be made based on genetic markers common between maps, could facilitate the comparison.

Aim and outline of the thesis

The aim of the work described in this thesis was to identify QTL, in particular for meat quality and carcass traits in a commercial crossbred, and to identify favorable haplotypes by fine mapping using a combined linkage and linkage disequilibrium (LDLA) analysis method towards the development of markers to be used in marker assisted selection (MAS). The emphasis of the genome scan was on regions known for harboring meat quality QTL based on literature. The subsequent fine mapping focused on a QTL for meat color on SSC4.

Initially pig breeding focused on performance traits. Due to the growing importance of non-production trait and quality traits in particular, there is a need for information regarding the genetics of those traits in commercial breeding lines of pigs. Chapter 2 describes the estimation of genetic parameters for carcass composition and pork quality characteristics based on measurements in a commercial crossbred population. Chapter 3 reports on the genome scan to detect QTL affecting carcass and meat quality traits. The regions included in the targeted genome scan were selected based on an extensive survey of the literature for QTL in the pig which also resulted in the addition of QTL information to PigAce.

The most promising QTL out of the genome scan were validated on a second set of families and using additional markers. Furthermore, the data set was re-analyzed using the LDLA analysis method (Chapter 4 & 5). The re-analysis resulted in the identification of some additional QTL. Also, additional evidence was gained for the meat color QTL on SSC4.

A serious contribution of LD may only be expected with higher marker densities. In order to increase the marker density in the region of the color QTL on SSC4, additional markers were developed in the region of interest based on the physical map of the pig in combination with pig-human comparative map information. The development and mapping of new microsatellite markers is described in Chapter 6. The new markers were subsequently used for additional fine mapping of the color QTL (Chapter 7).

In Chapter 8, the general discussion, the results as described in this thesis are discussed and placed in a broader perspective. The final part of this chapter reports on future directions taking into account new developments, in particular sequencing of the porcine genome, and their meaning for pig breeding.

Literature cited:

Andersson L., C.S. Haley, H. Ellegren, S.A. Knott, M. Johansson, K. Andersson, L. Andersson-Eklund, I. Edfors-Lilja, M. Fredholm, I. Hansson, J. Håkansson, and K. Lundström. 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science 263: 1771-1774.

Bidanel J.P., and M. Rothschild. 2002. Current status of quantitative trait locus mapping in pigs. Pig News and Information 23: 39-53.

Cameron N.D. 1993. Selection for meat quality: objectives and criteria. Pig News and Information 14: 161-168.

Cassens R.G., D.N. Marple, and G. Eikelenboom. 1975. Animal Physiology and Meat Quality, Advances in Food Research 21 (Eds. E. Michester, M. Mrak, C.F. Steward). Academic Press, New York, San Francisco, London 71-155.

Dekkers J.C. 2004. Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. J. Anim. Sci. 82: E313-328.

De Vries A.G., A. Sosnicki, J.P. Garnier, and G.S. Plastow. 1998. The role of major genes and DNA technology in selection for meat quality in pigs. Meat Sci. 49, Supplement 1: 245-255.

Essen-Gustavsson B., K. Karlström, K. Lundström, and A.C. Enfält. 1994. Intramuscular fat and muscle fiber lipid content in halothane-gene-free pigs fed high or low protein diets and its relation to meat quality. Meat Sci. 38: 269-277.

Flint J., W. Valder, S. Shifman, and R. Mott. 2005. Strategies for mapping and cloning quantitative trait genes in rodents. Nature Review Genetics 6: 271-285.

Fujii J., K. Otsu, F. Zorzato, S. De Leon, V.K. Khanna, J.E. Weiler, P.J. O'Brien, and D.H. Maclennan. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253: 448-451.

Geldermann H., E. Müller, G. Moser, G. Reiner, H. Bartenschlager, S. Cepica, A. Stratil, J. Kuryl, C. Moran, R. Davoli, and C. Brunsch. 2003. Genome-wide linkage and QTL mapping in porcine F_2 families generated from Piétrain, Meishan and Wild Boar crosses. J. Anim. Breed. Genet. 120: 363-393.

George A.W., P.M. Visscher, and C.S. Haley. 2000. Mapping quantitative trait loci in complex pedigrees: a two-set variance component approach. Genetics 156: 2081-2092.

Heuven H.C.M., H.J. van Wijk, and J.A.M. van Arendonk. 2003. Combining traditional breeding and genomics to improve pork quality. Outlook in Agriculture Vol.32 no.4: 235-239.

Heyer A. 2004. Performance, carcass and meat quality in pigs – influence of rearing system, breed and feeding. Doctoral thesis. Swedish University of Agricultural Sciences. Department of Food Science. Uppsala.

Hovenier R., E.W. Brascamp, E. Kanis, J.H.J. van der Werf, and A.P.A.M. Wassenberg. 1993a. Economic values of optimum traits: the example of meat quality in pigs. J. Anim. Sci. 71: 1429-1433.

Hovenier R., E. Kanis, Th. van Asseldonk, and N.G. Westerink. 1993b. Breeding for pig meat quality in halothane negative populations – a review. Pig News and Information 14: 17-25.

Hu Z-L., S. Dracheva, W. Jang, D. Maglott, J. Bastiaansen, M.F. Rothschild, and J.M. Reecy. 2005. A QTL resource and comparison tool for pigs: PigQTLDB. Mamm. Genome 16: 792-800.

Kiessling K-H., K. Lundström, H. Petersson, and H. Stalhammar. 1982. Age and feed related changes of fiber composition. Swedish Journal of Agricultural Research 12: 69-75.

Klont R.E., G. Eikelenbroom, and L. Brocks. 1998. Muscle fiber type and meat quality. Proceedings of the 44th International Congress of Meat Science and Technology. August 30 – September 4. Barcelona. 1: 98-105.

Klosowska D., and B. Klosowski. 1985. Histological and histochemical investigations on heterogeneity of muscle in pigs. Tag.-Ber. Akademie Landwirschaft – Wissenschaft Berlin. 236: 191-197.

Knap P.W., A.A. Sosnicki, R.E. Klont, and A. Lacoste. 2002. Simultaneous improvement of meat quality and growth-and-carcass traits in pigs. Proc. 7TH World congress on genetics applied to livestock production, Vol 31, pp 339-346.

Lawrie R.A. 1985. Meat Science. 4th Edition. Pergamon Press, Oxfor, UK.

Le Roy P., J. Naveau, J.M. Elsen, and P. Sellier. 1990. Evidence for a new major gene influencing meat quality in pigs. Gen. Res. 55: 33-40.

MacDougall D. B. 1982. Changes in the colour and opacity of meat. Food Chemistry 9.

Martinez S.W., and K. Zering. 2004. Pork quality and the role of market organization. Agricultural economic report number 835. United States Department of Agriculture.

Meuwissen T.H.E., and M.E. Goddard. 2000. Fine scale mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. Genetics 155: 421-430.

Meuwissen T.H.E., and M.E. Goddard. 2001. Prediction of identity by descent probabilities from marker haplotypes. Genet. Sel. Evol. 33: 605-634.

Meuwissen T.H.E., A. Karlsen, S. Lien, I. Olsaker, and M.E. Goddard. 2002. Fine mapping of a quantitative trait locus for twinning rate using combined linkage and linkage disequilibrium mapping. Genetics 161: 373-379.

Milan D., J.T. Jeon, C. Looft, V. Amarger, A. Robic, M. Thelander, C. Rogel-Gaillard, S. Paul, N. Iannuccelli, L. Rask, H. Ronne, K. Lundström, N. Reinsch, J. Gellin, E. Kalm, P. Le Roy, P. Chardon, L. Andersson. 2000. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science 288(5469): 1248-1251.

Morita S., R.E. Cassens, E.J. Briskey. 1969. Localization of myoglobin in striated muscle of the domestic pigs, benzidine and NADH-TR reactions. Stain Technology 44: 238-286.

Offer G., and P. Knight. 1988. The structural basis of water-holding capacity in meat. Part 2: Drip losses. In: R.A. Lawrie (Ed.) Developments in Meat Science. pp 173 – 243. Elsevier Applied Science, London.

Ollivier L. 1998. 'Genetic improvement of the pig', In 'The genetics of the pig' edited by Rothschild, M.F., and Ruvinsky, A., CAB International, Wallingford, Oxon, pp 511-540.

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

Rosenvold K., and H.J. Andersen. 2003. Factors of significance for pork quality – a review. Meat Sci. 64: 219-237.

Sellier P. 1998. 'Genetics of meat and carcass traits', In 'The genetics of the pig' edited by Rothschild, M.F., and Ruvinsky, A., CAB International, Wallingford, Oxon, pp 463-510.

Tarrant P.V. 1989. The effects of handling, transport, slaughter and shilling on meat quality and yield in pigs – a review. Irish Journal of Food Science and Technology 13: 70-107.

Van Laere A.S., M. Nguyen, M. Braunschweig, C. Nezer, C. Collette, L. Moreau, A.L. Archibald, C.S. Haley, N. Buys, M. Tally, G. Andersson, M. Georges, and L. Andersson. 2003. A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature 425: 832-836.

Van Heugten E. 2000. Understanding pork quality. Swine news 24(3).

Van Wijk H.J., A.J. van Kampen, B. Harlizius, J.W. Merks, and M.A.M. Groenen. 2003. PigAce; a pig genome database. Plant and Animal Genome XI, San Diego.

Verbeke W., M.J. van Oeckel, N. Warnauts, J. Viaene, and Ch.V. Boucqué. 1999. Consumer perception, facts and possibilities to improve acceptability of health and sensory characteristics of pork. Meat Sci. 53: 77-99.

Chapter 2

Genetic parameters for carcass composition and pork quality estimated in a commercial production chain

H.J. van Wijk[*], D.J.G. Arts[#], J.O. Matthews[+], M. Webster[+], B.J. Ducro[#], and E.F. Knol[*]

[*]IPG, Institute for Pig Genetics, P.O. Box 43, 6640 AA, Beuningen, The Netherlands; [#]Animal Breeding and Genetics Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands; [+]Premium Standard Farms Inc., P.O. Box 99, Milan, MO 63556, United States of America.

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Abstract: Breeding goals in pigs are subject to change and are directed much more towards retail carcass yield and meat quality because of the high economic value of these traits. The objective of this study was to estimate genetic parameters of growth, carcass, and meat quality traits. Carcass components included ham and loin weights as primal cuts, which were further dissected into boneless subprimal cuts. Meat quality traits include pH, drip loss, purge, firmness, and color and marbling of both ham and loin. Phenotypic measurements were collected on a commercial crossbred pig population (n = 1,855). Genetic parameters were estimated using REML procedures applied to a bivariate animal model. Heritability estimates for carcass traits varied from 0.29 to 0.51, with 0.39 and 0.51 for the boneless subprimals of ham and loin, respectively. Heritability estimates for meat quality traits ranged from 0.08 to 0.28, with low estimates for the water-holding capacity traits and higher values for the color traits; Minolta b* (0.14), Minolta L* (0.15), Minolta a* (0.24) and Japanese color scale (0.25). Heritability estimates differed for marbling of ham (0.14) and loin (0.31). Neither back fat nor ADG was correlated with loin depth (r_g = 0.0), and their mutual genetic correlation was 0.27. Loin primal was moderately correlated with ham primal (r_g = 0.31) and more strongly correlated with boneless ham (r_g = 0.58). BF was negatively correlated with (sub)primal cut values. Average daily gain was unfavorably correlated with subprimals and with most meat quality characteristics measured. Genetic correlations among the color measurements and water-holding capacity traits were high (average r_g = 0.70), except for Minolta a* (average r_g = 0.17). The estimated genetic parameters indicate that meat quality and valuable cut yields can be improved by genetic selection. The estimated genetic parameters make it possible to predict the response to selection on performance, carcass, and meat quality traits and to design an effective breeding strategy fitting pricing systems based on retail carcass and quality characteristics.

Key Words: Carcass composition, Genetic parameters, Heritability, Meat quality, Pig

Introduction

Consumer demands regarding food of animal origin are of growing importance. As a consequence, traits referring to meat quality are of increasing relevance for the pork industry. Sensory demands such as appearance and taste are translated in terms of technological carcass and meat quality characteristics. Furthermore, the technology for predicting ham and loin primal and subprimal (defatted dissectible meat) cut weights is developing (e.g. AutoVision and AutoFOM, SFK Technology A/S, Herlev, Denmark). Because of these developments, the pork industry is starting to use quality indicators in classification systems and is moving towards more refined value-based grading systems meeting the requirements of market segments (Brorsen et al., 1998).

For a long time, pig breeding programs focused mainly on the reduction of costs. Selection was aimed at increasing litter size, weight gain, decreasing back fat (**BF**), and improving feed conversion. Now breeding goals are subject to change and are directed much more towards retail carcass yield and meat quality because of the high economic value of these traits. Genetic improvement of valuable cuts of appropriate quality requires estimates of genetic parameters.
Genetic variation in meat quality has received attention in the past two decades (Cameron, 1990; Sellier, 1998; Lonergan et al., 2001). Research on genetic parameters of carcass dissection traits is limited and has received attention recently (NPPC, 1995; Newcom et al., 2002).

The objective of this study is to estimate genetic parameters for carcass and meat quality traits that are of practical relevance in combination with information from current or intended classification systems.

Materials and Methods

Genetic material

The commercial crossbred individuals used in this study were progeny from 20 sires of a synthetic Piétrain/Large White halothane-free boar line (TOPIGS, Vught, The Netherlands) bred to 239 sows of a single commercial line. Pedigree information of five ancestral generations of the boar line comprising 242 individuals was available.

Live animal evaluation and housing

Piglets were born over a two-month period, and at birth, all piglets were individually tagged and birth weights were recorded. Piglets were weaned at an average of 17 days of age, raised in a nursery for 6 to 7 weeks, and at an average weight of 22.7 kg, they were randomly allocated to one of three finishing sites (farm). Pigs were raised under commercial finishing conditions with ad libitum access to a corn-soybean diet and water. Metabolizable energy concentration of the diet ranged from 3.340 to 3.475 Mcal/kg of diet (as-fed basis). Males were castrated from 3 to 5 days after farrowing.

Slaughter and carcass evaluation

All pigs were slaughtered in one slaughterhouse at an average weight of 118 kg of live weight following a three-stage sampling system. In the first two successive sampling stages, all animals that had reached a visually assessed weight of 118 kg were designated for slaughter; the third stage of sampling contained the remaining individuals of a total of 1,855 pigs evaluated in this study. This system resulted in an average age at slaughter of 164, 172, and 185 days (**AGE**) for the first, second, and third stages of sampling, respectively. Before slaughter, the animals were held overnight in lairage with ad libitum access to water. Pigs were slaughtered on 17 different days over a 70-day period, and were grouped according to slaughter date (group). Groups could include pigs from different sites as well as from different sampling stages. Whole carcass data were recorded within 45 min. postmortem whereas the remaining measurements were collected 24 h postmortem. After bleeding, scalding, and dehairing, a preeviscerated carcass weight (**DCW**) was recorded. Final body weight was estimated from DCW using the following formula: final body weight = 106.5 * DCW. For the purpose of calculating average daily gain (**ADG**), all pigs were assumed to have a birth weight of 1.36 kg, and ADG was calculated using the following equation: ADG = (final body weight – birth weight)/AGE.

Measurements on the carcass were recorded using the left carcass half unless circumstances such as damage required using the right half. A Hennessy Grading Probe (model 4, Hennessy Grading Systems Ltd, Auckland, New Zealand) was used to record back fat (**BF**) and loin depth (**LD**), both in millimeters, at the 10^{th} rib, and a hot carcass weight (**HCW**) was recorded after evisceration. The percentage of muscle (**PLEAN**) was calculated using the following equation (SFK Technologies; Herlev, Denmark):

PLEAN = 58.86 - (0.61 * **BF**) + (0.12 * **LD**).

Cold carcass weight (**CCW**) was recorded after temperature equilibration (24 h). Primal cuts of ham and loin were weighed and further dissected into boneless subprimal cuts. The weight of the bone-in loin (**LOIN**) with skin removed and fat trimmed was recorded along with the bone-in, skin-on ham weight (**HAM**). Loins were processed to a boneless loin without fat cover (**BLOIN**). Hams were skinned and defatted and were fabricated into the boneless inside ham (semimembranosus, gracillis, and adductor), outside ham (semitendinosus and biceps femoris), knuckle (vastus intermedius, vastus lateralis, tensor fasciae, and vastus medialis), and light butt (gluteus medius) subprimals. The four subprimal weights added yielded a boneless ham weight (**BHAM**).

Meat quality measurements

Meat quality measurements were taken on both the loin and ham. Loin Minolta L*, a*, and b* (**LOINL, LOINA**, and **LOINB**; also referred to as the CIELAB color space) were taken on the fresh cut surface of a 2.5-cm chop removed from the sirloin end of the boneless center cut loin using a Minolta CR 300 colorimeter set at C illuminant (Minolta camera, Osaka, Japan). On the same chop, a subjective color score (1 to 6, with 1 = pale and 6 = very dark) was given to the cut surface (**JCScut**) using a Japanese color scale. The same system was used to score the rib surface of the loin (**JCSrib**). A subjective marbling score (**LMARB**; 1 to 5, with 1 = devoid, 2 = practically devoid, 3 = moderately abundant, 4 = abundant, 5 = overly abundant) was given to the chop based on National Pork Producers Council marbling standards (NPPC, 1991). Cores were taken from a second 2.5-cm chop, directly anterior to the first using a 25-mm coring device to determine drip loss percent (**DRIP**). The cores were placed in clean, dry, preweighed tubes and placed in a cooler for 24 hours. Upon removal from the cooler, the tubes were weighed with and without the sample to determine the amount of exudates (Christensen, 2003). An ultimate pH measurement (**pHU**) was taken in the boneless loin after the two chops for color and drip loss measurement were removed, approximately 24 to 28 hours postmortem. Purge loss (**PURGE**) was determined by cutting a 7.5- to 10-cm section from the sirloin end of the remaining boneless loin. Sections were placed in sealed plastic bags and refrigerated for 5 days. Upon removal from the cooler, samples were weighed in the bag. The sample was then removed, blotted dry with a paper towel and weighed again to determine the weight lost, expressed as a percentage of the

original sample. Subjective firmness scores (**FIRM**) on the NPPC scale (2000; 1 to 3, with 1 = soft [and exudative] and 3 = firm) were assigned to chops based on firmness of the loin and distortion of the loin interface surface on the sirloin end of the loin.

Meat quality measurements taken on the ham included Minolta L*, a*, and b* values on the fresh cut surface of the inside ham muscle (**HAML**, **HAMA**, and **HAMB**). A subjective ham marbling score (**HMARB**; scale 1 to 4, with 1 = devoid of marbling, 2 = moderate, 3 = abundant, and 4 = overly abundant marbling) was given to the outside ham muscle.

Statistical analysis

Analysis of variance was performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) to test for significant effects. Significant effects ($P < 0.05$) of sex (two classes), AGE (covariate), CCW (covariate), and GFP (20 classes) were found on almost all traits, where the latter effect represents the partly nested group (17 classes), farm (3 classes), and sampling stage (three classes) combinations. All first-order interactions were examined, but none was found to be significant.

Variance and covariance components were estimated using ASReml (version 1.0; Gilmour et al., 2002). Phenotypic (co)variances were computed by adding genetic, common environmental variances, and residual (co)variances. The following animal model was used for all traits except for ADG, where AGE and CCW was not used as a covariate:

$$\mathbf{Y_{ijkl} = SEX_i + GFP_j + b_1AGE_{ijkl} + b_2CCW_{ijkl} + a_k + c_l + e_{ijkl}}$$

where, Y_{ijkl} = trait under study;

SEX_i = fixed effect of i^{th} sex (two classes, barrow or gilt);

GFP_j = combined fixed effect of j^{th} group, farm, and sampling stages (20 classes);

AGE = age as a covariate;

CCW = cold carcass weight (kg) as a covariate;

a_k = additive genetic effect of k^{th} animal, $a_k \sim N(0, A \sigma_a^2)$;

c_l = random effect of l^{th} litter, $c_l \sim N(0, I \sigma_c^2)$;

e_{ijkl} = residual effect, $e_{ijkl} \sim N(0, I \sigma_e^2)$;

b_1 = regression coefficient of Y on age; and

b_2 = regression coefficient of Y on cold carcass weight.

Tests for allometric relationships between CCW and other carcass traits were made; however, no significant improvements in goodness of fit were obtained using log-transformed CCW in the model.

Results

Heritabilities and common environmental effects

Not all traits were measured on all individuals due to time limits, misreading, etc., during slaughter. The number of measurements per trait and summary statistics is shown in Table 1. Heritability estimates and common environmental effects with their standard errors and the genetic variances are presented in Table 2.

Heritability estimates for growth and fatness traits were within the range of published values from 0.03 to 0.74 (Clutter and Brascamp, 1998), although the heritability of ADG (h^2 = 0.19) was lower than the average of 0.31 presented by Clutter and Brascamp (1998). Meat percent (PLEAN) is a calculated composite measure of BF and LD, and the heritability of 0.43 reflects the weight of the underlying component traits.

Moderate to high heritability estimates were obtained for carcass traits (on average h^2 = 0.40). Ham primal weight was more heritable (h^2 = 0.40) than loin primal weight (h^2 = 0.29). Heritability estimates for HAM and ham without bones and fat (BHAM = 0.39) were nearly identical. This was not observed for the loin and BLOIN. The heritability of the individual boneless loin without fat (BLOIN = 0.51) was higher than LOIN heritability (0.29). The common environmental effects for the carcass traits averaged 0.14.

Heritability estimates for meat quality traits ranged from 0.08 (DRIP) to 0.31 (LMARB). The Minolta color reading measurements had a heritability of 0.17 on average. The heritabilities of the subjective color measurements, both taken on the loin, JCScut and JCSrib, were higher compared to the objective Minolta color readings and were 0.22 and 0.28 respectively. The heritability estimates for the traits related to water-holding capacity were low (h^2 = 0.08, 0.11 and 0.11 for DRIP, PURGE and pHU, respectively). Marbling in ham and loin was not heritable to the same extent; LMARB (h^2 = 0.31) showed a higher heritability than HMARB (h^2 = 0.14).

Table 1. Summary statistics for traits measured: abbreviations used in text, number of animals per trait (n), means, SD and minimum (Min.) and maximum (Max.) values.

Trait	Abbreviation	n	Mean	SD	Min.	Max.
Age, days	AGE	1,855	175.8	11.2	150	209
Growth and fatness traits						
Back fat, mm	BF	1,645	25.1	5.8	10.4	46.4
Loin depth, mm	LD	1,645	59.3	9.1	26.0	90.8
Meat, %	PLEAN	1,645	50.7	3.6	37.5	60.3
Average daily gain, g/day	ADG	1,818	649.5	78.1	353.3	919.6
Carcass traits						
Cold carcass weight, kg	CCW	1,791	86.2	8.1	48.9	113.1
Ham weight, kg	HAM	1,800	10.3	0.9	6.3	13.4
Loin weight, kg	LOIN	1,788	9.3	1.0	5.1	12.4
Boneless ham weight, kg	BHAM	1,673	5.2	0.5	2.9	6.8
Boneless loin weight, kg	BLOIN	1,781	3.3	0.4	1.6	4.4
Meat quality traits						
Minolta L* ham[a]	HAML	1,689	48.8	4.9	34.8	64.6
Minolta L* loin[a]	LOINL	1,792	48.3	3.8	37.3	65.0
Minolta a* ham[a]	HAMA	1,689	7.6	2.2	1.2	13.3
Minolta a* loin[a]	LOINA	1,792	6.9	2.0	1.7	13.5
Minolta b* ham[a]	HAMB	1,689	2.4	2.4	-3.1	10.6
Minolta b* loin[a]	LOINB	1,792	2.9	2.4	-2.4	12.2
Japanese color score cut[b]	JCScut	1,797	2.8	0.5	1	5.5
Japanese color score rib[b]	JCSrib	1,794	2.8	0.5	1	5
Drip loss, %	DRIP	1,790	3.10	2.18	0.02	16.23
Purge loss, %	PURGE	1,736	3.55	1.72	0.13	15.03
pH ultimate	pHU	1,651	5.66	0.14	5.26	6.58
Marbling score ham[c]	HMARB	1,690	1.6	0.7	1	4
Marbling score loin[d]	LMARB	1,797	2.5	0.7	1	5
Firmness score loin[e]	FIRM	1,797	1.8	0.6	1	3

[a] Minolta L* measured lightness of mean, Minolta a* measured redness, and Minolta b* measured yellowness.

[b] Japanese color scores were: 1 = very pale, light pink; and 6 = very dark red.

[c] Internal ham marbling scores: 1 = devoid of marbling, 2 = moderate marbling, 3 = abundant marbling, and 4 = overly abundant marbling.

[d] NPPC marbling scores: 1 = devoid, 2 = practically devoid, 3 = moderately abundant, 4 = abundant, and 5 = overly abundant (NPPC, 1991).

[e] NPPC firmness scores: 1 = soft, and 3 = firm (NPPC, 2000).

Correlations among traits

Phenotypic and genetic correlations are presented in Tables 3 to 5 for growth and fatness, and carcass and meat quality traits. Generally, estimates of genetic correlations were higher in absolute value than phenotypic correlations. In a few cases, the estimated correlation was outside the parameter space (i.e., larger then 1). Phenotypic correlations generally have limited interpretive value and are therefore not discussed.

Correlations among growth and fatness and carcass traits

The phenotypic and genetic correlations for growth and fatness and carcass traits are presented in Table 3. Generally, genetic correlations of ADG with the fatness traits recorded in this study were low. A low correlation was found between LD and PLEAN (0.19). Back fat was not correlated with LD (-0.01). Back fat, LD, and PLEAN had dissimilar correlations with growth rate (0.27, -0.01, and -0.29, respectively).

The primal and subprimal cut weights were moderately to highly correlated with each other (range in absolute value = 0.22 to 0.85), with the low correlation of HAM–BLOIN (0.22) being one exception. In contrast, LOIN was higher correlated with the boneless ham (BHAM), 0.58. The ham primal (HAM) was moderately correlated with the boneless ham (BHAM) (0.60). The loin primal (LOIN) and the boneless loin (BLOIN) were highly correlated (r_g = 0.85). A high correlation was found between the boneless ham (BHAM) and boneless loin weight (BLOIN) (0.74).

Table 2. Heritabilities (h^2) and common environment effects (c^2) with their standard errors (SE) and the genetic variance (σ^2_a) for meat quality, growth and fatness, and carcass traits.

Trait[a]	h^2	± SE	c^2	± SE	σ^2_a
Growth and fatness traits					
BF, mm	0.45	0.16	0.15	0.03	11.2
LD, mm	0.13	0.06	0.04	0.02	8.9
PLEAN, %	0.43	0.16	0.14	0.02	4.4
ADG, g/d	0.22	0.09	0.11	0.02	600
Carcass traits					
HAM, kg	0.40	0.15	0.12	0.02	0.066
LOIN, kg	0.29	0.11	0.11	0.02	0.079
BHAM, kg	0.39	0.15	0.13	0.02	0.056
BLOIN, kg	0.51	0.18	0.18	0.03	0.035
Meat quality traits					
HAML	0.11	0.06	0.09	0.02	2.324
LOINL	0.18	0.08	0.07	0.02	2.330
HAMA	0.26	0.11	0.09	0.02	0.492
LOINA	0.21	0.09	0.09	0.02	0.364
HAMB	0.12	0.06	0.06	0.02	0.381
LOINB	0.15	0.08	0.09	0.02	0.287
JCScut	0.22	0.09	0.09	0.02	0.059
JCSrib	0.28	0.11	0.09	0.02	0.054
DRIP, %	0.08	0.05	0.06	0.02	0.344
PURGE, %	0.11	0.07	0.14	0.02	0.302
pHU	0.11	0.07	0.15	0.03	0.002
HMARB	0.14	0.08	0.12	0.02	0.066
LMARB	0.31	0.12	0.16	0.02	0.123
FIRM	0.20	0.08	0.11	0.02	0.069

[a] See Table 1 for trait abbreviation definition

Growth is in general moderate and adversely correlated with the primal and subprimal cut weights, averaging -0.29, except for the correlation between HAM and ADG, which was found to be positive (0.39). Selection for higher growth could have a declining effect on the most valuable primal and subprimal weights. Moderately to highly negative correlations averaging -0.56 were estimated for BF with the ham and loin boneless and primal weights. Correlations of similar magnitude, although positive, were estimated for PLEAN and those primal and subprimal weights. Also loin depth was moderately to highly correlated with the loin and ham primals and subprimals, averaging 0.49.

Table 3. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) between growth and fatness and carcass traits[a].

Trait[b]	HMARB	LMARB	FIRM	BF	LD	PLEAN	ADG	HAM	LOIN	BHAM	BLOIN
HMARB		0.37	0.62	0.31	0.32	-0.27	0.12	0.66	-0.01	0.13	-0.05
LMARB	0.34		0.51	0.35	0.40	-0.28	0.16	0.18	0.15	-0.04	-0.29
FIRM	0.08	0.21		0.25	0.27	-0.21	-0.67	0.18	0.20	0.11	-0.01
BF	0.24	0.24	0.16		-0.01	-0.98	0.27	-0.38	-0.40	-0.86	-0.60
LD	-0.03	-0.06	0.03	0.00		0.19	0.00	0.20	0.78	0.34	0.65
PLEAN	-0.24	-0.24	-0.14	-0.95	0.31		-0.23	0.40	0.51	0.90	0.69
ADG	0.05	-0.05	-0.08	0.13	0.08	-0.01		0.39	-0.18	-0.25	-0.44
HAM	-0.07	-0.12	-0.01	-0.23	0.08	0.25	0.40		0.31	0.60	0.22
LOIN	-0.07	-0.06	0.09	-0.21	0.18	0.26	-0.21	0.01		0.58	0.85
BHAM	-0.28	-0.24	-0.04	-0.46	0.13	0.48	-0.02	0.61	0.22		0.74
BLOIN	-0.24	-0.24	-0.02	-0.45	0.27	0.51	-0.19	0.14	0.60	0.44	

[a] Standard errors of the genetic correlations presented in this table ranged from 0.01 to 0.38.
[b] See Table 1 for trait abbreviation definitions.

Correlations among meat quality traits

In Table 4, the genetic and phenotypic correlations among the meat quality traits are given. The Minolta color measurements on the loin were moderately to highly correlated genetically with the corresponding measurements on the ham, except for the Minolta a* values. Minolta L* measurements were highly correlated with the Minolta b* values for both ham and loin, averaging 0.81. Minolta a* measured at the loin (LOINA) was lowly correlated with Minolta b*

and L* values for both ham and loin (averaging 0.11 in absolute value). Minolta a* measured at the ham (HAMA), however, was moderately correlated with the Minolta b* and L* values on both ham and loin, averaging 0.55.

The correlations between the objective Minolta color readings and the subjective JCScut, averaging 0.66 (in absolute value), were generally high, except for HAMA (-0.16). In contrast, JCSrib was in general less correlated with the objective Minolta color readings (averaging 0.26 in absolute value), except for LOINA (0.77).

The genetic correlations estimated between the two water-holding capacity traits, DRIP and PURGE, approached 1. Generally, DRIP and PURGE were both highly correlated with HAML and HAMB (averaging 0.90). Correlations of PURGE with LOINL and LOINB were much lower (0.45 and 0.29). Estimated genetic correlations between DRIP and HAML (1), and DRIP and HAMB (1) were very high, whereas corresponding correlations between DRIP and LOINL, and DRIP and LOINB were high, but less extreme in magnitude (0.81 and 0.74, respectively). The subjective color value JCScut was highly negatively correlated with the water-holding capacity traits (averaging -0.82). Correlations with lower magnitudes were found for both DRIP and PURGE with HAMA and LOINA (averaging 0.17) and JCSrib (averaging 0.37). The correlations between pHU and the color traits showed a similar trend as observed for the water-holding capacity traits.

The marbling scores on ham and loin were moderately correlated with each other (0.37). Close to zero or low correlations were estimated between marbling and the JCS values.

Correlations of meat quality traits with growth and fatness and carcass traits

Table 5 summarizes genetic and the phenotypic correlations of meat quality traits with growth and fatness and carcass traits. High correlations were estimated among the meat quality traits Minolta L* and b*, JCScut, pHU, and the water-holding capacity traits (Table 4). Additionally, unfavorable correlations of high magnitude were estimated between those quality traits and growth rate, averaging 0.70 in absolute value. The correlation of ADG with pHU approaching -1 was noteworthy, as was the high correlation of ADG with DRIP (0.78). This suggests that single-trait selection on fast growth rate may lead to undesirable lower-pH meat, with decreased water-holding capacity and paler color. Daily growth (ADG) had low genetic correlations with both marbling measurements.

Table 4. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) among meat quality traits[a].

Trait[b]	HAML	LOINL	HAMA	LOINA	HAMB	LOINB	JCScut	JCSrib	DRIP	PURGE	pHU	HMARB	LMARB	FIRM
HAML		0.67	0.30	-0.03	0.95	0.56	-0.81	-0.37	1[c]	0.98	-0.83	0.28	0.02	-0.32
LOINL	0.31		0.60	-0.17	0.85	0.89	-0.76	-0.18	0.81	0.45	-0.60	0.08	0.48	-0.21
HAMA	0.09	0.14		0.39	0.52	0.76	-0.16	0.29	0.35	0.09	-0.30	0.68	0.30	0.16
LOINA	0.05	-0.23	0.34		-0.04	0.21	0.56	0.77	-0.13	-0.12	-0.03	0.51	0.13	0.32
HAMB	0.86	0.30	0.35	0.10		0.81	-0.75	-0.29	1[c]	0.79	-0.73	0.49	0.26	-0.07
LOINB	0.26	0.86	0.22	0.11	0.30		-0.43	0.16	0.74	0.29	-0.46	0.25	0.70	0.02
JCScut	-0.37	-0.53	0.06	0.18	-0.28	-0.39		0.53	-0.85	-0.79	0.80	-0.01	0.12	0.61
JCSrib	-0.27	-0.26	0.14	0.23	-0.19	-0.17	0.54		-0.34	-0.40	0.02	0.03	0.23	0.35
DRIP	0.36	0.38	0.16	0.10	0.32	0.32	-0.45	-0.21		1[c]	-0.86	0.10	0.01	-0.60
PURGE	0.34	0.33	0.10	0.08	0.31	0.29	-0.40	-0.23	0.46		-0.92	0.28	-0.09	-0.69
pHU	-0.46	-0.38	-0.22	-0.06	-0.45	-0.32	0.45	0.30	-0.50	-0.60		-0.12	0.24	0.79
HMARB	-0.01	0.16	0.15	0.14	0.02	0.23	0.02	0.04	-0.04	-0.03	0.03		0.37	0.62
LMARB	-0.09	0.26	0.14	0.09	-0.01	0.36	0.08	0.06	-0.14	-0.09	0.11	0.34		0.51
FIRM	-0.18	-0.16	-0.01	0.05	-0.15	-0.09	0.31	0.21	-0.19	-0.26	0.31	0.08	0.21	

[a] Standard errors of the genetic correlations presented in this table ranged from 0.05 to 0.38.

[b] See Table 1 for trait abbreviation definitions.

[c] Estimates outside the parameter space (1 < estimate < 1.06).

Correlations near zero were estimated for BF and PLEAN with water-holding capacity traits, the subjective meat color traits, and pHU (averaging 0.09 in absolute value). The estimated correlations for BF and PLEAN with the objective Minolta measurements averaging 0.43 were of favorable moderate magnitude. Back fat, LD, and PLEAN were similarly and moderately correlated with both marbling traits and FIRM (averaging 0.30 in absolute value) and were dissimilarly correlated with growth rate (0.35, -0.01 and, -0.29 respectively). Firmness (FIRM) was moderately correlated with JCS, DRIP, PURGE, and pHU. Low correlations were obtained between both marbling traits and the traits JCS, DRIP, PURGE, and pHU.

Both marbling traits were in general lowly correlated with the ham and loin primal and boneless weights, averaging 0.12 in absolute value, except for the correlation between HAM and HMARB (0.66). Favorable low to moderate correlations averaging 0.22 in absolute value (range -0.41 to 0.35) were obtained for DRIP, PURGE, and pHU, with both ham and loin primal and subprimal weights. In addition, the correlations for the subjective color traits with the ham and loin primal and subprimal weights averaging 0.44 were of favorable moderate to high magnitude. However, ham primal (HAM) and boneless ham (BHAM) weights were somewhat less correlated (averaging 0.32) with the subjective color readings than loin primal (LOIN) and boneless loin (BLOIN) weights (averaging 0.55). The Minolta color traits were also moderately and favorably correlated with the primals and boneless weights of both, ham, and loin (averaging in absolute value 0.34 and 0.43, respectively), except for HAMA, which was found unfavorably correlated with boneless weights of ham (BHAM) and loin (BLOIN).

Table 5. Genetic correlations (first row) and phenotypic correlations (second row) of meat quality with growth and fatness and carcass traits[a].

Trait[b]	HAML	LOINL	HAMA	LOINA	HAMB	LOINB	JCScut	JCSrib	DRIP	PURGE	pHU	HMARB	LMARB	FIRM
BF	0.17	0.43	0.68	0.42	0.37	0.61	-0.06	0.02	0.05	0.08	-0.24	0.31	0.35	0.25
	-0.01	0.19	0.12	0.09	0.03	0.24	0.01	0.03	0.00	-0.05	0.03	0.24	0.24	0.16
LD	0.06	-0.19	0.40	0.73	0.06	0.18	0.49	0.55	0.28	0.18	-0.04	0.32	0.40	0.27
	0.05	-0.05	0.01	0.04	0.04	-0.05	0.04	0.08	0.00	0.01	-0.03	-0.03	-0.06	0.03
PLEAN	-0.16	-0.46	-0.62	-0.30	-0.36	-0.58	0.14	0.06	-0.01	-0.05	0.23	-0.27	-0.28	-0.21
	0.02	-0.19	-0.11	-0.08	-0.02	-0.24	0.01	0.00	0.00	0.05	-0.04	-0.24	-0.24	-0.14
ADG	0.43	0.74	0.37	0.18	0.54	0.82	-0.68	-0.03	0.78	0.60	-1[c]	0.12	0.16	-0.67
	0.07	0.18	0.10	0.03	0.10	0.19	-0.11	-0.16	0.14	0.10	-0.14	0.07	-0.05	-0.08
HAM	-0.03	-0.19	0.14	0.36	0.09	0.01	0.21	0.51	0.00	0.16	-0.29	0.66	0.18	0.18
	0.03	-0.08	0.01	0.03	0.00	-0.08	-0.01	0.06	0.02	0.03	-0.04	-0.07	-0.12	-0.01
LOIN	-0.36	-0.35	-0.15	0.47	-0.41	-0.16	0.46	0.76	-0.17	-0.41	0.25	-0.01	0.15	0.20
	-0.10	-0.16	-0.01	0.07	-0.08	-0.12	0.11	0.16	-0.05	-0.05	0.06	-0.07	-0.06	0.09
BHAM	-0.19	-0.63	-0.60	-0.13	-0.34	-0.63	0.30	0.25	-0.25	-0.10	0.20	0.13	-0.04	0.11
	0.06	-0.17	-0.07	-0.05	0.03	-0.19	-0.02	0.01	0.04	0.04	-0.04	-0.28	-0.24	-0.04
BLOIN	-0.33	-0.78	-0.46	0.25	-0.54	-0.70	0.53	0.46	-0.20	-0.31	0.35	-0.05	-0.29	-0.01
	-0.01	-0.19	-0.09	-0.04	-0.02	-0.20	0.02	0.04	0.01	0.02	-0.04	-0.24	-0.24	-0.02

[a] Standard errors of the genetic correlations presented in this table ranged from 0.15 to 0.43.

[b] See Table 1 for trait abbreviation definitions.

[c] Estimate outside the parameter space (estimate = -1.08).

Discussion

Genetic material

This study was conducted on offspring of one sire line and one type of dam. The data were collected within a period of 70 days, and obtained at a high-speed slaughter line. Risks of such a setup are loss of animals through misreading, loss of cuts, and so forth; however, no indications of anything untrustworthy were found during analysis of the data. Advantages are the limited time and costs required and the large dataset representing commercial slaughter conditions.

Experimental setup

The experiment was set up to facilitate the identification and validation of QTL. The aim, therefore, was to get approximately 100 offspring per sire. The 20 sires were chosen as representatives for the line based on pedigree information. Pedigree of the dams was not available.

To avoid an overestimation of the additive genetic variances, the random effects of common litter environment were included in the statistical model. The common environmental effect comprised the effect of a shared environment by littermates (*i.e.* uterine and rearing effects) but also possible effects due to phenomena such as dominance and paternal imprinting. Estimates of common environmental effects were in the range from 0.04 to 0.18, which indicates that the contribution of the common environment is noticeable on the ultimate values at slaughter for some traits.

Heritabilities

Growth and fatness traits were in general moderately heritable (Table 2), except for ham marbling and loin depth, for which estimated heritabilities were of lower magnitude. The ADG heritability estimate in this study was low compared to literature values. No clear explanation can be offered for this finding. In addition, published estimates vary considerably, ranging from 0.13 to 0.57 (Lo et al., 1992; NPPC, 1995; Larzul et al., 1997; Gibson et al., 1998; Hermesch et al., 2000a). The heritability estimate of BF in this study is close to the average literature value of 0.49 (Clutter and Brascamp, 1998).

Overall, the estimated heritability for the brightness of meat, measured in an objective way at the loin (LOINL), was in agreement with the estimates found in earlier studies by Cameron (1990), De Vries et al. (1994), Larzul et al. (1997), Hermesch et al. (2000a), and Andersen and Pedersen (2001). For LOINA, the estimated heritability in this study was lower than the range of 0.54 to 0.57 published by Sonesson et al. (1998) and Andersen and Pedersen (2001). The estimated heritability for the meat yellowness of the loin (LOINB) was in agreement with the estimates published by Andersen and Pedersen (2001). Based on a different measurement method Sonesson et al. (1998) reported a higher heritability (0.54). No heritability estimates were found in the literature for objective color scores measured on the ham primal or subprimals.

Heritability estimates for the subjective color scores, JCScut and JCSrib, were of moderate magnitude. The estimates for both traits correspond to literature values (Hovenier et al., 1993; NPPC, 1995; Andersen and Pedersen, 2001). The heritabilities of the subjective color measurements were higher than those of the objective Minolta color readings. This was not as expected because the environmental effects may be less standardized for subjective measurements. One explanation may be the different methods of measuring; following a continuous scale for the objective (Minolta) measurements or the score in classes for the subjective (JCS) color measurements. Another reason may be that the JCS measurement represents an overall color score, whereas the three Minolta measurements represent single color spectra only. It seems worthwhile to investigate opportunities to combine the three Minolta values.

The estimated heritabilities for the water-holding capacity traits (DRIP and PURGE) were lower than most estimates presented in literature, which ranged from 0.08 to 0.30 (Hovenier et al., 1993; De Vries et al., 1994; Sonesson et al., 1998; Hermesch et al., 2000a). Differences between studies may be due to varying measurement methods and time points. The reviews of Hovenier et al. (1993) and Sellier (1998) reported large differences in heritability estimates due to varying measurement methods for the water-holding capacity traits. Moreover, large differences in heritability estimates between breeds for drip loss percent were observed by Hermesch et al. (2000a). Furthermore, the presence or absence of ryanodine receptor gene (halothane gene causing malignant hyperthermia syndrome) segregation influences the heritability estimate.

The estimated heritability for pHU of 0.11 was close to the range of published estimates of 0.13 to 0.20 (Cameron et al., 1990; Lo et al., 1992; De Vries et al., 1994; Larzul et al., 1997; Hermesch et al., 2000a; Andersen and Pedersen, 2001), although several high heritabilities (0.39 to 0.45) for pHU were found in literature as well (Hovenier et al., 1993; NPPC, 1995; Sonesson et al., 1998). In contrast to the published estimates, common environmental effects were corrected for in our study. The low heritability of pHU may limit genetic progress for this trait. When using pHU as an indicator trait, one needs to realize that with restricted amounts of genetic variation, the ability to consistently rank sires for their genetic potential is also limited.

The heritability estimates of marbling in ham (0.14) and loin (0.31) differed from each other, although not significantly. Most literature values are related to loin marbling and range from 0.13 to 0.31 (Lo et al., 1992; NPPC, 1995; Gibson et al., 1998; Sonesson et al., 1998).

The heritability estimates obtained for the carcass traits are among the highest in this study, with similar estimates for the ham primal and boneless ham weight, and an increasing value from loin primal and the boneless loin weight. Those high values offer good opportunities to select for higher ham and loin muscle yield, representing the most valuable parts of the carcass. Although high, the estimates in this study were lower than the estimates reported by Newcom et al. (2002). They presented heritabilities of 0.57 and 0.51 for ham primal weight and loin primal weight, and 0.76 and 0.72 for the boneless subprimals of ham and loin, respectively.

Correlations among growth and fatness and carcass traits

The hams and loins are the most valuable parts of the carcass. Although the quality of the meat is of increasing importance, meat yield remains decisive for carcass value, certainly in markets with pricing systems based on primal and subprimal cut weights. Ham weight (HAM) was lowly to moderately correlated (r_g = 0.31) with loin weight (LOIN), indicating that selection for high primal ham yield does not necessarily result in a high loin primal yield (Table 3). The boneless muscle yields from the ham and loin, BHAM and BLOIN, were more highly correlated (0.74). This implies that selection on high ham muscle yield will also lead to increased loin muscle yield. Both primals for ham and loin were highly correlated with their own subprimals, although the estimated correlation between loin primals and subprimals was higher, which may suggest a difference in fat deposition on ham and loin. Little is published about genetic correlation between primals and subprimals. Recently, Newcom et al. (2002) presented

correlations between primals and subprimals. They also showed high genetic correlations between ham primal and the boneless ham subprimal (0.89), and between both primal cuts for ham and loin (0.62).

Correlations among meat quality traits

Generally high correlations were found between most meat quality traits (Table 4). The water-holding capacity traits (DRIP and PURGE), pHU and the color traits (LOINL, HAML, LOINB, HAMB and JCScut) were especially highly correlated. In line with the phenomena of DFD or PSE meat, the estimates in this study indicate that meat with a high pHU tends to be darker and dry, whereas meat with a lower pHU tends to be more pale and exudative. The high genetic correlations are in agreement with previous studies by De Vries et al. (1994), Gibson et al. (1998), Sonesson et al. (1998), Hermesch et al. (2000b), and Andersen and Pedersen (2000). The high correlations offer possibilities to improve meat quality by measuring a limited number of traits only, which will decrease costs and labor in the slaughterhouse. Ultimate pH is quick and easy to measure and is therefore a good candidate trait that can be used as quality predictor trait.

The correlation between JCSrib and JCScut of r_g = 0.53 is reasonable for subjective measurements. Nonetheless, the differences in correlation of the two traits with the objective color readings suggest that both JCS measurements cannot be considered simply as identical traits.

Marbling scores on both ham and loin were moderately correlated with each other, indicating that marbling in ham or loin may be genetically different; this is supported by the difference in heritability estimates of both traits. However, small differences between both scores may be due to varying measurements, as loin marbling was judged at a chop and ham marbling at the outside surface of a muscle, and the measurements followed different score classes.

Correlations among meat quality and carcass traits

Most meat quality traits have a favorable relationship with the primal and subprimal weights of the ham and loin (Table 5), which implies that selection on individual cut weight will also result in improved meat quality. Correlations between subprimals of ham and loin (Table 3) also were favorably high (r_g = 0.74), implying that selection on ham only will positively affect loin

muscle development or vice versa. The favorable correlations between cut weights and meat quality are striking and in contrast to the general impression (see below). No literature was found presenting correlations of primal or subprimal cut weight with meat quality traits.

Correlations among main selection traits and carcass and meat quality traits

Average daily gain and BF have been the main selection traits (among the finishing traits) in the pig breeding industry. Based on estimates obtained in this study, selection on ADG will have a detrimental effect on meat quality traits. In particular, the unfavorable effect of ADG on pHU and DRIP is remarkable (Table 5), which implies that selection for fast growth rate will lead to paler pork with lower pH meat and a decreased water-holding capacity. Comparable estimates from other publications were inconsistent. De Vries et al. (1994) and Hermesch et al. (2000b) showed no clear relationship between growth rate and meat quality traits. Lo et al. (1992) and Hovenier et al. (1993) found a positive correlation between ADG and meat quality traits; however, the general impression is that selection for improved performance goes together with negative effects on meat quality characteristics. Sellier (1998) presented an average genetic correlation of –0.23 between meat quality index (IQV) and carcass lean percentage. Rosenvold and Andersen (2003) underlined this conclusion in their review.

The unfavorable correlations between ADG and quality traits approach unity. Checks of the results were performed by estimating the parameters on several subsets of the data. The additional analyses all obtained similar results. In addition, correction of ADG for back fat to create a “fat free” gain yielded similar results. However, heritabilities of both ADG and meat quality traits were in the range expected, as was the genetic correlation between ADG and back fat.

Back fat depth was not correlated with LD (-0.01) and can be considered as genetically different. Selection for leaner carcasses will not affect meat quality defined by the quality indicators as measured in this study (Table 5). Most of the objective and subjective color traits were favorably correlated with lean meat percent. Only a negative effect of PLEAN on Minolta a* values for both ham and loin was observed. Correlations close to zero were found between both water-holding capacity traits and PLEAN. Ultimate pH was favorably correlated with PLEAN. Genetic correlations between PLEAN with pHU and color traits vary substantially in

literature; Hovenier et al. (1993), De Vries et al. (1994), and Sonesson et al. (1998) presented generally negative or close to zero correlations between PLEAN and pHU or meat color traits.

The general impression that selection for improved performance goes together with negative effects on meat quality characteristics was confirmed in this study only when considering growth and fatness traits. When considering primal and subprimal cut weights, strikingly favorable correlation with meat quality was found.

Implications

We report genetic parameters for meat quality, growth, and carcass traits. The focus on carcass (sub)primal cuts and use of crossbred pigs make the results relevant for commercial pork production chains. The estimated parameters, together with genetic correlations between purebred parameter estimates, are valuable for the design of a breeding program focusing on an increase of the proportion of valuable cuts of appropriate quality. Selection for growth rate will have adverse consequences for meat quality based on the high unfavorable correlations found between average daily gain and most quality traits considered. Furthermore, selection for growth rate is negatively correlated with (sub)primal cut yield and will therefore not automatically lead to increased cut weights. However, selection towards increased carcass value by increasing (sub)primal weights with improved quality will clearly be feasible based on the correlations that were observed between most meat quality traits and (sub)primal cuts.

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Literature cited:

Andersen S., and B. Pedersen. 2000. Genetic parameters for colour traits and pH and correlations to production traits. Pages 129-133 in Proc. EAAP, publication No. 100, Zurich, Switzerland.

Brorsen B.W., J.T. Akridge, M.A. Boland, S. Mauney, and J.C. Forrest. 1998. Performance and alternative component pricing systems for pork. J. Agric. Appl. Econ. 302: 313-324.

Clutter A.C., and E.W. Brascamp. 1998. Genetics of Performance Traits. Pages 427-462 in Rothschild, M.F., and A. Ruvinsky, eds, The Genetics of the Pig, CAB International, New York.

Cristensen L.B. 2003. Drip loss sampling in porcine m. longissimus dorsi. Meat Sci. Vol. 63, Iss. 4: 469-477.

Cameron N.D. 1990. Genetic and phenotypic parameters for carcass traits, meat and eating quality traits in pigs. Livest. Prod. Sci. 26: 119-135.

De Vries A.G., P.G. van der Wal, T. Long, G. Eikelenboom, and J.W.M. Merks. 1994. Genetic parameters of pork quality and production traits in Yorkshire populations. Livest. Prod. Sci. 40: 277-289.

Gibson J.P., C. Aker, and R. Ball. 1998. Levels of genetic variation for growth, carcass and meat quality traits of purebred pigs. Proc. 6th World Cong. Genet. Appl. Livest. Prod., Armidale, Australia 23: 499-502.

Gilmour A.R., B.J. Gogel, B.R. Cullis, S.J. Welham, and R. Thompson. 2002. ASReml User Guide Release 1.0. VSN International Ltd, Hemel Hempstead, UK.

Hermesch S., B.G. Luxford, and H.U. Graser. 2000. Genetic parameters for lean meat yield, meat quality, reproduction and feed efficiency traits for Australian pigs 1. Description of traits and heritability estimates. Livest. Prod. Sci. 65: 239-248.

Hermesch S., B.G. Luxford, and H.U. Graser. 2000. Genetic parameters for lean meat yield, meat quality, reproduction and feed efficiency traits for Australian pigs 2. Genetic relationships between production, carcase and meat quality traits. Livest. Prod. Sci. 65: 249-259.

Hovenier R., E. Kanis, Th. van Asseldonk, and N.G. Westerink. 1993. Genetic parameters of pig meat quality traits in a halothane negative population. Livest. Prod. Sci. 32: 309-321.

Larzul C., L. Lefaucheur, P. Ecolan, J. Gogué, A. Talmant, P. Sellier, P. Le Roy, and G. Monin. 1997. Phenotypic and genetic parameters for longissimus muscle fiber characteristics in releation to growth, carcass and meat quality traits in Large white pigs. J. Anim. Sci. 75: 3126-3137.

Lo L.L., D.G. McLaren, F.K. McKeith, F.L. Fernando, and J. Novakofski. 1992. Genetic analysis of growth, real time ultrasound, carcass and pork quality traits in Duroc and Landrace pigs: II. Heritabilities and Correlations. J. Anim. Sci. 70: 2357-2396.

Lonergan S.M., E. Huff-Lonergan, L.J. Rowe, D.L. Kuhlers, and S.B. Jungst. 2001. Selection for lean growth efficiency in Duroc pigs influences pork quality. J. Anim. Sci. 79: 2075-2085.

Newcom D.W., T.J. Baas, J.W. Mabry, and R.N. Goodwin. 2002. Genetic Parameters for pork carcass components. J. Anim. Sci. 80: 3099-3106.

NPPC. 1991. Procedures to Evaluate Market Hogs. 3rd ed. Natl. Pork Prod. Counc., Des Moines, IA.

NPPC. 1995. Genetic Evaluation/Terminal Line Program Results. Ed. R. Goodwin and S. Burroughs. Natl. Pork Prod. Counc., Des Moines, IA.

NPPC. 2000. Pork composition and quality assessment procedures. Natl. Pork Prod. Counc., Des Moines, IA.

Rosenvold K. and H.J. Andersen. 2003. Factors of significance for pork quality – a review. Meat Sci. 64: 219-237.

Sellier P. 1998. Genetics of Meat and Carcass Traits. Pages 463-510 in Rothschild, M.F., and A. Ruvinsky, eds, The Genetics of the Pig, CAB International, New York.

Sonesson A.K., K.H. de Greef, and T.H.E. Meuwissen. 1998. Genetic parameters and trends of meat quality, carcass composition and performance traits in two selected lines of large white pigs. Livest. Prod. Sci. 57: 23-32.

Chapter 3

Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross

H.J. van Wijk[*], B. Dibbits[#], E.E. Baron[#1], A.D. Brings[#2], B. Harlizius[*], M.A.M. Groenen[#], E.F. Knol[*] and H. Bovenhuis[#]

[*]IPG, Institute for Pig Genetics, P.O. Box 43, 6640 AA, Beuningen, The Netherlands; [#]Animal Breeding and Genetics Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands.

[1] Current address: Department of Animal Production, Escola Superior de Agricultura "Luiz de Queiroz" – USP, Av.Padua Dias 11, 13418-900, Piracicaba-SP, Brazil.
[2] Current address: Institute of Animal Science, Animal Breeding and Husbandry Group, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany.

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Abstract: A quantitative trait loci (QTL) study for carcass composition and meat quality traits was conducted on finisher pigs from a cross between a synthetic Piétrain/Large White boar line and a commercial sow cross. The mapping population comprised 715 individuals evaluated for a total of 30 traits related to growth and fatness (4 traits), carcass composition (11 traits) and meat quality (15 traits). Offspring of eight sires (n = 715) were used for linkage analysis and genotyped for 73 microsatellite markers covering 14 chromosomal regions representing approximately 50% of the pig genome. The regions examined were selected based on previous studies suggesting the presence of QTL affecting carcass composition or meat quality traits. Thirty-two QTL exceeding the 5% chromosome-wise significance level were identified. Among these, five QTL affecting five different traits were significant at the 1% chromosome-wise level. The greatest significance levels were found for a QTL affecting loin weight on SSC11 and a QTL with an effect on the Japanese color scale score of the loin on SSC4. About one-third of the identified QTL were in agreement with QTL previously reported. Results showed that QTL affecting carcass composition and meat quality traits segregated within commercial lines. Use of these results for marker-assisted selection offers opportunities for improving pork quality by within-line selection.

Key Words: Carcass composition, Meat quality, Pig, Quantitative trait loci

Introduction

Production efficiency has been the focus of the swine industry during the past 30 yr. Selective breeding has contributed to the successful improvement of growth rate, reduced back fat thickness, and feed efficiency. The development of export markets and increased consumer demands for improved pork quality has led to a changed focus in selective breeding. The relative importance of pork quality has increased in relation to production traits; consequently, genetic improvement of pork quality has become the subject of several studies during the last decade (e.g. Sellier, 1998). The discovery of the Rendement Napole (Le Roy et al., 1990) and Halothane (Fujii et al., 1991) genes had by then already demonstrated the importance of allelic variation of single genes on pork quality.

Genetic improvement of meat quality by traditional breeding is difficult, and hampered by the need for extensive and expensive measurements of traits on slaughtered relatives. It is expected that for these types of traits, knowledge of the underlying genes will greatly contribute to the efficiency of selection. Many studies reported the identification of QTL in pigs for a variety of traits (e.g., Bidanel and Rothschild, 2002; Geldermann et al., 2003). However, QTL information for meat quality traits is relatively limited. In addition, most QTL studies were conducted on experimental crosses between divergent breeds. These QTL are not necessarily segregating in commercial breeds or the allelic effects might be different. Therefore, these QTL need to be verified within commercial lines before implementation. This was first done recently by Nagamine et al. (2003), Evans et al. (2003), and Vidal et al. (2005). However, information on QTL affecting body composition and meat quality traits in commercial crosses is still limited.

The objective of this study was to identify QTL for body composition and meat quality traits segregating within a commercial synthetic Piétrain/Large White boar line.

Materials and Methods

Genetic Material

Twenty sires of a synthetic Piétrain/Large White halothane-free boar line (TOPIGS, Vught, The Netherlands) were mated to 239 anonymous sows of a commercial sow cross. The synthetic sire line dates back to 1976 when Piétrain boars were crossed with Yorkshire/Large White gilts. The generation interval was on average 1.5 yr, indicating approximately 18 generations of

selection, which was consistently for lean gain, and from 1995 onwards, was additionally for piglet survival. Details of the management of the animals were described by Van Wijk et al. (2005). Phenotypic measurements were taken from approximately 100 offspring per sire, resulting in a total of 1,855 animals recorded. This initial study was performed on eight of the paternal half-sib families, which were randomly chosen, and encompassed 715 piglets with 77 to 103 animals per family. Their DNA was isolated from tissue samples collected from sires and offspring.

Phenotypic Records

Phenotypic measurements were recorded for 30 traits. These included traits for growth and fatness (4 traits), carcass composition (11 traits), and meat quality (15 traits; see Table 1). A detailed description of the phenotypic measurements was presented by Van Wijk et al. (2005). Summary statistics for traits measured, abbreviations used, and units of measurement are presented in Table 1.

Briefly, final body weight (BW) was estimated from a preeviscerated carcass weight (DCW) using the formula: Final BW = 106.5 * DCW. For calculating ADG, all pigs were assumed to have a birth weight of 1.36 kg, and ADG was calculated using the following equation: ADG = (Final BW – birth weight)/age at weighing. Hot carcass weight (HCW) was recorded after evisceration and the percentage of meat (**PLEAN**) was calculated [PLEAN = 58.86 – (0.61 * back fat) + (0.12 * LM depth)]. After cooling, the cold carcass weight (**CCW**) was recorded. The shrink of the carcass (**SHRINK**) was derived as the difference between hot and cold carcass weight. Back fat (**BF**) and LM depth (**LMd**) were measured at the 10^{th} rib using an ultrasonic probe, Hennessy Grading Probe Model 4 (Hennessy Grading System LTD, Auckland, New Zealand).

Primal cuts of loin and ham were weighed and further dissected into boneless subprimal cuts. For the loin, the weights for bone-in loins with skin removed and fat trimmed (**LOIN**) were recorded. Loins were further processed and the weights of the boneless loin with fat strap and fat cover left on (**ELOIN**) and the boneless loin with fat removed (**DLOIN**) were recorded. Hams were weighed with bone-in and skin on (**HAM**).

Table 1. Summary statistics of traits measured.

Trait	Abbreviation[1]	Mean	SD	Minimum	Maximum
Growth and fatness traits					
Back fat, mm	BF	26.3	5.9	12.8	45.2
LM depth, mm	LMd	59.6	9.1	28.4	90.8
Meat percentage, %	PLEAN	50.0	3.7	38.3	60.3
Average daily gain, g/d	ADG	655.4	77.2	385.1	878.8
Cold carcass weight, kg	CCW	86.5	8.2	50.5	113.1
Carcass traits					
Shrink, %	SHRINK	1.3	0.5	0.05	3.08
Ham weight, kg	HAM	10.3	0.9	7.3	13.4
Outside ham weight, kg	OHAM	1.97	0.22	1.40	2.90
Inside ham weight, kg	IHAM	1.85	0.20	1.13	2.36
Knuckle ham weight, kg	KHAM	1.18	0.13	0.77	1.63
Lite butt ham weight, kg	LBHAM	0.17	0.05	0.05	0.36
Boneless ham weight, kg	BHAM	5.2	0.5	3.8	6.6
Loin weight, kg	LOIN	9.3	1.0	5.2	12.1
Fat covered loin weight, kg	ELOIN	4.5	0.5	2.5	6.2
Fat removed loin weight, kg	DLOIN	3.3	0.4	1.8	4.4
Meat quality traits					
Minolta L* ham	HAML	49.3	4.9	35.8	64.6
Minolta a* ham	HAMA	7.7	2.1	1.4	13.3
Minolta b* ham	HAMB	2.5	2.4	-3.1	10.6
Minolta L* loin	LOINL	48.8	3.8	39.5	65.0
Minolta a* loin	LOINA	6.9	2.0	1.7	13.5
Minolta b* loin	LOINB	3.0	2.4	-1.8	10.6
Japanese color score cut[2]	JCScut	2.7	0.5	1	5.5
Japanese color score rib[2]	JCSrib	2.7	0.5	1	5
Drip loss	DRIP	3.29	2.20	0.02	16.01
Purge loss	PURGE	3.74	1.81	0.22	15.03
pH initial	PHI	6.32	0.23	5.57	6.83
pH ultimate	PHU	5.65	0.13	5.35	6.40
Marbling score ham[3]	HMARB	1.6	0.7	1	4
Marbling score loin[4]	LMARB	2.6	0.7	1	5
Firmness score loin[5]	FIRM	1.8	0.6	1	3

[1] Abbreviations are as defined in the text.

[2] Subjective color score, with 1 = pale and 6 = very dark.

[3] Subjective ham marbling score, with 1 = devoid of marbling, 2 = moderate, 3 = abundant, and 4 = overly abundant.

[4] Subjective loin marbling score, with 1 = devoid, 2 = practically devoid, 3 = moderately abundant, 4 = abundant, and 5 = overly abundant.

[5] Subjective firmness score, with 1 = soft and exudative, and 3 = firm.

Hams were subsequently skinned and defatted to obtain a boneless 4-muscle ham weight (**BHAM**), and further processed into 4 subprimal cuts, the inside, outside, knuckle, and lite butt (**IHAM**, **OHAM**, **KHAM**, and **LBHAM**, respectively), which were weighed individually.

Meat quality measurements were taken on both the loin and ham. Loin Minolta L*, a*, and b* measures (**LOINL**, **LOINA**, and **LOINB**) were taken in the *longissimus thoracis* muscle on a fresh cut surface of a 2.5-cm thick chop removed from the sirloin end of the boneless centre cut loin. Ham Minolta measurements were taken on the fresh cut surface of the inside ham muscle (**HAML**, **HAMA**, and **HAMB**). Japanese colour scores also were taken on the cut surface of the loin (**JCScut**) and the rib-surface (**JCSrib**) of the defatted loin.

A marbling score was given to chops of loin (**LMARB**) and the outside ham (**HMARB**) based on National Pork Producers Council marbling standards (NPPC, 1991). Firmness scores (**FIRM**) were assigned to the loin chops following NPPC 1-to-3 scale (NPPC, 2000). Drip loss (**DRIP**) was expressed as a percentage loss of exudates, during 24 h cooling of a 25-mm core taken from a second 2.5-cm loin chop. Initial pH (**pHI**) was measured between the 10^{th} and 11^{th} ribs, and an ultimate pH (**pHU**) score was taken 24 to 28 h postmortem in the boneless loin. Purge loss (**PURGE**) was determined as the percentage loss of exudates during 6-d refrigeration, of a 7.5-cm section defatted loin.

DNA Isolation

The DNA was extracted from ear or loin tissue samples using the Puregene DNA isolation kit (D-70KA, Gentra Systems, Minneapolis, MN) with minor adaptations to the manufacturer's protocol. The isolated DNA was checked on 1.2% agarose gels for quality and adjusted in Sodium Chloride-Tris-EDTA (STE) buffer (5mL 1 *M* Tris-HCL (pH = 8) + 200 µL 0.5 *M* EDTA (pH = 8) in 1 L MilliQ) to a final concentration of 15 ng/µL.

Genotyping and Map Construction

Before initiation of this study, an inventory was made of the QTL mapping results in pigs. Approximately 30 publications related to about 15 experimental crosses described approximately 350 significant QTL for a variety of traits (data not shown). Information from this QTL survey was made available through PigAce, which is accessible at https://acedb.asg.wur.nl. The regions

examined in this study were selected with emphasis on previously described QTL affecting carcass composition and meat quality traits (Table 2). These 14 autosomal chromosome regions together represented approximately 50% of the genome.

Seventy-three microsatellite markers were selected to cover the 14 regions uniformly. Markers were individually amplified by standard PCR protocols. Compatible amplification products were pooled before electrophoresis using automated sequencers (ABI PRISM 377 or ABI PRISM 3100) and analyzed using GeneScan or GeneMapper software (Applied Biosystems, Foster City, CA). Genotypes were checked against pedigree information and a second examiner evaluated all marker genotypes before further analysis of the data. Genotypes that could not be scored unambiguously were treated as missing data. The linkage map was constructed using CriMap, version 2.4 (Green et al., 1990), and using the Kosambi mapping function. The sex-average linkage map was used in the QTL analysis.

Statistical Analysis

Before the QTL analyses, the phenotypic data were adjusted for systematic effects, using phenotypic data of the whole population (n = 1,855; Van Wijk et al., 2005). Effects were estimated using the ASReml software package (Gilmour et al., 2002). The following model was used to describe all phenotypic traits except ADG, where AGE and CCW were excluded:

$$Y_{ijk} = SEX_i + GFP_j + b_1AGE_{ijk} + b_2CCW_{ijk} + c_k + e_{ijk,} \quad [1]$$

where,

Y_{ijk} = trait under study;

SEX_i = fixed effect of i^{th} sex (two classes, barrow or gilt);

GFP_j = the combined fixed effect of j^{th} group, farm, and sample stages, (20 classes);

AGE = age as a covariate;

CCW = cold carcass weight (kg) as a covariate;

c_k = the random effect of k^{th} litter, $c_k \sim N(0, I\,\sigma_c^2)$;

e_{ijk} = the residual effect, $e_{ijk} \sim N(0, I\,\sigma_e^2)$;

b_1 = the regression coefficient of Y on age;

b_2 = the regression coefficient of Y on cold carcass weight.

Table 2. Covered genome regions shown by chromosome number, size of the region covered, number of markers, average marker interval (Int), information content (IC), and references with the main QTL reported by 1 of the 3 trait classes along with the observed (Obs) number of QTL in this study.

SSC	Region	Size, cM	No. of SSRs	Int., cM	IC	QTL trait class[1]					
						Growth and fatness	Obs[2]	Carcass comp.	Obs[2]	Meat quality	Obs[2]
1	whole	135	8	19	0.50	1a, 6a, 7b, 11, 16	-	1b, 6b, 11, 16	-	6b	-
2	whole	148	10	16	0.63	4, 7b, 16	-	2, 16, 19, 20	2	4, 6b	2
4	q-arm	97	6	19	0.34	3, 6a, 7b, 8, 9, 12, 16	-	2, 6b, 12, 16	1	4, 6b, 7a, 9, 14	3
5	q-arm	77	5	19	0.60	6a, 7b	-	13	3	6b	-
6	whole	157	6	31	0.48	6a, 7b, 14, 16	-	9, 15, 16, 18a	3	5, 6b, 9, 14, 16	1
8	p-arm	67	4	22	0.63	7b, 8	-	2	-	6b, 14	1
10	p-end	50	4	17	0.63	-	-	17a	1	6b	1
11	p-arm	53	4	18	0.53	-	1	1b, 6a, 17b	2	6b	-
12	q-mid	37	3	19	0.56	6a, 7b	-	-	1	6b	-
13	q-end	73	5	18	0.59	6a, 7b, 8, 11, 18b	1	18b	3	6b, 7a, 17c	2
14	whole	101	7	17	0.64	6a, 7b	-	1b, 16	1	6b	1
15	q-end	61	4	20	0.64	-	-	-	-	6b, 10	1
17	q-end	49	4	16	0.46	-	-	-	-	6b	-
18	q-end	37	3	19	0.71	6a	-	-	-	6b, 17c	1
Total	**14**	**1,129**	**73**	**19**			**2**		**17**		**13**

[1] The numbers correspond to the following publications and crosses: (1a/b) Rohrer et al. (1998a/b), MxWC; (2) Andersson-Eklund et al. (1998), EWBxLW; (3) Walling et al. (2000), MxELW; (4) De Koning et al. (1999), MxDc; (5) De Koning et al. (2000), MxDc; (6a/b) Malek et al. (2001a/b), BxY; (7a/b) De Koning et al. (2001a/b), MxDc; (8) Bidanel et al. (2001), MxLW; (9) Grindflek et al. (2001), NL/DxNL/Y; (10) Ciobanu et al. (2001), BxY; (11) Nezer et al. (2002), PxLW, WxP, WxM; (12) Wimmers et al. (2002), DxBM; (13) Bidanel et al. (2002), MxLW; (14) Ovilo et al. (2002), IxL; (15) Varona et al. (2002), IxL; (16) Geldermann et al. (2003); (17a/b/c) Dragos-Wendrich et al. (2003); (18a/b) Yue et al. (2003a), MxP, EWBxP, EWBxM; (19) Jeon et al. (1999), EWBxLW; and (20) Nezer et al. (1999), LWxP. Breeds used in crosses are abbreviated as follows: M, Meishan; B, Berkshire; Y, Yorkshire; LW, Large white; Dc, Dutch commercial; P, Piétrain; WC, White composite; EWB, European wild boar; ELW, European large white; D, Duroc; BM, Berlin miniature pig; NL, Norwegian landrace; I, Iberian; and L, Landrace.

[2] The number indicates the number of QTL within the trait class to the left that were observed in this study on the particular chromosome.

The adjusted trait score (Y^*) used in the QTL analysis represents the residual effect (i.e., the phenotypic data adjusted for the non-genetic and litter effects estimated under Model [1]). The litter effect included genetic effects of the dams along with common environmental effects.

The QTL analysis was performed using the multimarker regression approach for interval mapping in half-sib populations as applied by Knott et al. (1996) and De Koning et al. (1999). This method estimates the difference between alternative alleles transmitted by the sire. Sire haplotypes were reconstructed based on the frequency of the paternal marker allele combinations in the half-sib offspring. The most frequent haplotypes in the offspring were considered to represent the parental haplotypes. For each half-sib offspring, the probability of inheriting one of the sire's haplotypes was calculated at 1-cM intervals along the genome, conditional upon the flanking marker genotypes. For QTL detection, the phenotypic trait scores were regressed on these probabilities. Regression was within half-sib families. An *F*-test statistic was calculated along the chromosome at every 1-cM interval across the half-sib families.

Chromosome-wise significance thresholds (P_{chr}) were determined empirically for each trait by chromosome combination using permutation as described by Churchill and Doerge (1994). The term "chromosome-wise significance" is used in this study but may not be entirely appropriate because not all regions represent whole chromosomes. Thresholds were obtained based on 10,000 permutations. Genome-wide significance thresholds were calculated by applying the Bonferroni correction following the formula: $P_{gen} = 1 - (1 - P_{chr})^{1/r}$, where r is the

chromosome length divided by the total length of the 14 regions covered (De Koning et al., 1999). In this paper we report QTL exceeding the 5% chromosome-wise significance level.

Results

Means and SD of phenotypic traits of the mapping population are presented in Table 1. For most of the traits, heritability and phenotypic and genetic correlation estimates were described in Van Wijk et al. (2005).

Genotyping and Map Construction

The heterozygosity of the markers ranged from 0.2 to 1 with an average of 0.7. Marker order was identical to the USDA Meat Animal Research Centre (**MARC**) map (Rohrer et al., 1996). Marker Sw2512 at the distal end of SSC1 could not be positioned unambiguously but the best order was in agreement with USDA-MARC map (Rohrer et al., 1996). The sex-averaged linkage map spanning the 14 regions had a cumulative length of 1,129 cM (Kosambi), which is only slightly larger than the USDA-MARC map (Rohrer et al., 1996). The number of markers per selected chromosomal region varied between 3 and 10, with an average interval size of 19 cM (vs. 17 cM in USDA-MARC map). Four marker brackets exceeded 30 cM.

QTL Results

Trait by chromosome significance thresholds of the *F*-statistic were estimated empirically based on 10,000 permutated data sets. Thresholds differed between trait by chromosome combinations and were on average 2.29, 2.88 and 3.65 respectively for the 5%, 1% and 0.1% significance level.

The QTL mapping results are summarized in Table 3. Thirty-two QTL affecting 20 of the 29 traits analyzed were identified at the 5% chromosome-wise significance level. Among these QTL, five were significant at the 1% chromosome-wise level. Genome-wide significance levels of all 32 QTL were in the range of $P = 0.06 - 0.67$. The QTL were identified for 12 of the 14 regions under study and the number of QTL per trait varied from one to three. Three QTL were identified for the traits knuckle ham weight, inside ham weight, and weight of the loin with fat removed (KHAM, IHAM, and DLOIN).

Table 3. Summary of QTL mapping results by chromosome.

SSC	Trait[1]	Pos[2], cM	Marker bracket		P_{chr}[3]	R^2	No. of families[4]	QTL effect[5], σ_p	Known[6]
			Left	Right					
2	HAMB	112	SwR2157	Sw2514	0.0159	0.0424	3	0.36	6b
2	OHAM	112	SwR2157	Sw2514	0.0312	0.0329	3	0.29	-
2	KHAM	1	SwC9	Sw2623	0.0399	0.0318	1	0.56	2, 19, 20
2	DRIP	62	SwR783	Sw240	0.0513	0.0297			6b
4	JCScut	97	Sw445	S0097	0.0060	0.0332	1	0.84	6b,[7] 7a, 14
4	HAML	89	Sw445	S0097	0.0392	0.0274	2	0.61	6b, 7a, 14
4	PHU	97	Sw445	S0097	0.0401	0.0204	1	0.50	-
4	KHAM	81	Sw445	S0097	0.0494	0.0267			-
5	ELOIN	27	S0018	Sw995	0.0253	0.0295	2	0.40	-
5	IHAM	5	S0005	S0018	0.0351	0.0295	2	0.42	-
5	DLOIN	25	S0018	Sw995	0.0473	0.0269	1	0.46	-
6	OHAM	70	Sw1353	Sw1067	0.0129	0.0363	2	0.45	9, 18a
6	IHAM	1	Sw2535	Sw1353	0.0135	0.0366	1	1.96	-
6	BHAM	72	Sw1353	Sw1067	0.0224	0.0347	1	0.41	9, 18a
6	HMARB	58	Sw1353	Sw1067	0.0477	0.0318	1	0.90	-
8	HAMA	1	Sw2410	Sw905	0.0241	0.0311	2	0.54	-
10	LOINA	42	Sw1894	Sw497	0.0215	0.0294	1	0.36	-
10	KHAM	48	Sw1894	Sw497	0.0286	0.0296	2	0.46	17a
11	ELOIN	53	Sw1632	S0071	0.0031	0.0371	2	0.57	1b,[8] 6a, 17b
11	LMd	41	Sw1632	S0071	0.0096	0.0361	2	0.96	1b,[8] 6a, 17b
11	DLOIN	53	Sw1632	S0071	0.0266	0.0281	2	0.45	1b,[8] 6a, 17b
12	SHRINK	1	Sw874	Sw168	0.0081	0.0332	2	0.68	-
13	PHI	33	SwR428	Sw864	0.0100	0.1088	1	1.40	6b,[9]17c
13	HAMA	62	S0068	Sw398	0.0182	0.0326	1	0.84	7a
13	IHAM	21	SwR428	Sw864	0.0191	0.0331	2	0.41	18b
13	PLEAN	15	S0282	SwR428	0.0250	0.0332	2	0.78	6a, 11, 18b
13	DLOIN	72	S0068	Sw398	0.0328	0.0293	2	0.51	-
13	BHAM	34	SwR428	Sw864	0.0448	0.0292	2	0.45	18b
14	HMARB	1	Sw857	Sw1027	0.0223	0.0339	1	0.79	-
14	LBHAM	67	Sw77	Sw1557	0.0321	0.0319	2	0.44	16
15	PURGE	24	Sw1683	Sw906	0.0221	0.0312	2	0.62	RN gene
18	PHI	37	Sw787	S0062	0.0355	0.0866	2	0.87	6b,[9]17c

[1] See Table 1 for trait abbreviations.

[2] Position with greatest *F*-statistic.

[3] Chromosome-wise *P* value.

[4] Number of informative families (families with a *F*-statistic exceeding the $P = 0.05$ threshold in an individual family analysis and inferred to be segregating).

[5] QTL effect, in σ_p (the average of estimated allele substitution effect/σ_p of the individual families that contributed significantly to the across-family effect).

[6] Known QTL previously described. References reporting similar QTL are listed (see Table 2 for the numbering scheme).

[7] QTL for related traits (i.e., Minolta or Hunter color measurements).

[8] QTL for related traits more proximal located on SSC11.

[9] QTL for water-holding capacity, a correlated trait.

Figure 1 shows the profile of the test statistics for the four chromosomes carrying QTL significant at the 1% chromosome-wise level.

For the growth and fatness traits, two QTL were detected; a QTL for LM depth (LMd) on SSC11 between markers Sw1632 and S0071 significant at the 1% chromosome-wise level, and a QTL for meat percentage (PLEAN) on SSC13 near markers S0282 and SwR428.

For the carcass composition traits in total, 16 QTL were identified. Eleven QTL affecting ham primal or sub-primal weights were found on seven chromosomes [i.e., SSC2 (two QTL), SSC4, SSC5, SSC6 (three QTL), SSC10, SSC13 (two QTL), and SSC14 (one QTL)]. Five QTL on chromosomes SSC5 (two QTL), SSC11 (two QTL), and SSC13 affected loin primal or sub-primal weights. This included the most significant result obtained (i.e., the QTL for ELOIN on SSC11). The three QTL on SSC11 are consistent in position across the different loin traits, which also are genetically correlated with each other.

Fourteen QTL were detected for 11 meat quality traits on 10 different chromosomes (Table 3). The QTL for pH (PHI and PHU) were detected on SSC4, SSC13, and SSC18, of which the QTL on SSC13 for PHI was significant at the 1% chromosome-wise level. Regions affecting water-holding capacity traits (DRIP and PURGE) were found on SSC2 and SSC15. Five regions were found to affect meat color. Ham color was affected by QTL on SSC2, SSC4, SSC8, and SSC13. The QTL on SSC4 and SSC10 affected loin color, and the QTL on SSC4 for JCScut was significant at the 1% chromosome-wise level. Two suggestive QTL for ham marbling (HMARB) were found on SSC6 and SSC14.

For the regions showing significant evidence for the presence of a QTL at the chromosome-wise level, individual family analyses were performed. These analyses provide family-specific *F*-statistics and thresholds, allowing us to determine which families contributed to the overall effects; in general, one or two of the eight half-sib families contributed significantly. These families, which were inferred to be heterozygous for the QTL, showed average effects in the order of 0.29 to 1.96 σ_p (Table 3). The proportion of variance explained (R^2) by individual QTL were in the range of 2.0 to 10.1%, with an average of 3.6% (Table 3). Summation of the variance explained by individual QTL affecting a specific trait showed that, on average, the detected QTL explained 9% of the variance; for pH initial (PHI), this was 19%.

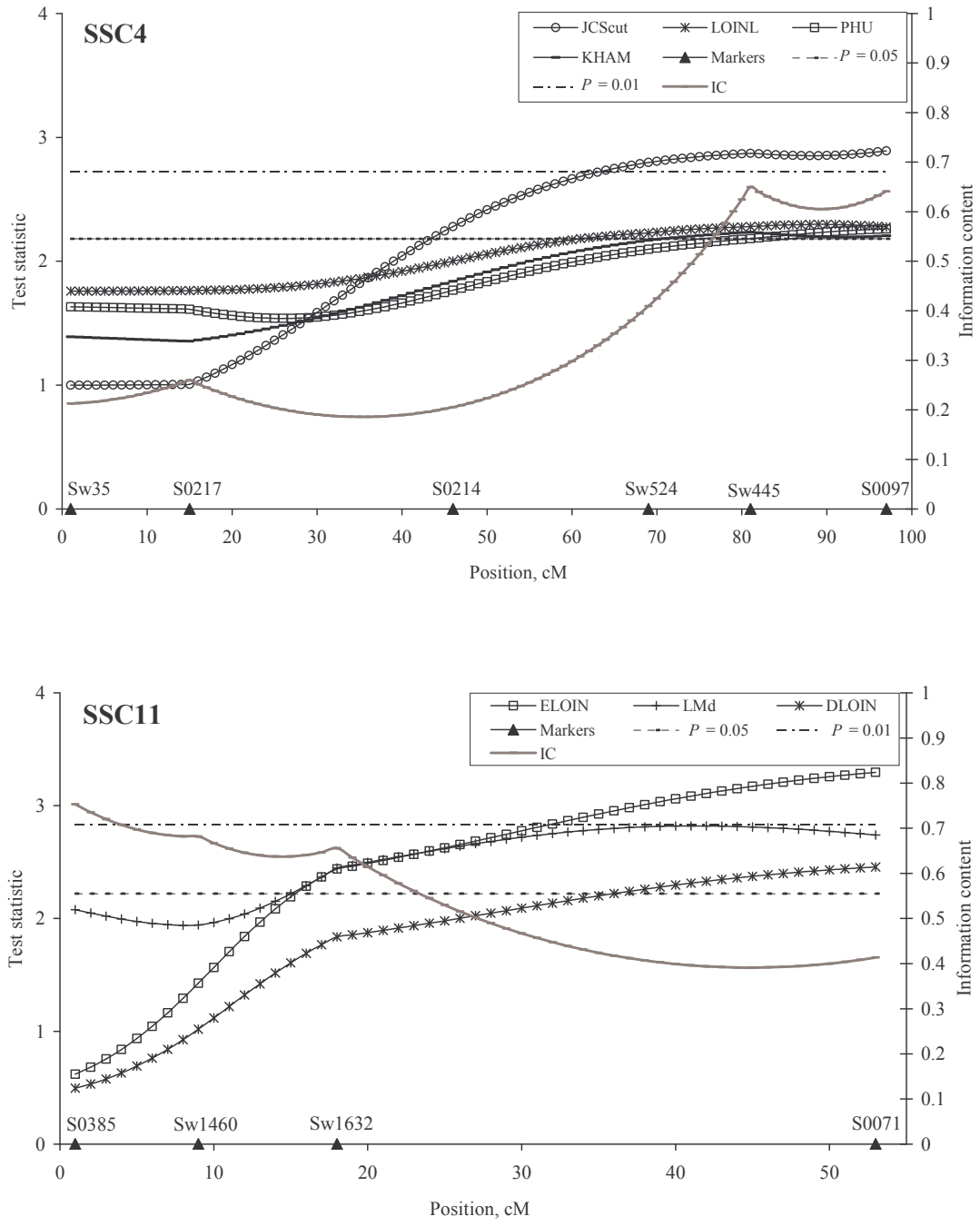

Figure 1. *F*-statistic profiles and information content (IC), for the four *Sus scrofa* chromosomes carrying QTL with $P < 0.01$ chromosome-wise significance. Traits abbreviations are given in Table 1.

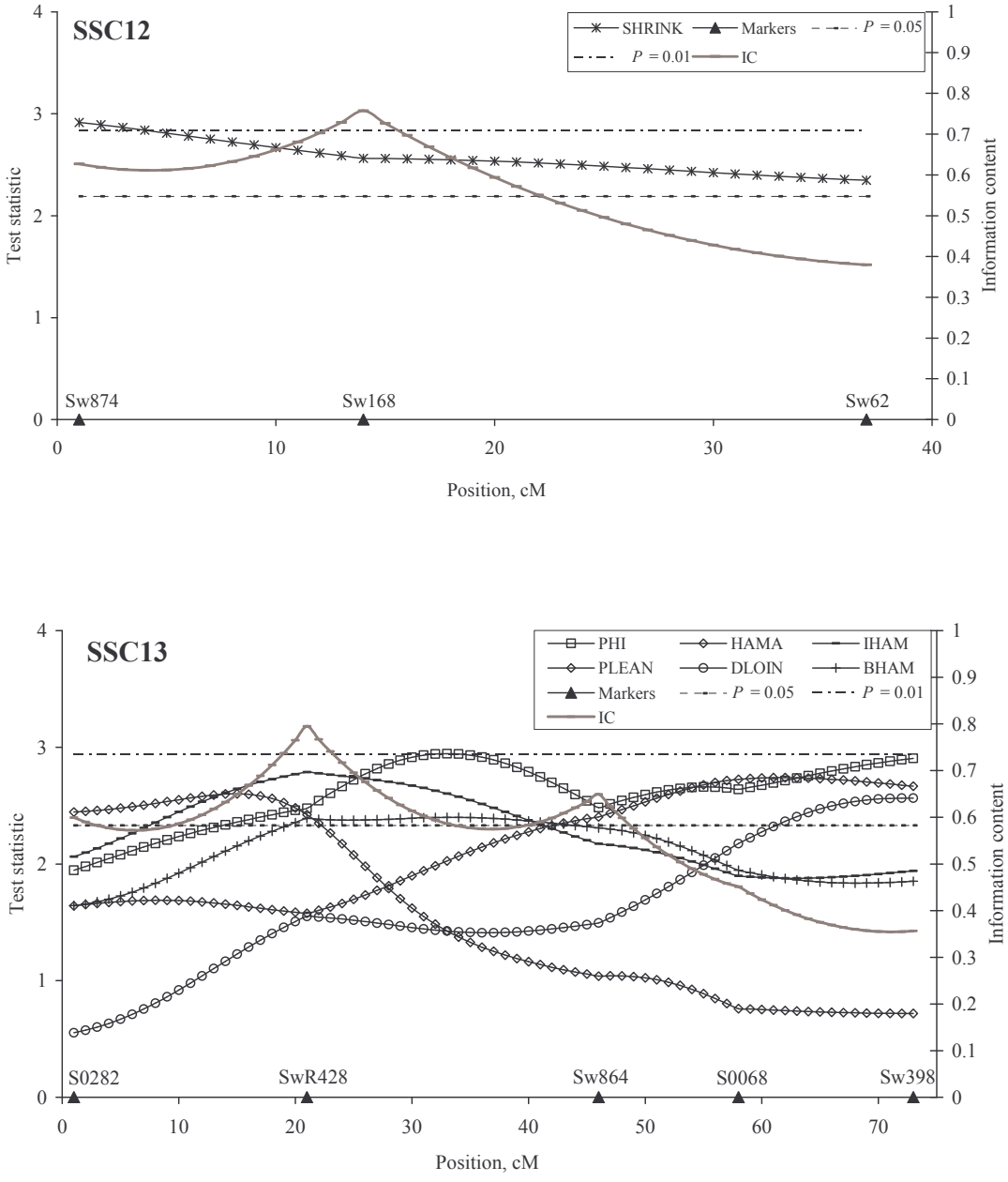

Figure 1. Continued.

Discussion

Significance

In this paper we report QTL exceeding the 5% chromosome-wise significance level. Of the 406 tests, 32 were found to be significant at the 5% level, which is above the 20 that could be expected by chance only. The limited power together with the results obtained implies that further studies are required to distinguish true QTL and false positives.

None of the identified QTL reached the 5% genome-wide level, although the QTL for Japanese color score on SSC4 and loin weight on SSC11 almost reached that threshold (P_{gen} = 0.07). These results suggest that QTL with very large effect do not seem to be segregating within this commercial line for the regions examined. This might be inherent to reduced within-breed phenotypic differences compared with between-breed differences underlying the QTL in most previous studies. Although this is expected to be true for traits that were subject to intense selection, this does not necessarily apply for meat quality traits, which were not included in the breeding goal until recently. Nevertheless, when designing studies for identification of QTL segregating within commercial pig lines it seems sensible to take into account that the probability of boars being heterozygous for the QTL is relatively low. Geldermann et al. (2003) summarized the results from QTL mapping studies for a number of traits. Table 5 of Geldermann et al. (2003) contains details of 195 QTL described in 28 publications together representing 15 different experimental crosses. Of the QTL listed 68, 23, and 9% were significant at the $P_{chr} < 0.05$, $P_{gen} < 0.01$, and $P_{gen} < 0.05$ levels, respectively, corresponding to the identification of approximately nine QTL at the $P_{gen} < 0.01$ per cross. Of the few studies on commercial lines available to date (De Koning et al., 2003; Evans et al., 2003; Nagamine et al., 2003; Vidal et al., 2005, this study), the majority of the QTL reported were significant at the 5% chromosome-wise level. Vidal et al. (2005) came to a similar conclusion based on a QTL mapping study in a purebred Landrace population; a small number of QTL with relatively small effects were identified.

Growth and Fatness Traits

For the growth and fatness traits, two QTL were identified; a QTL for LMd on SSC11 and a QTL for meat percentage (PLEAN) on SSC13. No QTL for similar traits were reported previously. However, Rohrer and Keele (1998b), Malek et al. (2001a), and Dragos-Wendrich et

al. (2003b) reported QTL for LM area, and loin and neck meat weight in the same region of SSC11. In this study, the QTL on SSC11 also affected the trimmed loin weights ELOIN and DLOIN. Meat percentage (PLEAN) was calculated based on loin depth and back fat measurements, in which the latter accounts for the largest part of the variance. The QTL for back fat thickness on SSC13 were found by Malek et al. (2001a), Nezer et al. (2002), and Yue et al. (2003b). Yue et al. (2003b) also reported a QTL for fat to meat ratio in the same region.

Carcass Composition

The QTL affecting different ham weights were identified on seven chromosomes. Although these traits have genetic correlations of up to 0.89 (Van Wijk et al., 2005), most QTL affect a single ham sub-primal weight only. The knuckle ham weight (KHAM) QTL on SSC2 corresponds to the region of the IGF2 locus, which is known to affect muscle mass (Jeon et al., 1999; Nezer et al., 1999; Van Laere et al., 2003). Analysis of IGF2, however, revealed that all boars were homozygous for the favorable IGF2 allele (IGF2-intron3-A3072A) suggesting that either an unknown mutation in the IGF2 gene or a linked gene may exist that affects ham weight.

The knuckle ham weight (KHAM) QTL at the end of SSC4q does not correspond to the ham weight QTL found in previous studies on SSC4, which are more centrally located near the candidate genes V-ATPase, ATP1A2, and ATP1B1 (Cepica et al., 2003). However, because of the low information content on SSC4, the estimated position of the QTL is not very precise.

The inner ham weight (IHAM) QTL on SSC5 and the telomeric region of SSC6p were not described previously. Two other QTL on SSC6 affecting outside ham and boneless ham weights (OHAM and BHAM) are located near the RYR1 locus. These findings agree with Grindflek et al. (2001) and Yue et al. (2003a), who both reported suggestive evidence for a QTL affecting ham weight. Because halothane-free boars were used in this study the QTL could indicate that unknown RYR1 alleles might exist.

On SSC10, a third QTL affecting knuckle ham weight (KHAM) was identified. There is little evidence for a QTL affecting ham weight in the literature other than a chromosome-wise evidence ($P < 0.05$) reported by Dragos-Wendrich et al. (2003a).

A QTL affecting boneless and inside ham weights (BHAM and IHAM) was identified on SSC13 proximal to the PIT1 gene region where a suggestive ham meat weight QTL was found in a Wild Boar x Meishan cross by Yue et al. (2003b).

Three QTL affecting loin weights were identified on chromosomes 5, 11, and 13. There is no evidence in the literature for a QTL on SSC5 affecting loin weight. The QTL on SSC11 corresponded to previous findings, although only weak evidence was reported (Rohrer and Keele, 1998b; Dragos-Wendrich et al., 2003b). A third QTL affecting loin weight was found on SSC13 near the PIT1 gene region. No previous studies reported an effect solely on loin weight, whereas several studies reported QTL affecting ham weight, carcass weight, fatness, and growth in this region (Yu et al., 1999; De Koning et al., 2001b; Malek et al., 2001a; Geldermann et al., 2003).

Meat Quality

Thirteen QTL on nine chromosomal regions affecting 10 different meat quality traits were identified.

Ham marbling (HMARB) QTL were identified on SSC6 and SSC14. No previous studies reported marbling QTL in these two regions. On SSC6, the QTL is located in the region of RYR1 and H-FAB known from QTL for fatness and intramuscular fat.

Six QTL related to meat color were identified from which the two QTL on SSC8 and SSC10 were not described in previous studies. The Minolta b^* ham (HAMB) QTL on SSC2 corresponded with meat color QTL found by Malek et al. (2001b). At the telomeric region of SSC4q, QTL were found for Minolta L^* ham (HAML, lightness) and JCS loin. The latter was among the most significant QTL found in this study and the findings correspond with findings of Malek et al. (2001b), De Koning et al. (2001a), and Ovilo et al. (2002). Ovilo et al. (2002) already proposed the protoporphyrinogen oxidase gene (PPO), which participates in the heme biosyntheses pathway, as a possible candidate gene. The glutamate-cysteine ligase modifier subunit (GCLM) gene, which is involved in the glutathione synthesis (GSH) pathway, is another candidate gene located in the region. Myoglobin serves as an intracellular storage site for oxygen in muscle tissue. The oxidation of oxymyoglobin to metmyoglobin causes fresh meat discoloration; glutathione may have a reducing effect on the oxidation (Tang et al., 2003).

The Minolta a^* ham (HAMA) QTL on SSC13 corresponds to the region where De Koning et al. (2001a) reported a QTL for lightness of the meat.

Based on the results of this study there seems little agreement between the different color measurements at the loin or ham. None of the color QTL reported affected both loin and ham

color. A similar conclusion could be draw from the publication of Malek et al. (2001b), except for the region of the PRKAG3 (Rendement Napole) gene on SSC15. We did not find any effect on color for the region of the PRKAG3 locus but found an effect on purge loss. Reduced water-holding capacity, pH, and lighter colored meat are well known effects of the unfavorable allele of the Rendement Napole gene (Le Roy et al., 1990). The unfavorable R200Q mutation however, is Hampshire-specific (Milan et al., 2000), and it is not expected that the mutation is segregating in our cross. Ciobanu et al. (2001) reported additional alleles of the gene with an effect on meat quality, which may segregate in our population.

A second drip loss (DRIP) QTL was found on SSC2. This finding corresponds to findings of Malek et al. (2001b) who reported a similar QTL with the greatest *F*-value close to marker Sw766.

Loss of moisture is often associated with paler color, reduced firmness, and lower pH. In this study we did not find QTL for pH or color in the regions of the DRIP and PURGE QTL, although this may be expected based on the high genetic correlations between the traits (i.e., r_g between -0.86 and -1 were found between water-holding capacity traits and pH in our data set).

Additional QTL with effects on pH were found on other chromosomes. Two QTL for PHI were found on SSC13 and SSC18 and a QTL for PHU was found on SSC4. The PHI QTL are located in a region where QTL related to water-holding capacity were described by others (Malek et al., 2001b; Bidanel and Rothschild, 2002; Dragos-Wendrich et al., 2003c). The PHU QTL in the telomeric region of SSC4q was not reported previously. This QTL is located at the same position as the JCS color QTL we identified. Several QTL in the central region of SSC4 with an effect on growth, fatness and carcass composition traits have been described (reviewed by Bidanel and Rothschild, 2002; Geldermann et al., 2003). The results described in our study put further emphasis on the importance of this chromosome for the pig breeding industry. Evidence for meat quality QTL in the telomeric region of SSC4q as obtained in this study and reported by Ovilo et al. (2002) expand the region of interest on SSC4.

Experimental Design

Information obtained on within-line variation is relevant for traits under selection and could, in principal, be directly applied in marker-assisted selection programs. This makes the use of commercial populations for QTL mapping studies preferable. However, several factors

negatively affect the power of QTL mapping in commercial pig populations. Analyses have to be performed within existing families. These families are often of limited size and not all of them may be segregating for the QTL.

To achieve sufficient power for the detection of QTL, we collected phenotypes of large half-sib families. Following the formula of Weller et al. (1990) and Van der Beek et al. (1996), the power of detecting QTL with effect of > 0.35 σ_p (or explaining > 15% of σ_a^2) is >0.50 in the present experiment (under the assumptions of full marker informativeness, eight sires with 100 progeny each, a trait with h^2 of 0.30, marker-QTL recombination fraction of 0.10, heterozygosity of the QTL of 0.5, and Type I error of 0.05%). Therefore, the design of the experiment allowed for the detection of QTL with moderate to large effect. The probability of detecting QTL with effects smaller than 0.35 σ_p was low. Although genotyping of all families could have enhanced the power of the study, eight families were typed in this initial stage to identify the most interesting regions in this population. In a second stage, the remaining families may be typed for the most promising regions. Such a two-stage approach saves genotyping and, with the type II error controlled at a low level, such preselection of promising regions has a very low chance of eliminating regions with a true QTL.

These results show the feasibility of detecting QTL within a selected commercial breeding line. The examined regions were selected because of described QTL affecting carcass composition and meat quality traits in those regions. This study provides additional information on whether QTL that have been identified using experimental crosses between divergent lines segregate within commercial pig populations as assessed recently by Evans et al. (2003), Nagamine et al. (2003) and Vidal et al. (2005). However, because of the limited power of the study, QTL may remain undetected. Therefore, this study may not be considered as a conclusive confirmation of previous findings. Approximately half of the identified QTL are consistent with previous findings (Table 3). These QTL suggest the existence of locus and allelic homogeneity (*i.e.* segregation for the same QTL and the same alleles within the QTL across populations). This is consistent with the findings of Nagamine et al. (2003) and Evans et al. (2003) who confirmed that the same chromosomal regions may account for between-breed and within-breed variation. Because of the limited power of the study, QTL with smaller effects may remain undetected. In addition to confirmation of known QTL, new QTL were identified (half of the QTL identified in this study were not already known from previous studies, Table 3). Several of the regions

covered showed significant QTL for a few, often correlated, traits. This is most striking for SSC11 and SSC6. These regions may carry a single QTL with pleiotropic effects on those traits. This study is one of a limited numbers of studies presenting carcass composition and meat quality traits recorded in a commercial slaughter cross. Thus, the study provides additional information about segregating genome regions of the studied traits within a commercial breed. The study included some less studied meat quality and carcass composition traits of relevance to the pork industry.

Implications

This study is one of the few quantitative trait loci mapping studies conducted on a commercial slaughter pig cross. The study provides information on within-line segregation of quantitative trait loci, which is needed to allow implementation of marker-assisted selection on a within-line basis. The results obtained show that within-line quantitative trait loci are segregating with significant effect on phenotypic variation in traits of commercial interest. Despite long-term selection on growth and feed efficiency, these quantitative trait loci did not reach fixation, which offers opportunities for genetic improvement of pork by altering quantitative trait loci allele frequencies by means of marker-assisted selection.

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Literature cited:

Andersson-Eklund L., L. Marklund, K. Lundstrom, C.S. Haley, K. Andersson, I. Hansson, M. Moller, and L. Andersson. 1998. Mapping quantitative trait loci for carcass and meat quality traits in a wild boar x Large White intercross. J. Anim. Sci. 76: 694-700.

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

Bidanel J.P., and M. Rothschild. 2002. Current status of quantitative trait locus mapping in pigs. Pig News and Information. Vol. 23 No. 2: 39-53.

Cepica S., A. Stratil, M. Kopecny, P. Blazkova, J. Schröffel Jr, R. Davoli, L. Fontanesi, G. Reiner, H. Bartenschlager, G. Moser, and H. Geldermann. 2003. Linkage and QTL mapping for *Sus scrofa* chromosome 4. J. Anim. Breed. Genet. 120 (Suppl. 1): 28-37.

Churchill G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138: 963-971.

Ciobanu D., J. Bastiaansen, M. Malek, J. Helm, J. Woollard, G. Plastow, and M. Rothschild. 2001. Evidence for new alleles in the protein kinase adenosine monophosphate-activated γ_3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics 159: 1151-1162.

De Koning D.J., L.L. Janss, A.P. Rattink, P.A. van Oers, B.J. de Vries, M.A.M. Groenen, J.J. van der Poel, P.N. de Groot, E.W. Brascamp, and J.A. van Arendonk. 1999. Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs (*Sus scrofa*). Genetics 152: 1679-1690.

De Koning D.J., A.P. Rattink, B. Harlizius, J.A. van Arendonk, E.W. Brascamp, and M.A.M. Groenen. 2000. Genome-wide scan for body composition in pigs reveals important role of imprinting. Proc. Natl. Acad. Sci. 97: 7957-7950.

De Koning D.J., B. Harlizius, A.P. Rattink, M.A.M. Groenen, E.W. Brascamp, and J.A. van Arendonk. 2001a. Detection and characterization of quantitative trait loci for meat quality traits in pigs. J. Anim. Sci. 79: 2812-2819.

De Koning D.J., A.P. Rattink, B. Harlizius, M.A.M. Groenen, E.W. Brascamp, and J.A.M. van Arendonk. 2001b. Detection of characterization of quantitative trait loci for growth and reproduction traits in pigs. Livest. Prod. Sci. 72: 185-198.

De Koning D.J., R. Pong-Wong, L. Varona, G.J. Evans, E. Giuffra, A. Sanchez, G. Plastow, J.L. Noguera, L. Andersson, and C.S. Haley. 2003. Full pedigree quantitative trait locus analysis in commercial pigs using variance components. J. Anim. Sci. 81: 2155-2163.

Dragos-Wendrich M., G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. 2003a. Linkage and QTL mapping for *Sus scrofa* chromosome 10. J. Anim. Breed. Genet. 120 (Suppl. 1): 82-88.

Dragos-Wendrich M., G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. 2003b. Linkage and QTL mapping for *Sus scrofa* chromosome 11. J. Anim. Breed. Genet. 120 (Suppl. 1): 89-94.

Dragos-Wendrich M., A. Stratil, J. Hojny, G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. 2003c. Linkage and QTL mapping for *Sus scrofa* chromosome 18. J. Anim. Breed. Genet. 120 (Suppl. 1): 138-143.

Evans G.J., E. Giuffra, A. Sanchez, S. Kerje, G. Davalos, O. Vidal, S. Illán, J.L. Noguera, L. Varona, I. Velander, O.I. Southwood, D.J. de Koning, C.S. Haley, G.S. Plastow, and L. Andersson. 2003. Identification of quantitative trait loci for production traits in commercial pig populations. Genetics 164: 621-627.

Fujii J., K. Otsu, F. Zorzato, S. de Leon, V.K. Khanna, J.E. Weiler, P.J. O´Brien, and D.H. Meaclennan. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253: 448-451.

Geldermann H., E. Müller, G. Moser, G. Reiner, H. Bartenschlager, S. Cepica, A. Stratil, J. Kuryl, C. Moran, R. Davoli, and C. Brunsch. 2003. Genome-wide linkage and QTL mapping in porcine F_2 families generated from Piétrain, Meishan and Wild Boar crosses. J. Anim. Breed. Genet. 120: 363-393.

Gilmour A.R., B.R. Cullis, S.J. Welham, and R. Thompson. 2002. ASReml reference manual 2nd edition, Release 1.0. NSW Agriculture Biometrical Bulletin 3, NSW Agriculture, Locked Bag, Orange, NSW 2800, Australia.

Green P., K. Falls, and S. Crook. 1990. Documentation for CriMap, Version 2.4. Washington University School of Medicine, St. Louis, MO.

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

Jeon J.T., Ö. Carlborg, A. Törnsten, E. Giuffra, V. Amarger, P. Chardon, L. Andersson-Eklund, K. Andersson, I. Hansson, K. Lundström, and L. Andersson. 1999. A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. Nat. Genet. 21: 157-158.

Knott S.A., J.M. Elsen, and C.S. Haley. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theor. Appl. Genet. 93: 71-80.

Le Roy P., J. Naveau, J.M. Elsen, and P. Sellier. 1990. Evidence for a new major gene influencing meat quality in pigs. Genet. Res. 55: 33-40.

Malek M., J.C.M. Dekkers, H.K. Lee, T.J. Baas, and M.F. Rothschild. 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., J.C.M. Dekkers, H.K. Lee, T.J. Baas, K. Prusa, E. Huff-Lonergan, and M.F. Rothschild. 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.

Milan D., J-T. Jeon, C. Looft, V. Amarger, A. Robic, M. Thelander, R-G. Claire, S. Paul, N. Iannuccelli, L. Rask, H. Ronne, K. Lundström, N. Reinsch, J. Gellin, E. Kalm, P. Le Roy, P. Chardon, and L. Andersson. 2000. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science 288: 1248-1251.

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

Nezer C., L. Moreau, B. Brouwers, W. Coppieters, J. Detilleux, R. Hanset, L. Karim, A. Kvasz, P. Leroy, and M. Georges. 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., L. Moreau, D. Wagenaar, and M. Georges. 2002. Results of a whole genome scan targeting QTL for growth and carcass traits in a Piétrain x Large White intercross. Genet. Sel. Evol. 34: 371-387.

NPPC. 1991. Procedures to Evaluate Market Hogs. 3rd ed. Natl. Pork Prod. Counc., Des Moines, IA.

NPPC. 2000. Pork composition and quality assessment procedures. Natl. Pork Prod. Counc., Des Moines, IA.

Ovilo C., A. Clop, J.L. Noguera, M.A. Oliver, C. Barragán, C. Rodriquez, L. Silió, M.A. Toro, A. Coll, J.M. Folch, A. Sánchez, D. Babot, L. Varona, and M. Perez-Enciso. 2002. Quantitative trait locus mapping for meat quality traits in an Iberian x Landrace F2 pig population. J. Anim. Sci. 80: 2801-2808.

Rohrer G.A., L.J. Alexander, Z.L. Hu, T.P.L. Smith, J.W. Keele, and C.W. Beattie. 1996. A comprehensive map of the porcine genome. Genome Res. 6: 371-391.

Rohrer G.A., and J.W. Keele. 1998a. Identification of quantitative trait loci affecting carcass composition in Swine: I. Fat deposition traits. J. Anim. Sci. 76: 2247-2254.

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

Sellier P. 1998. 'Genetics of meat and carcass traits', In 'The genetics of the pig' edited by Rothschild, M.F., and Ruvinsky, A., CAB International, Wallingford, Oxon, pp 463-510.

Tang J., F. Cameron, S. Lee and T.A. Hoagland. 2003. Effect of glutathione on oxymyoglobin oxidation. J. Agric. Food Chem. 51: 1691-1695.

Van der Beek S., J.A.M. van Arendonk, and A.F. Groen. 1996. Power of two- and three-generation QTL mapping experiments in an outbred population containing full-sib or half-sib families. Theor. Appl. Genet. 91: 1115-1124.

Van Leare A.S., M. Nguyen, M. Braunschweig, C. Nezer, C. Collette, L. Moreau, A.L. Archibald, C.S. Haley, N. Buys, M. Tally, G. Andersson, M. Georges, and L. Andersson. 2003. A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature 425: 832-836.

Van Wijk H.J., D.J.G. Arts, J.O. Matthews, M. Webster, B.J. Ducro, and E.F. Knol. 2005. Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. J. Anim. Sci. 83: 324 – 333.

Varona L., C. Ovilo, A. Clop, J.L. Noguera, M. Perez-Enciso, A. Coll, J.M. Folch, C. Barragan, M.A. Toro, D. Babot, and A. Sanchez. 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., J.L. Noguera, M. Amills, L. Varona, M. Gil, N. Jiménez, G. Dávalos, J.M. Folch, and A. Sánchez. 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.

Walling G.A., P.M. Visscher, L. Andersson, M.F. Rothschild, L. Wang, G. Moser, M.A.M. Groenen, J.P. Bidanel, S. Cepica, A.L. Archibald, H. Geldermann, D.J. de Koning, D. Milan, and C.S. Haley. 2000. Combined analyses of data from quantitative trait loci mapping studies. Chromosome 4 effects on porcine growth and fatness. Genetics 155: 1369-1378.

Weller J.I., Y. Kashi, and M. Soller. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. J. Dairy Sci. 73: 2525-2537.

Wimmers K., E. Murani, S. Ponsuksili, M. Yerle, and K. Schellander. 2002. Detection of quantitative trait loci for carcass traits in the pig by using AFLP. Mamm. Genome 13: 206-210.

Yu T.P., L. Wang, C.K. Tuggle, and M.F. Rothschild. 1999. Mapping genes for fatness and growth on pig chromosome 13: a search in the region close to the PIT1 gene. J. Anim. Breed. Genet. 116: 269-280.

Yue G., A. Stratil, M. Kopecny, D. Schröffelova, J. Schröffel Jr, J. Hojny, S. Cepica, R. Davoli, P. Zambonelli, C. Brunsch, I. Sternstein, G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. 2003a. Linkage and QTL mapping for *Sus scrofa* chromosome 6. J. Anim. Breed. Genet. 120 (Suppl. 1): 45-55.

Yue G., V. Russo, R. Davoli, I. Sternstein, C. Brunsch, D. Schröffelova, A. Stratil, G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. 2003b. Linkage and QTL mapping for *Sus scrofa* chromosome 13. J. Anim. Breed. Genet. 120 (Suppl. 1): 103-110.

Chapter 4

Variance component analysis of QTL for pork carcass composition and meat quality on SSC4 and SSC11

H.J. van Wijk,[*] H. Buschbell,[#1] B. Dibbits,[#] S.C. Liefers,[#] B. Harlizius,[*] H.C.M. Heuven,[#] E.F. Knol,[*] H. Bovenhuis[#], and M.A.M. Groenen[#]

[*]IPG, Institute for Pig Genetics, P.O. Box 43, 6640 AA, Beuningen, The Netherlands;
[#]Animal Breeding and Genetics Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands.

[1]Current address: Institute of Animal Science, Animal Breeding and Husbandry Group, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany.

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Abstract: In a previous study, QTL for carcass composition and meat quality were identified in a commercial finisher cross. The main objective of the current study was to accumulate more evidence and fine map the QTL on SSC4 and SSC11 by genotyping an increased number of individuals and markers, and analyze the data using a combined linkage and linkage disequilibrium analysis (LDLA) method, which will likely result in the identification of new QTL as well. A modified version of the method excludes linkage disequilibrium (LD) information from the analysis, enabling the comparison of results based on linkage information only or results based on combined linkage and linkage disequilibrium information. A total of 1,855 animals were genotyped for 15 and 13 markers on SSC4 and SSC11, respectively. Accumulated evidence was obtained for the QTL affecting meat color on SSC4, whereas the QTL affecting loin weight could not be confirmed. The LDLA resulted in the identification of new significant effects for 8 traits on the two chromosomes compared to analyses without using LD information. Heritabilities of the QTL effects ranged from 1.8 to 13.2 percent. The analysis contributed to a more accurate positioning of QTL, and further characterized their phenotypic effect. However, results showed that even higher marker densities are required to take full advantage of linkage disequilibrium information, and to identify haplotypes associated with favorable QTL alleles.

Key words: Meat quality, Pig, Quantitative trait loci, SSC4, SSC11, Variance component

Introduction

Mapping of QTL has become common practice in farm animal research as is illustrated by the numerous QTL reported (see databases by Polineni, 2004; Hu et al., 2005; Wang et al., 2005).

The analysis methods usually applied are based on regression (Knott and Haley, 1992; Knott et al., 1996), and are aimed at detecting linkage between markers and QTL. Unless the genotyped population is very large, the linkage analysis approach generally results in large confidence intervals for the QTL due to a relatively small number of meioses. Implementation of QTL in marker-assisted breeding programs often requires a higher map resolution. Genotyping of additional markers, eventually on additional families, might be used as a first step to increase the map resolution of QTL.

Recently, linkage disequilibrium (LD) mapping methods have been proposed for fine mapping, which aim at capitalizing on historical recombination events (Riquet et al., 1999; Meuwissen and Goddard, 2000). The classical linkage and LD analysis are complementary and methods have been proposed that simultaneously models linkage and LD information (Meuwissen et al., 2002; Farnir et al., 2002). The method of Meuwissen et al. (2002) allows for simultaneous estimation of variance components for systematic, polygenic and QTL variance. Compared to a classical regression analysis the method can deal with additional relationships within and between families, increasing the power to detect QTL. Furthermore, utilizing LD information may result in a more accurate mapping of QTL. Whether LD will be of added value depends upon the extent of LD in the population under study and the marker density. It has been shown that LD can extent over map distances of >10 centiMorgan in the pig (Nsengimana et al., 2004).

The aim of the present study was to accumulate more evidence and fine map QTL on SSC4 and SSC11 by applying a combined linkage and linkage disequilibrium analysis method on an increased data set, which will likely also result in the identification of new QTL.

Materials and Methods

Genetic Material and Phenotype Measurements

The population used in this experiment was created by mating 17 sires of a synthetic Piétrain/Large White halothane free boar line (TOPIGS, The Netherlands) to 239 commercial crossbred sows with unknown pedigrees. All piglets were born over a 2 mo period. Phenotypic measurements were taken for 30 traits on approximately 100 offspring per sire, resulting in a total of 1,855 animals recorded. A detailed description of the population and traits was presented by Van Wijk et al. (2005, 2006).

To obtain a better phenotypic description of traits of interest nine additional variables were calculated based on the primary phenotypic measurements. These variables will be termed derived variables. Japanese color scale is a subjective color score on a scale of 1 to 6. Subjective scoring in classes may add to the error of the measurement. Repeated observations may reduce the error. Assuming that the Japanese color score measured at the rib (**JCSrib**) and cut (**JCScut**) surface of the loin could be considered as repeated measurements of the same trait an average Japanese color socre (**AvgJCS**) was calculated. Alternatively, the error of the color measurement may be reduced by taking objective Minolta measurements. The Minolta measures three color components, which are the lightness or luminescence (Minolta L*), the redness (Minolta a*), and the yellowness (Minolta b*). The Minolta measurements were then regressed against the AvgJCS and predicted values were calculated (**EstCol**) following the formula: EstCol = (5.48-0.067 * Minolta L* loin – 0.056 * Minolta b* ham + 0.080 * Minolta b* loin + 0.049 * Minolta a* ham). Finally these predictions (EstCol) were averaged with the AvgJCS to obtain a mean value for the subjective and objective color measurement as total (aggregated) color score (**TotCol**) following the formula: TotCol = (EstCol + AvgJCS)/2. Following this approach all available color information was used in one aggregated color trait in an attempt to decrease the error on the individual measurements.

The chroma (C) and hue (H) values were calculated for both the ham (**hamC** and **hamH**) and loin (**loinC** and **loinH**) measurements as $C = \sqrt{(\text{Minolta a}^{*2} + \text{Minolta b}^{*2})}$, and $H = \tan^{-1}$ (Minolta b*/Minolta a*). Chroma is a measure of colour intensity which increases when Minolta a* and/or Minolta b* increases. Hue indicates the degree of colour change from red (low values of hue) to yellow (high values of hue) (Setser, 1984). Finally, ham and loin gain (**HamG** and

LoinG) were calculated as: (boneless ham weight / cold carcass weight) * average daily gain and (domestic loin weight / cold carcass weight) * average daily gain.

Genotyping and Linkage Map Construction

Eight paternal half-sib families had been used in the initial genome scan with six and four markers on SSC4 and SSC11, respectively (Van Wijk et al., 2006). Additional genotypes were generated in two steps. First, nine additional paternal half-sib families were typed for the 10 markers on SSC4 and SSC11 that already had been used in the initial genome scan. Second, the whole population of 17 paternal half-sib families was typed for 18 additional markers on the two regions of interest. Nine of the markers are located on SSC4q and nine on the p-arm and centromeric region of SSC11. The typed markers were selected based on their position in the two regions of interest, their informativeness, and scoring ability. For SSC11, no informative markers were available for the ~25 cM interval between markers Sw1632 and S0071. Furthermore, seven markers were included located distal from marker S0071 to cover a larger genome region on SSC11. The reason for that was the QTL for loin weight which had the highest *F*-statistic at marker S0071 in the initial genome scan. Genotypes were scored in duplo and checked against pedigree information. The chrompic option of CriMap (Green et al., 1990) was used to check for double recombinants prior to final linkage map construction based on the Kosambi mapping function. Sex-average linkage maps with 15 and 13 markers on SSC4 and SSC11, respectively, were used in the QTL analysis.

Statistical Analysis

Regression Analysis: Paternal half-sib linkage analysis (PHS) was performed using a classical regression interval analysis nested within half-sib families (Knott et al., 1996; De Koning et al., 1999) on phenotypic trait data pre-corrected for systematic effects as described in Van Wijk et al. (2006). Segregating sire families were identified by individual family analysis. Significance thresholds were determined empirically for each trait by chromosome combination by performing 10,000 permutations (Churchill and Doerge, 1994).

Variance Component Analysis: Variance component based linkage analysis (denoted as VC LA) and combined linkage and linkage disequilibrium analyses (LDLA) were performed using the method proposed by Meuwissen and Goddard (2000). The method models expected

covariances between haplotype effects, which are proportional to linkage disequilibrium in the population, at a postulated QTL position. The method involves the following steps. 1) Construction of haplotypes of parents and offspring. Haplotypes were constructed using the SimWalk program (Sobel and Lange, 1996). 2) Calculation of identity by descent probabilities of pairs of haplotypes as described by Meuwissen and Goddard (2001) using the LDLA package (Janss and Heuven, 2005). Calculation of identity by descent (IBD) probabilities for each putative QTL position given the marker scores results in a series of IBD probability matrices. Different from Meuwissen and Goddard (2001), pedigree information was not included when calculating IBD probabilities between base (parental) haplotypes. Identity by descent probabilities and likelihood's were evaluated at four points (putative QTL positions) within each marker bracket (*i.e.* evaluation points at 1, 25, 50 and 75 % of the distance from the first marker to the next marker). 3) The final step calculates the maximum likelihood estimates of the variance components at each evaluation point. In this step systematic, polygenic, QTL and residual variances are estimated simultaneously. The program ASReml (Gilmour et al., 1998) was used to calculate the maximum likelihood at each evaluation point using the appropriate IBD matrix. Phenotypes were analyzed using the following model:

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Wv} + \mathbf{Sc} + \mathbf{e} \quad [1]$$

Where **Y** is the vector of phenotypes, **b** is a vector of systematic effects, **u** is a vector of random additive polygenic effects of background loci, **v** is a vector of random additive effects due to the QTL, **c** is the vector of random litter effects, and **e** are the random residuals. The random effects were assumed normally distributed with mean zero and variances $\boldsymbol{\sigma}^2_{\mathbf{u}}$, $\boldsymbol{\sigma}^2_{\mathbf{v}}$, $\boldsymbol{\sigma}^2_{\mathbf{c}}$ and $\boldsymbol{\sigma}^2_{\mathbf{e}}$ respectively. **X**, **Z**, **W** and **S** are known incidence matrices for the effects of **b**, **u**, **v** and **c** respectively. The assumption that QTL effects are normally distributed may not be true for bi- or tri-allelic QTL, but REML analysis with an expected covariance matrix of random QTL effects is robust to the number of alleles at the QTL, and as a REML method it should be robust to deviations from normality in general (Hoeschele et al., 1997). In model [1], denoted as the full model, **v** represents the combined maternal and paternal haplotype effects as a single additive component (a single variance component). Maternal ($\mathbf{v_d}$) and paternal ($\mathbf{v_s}$) haplotype effects can be modeled separately (2 variance components) to allow for differences in effect (*i.e.* fitting parent-of-origin and/or breed specific effects):

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{W_d v_d} + \mathbf{W_s v_s} + \mathbf{Sc} + \mathbf{e} \quad [2]$$

Considering uncorrelated maternal ($\mathbf{W_d v_d}$) or paternal ($\mathbf{W_s v_s}$) components separately allows for a maternal- or paternal-only analysis.

Assuming unrelated base (parental) haplotypes (ignoring LD information by adjusting the IBD probabilities between base haplotypes to zero) results in a variance component based linkage analysis (VC LA). Comparison of VC LA results with LDLA results will give insights in the contribution of LD. A paternal VC LA analysis resembles a paternal half-sib regression analysis.

Significance testing was based on a likelihood ratio test (LRT). The LRT was calculated as twice the difference between the lnL of the QTL model versus the base model (model without a QTL effect). For the variance component analyses no adjustment for multiple testing was made. Permutations, as applied for the regression analysis, is computationally too demanding for variance component analysis. The 5% nominal significance level of the LRT was determined from a chi-square distribution of 1 d.f. (model [1]) or 2 d.f. (model [2]) (degrees of freedom equal to the number of associated QTL effects) following Grignola et al. (1997) and Zhang et al. (1998). Applying this test statistic seems to be reasonable as Grignola et al. (1996) showed that the variance component LRT statistic for the QTL model versus the base model is in between a 1- and a 2-d.f. chi-square distribution. Furthermore, the number of tests in this study is much more limited compared to a whole genome scan.

Results and Discussion

Genotyping and Map Construction

Genetic linkage maps for SSC4 and SSC11 are presented in Table 1. The 15 markers on SSC4 span 91 cM, with an average marker interval of 6.1 cM. The 13 markers on SSC11 span 87 cM, with an average marker interval of 6.7 cM. The average information content along the chromosome was 0.69 for SSC4 and 0.68 for SSC11. The map of SSC11 contains a large gap of 31 cM. Available microsatellites in that region were tested, but not informative on the sires. Generally, marker order was in good agreement with the USDA-MARC.2 genetic linkage map (Rohrer et al., 1996) except for SSC11 where marker Sw486 was positioned ahead of markers Sw1452 and Sw435. The three markers in the USDA map span an interval of only 2 cM, indicating tight linkage and a different order is therefore possible. Map lengths were slightly

higher in our data as compared to the USDA genetic linkage map, *i.e.* 21 and 10 cM for SSC4 and SSC11, respectively.

The average number of alleles per marker was 3.5 in sires and 6.5 in dams, with unique alleles specific for sires (~10% of the alleles) and dams (~45% of the alleles). Sire or dam specific alleles could reveal breed specific effects. Seven sire specific alleles were observed in six markers on SSC4, with a total of 53 alleles (SSC4: 7/53 in 6 markers). Three markers on SSC11 showed 4 sire specific alleles of 41 alleles in total (SSC11: 4/41 in 3 markers). The number of dam specific alleles was 42/95 in 13 SSC4 markers, and 30/68 in 10 SSC11 markers.

Table 1. Calculated linkage maps compared with the USDA map.

SSC4			**SSC11**		
Marker[1]	**cM**	**cM**$_{USDA}$[2]	**Marker**[1]	**cM**	**cM**$_{USDA}$
Sw35	0	56 (0)	S0385	0	0
S0217	10	70 (14)	*S0391*	6	5
Sw270	26	79 (23)	*Sw1486*	7	8
S0214	28	79 (23)	Sw1460	9	9
Sw512	33	81 (25)	Sw1632	16	17
Sj551	36		S0071	47	44
Sw524	47	99 (43)	*Sw486*	54	54
Sj673	48		*Sw1452*	57	52
S0067	53	103 (47)	*Sw435*	58	53
Sw445	59	106 (50)	*S0230*	61	56
Sw58	62	108 (52)	*S0009*	64	60
S0097	78		*Sw1377*	75	69
Sw2066	82	121 (65)	*Sw903*	87	77
Sj672	85				
SwR153	91	126 (70)			
Avg.	6.1	6.4		6.7	5.9

[1] Markers in *italics* were genotyped for the first time in this study.

[2] Numbers in parentheses are the cM position if Sw35 is adjusted to a position of 0.

Regression Analysis

The paternal half-sib regression (PHS) analysis on the increased data set identified fifteen QTL at the nominal $P = 0.05$ threshold, 9 on SSC4 and 6 on SSC11 (Tables 2 and 3). The nominal significance level was used to facilitate comparison of results between regression and

VC analysis. Two of the QTL were significant at the $P = 0.005$ chromosome-wise level (JCScut on SSC4 and LMARB on SSC11).

The PHS analysis confirmed the existence of the QTL for JCScut on SSC4 (nominal $P = 0.001$), with the highest *F*-statistic at 55 cM near markers S0067 and Sw445 (Figure 1). The *F*-statistic profile was relatively flat with a wide interval of approximately 30 cM above the threshold. The extended population contributed to additional evidence for the presence of the QTL for JCScut on SSC4. Individual family analyses revealed that only three sires were heterozygous for the QTL affecting JCScut.

The second QTL, significant at the chromosome-wise level, was a QTL for loin marbling (LMARB) on SSC11. The highest *F*-statistic was found at marker S0385 at the tip of SSC11p (nominal $P = 0.004$). This QTL had not been identified in the initial genome scan (Van Wijk et al., 2006).

Although the power of the joint analysis increased considerably (~25%) compared to the initial genome scan (Van Wijk et al., 2006), decreased evidence ($P = 0.042$) was found for the QTL for loin weight at the centromeric region of SSC11 which was one of the most significant QTL identified in the initial genome scan (nominal $P = 0.001$ in the initial genome scan).

Table 2. QTL mapping results for SSC4, with position (centi-morgan, cM) with highest likelihood ratio test (LRT) value, the significance level (*P*), and the total variance explained by the QTL (h^2_{qtl}) with the SE.

		PHS[4]		VC LA[4]					LDLA[4]				
Trait	**Abbr.**	**cM**	***P*[1]**	**cM**	**LRT**	***P*[2]**	**h^2_{qtl}**	**SE**	**cM**	**LRT**	***P*[2]**	**h^2_{qtl}**	**SE**
Loin depth, mm	LMd[3]	-	-	-	-	--	-	-	0	6.36	*	0.045	0.036
Minolta L* ham	HAML	-	-	14	9.66	**	0.060	0.048	14	9.84	**	0.083	0.046
		71	0.029	*69*	*5.98*	*ns*	-	-	-	-	-	-	-
Minolta a* ham	HAMA	-	-	61	5.99	*	0.074	0.036	*77*	*5.28*	*ns*	-	-
Minolta a* loin	LOINA	-	-	-	-	-	-	-	29	7.76	*	0.045	0.028
		79	*0.076*	-	-	-	-	-	77	7.26	*	0.024	0.021
pH ultimate	pHU	74	0.027	-	-	-	-	-	-	-	-	-	-
Jap. color score cut, 1 to 6	JCScut	56	0.001	*54*	*4.69*	*ns*	-	-	54	6.15	*	0.042	0.029
Purge loss, %	PURGE	69	0.018	89	5.99	*	0.071	0.032	*83*	*4.90*	*ns*	-	-
Outside ham weight, kg	OHAM	-	-	55	6.56	*	0.078	0.035	-	-	-	-	-
Knuckle ham weight, kg	KHAM	60	0.040	*58*	*4.54*	*ns*	-	-	*35*	*5.60*	*ns*	-	-
Boneless ham weight, kg	BHAM	-	-	57	7.14	*	0.079	0.034	69	6.02	*	0.132	0.054
Total color	TotCol	89	0.029	*89*	*4.69*	*ns*	-	-	-	-	-	-	-
Estimated color	EstCol	-	-	18	6.92	*	0.038	0.025	14	6.66	*	0.060	0.039
		91	0.012	*89*	*4.58*	*ns*	-	-	-	-	-	-	-
Chroma ham	HamC	-	-	-	-	-	-	-	78	6.72	*	0.043	0.031
Chroma loin	LoinC	-	-	-	-	-	-	-	33	8.56	*	0.018	0.015
		86	*0.086*	-	-	-	-	-	80	9.04	*	0.047	0.029
Ham gain per day, g/d	HamG	74	0.018	-	-	-	-	-	-	-	-	-	-
Loin gain per day, g/d	LoinG	86	0.014	-	-	-	-	-	-	-	-	-	-

[1] nominal significance level $P < 0.05$.

[2] ns = not significant, LRT > 5.99 for $P < 0.05$(*), LRT > 9.21 for $P < 0.01$(**) and LRT > 10.60 for $P < 0.005$(***).

[3] Abbreviated as LD in Van Wijk et al. (2005).

[4] Traits that were nearly significant (ns) ($P < 0.10$ for PHS; LRT > 3.0 for VC LA and LDLA) are italicized and inlucded to facilitate comparisons.

Table 3. QTL mapping results for SSC11, with centi-morgan (cM) position with highest likelihood ratio test (LRT) value, the significance level (*P*), and the total variance explained by the QTL (h^2_{qtl}) with the SE.

		PHS[4]		VC LA[4]					LDLA[4]				
Trait	**Abbr.**	**cM**	***P*[1]**	**cM**	**LRT**	***P*[2]**	**h^2_{qtl}**	**SE**	**cM**	**LRT**	***P*[2]**	**h^2_{qtl}**	**SE**
Loin depth, mm	LMd[3]	34	0.028	-	-	-	-	-	59	6.20	*	0.035	0.029
Meat, %	PLEAN	62	0.037	-	-	-	-	-	57	7.30	*	0.044	0.029
Average daily gain, g/d	ADG	48	0.025	58	8.54	*	0.031	0.018	61	9.32	**	0.051	0.031
Minolta a* ham	HAMA	-	-	-	-	-	-	-	14	6.06	*	0.072	0.041
Minolta b* ham	HAMB	- *88*	- *0.070*	59 85	8.34 7.12	* *	0.089 0.107	0.026 0.032	- 85	- 6.66	- *	- 0.109	- 0.058
Marbling score ham, 1 to 4	HMARB	*7*	*0.070*	-	-	-	-	-	10	7.34	*	0.043	0.031
Marbling score loin, 1 to 5	LMARB	1	0.004	0	10.70	***	0.035	0.031	0	12.27	***	0.037	0.028
Outside ham weight, kg	OHAM	-	-	-	-	-	-	-	57	7.14	*	0.033	0.025
Boneless ham weight, kg	BHAM	48	0.017	-	-	-	-	-	*58*	*5.44*	*ns*	-	-
Boneless loin weight, kg	ELOIN	24	0.042	*25*	*3.24*	*ns*	-	-	*17*	*5.88*	*ns*	-	-
Trimmed loin weight, kg	DLOIN	-	-	-	-	-	-	-	10	7.82	*	0.053	0.034

[1] nominal significance level $P < 0.05$.

[2] *ns* = not significant, LRT > 5.99 for $P < 0.05$(*), LRT > 9.21 for $P < 0.01$(**) and LRT > 10.60 for $P < 0.005$(***).

[3] Abbreviated as LD in Van Wijk et al. (2005).

[4] Traits that were nearly significant (ns) (P < 0.10 for PHS; LRT > 3.0 for VC LA and LDLA) are italicized and inlucded to facilitate comparisons.

Variance Component Linkge Analysis

The variance component QTL results (VC LA and LDLA) are presented in Tables 2 and 3 for SSC4 and SSC11, respectively. For the VC method we used a likelihood ratio test without taking into account that multiple traits were analyzed and multiple tests were performed along the chromosome. In this study the nominal 5% significance level was applied. This threshold is used in similar studies (De Koning et al., 2003; Olsen et al., 2004; Uleberg et al., 2005; Gautier et al., 2006) and facilitates comparison of results. Estimates of the total variance explained by the QTL (h^2_{qtl}) are also presented in Tables 2 and 3. The h^2_{qtl} were considerable and ranged from 1.8 to 13.2 percent. However, no clear relationship was observed between the significance and estimated QTL effects.

The VC LA resulted in the identification of 10 effects for nine traits (six on SSC4 and four on SSC11) at the nominal level ($P = 0.05$).

On SSC4, the most significant effect ($P < 0.01$) was found for Minolta L* ham (HAML). Maternal- and paternal-only analyses revealed that the effect was segregating in the sire line (data not shown). This QTL, however, has not been identified with the paternal half-sib regression analysis. In this case, the two methods that were expected to give very similar results, PHS and VC LA paternal model, differed.

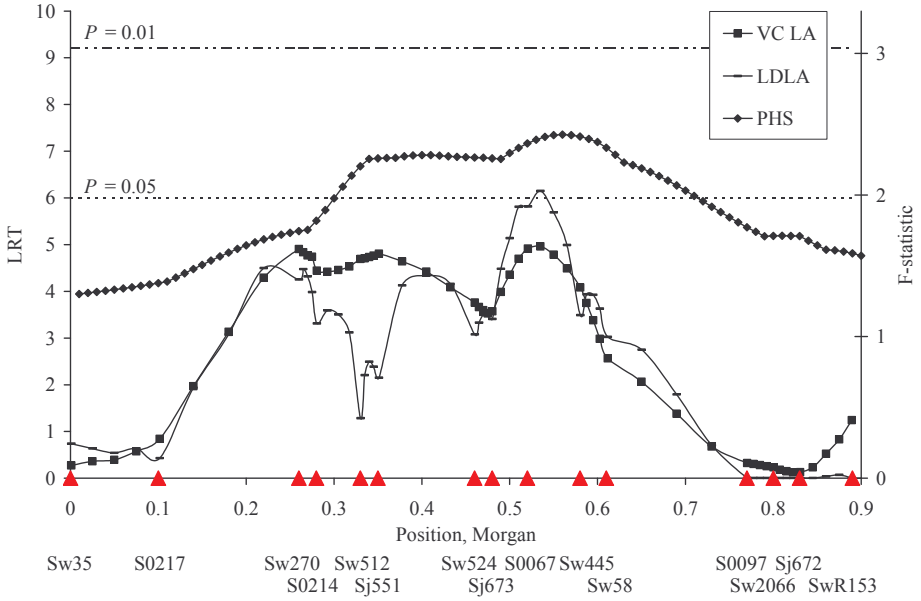

Figure 1. Regression (PHS) and variance component (VC LA and LDLA) analyses results for Japanese color score cut surface (JCScut) on SSC4. Triangles indicate marker positions.

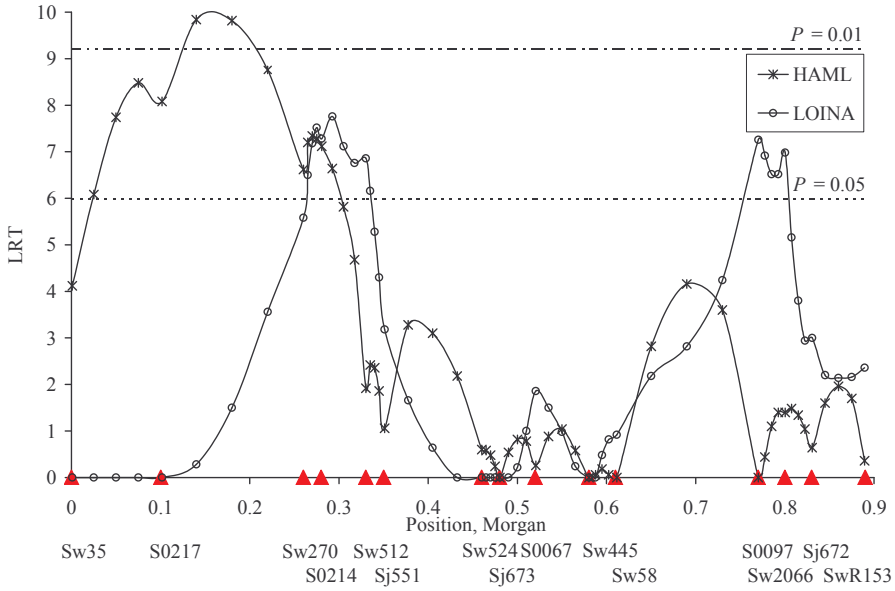

Figure 2. LDLA profiles for Minolta L* ham (HAML) and Minolta a* loin (LOINA) on SSC4. Triangles indicate marker positions.

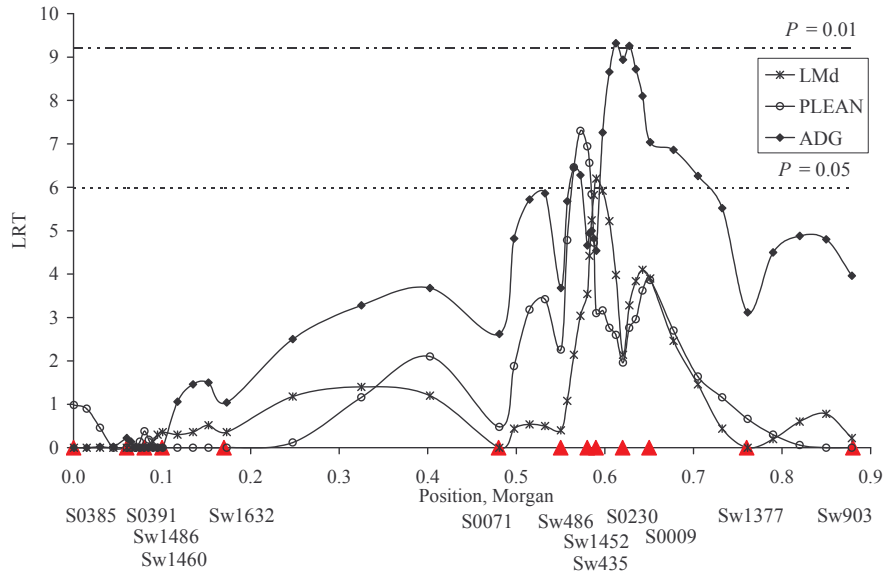

Figure 3. LDLA profiles for loin depth (LMd), percentage lean meat (PLEAN), and average daily gain (ADG) on SSC11. Triangles indicate marker positions.

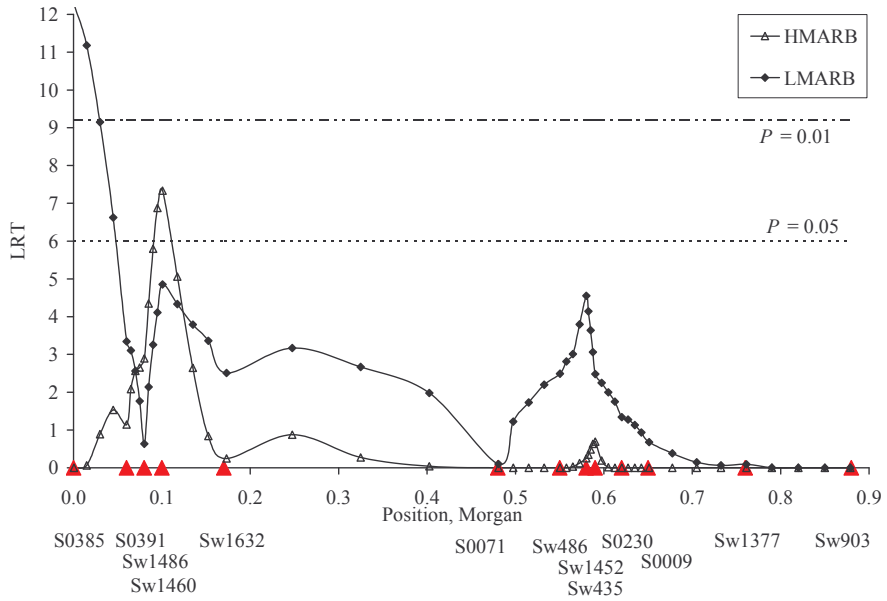

Figure 4. LDLA profiles for ham (HMARB) and loin marbling (LMARB) on SSC11. Triangles indicate marker positions.

The QTL for JCScut on SSC4, which was most significant in the regression analysis, did not reach the significance threshold under the full model (Table 1 in italics, and Figure 1) but passed the threshold under the paternal only model (not shown). The *F*-statistic and LRT profiles were very similar, with the latter one having an increased resolution (Figure 1).
On SSC11, the most significant effect was found for loin marbling (LMARB). This result corresponds with the regression analysis.

The VC LA analysis resulted in additional QTL compared to the regression linkage analysis due to combining paternal and maternal information. The increased number of significant effects under the full model nicely corresponded to results obtained with a maternal or paternal model (data not shown), which showed that the effects were generally either of maternal or paternal origin. Independent modeling of the paternal or maternal haplotype effects reveals the considerable contribution of the dam haplotypes to the variance of the measured traits. This is in agreement with the observed higher allelic diversity within the dams which fits with the use of three-way crossbred individuals as dams mated with pure line sires.

The LDLA analysis resulted in identification of 19 significant effects for 17 traits (Tables 2 and 3). The increased number of significant effects shows a considerable contribution of LD. For Chroma (HamC and LoinC) combining of information showed a positive effect on the power, resulting in increased LRT values compared to the individual underlying traits. However, LRT profiles of the derived variables were in general very similar to the LRT profiles of the underlying traits and are therefore not discussed. Accumulation of the different colour measurements into compound variables did not lead to new disclosures.

The analysis for SSC4 revealed 10 effects ($P < 0.05$) for eight traits (Table 2). Similar to the VC LA analysis, the most significant effect was found for Minolta L* ham (HAML). Two related traits Minolta a* loin (LOINA) and chroma loin (LoinC) very clearly showed a two-peak profile, suggesting two QTL affecting those traits. Likelihood ratio test profiles for JCScut and the two significant Minolta traits (HAML and LOINA) are presented in Figures 1 and 2, respectively.

The QTL for JCScut with a LRT of 6.15 is in agreement with the QTL identified in the regression analysis. The highest LRT value within the ninth-marker bracket corresponds to the

position with the highest *F*-statistic from the regression analysis (Figure 1). The LRT profiles for the different color traits (Figures 1 and 2) may suggest the presence of two QTL on the chromosome, with one of them located around the third marker with pleiotropic effect on the different color measurements. However, a two QTL model did not provide evidence for the presence of two QTL affecting the color traits (data not shown).

The QTL for JCScut is in agreement with previous findings. Ovilo et al. (2002) and Nii et al. (2005) reported meat colour QTL in the same region. De Koning et al. (2001) and Malek et al. (2001) published meat colour QTL on SSC4 positioned more to the centromere or distal on SSC4, although confidence intervals are usually large in a regression analysis with marker intervals that are commonly used in a genome scan.

The QTL for boneless ham weight (BHAM) is in line with the many QTL affecting different muscle mass and back fat measurements reported on SSC4 (see PigQTLDB; Hu et al., 2005).

The LDLA results for SSC11 are presented in Table 3. A total of nine effects were identified at the nominal $P < 0.05$ level. Likelihood ratio test profiles for the meatiness traits loin depth (LMd), meat percentage (PLEAN), and ADG are presented in Figure 3. Profiles for the marbling traits are presented in Figure 4.

The most significant effect was found for marbling score loin (LMARB), which is in agreement with the PHS regression and VC LA analyses. A QTL affecting marbling on SSC11 was not reported in the literature.

The LRT profiles for the meatiness traits loin depth (LMd), meat percentage (PLEAN), and ADG (Figure 3) are very similar and suggest a single QTL affecting the different traits.
Most effects on SSC11 were not identified in the preceding genome scan (Van Wijk et al., 2006) because the 40 cM region beyond marker S0071 was not covered.

Comparison of Analysis Methods

Few studies have reported on a comparison of data analyzed with the different methods used in this study (De Koning et al., 2003; Nagamine et al., 2004; Grapes et al., 2004; Kolbehdari et al., 2005). The regression and variance component methods have similar power in a simple pedigree structure. In complex pedigrees, the variance component method is thought to achieve greater power to detect QTL. Furthermore, the variance component method uses potential information from segregation on the maternal side (Nagamine et al., 2004). Other advantages of

the VC method are simultaneous estimation of (non-)genetic effects, as well as less sensitivity for small family sizes and/or less informative markers (Kolbehdari et al., 2005) Also, the VC method provides estimates of the effect of each haplotype, which links up with breeding value estimation.

Significant QTL obtained with the PHS regression analysis were not necessarily significant in the VC LA analysis or vice versa, although results of both methods were comparable. Ten effects were (near) significant in both analyses, whereas, six effects were significant in the regression analysis and not in the VC LA analysis (Tables 2 and 3). Another six effects were significant in the VC LA analysis and not in the regression analysis; all segregating on the maternal side which explains why these were not found in the regression analysis. The comparable results between the PHS and VC LA analysis also indicate that the VC test statistic as applied was reasonable and not too liberal. A warning is justified however; since no permutation was performed for the VC analysis it remains difficult to judge whether the reported values implies a good hint or strong evidence for the presence of a QTL.

The LDLA analysis resulted in the identification of an increased number of significant effects compared to the VC LA or regression analysis. Sixteen effects showed an increase of LRT value for LDLA compared to VC LA. Despite the higher number of significant effects found with the LDLA analysis, LD did not always positively influence the LRT value. Six effects were reduced by inclusion of LD. A reason might be that the LDLA method assumes that all individuals are descendants from a common (ancient) base population. This is not necessarily true for a cross between different breeds. Uleberg et al. (2005) presented an adjusted version of the LDLA method accounting for the fact that parents from different breeds do not (necessarily) descend from a common base population. Uleberg et al. (2005) ignored the IBD probabilities between base individuals of the two parental breeds. In this way base individuals of the different breeds are considered completely unrelated despite very similar haplotypes. However, it is questionable whether haplotypes in different breeds can be considered completely unrelated in reality. To use crossbred data more correctly further developments of the LDLA method may be required. In the current method, alike in state (AIS) probabilities estimated based on the genotypes are used in calculation of IBD probabilities. In crossbred data AIS probabilities estimated based on sire or dam genotypes could be used to calculate IBD probabilities between pairs of haplotypes of paternal or maternal origin.

The expectation is that LD in particular will contribute in case of higher marker densities. The effective marker density will depend on the LD in the population. Nsengimana et al. (2004) investigated the extent of LD in two genomic regions on SSC4 and SSC7 and concluded that genome-wide association studies are feasible in commercial pig populations at marker densities of 5 to 10 cM. In our study, no common haplotypes, shared by segregating sires, could be identified associated with the effects for JCScut or LMARB. Based on this study, it can be concluded that higher marker densities are required for the identification of common haplotypes across families associated with segregating effects.

Implications

The variance component QTL mapping method using combined linkage and linkage disequilibrium information results in improved QTL detection power and contributes to a more accurate positioning of QTL. The method allows modeling of maternal and paternal haplotype effects, contributing to a better characterization of QTL. Furthermore, the variance component method provides estimates of haplotype effects, which can be used in marker-assisted selection. Significant effects for several traits were obtained, including a QTL for loin marbling on SSC11, which was consistent in the different analysis methods. Also, the QTL affecting meat color on SSC4 was confirmed in this study. Meat quality QTL are of importance for the pork production chain. Color is an important component of the visual appearance of pork and related to the wholesomeness of pork along with texture and flavor. However, prior to application in marker-assisted breeding further fine mapping of the identified QTL is required.

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Literature cited:

Churchill G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138: 963-971.

De Koning D.J., L.L. Janss, A.P. Rattink, P.A. van Oers, B.J. de Vries, M.A.M. Groenen, J.J. van der Poel, P.N. de Groot, E.W. Brascamp, and J.A.M. van Arendonk. 1999. Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs (*Sus scrofa*). Genetics 152: 1679-1690.

De Koning D.J., B. Harlizius, A.P. Rattink, M.A.M. Groenen, E.W. Brascamp and J.A.M. van Arendonk. 2001. Detection and characterization of quantitative trait loci for meat quality traits in pigs. J. Anim. Sci. 79: 2812-2819.

De Koning D.J., R. Pong-Wong, L. Varona, G.J. Evans, E. Giuffra, A. Sanchez, G. Plastow, J.L. Noguera, L. Andersson, and C.S. Haley. 2003. Full pedigree quantitative trait locus analysis in commercial pigs using variance components. J. Anim. Sci. 81: 2155-2163.

Farnir F., B. Grisart, W. Coppieters, J. Riquet, P. Berzi, N. Cambisano, L. Karim, M. Mni, S. Moisio, P. Simon, D. Wagenaar, J. Vilkki, and M. Georges. 2002. Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. Genetics 161: 275-287.

Gautier M., R.R. Barcelona, S. Fritz, C. Grohs, T. Druet, D. Boichard, A. Eggen, and T.H.E. Meuwissen. 2006. Fine mapping and physical characterization of two linked QTLs affecting milk fat yield in dairy cattle on BTA26. Genetics 172: 425-436.

Gilmour A.R., B.R. Cullis, S.J. Welham, and R. Thompson. 1998. ASREML: User manual. Orange Agric. Inst., NSW, Australia.

Grapes L., J.C.M. Dekkers, M.F. Rothschild, and R.L. Fernando. 2004. Comparing linkage disequilibrium-based methods for fine mapping quantitative trait loci. Genetics 166: 1561-1570.

Green P., K. Falls, and S. Crook. 1990. Documentation for CriMap, Version 2.4. Washington University School of Medicine, St. Louis, MO, USA.

Grignola F.E., I. Hoeschele, Q. Zhang, and G. Thaller. 1996. Mapping quantitatitve trait loci in outcross populations via residual maximum likelihood. II. A simulation study. Genet. Sel. Evol. 28:491-504.

Grignola F.E., Q. Zhang, and I. Hoeschele. 1997. Mapping linked quantitative trait loci in outcross populations via residual maximum likelihood. Genet. Sel. Evol. 29:529-544.

Hoeschele I., P. Uimari, F.E. Grignola, Q. Zhang, and K.M. Gage. 1997. Advances in statistical methods to map quantitative trait loci in outbred populations. Genetics 147: 1445-1457.

Hu Z-L., S. Dracheva, W. Jang, D. Maglott, J. Bastiaansen, M.F. Rothschild, and J.M. Reecy. 2005. A QTL resource and comparison tool for pigs: PigQTLDB. http://www.animalgenome.org/QTLdb/. Mamm. Genome 16(10): 792-800.

Janss L.L.G., and H.C.M. Heuven. 2005. LDLA, a package to compute IBD matrices for QTL fine mapping by variance component methods. Abstract of 56th annual meeting of EAAP, Uppsala, Sweden.

Knott S.A., and C.S. Haley. 1992. Maximum likelihood mapping of quantitative trait loci using full-sib families. Genetics 132(4): 1211-1222.

Knott S.A., J.M. Elsen, and C.S. Haley. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theor. Appl. Genet. 93: 71-80.

Kolbehdari D., G.B. Jansen, L.R. Schaeffer, and B.O. Allen. 2005. Power of QTL detection by either fixed or random models in half-sib designs. Genet. Sel. Evol. 37(6): 601-614.

Malek M., J.C.M. Dekkers, H.K. Lee, T.J. Baas, K. Prusa, E. Huff-Lonergan, and M.F. Rothschild. 2001. 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.

Meuwissen T.H.E., and M.E. Goddard. 2000. Fine scale mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. Genetics 155: 421-430.

Meuwissen T.H.E., and M.E. Goddard. 2001. Prediction of identity by descent probabilities from marker-haplotypes. Genet. Sel. Evol. 33: 605-634.

Meuwissen T.H.E., A. Karlsen, S. Lien, I. Olsaker, and M.E. Goddard. 2002. Fine mapping of a quantitative trait locus for twinning rate using combined linkage and linkage disequilibrium mapping. Genetics 161: 373-379.

Nagamine Y., P.M. Visscher, and C.S. Haley. 2004. QTL detection and allelic effects for growth and fat traits in outbred pig populations. Genet. Sel. Evol. 36: 83-96.

Nsengimana J., P. Baret, C.S. Haley, and P.M. Visscher. 2004. Linkage disequilibrium in the domestic pig. Genetics 166: 1395-1404.

Nii M., T. Hayashi, S. Mikawa, F. Tani, A. Niki, N. Mori, Y. Uchida, N. Fujishima-Kanaya, M. Komatsu, and T. Awata. 2005. Quantitative trait loci mapping for meat quality and muscle fiber traits in a Japanese wild boar x Large White intercross. J. Anim. Sci. 83: 308-315.

Olsen H.G., S. Lien, M. Svendsen, H. Nilsen, A. Roseth, M. Aasland Opsal, and T.H.E. Meuwissen. 2004. Fine mapping of milk production QTL on BTA16 by combined linkage and linkage disequilibrium analysis. J. Dairy Sci. 87: 690-698.

Ovilo C., A. Clop, J.L. Noruera, M.A. Oliver, C. Barragán, C. Rodriquez, L. Silió, M.A. Toro, A. Coll, J. M. Folch, A. Sánchez, D. Babot, L. Varona and M. Perez-Enciso. 2002. Quantitative trait locus mapping for meat quality traits in an Iberian x Landrace F2 pig population. J.Anim.Sci. 80: 2801-2808.

Polineni P. 2004. Developing a web accessible integrated database and visualization tool for bovine quantitative trait loci. http://bovineqtl.tamu.edu/. Thesis, Texas A&M University, Texas.

Riquet J., W. Coppieters, N. Cambisano, J.–J Arranz, P. Berzi, S.K. Davis, B. Grisart, F. Fanir, L. Karim, M. Mni, P. Simon, J.F. Taylor, P. Vanmanshoven, D. Wagenaar, J.E. Womack, and M. Georges. 1999. Fine mapping of quantitative trait loci by identity by descent in outbred populations: application to milk production in dairy cattle. Proc. Natl. Acad. Sci. 96: 9252-9257.

Rohrer G.A., L.J. Alexander, Z.L. Hu, T.P.L. Smith, J.W. Keele, and C.W. Beattie. 1996. A comprehensive map of the porcine genome. Genome Res. 6: 371-391.

Setser C.S. 1984. Color: reflections and transmissions. J. Food Quality 6: 183-186.

Sobel E., and K. Lange. 1996. Descent graphs in pedigree analysis: applications to haplotyping. Location scores, and marker sharing statistics. Am. J. Hum. Genet. 58: 1323-1337.

Uleberg E., I.S. Widerøe, E. Grindflek, J. Szyda, S. Lien, and T.H.E. Meuwissen. 2005. Fine mapping of a QTL for intramuscular fat on porcine chromosome 6 using combined linkage and linkage disequilibrium mapping. J. Anim. Breed. Genet. 122: 1-6.

Van Wijk H.J., D.J.G. Arts, J.O. Matthews, M. Webster, B.J. Ducro, and E.F. Knol. 2005. Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. J. Anim. Sci. 83: 324-333.

Van Wijk H.J., B. Dibbits, E.E. Baron, A.D. Brings, B. Harlizius, M.A.M. Groenen, E.F. Knol, and H. Bovenhuis. 2006. Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross. J. Anim. Sci. 84: 789–799.

Wang J., X. He, J. Ruan, M. Dai, J. Chen, Y. Zhang, Y. Hu, C. Ye, S. Li, L. Cong, L. Fang, B. Liu, S. Li, J. Wang, D.W. Burt, G.K-S. Wong, J. Yu, H. Yang, and J. Wang. 2005. ChickVD: a sequence variation database for the chicken genome. http://chicken.genomics.org.cn/. Nucleic Acids Res. 33: 438-441.

Zhang Q., D. Boichard, I. Hoeschele, C. Ernst, A. Eggen, B. Murkve, M. Pfister-Genskow, L.A. Witte, F.E. Grignola, P. Uimari, G. Thaller, and M.D. Bishop. 1998. Mapping quantitative trait loci for milk production and health of dairy cattle in a large outbred pedigree. Genetics 149:1959-1973.

Chapter 5

Carcass and meat quality QTL on *Sus scrofa* chromosome 2, 13, and 14 of commercial finishing pigs

H.C.M. Heuven[#], H.J. van Wijk[*], B. Dibbits[#], A.J.A. van Kampen[#], and H. Bovenhuis[#]

[*]IPG, Institute for Pig Genetics, P.O. Box 43, 6640 AA, Beuningen, The Netherlands;

[#]Animal Breeding and Genetics Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands.

Abstract: Quantitative trait loci (QTL) affecting carcass- and meat quality located on SSC2, SSC13 and SSC14 were identified using variance component methods for QTL detection. Data was obtained on a commercial crossbred population sired by 17 boars from a synthetic line.
QTL which significantly affected loin muscle mass were found on SSC2 and SSC14. On SSC13 a QTL affecting inner-ham muscle mass was identified. QTL affecting meat quality traits, such as Minolta L*, a* and b*, ultimate pH and Japanese color score were detected on SSC2. These results agreed well with previous QTL-studies involving SSC2.
Due to the low marker density, *i.e.* an average of 23 cM between markers, linkage disequilibrium information did not add power and therefore did not add to the results. The same QTL were identified using a Mendelian model compared to a model allowing for separate segregation of sire and dam haplotypes. However, fitting a sire and a dam component showed that QTL segregated either from the sire or from the dam or from both parental lines.

Key words: Meat quality, Pigs, QTL, SSC2, SSC13, SSC14

Introduction

Pig breeding programs aim at improving the genetic composition of pigs for economically important traits. Carcass quality has been successfully improved in most selection programs due to the ease of obtaining phenotypes on live animals and to relative high heritability for these traits. Breeding for meat quality, however, has received much attention over the past two decades but has not been emphasized in most selection programs (De Vries et al., 1992; Hovenier et al., 1993; Sellier, 1998; Knap et al., 2002). Meat quality traits can only be measured on relatives of selection candidates at a relative late point in selection programs. Therefore, successful improvement of meat quality might be realized by combining molecular information and traditional measurements because marker data can be obtained on all animals at an early age (Heuven et al., 2003).

Molecular information has increased rapidly through genome scans of experimental cross-bred populations (reviewed by Bidanel and Rothschild, 2002). QTL studies using commercial populations, however, are limited (Nezer et al., 1999; Grindflek et al., 2001; Malek et al., 2001; Evans et al., 2003; De Koning et al., 2003; Van Wijk et al., 2006).

In fore mentioned studies the statistical linkage analyses method employed was 'paternal half-sib regression' which models the segregation of paternal QTL (Knott et al., 1996). Variance component methods, based on theory developed by Fernando and Grossman (1989), are currently becoming the method of choice because they allow for much greater flexibility in modeling of QTL in arbitrary pedigrees while taking all source of variation simultaneously into account (De Koning et al., 2003).

In a preliminary analysis using eight of the 17 half-sib families, putative QTL were shown on (parts of) SSC2, SSC13, and SSC14 (Van Wijk et al., 2006). Based on these results it was decided to genotype the remaining nine families and analyze the overall data set.

The goal of this paper is to confirm QTL affecting meat and carcass quality of commercial finishers located on SSC2, SSC13, and SSC14 using variance component methods.

Material and Methods

Population and phenotypes

The commercial finishers were a cross product of 17 boars of a synthetic sire line (Piétrain/Large White/, TOPIGS, The Netherlands) and 239 anonymous hybrid sows. The piglets were born during a 2 month period in 2002. Piglets were individually tagged at birth and males were castrated 3 to 5 days after farrowing. The pigs were weaned on average at 17 d of age and raised till an average weight of 22.7 kg before being moved to the finishing barns. Commercial diets were fed ad lib and pigs had free access to water.

Pigs were loaded in three batches per compartment at an average weight of 118 kg live weight and kept overnight in a lairage at the slaughterhouse. The average age (**AGE**) of each batch was 164, 172 and 185 days respectively. During a 70 d period pigs were slaughtered on 17 different days. Measurements on the carcass were recorded on one half of the carcass. Back fat (**BF**) and loin depth (**LD**) were measured at the 10^{th} rib using the Hennessy grading probe HGP Systems Ltd, Auckland NZ). Lean percentage (**PLEAN**) was calculated as: PLEAN= 58.86 – (0.61 x BF) + (0.12 x LD).

Cold carcass weight (**CCW**) was recorded after temperature equalization. Primal cuts of ham (**HAM**) and loin (**LOIN**) were weighed and further dissected into boneless subprimals and individual muscles. Hams had the skin and fat removed and four subprimals were weighed:

inside ham (**IHAM**), outer ham (**OHAM**), knuckle ham (**KHAM**) and the lite butt ham (**LBHAM**, *i.e.* part of the *gluteus medius* muscle). Together they summed up to boneless ham muscle weight (**BHAM**). Loins were processed to a boneless loin without the fat cover (**DLOIN**).

Meat quality measurements were taken both on the loin and the ham. Ultimate pH (**pHu**) was measured in the boneless loin 24-28 hrs post mortem. Loin Minolta L*, a* and b* (**LOINL**, **LOINA** and **LOINB**) were taken on the fresh cut surface of a 2.5-cm chop removed from the sirloin end using a Minolta CR 300 (Minolta, Osaka, Japan). The same chop was used for a subjective color score (score 1 to 6, with 1 = pale, and 6 = very dark) using the Japanese color scale (**JCScut**). The side view of the loin was also scored using this scale (**JCSrib**). A subjective marbling score (**LMARB**; 1 to 5, with 1 = devoid, and 5 = overly abundant) was given to the chop based on National Pork Producers Council marbling standards (NPPC, 1991). Cores were taken from a second 2.5-cm chop using a 25-mm coring device to determine drip loss percentage (**DRIP**). Samples were weighed and put in pre-weighed tubes and stored in a cooler. After 24 hrs samples were reweighed and drip loss was calculated (Christensen, 2003). Purge loss (**PURGE**, %) was determined by weighing a 7.5- to 10-cm piece of the remainder of the boneless loin, cooling it for 5 days in plastic bags and reweighing. Subjective firmness scores (**FIRM**; 1 to 3, 1 = soft and exudative, and 3 = firm) were obtained using NPPC standards (NPPC, 2000).

Meat quality measurements taken on the ham included Minolta L*, a*, and b* values on the fresh cut surface of the inside ham muscle (**HAML**, **HAMA** and **HAMB**). A subjective marbling score (**HMARB**; 1 to 4; 1 = devoid, and 4 = abundant) was assigned to the outside ham muscle.

Genotyping and linkage map construction

DNA was extracted from ear or loin tissue samples using the Puregene® DNA Isolation kit (D-70KA, Gentra Systems, Minneapolis, USA). Isolated DNA was tested on 1.2% agarose gel for quality and adjusted in Sodium Chloride-Tris-EDTA (STE) buffer to a final concentration of 15 ng/µL.

Genotyping was performed in two batches. First eight half-sib families were typed for 10, 5, and 7 microsatellite markers on SSC2, SSC13, and SSC14 respectively (Van Wijk et al., 2006). Subsequently, 9 additional families were genotyped for 8, 5, and 5 markers respectively. The markers included in the statistical analysis are given in Table 1. Genotypes were scored in

duplicate and checked against pedigree information. CriMap 2.4 (Green et al., 1990) was used to construct a sex-average linkage map. The resulting recombination fractions/cM distances were used in SimWalk version 2.89 (Sobel and Lange, 1996) to reconstruct haplotypes and in the QTL analyses.

Statistical analysis

QTL were mapped based on a segregation analysis using the variance component method because it uses both the segregation from the sires and the dams, it allows for simultaneously estimation of polygenic-, QTL-, litter-, and fixed-effects and it allows for complex pedigrees (half- and full-sib structure). Identity by descent (IBD) of parent-offspring haplotypes, using reconstructed haplotypes, was calculated using the LDLA package (Janss and Heuven, 2005). The package is based on theory developed by Meuwissen and Goddard (2001). IBD probability matrices were calculated for five evaluation points in each bracket, *i.e.* at 10, 30, 50, 70, and 90% of the distance from the left marker to the right marker in each bracket. The likelihood at each evaluation point was determined by using ASREML (Gilmour et al., 1998).

Phenotypes were analyzed according to the following model (Mendelian model). Since the pedigree of the sows was not available a sire model seemed more appropriate than an animal model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Sc} + \mathbf{Wv} + \mathbf{e} \qquad [1]$$

Where **y** is a vector containing phenotypic values, **b** is a vector containing non-genetic effects, **u** is a vector containing polygenic effects of the sires, **c** is a vector containing common litter and dam effects, **v** is a vector containing haplotype effects due to a putative QTL and **e** contains the residual effects. Non-genetic effects considered were a barn-group-batch, and sex as class variables and 'cold carcass weight' and 'days in the finishing barn' as linear covariables. The random effects of **u**, **c**, **v**, and **e** were assumed to be normally distributed with zero mean and variances $\mathbf{A}\sigma^2_u$, $\mathbf{I}\sigma^2_c$, $\mathbf{G_p}\sigma^2_v$, and $\mathbf{I}\sigma^2_e$ respectively, where **A** is the genetic relationship matrix among the sires including five generations of known pedigree, $\mathbf{G_p}$ is the IBD matrix among the haplotypes at evaluation point **p**, and **I** is an identity matrix. **X**, **Z**, **S**, and **W** are incidence matrices relating effects to phenotypes.

To relax the assumption of equal variance among the paternal and maternal haplotypes in model 1 the following model (2) was applied:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Sc} + \mathbf{W_s v_s} + \mathbf{W_d v_d} + \mathbf{e} \quad [2]$$

In model 2 a separate variance component is fitted for the paternal ($\mathbf{v_s}$) and maternal ($\mathbf{v_d}$) haplotypes (two components model). Sire and the anonymous hybrid dams originated from different populations which could be segregating for different QTL-alleles.

Test statistic and significance threshold

To test the hypothesis of the presence of a QTL (H_1) versus no QTL (H_0) the likelihood ratio test (LRT) was applied. The LRT statistic at each evaluation point is calculated as twice the difference between the log likelihood of the model 1 (or 2) minus the log likelihood of a model without a QTL effect. The test statistic plotted along the chromosome results in a LRT-profile. Given this profile, thresholds were calculated which take multiple testing across the chromosome into account (Piepho, 2001).

Results

Map construction

Genetic linkage maps are presented in Table 1. The order of the markers and the distance among markers is in close agreement with the USDA-MARC.2 genetic linkage map (Rohrer et al., 1996) except for marker pair SwR1910-SwR783 on SSC2 which is reversed and separated by 14 cM instead of 1 cM. The average distance among the markers is 23, 21 and 25 cM for SSC2, SCC13 and SSC14 respectively. Distances, using the Haldane linkage function, obtained in this study were used in the QTL analysis.

QTL on SSC2, SSC11, and SSC14

General statistics regarding the data is given in Van Wijk et al. (2005). The LRT statistics for traits that exceeded a threshold value of 0.05 and the position of their maximum value are given in Table 2. Results are shown for the Mendelian model (applying a single variance component) as well as for the two components model, *i.e.* allowing for different variances among paternal and among maternal haplotypes.

Table 1. Linkage maps for SSC2, SSC13, and SSC14 compared to the USDA-MARC map using the Kosambi mapping function and average distances among the markers.

SSC2			SSC13			SSC14		
Marker	**cM**	**USDA**	**Marker**	**cM**	**USDA**	**Marker**	**cM**	**USDA**
SwC9[1]	0	0	S0282	0	0	Sw857[2]	0	0
Sw2623	11	0	SwR428	24	23	Sw1027	15	15
SwR1910	29	24	Sw864	53	43	Sw2519	39	44
SwR783	43	23	S0068	68	62	Sw77	60	62
Sw240	72	41	Sw398	84	79	Sw2515	100	102
Sw2167	89	56						
Sw2514	135	103						
SwR308	161	127						
Avg. dist.	23	18		21	20		25	26

[1] on the USDA-map SwC9 is at 1 cM on SSC2.

[2] on the USDA map Sw857 is at 7 cM on SSC14.

The LRT statistics and position of the QTL were very similar for the Mendelian and the two components model. The significance is slightly lower for the two components model because it estimates one additional parameter and therefore requires an additional degree of freedom. LRT-profiles for the most significant traits on SSC2 are given in Figure 1.

Color

A near significant QTL was observed for HAML at 36 cM on SSC2. Malek et al. (2001) also found a QTL for this trait on SSC2. However they located them at 72 and 116 cM. (The locations of QTL from other studies are taken from the pigQTLdb (Hu et al., 2005) where all the map distances are converted to the USDA-MARC map). For HAMA and HAMB no QTL have previously been reported on SSC2. The JCSrib QTL is in accordance with the QTL for a similar subjective color score observed by Malek et al. (2001) although their score was on the cut surface of the loin instead of the side-rib-view. The QTL for HAMB and JCSrib were found at almost the same position which might indicate that it is the same QTL affecting both traits.

Table 2. LRT statistic and position of traits with a (near) significant QTL-effect on SSC2, SSC13, and SSC14.

Model	Mendelian model [1]				Two components model [2]			
Trait	LRT	Signf.[1]	cM	Eval.[2]	LRT	Signf.[1]	cM	Eval.[2]
				SSC2				
HAML	7.1	ns	36	13	9.9	ns	27	9
HAMA	15.0	<.005	10	5	15.0	<.005	10	5
HAMB	6.5	ns	158	35	8.7	ns	158	35
PHu	13.1	<.005	112	28	13.4	<.01	112	28
JCSrib	11.1	<.01	153	34	11.3	<.05	153	34
DLOIN	11.7	<.01	103	27	11.9	<.05	103	27
				SSC13				
INHAM	7.6	<.05	27	6	9.0	ns	21	5
				SS14				
DLOIN	9.1	<.05	50	13	14.3	<.01	50	13

[1] ns = nearly significant.

[2] eval. = evaluation point, *i.e.* at 10, 30, 50, 70 or 90% of each bracket.

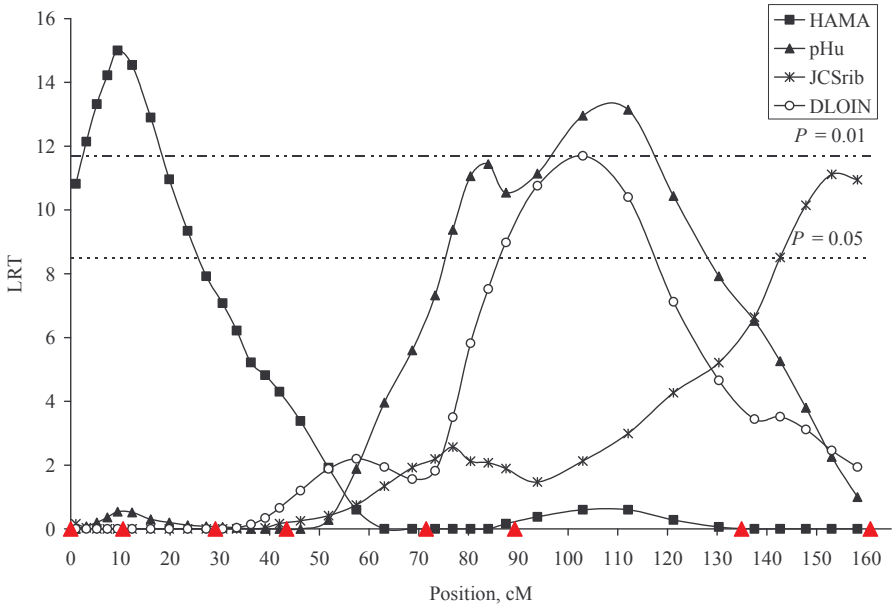

Figure 1. LRT –profiles for the most significant traits on SSC2 using the Mendelian model. Triangles indicate marker positions.

pH

The QTL for pHu on SSC2 was observed in a large bracket and could be the result of two QTL. Lee et al. (2003) observed two QTL for pHu on SCC2 at 42 and 64 cM in a F2-cross between Meishan and Piétrain. Su et al. (2004) observed a QTL for pHu at 67 cM. The ultimate pH usually is a good predictor of water holding capacity. Malek et al. (2001) showed that two QTL are segregating for this trait on SSC2 (around 75 and 114 cM). However, in this study no significant QTL were found for drip or purge on SSC2.

Estimated haplotype effects on pHu were obtained using the Mendelian model for each haplotype of each sire. The absolute difference between the two haplotype effects of each sire is shown in Figure 2. The average standard error for these effects is 0.026. Five sires have an absolute difference that is more than two times the standard error indicating that these families are most likely segregating for a QTL affecting pHu. Four of these sire families were already indicated as segregating in a preliminary analysis using paternal half-sib regression.

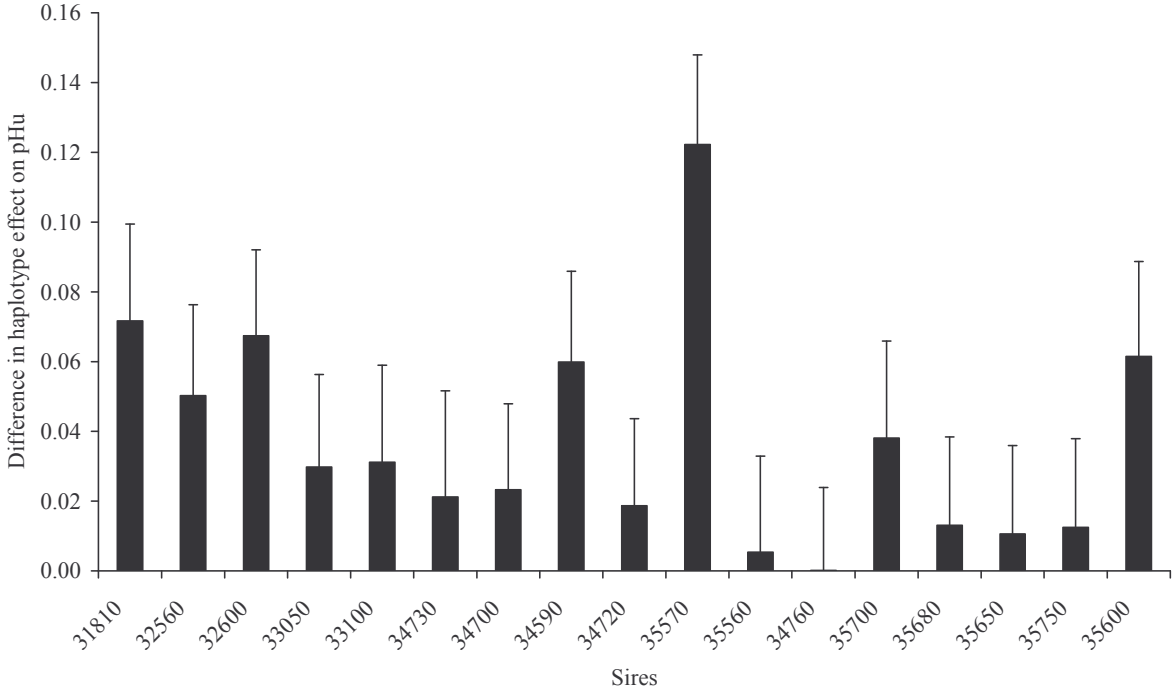

Figure 2. Absolute difference between haplotype effects on pHu for each sire and their standard error.

Carcass traits

A QTL for DLOIN at 103 cM on SSC2 has not been reported previously. Milan et al. (2002), Jeon et al. (1999), Nezer et al. (1999) and Lee et al. (2003) all report a QTL involving amount of loin at the beginning of SSC2, which is most likely associated with the *IGF2*-gene. However, Varona et al. (2002) and also Lee et al. (2003) reported a QTL for loin depth and percentage lean cuts around 65 cM.

Rohrer and Keele (1998) observed a QTL for a similar trait as DLOIN on SSC14, *i.e.* 'trimmed whole sale product' which had a correlation of .81 with loin eye area. Milan et al. (2002) found a suggestive QTL for loin weight on SSC14 at 96 cM. The QTL for IHAM on SSC13 is in accordance with a QTL for ham muscle weight observed by Yue et al. (2003) in a F2-cross between 'White' and Meishan.

In Table 3 proportion of total variance due to polygenic (h^2), litter (c^2) and QTL (v^2) as well as residual and total variance is given for traits mentioned in Table 2 at the evaluation point where the LRT for the QTL was at its maximum.

Table 3. Total and residual variance and percentage of variance associated with polygenic-, litter- and QTL-effect (h^2, c^2 and v^2) for the significant traits.

		Mendelian model				**Two components model**[a]					
	Total variance	**Residual variance**	**h^2**	**c^2**	**v^2**	**Residual variance**	**h^2**	**c^2**	**v^2**	**v_s^2**	**v_d^2**
SSC2											
HAML	20.94	17.56	0.07	0.06	0.09	17.16	0.11	0.03	0.12	0.02	0.10
HAMA	1.897	1.524	0.08	0.06	0.12	1.525	0.07	0.06	0.12	0.06	0.05
HAMB	3.106	2.762	0.10	0.03	0.05	2.780	0.07	0.05	0.04	0.04	0.00
PHu	0.018	0.013	0.00	0.11	0.18	0.013	0.02	0.09	0.21	0.08	0.13
JCSrib	0.184	0.146	0.28	0.05	0.08	0.146	0.26	0.06	0.08	0.05	0.03
DLOIN	0.068	0.042	0.53	0.13	0.13	0.042	0.50	0.14	0.12	0.07	0.05
SSC13											
INHAM	0.023	0.017	0.25	0.14	0.05	0.017	0.24	0.15	0.03	0.03	0.00
SSC14											
DLOIN	0.069	0.042	0.58	0.15	0.08	0.042	0.65	0.12	0.11	0.01	0.10

[a] for the two components model, QTL variance (v^2) was split in a paternal (v_s^2) and maternal (v_d^2) component.

In general the proportions of variance due to polygenic and litter effects are in close agreement with Van Wijk et al. (2005) who analyzed the same data set before marker data was available, *i.e.* they applied a model without QTL effects. Disagreement is apparent for HAMA and pHu. For HAMA the estimate of polygenic variance (h^2) dropped from 0.26 to 0.08 and 0.07 for the Mendelian and the two components model respectively. Similarly the h^2 for pHu dropped from 0.11 to 0.00 and 0.02. In both cases the QTL variance (v^2) is relatively high, indicating that the genetic variance has shifted from polygenic to QTL variance. This might be the result of the specific data set analyzed. Since it is unlikely that a single QTL explains most of the genetic variance the QTL variance is most likely overestimated.

For DLOIN on SSC14 some of the litter variance has shifted towards the polygenic variance when compared to Van Wijk et al. (2005), the analysis of DLOIN on SSC2 resulted in a higher polygenic variance.

Discussion

To correct for multiple testing of QTL on many different evaluation points the method described by Piepho (2001) was applied. Depending on the trait analyzed, the 0.05 threshold thus obtained correspond with a nominal p-value of around 0.005. Few false positive QTL will be found at the expense of false negatives using these strict thresholds. However, budget limitations allow for fine mapping of only the most promising QTL in terms of significance and economic importance.

In this study linkage disequilibrium (LD) information was not included when calculating the IBD matrices, although this is optional in the LDLA-package. Although it was thought to be improper to calculate IBD among parental haplotypes it was applied for the sake of comparison. The results were very similar to those obtained with using linkage information only (data not shown). This was not surprising given the relative large distances among the markers (20-25 cM). It is also not clear how IBD due to LD should be calculated for crossbred populations. The theory to calculate this IBD developed by Meuwissen and Goddard (2001) assumes a single population some 100 generations ago which is not very likely for widely different pig breeds. Assuming no IBD due to LD between base-haplotypes of different breeds as was applied by Uleberg et al. (2005), seems to be too extreme as well because most likely all pigs originate from

a single population. Biodiversity studies, e.g. Eding and Meuwissen (2001), might provide theory on how to determine IBD within and between breeds simultaneously.

Given the hybrid origin of the population used in this study, *i.e.* a single strain sire line was crossed with a 3-way cross sow, the two components model is probably more appropriate than the Mendelian model because in the two components model the segregation of the paternal and maternal haplotypes are modeled as independent effects. This is illustrated in Table 3 where contribution of paternal and maternal components is given, *i.e.* v^2_s and v^2_d. It can be observed that QTL for HAML and DLOIN on SSC14 are mainly segregating from the dam side, HAMB and INHAM are segregating from the sire side while the contributions are similar for all other traits.

Conclusions

QTL affecting carcass quality were found on SSC2, SS13 and SSC14 while QTL affecting meat quality were found on SSC2. QTL effects were (highly) significant even after correction for multiple testing. These results will be followed up by fine mapping one or more regions on SSC2 and/or SSC14.

The variance component method to detect QTL allows for different models, e.g. Mendelian, two components, paternal and maternal models, which might indicate that some of the QTL are segregating in maternal or paternal lines.

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Literature cited:

Bidanel J.P., and M. Rothschild. 2002. Current status of quantitative trait locus mapping in pigs. Pig News and Information. Vol. 23 No. 2: 39-53.

Christensen L.B. 2003. Drip loss sampling in porcine *m. longissimus dorsi*. Meat Sci. Vol. 63(4): 69-477.

De Koning D.J., R. Pong-Wong, L. Varona, G.J. Evans, E. Giuffra, A. Sanchez, G. Plastow, J.L. Noguera, L. Andersson, and C.S. Haley. 2003. Full pedigree quantitative trait locus analysis in commercial pigs using variance components. J. Anim. Sci. 81: 2155-2163.

De Vries A.G., P.G. van der Wal, G. Eikelenboom, and J.W.M. Merks. 1992. Genetische variatie in varkensvleeskwaliteit. I.V.O. report B-376, I.V.O. Zeist, The Netherlands.

Eding H., and T.H.E. Meuwissen. 2001. Marker based estimates of between and within population kinships for the conservation of genetic diversity. J. of Anim. Breeding and Genetics 118: 41-159.

Evans G.J., E. Giuffra, A. Sanchez, S. Kerje, G. Davalos, O. Vidal, S. Illan, J.L. Noguera, L. Varona, I Velander, O.I. Southwood, D.J. de Koning, C.S. Haley, G.S. Plastow, and L. Andersson. 2003. Identification of quantitative trait loci for production traits in commercial pig populations. Genetics 164: 621-627.

Fernando R.L., and M. Grossman. 1989. Marker selection using best linear unbiased prediction. Genet. Sel. Evol. 21: 467-477.

Gilmour A.R., B.R. Cullis, S.J. Welham, and R. Thompson. 1998. ASREML: User manual. Orange Agric. Inst., NSW, Australia.

Green P., K. Falls, and S. Crook. 1990. Documentation for CriMap, Version 2.4. Washington University School of Medicine, St. Louis, MO, USA.

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

Heuven H.C.M., H. Bovenhuis, L. G. Janss, and J.A.M. van Arendonk. 2005 Efficiency of population structures for mapping of Mendelian and imprinted quantitative trait loci (QTL) in outbred pigs using variance component methods. Genet. Sel. Evol. 37: 635-655.

Heuven H.C.M., H.J. van Wijk, and J.A.M. van Arendonk. 2003. Combining traditional breeding and genomics to improve pork quality. Outlook on Agriculture 32 (4): 235-239.

Hovenier R., E. Kanis, Th. van Asseldonk, and N.G. Westerink. 1993. Breeding for pig meat quality in halothane-negative populations – a review. Pig News and Information 14: 17N-25N.

Hu Z-L., S. Dracheva, W. Jang, D. Maglott, J. Bastiaansen, M.F. Rothschild, and J. Reecy. 2005. A quantitative trait loci resource and comparison tool for pigs: PigQTLDB. Mamm. Genome 15: 792-800.

Janss L.L.G., and H.C.M. Heuven. 2005. LDLA, a package to compute IBD matrices for QTL fine mapping by variance component methods. Abstracts of 56th annual meeting of EAAP, Uppsala, Sweden.

Jeon J.T., O. Carlborg, A. Tornsten, E. Giuffra,V. Amargar, P. Chardon, L. Andersson-Eklund, K. Andersson, I. Hansson, K. Lundstrom, and L. Andersson. 1999. A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the *IGF2* locus. Nat. Genet. 21: 157-158.

Knap P.W., A.A. Sosnicki, R.E. Klont, and A. Lacoste. 2002. Simultaneous improvement of meat quality and growth-and-carcass traits in pigs. Proc. of the 7th World Congres on Genetics applied to Livestock Production, Vol. 31: 339-346.

Knott S.A., J.M. Elsen, and C.S. Haley. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theor. Appl. Genet. 93: 71-80.

Lee S.S., Y. Chen, C. Moran, S. Cepica, G. Reiner, H. Bartenschlager, G. Moser, and H. Geldermann. 2003. Linkage and QTL mapping for *Sus scrofa* chromosome 2. J. Anim. Breeding and Genetics 120 (suppl.1): 11-19.

Malek M., J.C.M. Dekkers, H.K. Lee, T.J. Baas, K. Prusa, E. Huff-Lonergan, and M.F. Rothschild. 2001. 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.

Meuwissen T.H.E., and M.E. Goddard. 2001. Prediction of identity by descent probabilities from marker-haplotypes. Genet. Sel. Evol. 33: 605-634.

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

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

NPPC. 1991. Procedures to evaluate market hogs. 3rd ed. Natl. Pork Prod. Counc., Des Moines, IA, USA.

NPPC. 2000. Pork composition and quality assessment procedures. Natl. Pork Prod. Counc., Des Moines, IA, USA.

Piepho H.P. 2001. A quick method for computing approximate thresholds for quantitative trait loci detection. Genetics 157: 425-432.

Rohrer, G. A., L. J. Alexander, Z. L. Hu, T. P. L. Smith, J. W. Keele, and C.W. Beattie. 1996. A comprehensive map of the porcine genome. Genome Res. 6: 371-391.

Rohrer G.A., and J. W. Keele. 1998. Identification of quantitative trait loci affecting carcass composition in swine: II Muscling and wholesale product yield traits. J. Anim. Sci. 76: 2255-2262.

Sellier P.1998. Genetics of meat and carcass traits. Pp 463-510 in 'The genetics of the pig' edited by M.F. Rothschild and A. Ruvinsky. CAB International, Oxon, UK.

Sobel E., and K. Lange. 1996. Descent graphs in pedigree analysis: applications to haplotyping. Location scores, and marker sharing statistics. Am. J. Hum. Genet. 58: 1323-1337.

Su Y., B. Ma, and Y. Xiong. 2004. Genetic location of body composition traits in pigs. Hereditas (Beijing) 26(2): 163-166.

Uleberg E., I.S. Wideroe, E. Grindflek, J. Szyda, S. Lien, and T.H.E. Meuwissen. 2005. Fine mapping of a QTL for intra muscular fat on porcine chromosme 6 using combined linkage and linkage disequilibrium mapping. J. Anim. Breed. Genet. 122: 1-6.

Van Wijk H.J., D.J.G. Arts, J.O. Matthews, M. Webster, B.J. Ducro, and E.F. Knol. 2005. Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. J. Anim. Sci. 83: 324- 333.

Van Wijk H.J., B. Dibbits, E.E. Baron, A.D. Brings, B. Harlizius, M.A.M. Groenen, E.F. Knol, and H. Bovenhuis. 2006. Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross. J. Anim. Sci. 84: 789-799.

Van Wijk H.J., H. Buschbell, B. Dibbits, S.C. Liefers, B. Harlizius, H.C.M. Heuven, E.F. Knol, H. Bovenhuis, and M.A.M. Groenen. 2006. Fine mapping of QTL for pork carcass composition and meat quality on SSC4 and SSC11. Submitted.

Verona L., C. Ovilo, A. Clop, J.L. Noguera, M. Perez-Enciso, A. Coll, J.M. Folch, C. Barragan, M.A. Toro, D. Babot, and A. Sanchez. 2002. QTL mapping for growth and carcass traits in an Iberian by Landrace pig intercross: additive, dominant and epistatic effects. Genet. Res. Camb. 80: 145-154.

Yue G., V. Russo, R. Davoli, I. Sternstein, C. Brunsch, D. Schroffelova, A. Stratil, G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. 2003. Linkage and QTL mapping for *Sus scrofa* chromosome 13. J. Anim. Breeding and Genetics 120 (suppl.1): 103-110.

Chapter 6

In silico identification and mapping of microsatellite markers on *Sus scrofa* chromosome 4

H.J. van Wijk[*1], S. Liefers[#1], H. Buschbell[#1], B. Dibbits[#], B. Harlizius[*], and M.A.M. Groenen[#]

[*]IPG, Institute for Pig Genetics, P.O. Box 43, 6640 AA, Beuningen, The Netherlands;

[#]Animal Breeding and Genetics Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands.

[1] Equal contribution

[2] Current address: Institute of Animal Science, Animal Breeding and Husbandry Group, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany.

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Abstract: Marker density of a QTL region on pig chromosome 4 was increased. New microsatellites were identified by *in silico* mining of BAC-end and genomic shotgun sequences. Among 8,784 BES predicted within the region, 148 SSRs were identified. In addition, 27,450 CA/TG repeats were identified within the genomic shotgun sequences, of which 157 were most likely located on SSC4q. A selection of 61 new microsatellites was mapped, together with previously mapped markers. The results showed that the human-pig comparative map in combination with BES and genomic sequence resources provides an excellent source for a highly efficient and targeted development of markers.

Key words: Linkage map; Microsatellite marker; Porcine; SSC4; SSR

Introduction

Simple sequence repeats (SSRs or microsatellites) are multi-allelic and have been proven to be the markers of choice in molecular genetic studies or breeding research of farm animals, due to their high level of polymorphism. Simple sequence repeats are abundant and randomly or nearly randomly distributed across genomes (Li et al., 2002). In pig, Rohrer et al. (1994) developed the first molecular marker map consisting of SSRs. Since then, many more SSRs were mapped and the current porcine linkage map contains approximately 1,400 SSRs (http://www.marc.usda.gov/). However, SSR marker development is expensive, labor intensive and time consuming when developed from small insert genomic DNA libraries or direct-sequencing using primers annealed with di-nucleotide repeats and specific flanking nucleotides (Krause et al., 2002; Fujishima-Kanaya et al., 2003). For the purpose of fine mapping QTL, additional informative markers are required. Efforts to develop new markers of different type are therefore widespread. In the context of large scale sequencing and genomics programs in various farm animal species, including pig, increasing genome resources are becoming available. Recently, substantial amounts of porcine sequence data have become available, which are a rich resource of SSRs (Porcine Genome Physical Mapping Project and Sino-Danish Pig Genome Sequencing Consortium, Humphray et al., 2005; Wernersson et al., 2005). The identification of new SSRs by *in silico* mining is a relatively cost and time efficient approach compared to SSRs being developed from genomic libraries. There is an extensive conserved homology between the pig and human genome allowing for positioning BAC contigs and sequences onto the porcine genome map through comparative mapping information and blast hits anchored to the human reference sequence. These sequences positioned onto the porcine genome map are a valuable source for the development of new markers in specific genome regions of interest.

In a previous study a QTL on SSC4q was found (Van Wijk et al., 2006). In order to increase the map density of the QTL region more informative markers were required. According to comparative mapping information (Fujishima-Kanaya et al., 2003; Jiang et al., 2003; Moller et al., 2004; Hamasima et al., 2005) the QTL region compares approximately to the regions on HSA1 (84Mb-183Mb) and HSA8 (51Mb-93Mb) (HSA build 35).

The objectives of the current study were (1) to search the BAC-end and Sino-Danish Pig Genome Project (SDPGP) sequences for di-nucleotide SSRs, and (2) to genotype a set of new and informative SSRs located in the region of interest.

Materials and Methods

Sequence data sources and detection of SSRs

BAC-end sequences were downloaded from the Porcine Genome Physical Mapping Project site (http://www.sanger.ac.uk/Projects/S_scrofa/, accessed March 2005). Marra et al. (1997) constructed and fingerprinted a swine genomic BAC library resulting in an integrated physical map of the pig. At the date of access, a total of 468,078 BAC-end sequence reads were available of 267,894 BAC fingerprints assembled into 561 BAC contigs (Humphray et al., 2005). The BAC-end sequences represent approximately 0.1x coverage of the pig genome sequence. The perl script MISA (Thiel et al., 2003) was used to search the BESs to identify SSRs with a minimum of 12 di-nucleotide repeats. It has been shown previously that only di-nucleotide repeat motifs yield a sufficient number of informative markers (Rohrer et al., 2002).

A second source of genome sequences came from the Sino-Danish Pig Genome Sequencing Consortium who has generated about 3.84 million sequence reads, which is an equivalent to 0.66x coverage of the pig genome (Wernersson et al., 2005). The FASTA sequences of these shotgun sequences were downloaded and searched for the presence of a CA/TG repeat at least 12 bp in size. The CA/TG positive sequences obtained, including their respective mates, were compared against the sequence of human chromosome 1 from position 100 Mbp to 166 Mbp (HSA build 35). Towards this end, the sequence of human chromosome 1 for the region indicated, was broken up into fragments of 10 Kbp in size, repeat-masked and aligned against the porcine sequences using the BLASTn program. The resulting porcine sequences were repeat-masked and compared to the complete human genome sequence. Only sequences showing a bi-directional best hit were eventually used for further analysis.

Once SSR containing sequences were identified the sequences were compared to each other as well as to previously mapped SSRs to identify duplicates. Simple sequence repeat flanking polymerase chain reaction (PCR) primers were designed using PRIMER 3.0 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi, Rozen and Skaletsky, 2000), with targeted

amplicon sizes between 100 and 450 bp to facilitate analysis of the SSRs in relatively large multiplex sets on a 3730 ABI-sequencer. For the primers the optimal melting temperature was set to 55 °C, and the optimal primer length was set to 20 bases.

Primer pairs located in the region of interest on SSC4 were tested using a single PCR profile (35 cycles of 30s 72˚C, 30s 55˚C, 30s 96˚C) for obtaining a single clear PCR product checked by electrophoreses in 2% agarose. Finally, primer pairs giving a clear product were tested for polymorphism by sequencing PCR products obtained from eight founder individuals of one of the mapping populations (B&D).

Mapping populations

The first population (denoted as PUP) used consisted of crossbred individuals from a cross between sires of a synthetic Piétrain/Large White halothane free boar line (TOPIGS, The Netherlands) and commercial crossbred sows with unknown pedigree. The population consisted of 17 paternal half-sib families with a total number of piglets of 1,855. A detailed description of the population has been presented by Van Wijk et al. (2005).

The second population (denoted as B&D) consisted of 26 purebred paternal half-sib families of the above mentioned synthetic sire line with in total 555 piglets from 297 sows. Sows were not genotyped. Progeny was from subsequent litters of dams mated with different sires resulting in overlapping generations. The number of piglets per paternal half-sib family ranged from 13 to 35.

Genotyping and map construction

Markers were individually amplified using standard PCR protocols. Compatible amplification products were pooled before electrophoreses on a 3730 ABI® sequencer and raw data were analyzed using GeneMapper software (Applied Biosystems, Foster City, CA). A second examiner evaluated all marker genotypes and genotypes were checked against pedigree information prior to further analysis of the data. Marker order and map distances, following Kosambi's mapping function, were computed using CriMap 2.4 software (Green et al., 1990). The chrompic option was used to identify unlikely double crossovers. Genotypes which could not be scored unambiguously were treated as missing data.

Results and Discussion

Identification of SSRs

In the 225,241 BES with a blast hit against the human genome we identified 4,428 di-nucleotide SSRs consisting of at least 12 repeats. Good primer pairs could be designed for a total of 3,529 SSRs. Numbers per human chromosome are summarized in Table 1. Assuming that the markers are equally distributed throughout the pig genome, with a size similar to that of human (estimated pig genome size is 2,700 Mb), the estimated density of SSRs with a good primer design in the pig genome is about 1.3 SSRs per Mb. Given the fact that the 225,241 BES screened represent approximately 5 % of the porcine genome, this indicates that the di-nucleotide SSR>12 in the porcine genome is, on average, one per ~40 Kbp which is similar to the frequency found in human and mice (IHGSC, 2001; MGSC, 2002). Of the 225,241 BES, a total of 8,784 were predicted within the genome region of interest, in between markers *Sw35* and *SwR153* (HSA1: 84 Mb to 183 Mb and HSA8: 51 Mb to 93 Mb), containing 147 SSRs that satisfied the search criteria.

The second resource used to identify SSRs was the collection of genome shotgun sequences from the Sino-Danish collaboration (Wernersson et al., 2005). This resource was only searched for the more abundant CA/TG repeat with a minimum size of 12 bp. Within the 3 million shotgun sequences, 27,450 CA/TG>12 containing sequences were identified.

This indicates, on average, a CA>12 repeat every ~80 Kbp in the porcine genome which again is similar to the frequencies found in human and mice. In order to identify the porcine sequences among these 27,450 sequences that most likely are located on SSC4q, the sequences and their mates were compared to the homologous human region on HSA1 from position 100 Mbp to 166 Mbp. The resulting selection of 402 sequences was again compared to the complete human genome. Only the 157 sequences that showed a bi-directional best hit were eventually selected for marker development.

Table 1. The number of identified SSRs per human chromosome for BES sequences.

HSA	No. of SSRs[1]	Primer 3 SSRs[2]
1	308	249
2	345	267
3	307	242
4	314	254
5	287	234
6	302	235
7	223	184
8	224	174
9	207	171
10	219	176
11	230	183
12	247	196
13	149	122
14	150	122
15	120	93
16	121	96
17	111	83
18	117	84
19	49	41
20	83	71
21	57	45
22	34	28
X	224	179
Y	-	-
Total	4428	3529

[1] Criteria SSR: di-nucleotide motifs, minimal 12 repeats.
[2] Criteria primer design: targeted amplicon size between 100 and 450 bp.

Primer design

Mutual comparison of the identified SSR containing sequences showed that 137 of the 147 (BES) and 114 of the 157 (shotgun sequences) were unique. Three of the 114 shotgun sequence derived repeats showed similarity with existing microsatellite markers. Good primer pairs could be designed for 108 of the 137 BES and all 111 of the shotgun sequence identified repeats. The primers were tested and 63 of the 108 and 53 of the 111 pairs met the requirements of giving a clear PCR product. These remaining 63 and 53 markers respectively were tested for polymorphism. A total of 72 (with 40 BES derived and 32 shotgun sequence derived) SSRs were found sufficiently informative.

Genotyping and map construction

The PUP population was genotyped for 30 of the BES derived markers. The B&D population was genotyped for 31 of the shotgun sequence derived markers together with a selection of 15 of the BES derived markers. Marker characteristics of the 30 genotyped BES derived SSRs are listed in Table 2. Table 3 presents the characteristics of the 31 genotyped shotgun derived markers. On both populations genotype data was already available for 15 (PUP) and 10 (B&D) public microsatellite markers. The number of markers overlapping between the two populations is 22. The calculated genetic linkage maps are presented in Table 4. The PUP population was typed for a total of 45 markers, displaying on average 1,305 informative meioses. Mapping resulted in a sex-average linkage map with 40 markers spanning 90 cM of the q-arm of SSC4. The genetic map distance corresponds to a region of 103 Mb of the human genome which means an average of 1.14 Mb per cM. The new markers are positioned with regard to the 15 framework markers on SSC4 (USDA-MARC.2; Rohrer et al., 1996). The new markers are nicely distributed over the region and the mapping order of the markers is in excellent agreement with the order of the BES positioned on the human genome. Two closely linked marker pairs were mapped in reversed order compared to their position within the human genome (*S0817 – S0830* and *S0825 - S0829*). The markers in both pairs were closely linked to each other and a more accurate relative positioning requires additional recombination events (mapping information).

Five markers from successive BES were unlinked and together formed a small linkage group spanning 19 cM representing a region that is not synthenic between HSA1 and SSC4. The genomic range we used to select the BES for the identification of markers in the region of interest was chosen somewhat wider in order to be sure to obtain markers from around the breakpoint on SSC4 between HSA1 and HSA8 and to prevent a gap in the genetic map around the breakpoint. It is likely that those 5 markers represent a region located on SSC9. In this study however we were not able to confirm this. In our initial genome scan on the PUP population 14 chromosomal regions were screened using 73 markers covering approximately 50% of the genome (Van Wijk et al., 2006) with SSC9 not included.

Table 2. Characteristics of the 30 BES derived microsatellite markers.

SSR	Name BES[1]	Repeat type	# alleles[2]	Fragment size (bp)[2]	Position HSA (chr: bp)	Forward primer (5'-3')	Reverse primer (5'-3')
S0815	bE45M15T7	CA(13)	3	410-412	8:88845404	TGGTGTTTTAGGCTTCTGTTCA	TGTCCTCACAATTCAGCAATTC
S0826	bE46P24SP6	GT(25)	7	370-398	8:80800279	TCCCTAGATCCAGCATCCAC	ATCAGCAAGGAAGCCAAAAA
S0814	bT93P14SP6	CA(15)	3	373-377	8:75996844	TGTTAGTGCACCTGCTCCAT	TGAACAATTCCATCTTCGATTTT
S0808	RPCI44_0287M.f	GT(24)	7	310-327	8:74160631	TATACACGAGGAAAGGGGGA	TCTTTGTAATCTGGCCCTGC
S0828	bT97I9T7	GT(19)	3	391-406	8:72023897	TCTGCACCATTGCCAAATAA	TGTGTCTCATCACCTCCCAA
S0805	bT5C13SP6	CA(18)	5	250-264	8:70108482	AGCCAAAACTTCCCCTCAAT	TTTGCAATTTCTCTTTGGGG
S0804	RPCI44_0323M4.f	CA(33)	9	226-282	8:59538188	ATGGAAATCCAAGCGATCAG	ACAGCACCTGGATGCTCTCT
S0824	bE230K3T7	TC(13)	3	356-360	1:167372082	AACTCCTATGCAGGAATGCG	AGGCTCGCAAGTATCTTCCA
S0817	bT38G11T7	GT(22)	8	227-253	1:160127667	CCACACTTGCTAGGCACTCA	CCACAACAGGAACTCAGCAA
S0830	RPCI44_0449A14.f	TG(21)	6	435-457	1:160938182	CTCTGGGCTGAGCCTTACAC	TTTGCATAACACACCTTTCCC
S0819	bE209A4T7	TG(17)	8	278-296	1:153697443	GTTCCATCGTGGGAGACACT	GGGCTGCCTCTCTGCTAAAT
S0818	RPCI44_0242G20.r	CA(21)	3	246-272	1:149953277	GCCCACCTGACAATGGAATA	GTTAAGGATCTGGCACTGGC
S0809	bT222B24T7	TG(23)	9	315-344	1:147118063	TCAAACAGATGATGGAGGCA	GGAGTCTTTGGCTGTGAGAGA
S0811	bT150I9T7	AT(17)	4	321-333	1:119890510	TCCAACCTAAAGTGGGAACG	GCCCAAGCTGACAAAATGAT
S0822	bT45L19SP6	CA(22)	6	330-358	1:118250179	TCTTTTCTCTGGTTGGTGGG	GACAGAACATGATGGGGGAT
S0802	bE131P13SP6	TG(10)AG(11)	3	181-190	1:117008015	CGATGGAACATGATGAAGGA	TGGGTCTCTGTGCCTCTTCT
S0813	bE232A18SP6	AC(17)	5	366-384	1:116759844	AGCTGCAAAGGGCTGTTAAA	TACAAACCTCCACTTTCCCG
S0812	bT248B11SP6	CA(23)	7	353-457	1:115556127	ATCCAGGGACCCAGAACAT	AGGTCACACGGCTGCTAAGT
S0821	CH242_015M15.r	CA(23)	7	299-326	1:106822191	TGCAGAAGAATTGAAGCCAC	CAGTATTGGGGATGGAATGG
S0803	bE195H12SP6	AC(17)	7	203-230	1:102998094	GTTCCCCCTTCAACACACAG	AGCAGATTCCCGCAGAGTAA
S0806	bE163E15T7	AC(20)	8	255-280	1:100804729	GCTAACATTGGTGCAACCCT	CACGAACACACCGACCTATG
S0810	RPCI44_0251I12.r	AC(12)	5	316-346	1:99180857	GAAGCAGGACAACAGGAAGG	CAGTGCTTGCTGAAAAGCAG
S0807	bT88C15T7	AC(20)	8	274-288	1:95499604	AGAGGCTGCCTGTGACTCAT	AACAATAGGCTGCATTTGGG
S0825	bT171P18SP6	GT(23)	7	356-376	1:93094985	TTGCACTATGGGGTGTGTGT	CTTTGGTCAGGTCACAGGGT
S0829	bE197O11T7	CA(17)	6[3]	419-435	1:93537469	GGTGGGACCTTAAAAAGCAA	CATGCAATAGGGCATGTGAC
S0820	bT235M4T7	GT(16)	4	296-307	1:180204021	CGTACAGCAAAGTGACCCAA	GATCCTTCCTGGAGCAACAC
S0827	bE212D15SP6	GT(17)	7	381-412	1:180076952	CTTACTTGGGAGCTTGGCTG	AGGGAGGCACTGGAGAAAAT
S0801	bE63M11SP6	AC(27)	7	180-202	1:174274982	GTCCTGTTTTCTACGGGGGT	GGCTGTGTCCCAGAAGAGTC
S0831	bT238B21T7	AT(18)	4	446-452	1:172728081	TGCTTGCCACTGAGTTGTCT	TGCCTGTGTGTGTGAGATTC
S0816	bT56H20T7	AC(15)	3	202-232	1:171764429	CTGGCATTGGTGGTGTAGTG	TGTATGACCCAACTCCTCCC

[1] BES information can be found on http://www.sanger.ac.uk/Project/S_scofa.

[2] Number of alleles and fragment size ranges as observed in the PUP population.

[3] Marker with 0-allele.

Table 3. Characteristics of the 31 shotgun sequence derived microsatellite markers.

SSR	Sequence ID	Repeat type	# alleles[1]	Fragment size (bp)[1]	Position HSA (chr: Mbp)	Forward primer (5'-3')	Reverse primer (5'-3')
M41	bd_84811	CA(12)	3	280-310	1:146.78	TGGGACTCATTTTCCTCACC	TGGAATTTGGACAAGATGCTC
M42	bda_15511	GT(10)	5	280-305	1:103.35	TAAAACCTCATGGGACAGGC	CCATCTCATCCATAGCCCAG
M43	bdd_64131	CA(20)	3	370-405	1:155.92	TCATTATGGAGCTGCCACTG	CTGATCCCGAGACAAAGGAG
M44	byf_45401	GT(17)	3	355-375	1:142.75	GCCAACCCACATGGAGTAAG	TTTCAGCCCAAACCATAAGC
M45	cpg0_057524	GT(16)	5	190-235	1:162.24	AATCCACTGCACCTCCTTTG	TTTGTGATGTCAGCAGCCTC
M46	cpg0_081158	GT(22)	5	310-340	1:157.57	GGAGGAAGGTTGTAGAGCCC	CCAGTCTCCTGAGGCTGAAG
M47	dpcxa0_013458	GT(18)	4	295-325	1:110.43	GAGGGAAAAGGATTCCCAAC	CTGCCTGTCTATTGCCCTTC
M48	dpcxb0_017485	CA(15)	4	375-395	1:117.14	CTTCTGAAGGCAGGTCCATC	CTGAAGCAAGGCAGTAAGCC
M49	dpcxb0_026687	CA(16)	3	170-195	1:111.86	TGTTTCCACTTTCTGGCCTC	TCCATCTTTCCCATTTCCAG
M50	dpcxb0_120612	GT(18)	3	180-210	1:111.67	TGTTCCAGCATAGGGTTTCC	TAAGAGGTTTGGGCCAGATG
M51	dpcxb0_208952	GT(15)	2	330-345	1:112.28	CAGAGAAACCAAGCAGGGTC	TCAACAGCTGTGAAACCAGC
M52	dpcxb0_315534	CA(19)	3	270-295	1:148.50	GATTAGGAGGCAAGGGAAGG	TGAAGCCACATTCTGACAGG
M53	dpcxb0_109087	GT(18)	5	380-425	1:162.14	TTGCAAGAGTCCTACCCCAC	TTCCCCCAGTTGCTATTCAG
M54	byd_28580	CA(12)	4	280-320	1:100.90	AAATCAAACCTCACCTCCCC	TTTGCTTAAGCTCCAGCTCC
M55	dpcxa0_056278	GT(14)	3	310-340	1:148.60	GAGACTAGGCCAAGCCACAG	AGGTCCAAACGCTGGTACAC
M56	cpg0_030745	GT(13)	3	210-230	1:107.98	GAAGGAGAAGGCTCAGGGTC	AAGAAGCCAGCGTCAGTCTC
M57	dpbxa0_017470	CA(18)	5	245-285	1:161.93	TGCGTCTGTCTGTCCTGTTC	GTAATGCCACGGTAAATCCG
M58	cpg0_100432	GT(15)	7	290-320	1:118.15	AGTAGCCAGGGCAAACACAC	CAGCGTTGCAGCTTATTGAG
M59	byb_40562	CA(14)	3	375-395	1:146.79	TCAGTCATTCTCCCTCCCTC	CCCTTACTGTGCCTTGCTTC
M60	byc_88771	GT(20)	4	245-280	1:111.60	TACCTTGGATCTCTGCCCAC	TGGTACATGCCTGGGTAAGG
M61	byc_98867	GT(24)	6	295-335	1:110.58	AGCATGCTTCTGCTAATCCC	TGTCCACCGACAGATGAATG
M62	bd_41353	CA(13)	3	340-365	1:156.71	GGACACAGAAAGCTTTGACG	ACTTCTCACCACAACCCCTG
M63	byb_28486	GT(17)	3	385-405	1:155.36	GCTACCAAAAGGGATAGTGGG	GGATTGTCCGGTTGATCTTC
M64	bdd_73749	CA(23)	6	260-310	1:152.24	TGCTTCAACCATTATGCTTCC	TTTCAGCTCATGGCAATCTG
M65	dpbxa0_046575	GT(18)	3	325-350	1:162.00	TGACCAGGTGTAGAGGAGGC	CTGGTTTCAGCCTGGAGTTC
M66	bye_53969	CA(16)	2	350-375	1:156.53	GGCATGGCAACCTCTCTATC	ACATCTGTCCCAGTGAAGGC
M67	dpcxb0_236111	CA(15)	3	385-400	1:102.91	TCACCCTCTCCCTCAATCAC	CTCTGTGCCACCACAAGAAC
M68	dpbxa0_032103	CA(13)	2	260-285	1:115.67	ATCCCTTTGGAAAGAATGGG	TCCACCTCAGAATGGGAGAG
M69	bdb_57074	GT(12)	3	300-315	1:114.87	TGCTCATGCAAGAAAAATGC	CTCCGAATGGAAATTCTTGG
M70	bye_34985	GT(19)	3	345-370	1:103.73	ATCCCCCAAAATTTCAATGG	TTGAAAGTACACACACACACACG
M71	bl_47102	GT(14)	5	385-410	1:117.20	GGCTGAGACTATCACCCAGC	ACTTTCCCACAGGAACAACG

[1] Number of alleles and fragment size ranges as observed in the B&D population.

The B&D population was typed for a total of 56 markers with on average 191 informative meioses. Mapping resulted in a male-specific linkage map with 52 markers spanning 89 cM (*i.e.* average marker interval of 1.7 cM). Four markers (M61, M62, M64 and M71) were discarded from the data set prior to the mapping because they could not be scored unambiguously. Respectively, 23 and 8 markers were in common with the PUP map and the USDA-MARC map. Marker order was in good agreement with those maps, except for marker pair *S0830 – S0817*, which are in reversed order compared to the PUP map. This marker pair was already mentioned above and the order on this map (B&D) is in agreement with the positioning of the sequences on the human genome. Generally marker order was also in good agreement with the order of the sequences positioned on the human genome. Deviations from the order on the human genome were found for markers M44 and M52. The markers in the region around M44 and M52 are closely linked and their relative positions could not be considered as very reliable based on this single mapping, also due to the limited number of informative meioses. The map inflation compared to the PUP map may also be explained by unidentified genotyping errors, which could not be observed because the dams were not genotyped.

Conclusion

In conclusion, we mapped 61 new microsatellites on SSC4. The microsatellites were developed by *in silico* mining of porcine BES and genomic shotgun sequences positioned on the human genome. We utilized physically anchored porcine BESs, genomic shotgun sequences and comparative human-porcine map information. Using these resources, we showed that the targeted development of new markers from sequences positioned on the genome is a very efficient approach to increase map resolution of a specific genome region of interest. The direct linkage to known human homologues genome regions will further facilitate the identification of candidate genes for QTL.

Table 4. Marker positions in the PUP, B&D, and USDA-MARC genetic linkage maps, the human chromosome number (HSA), and position on the human chromosome in mega-bases (Mb).

Marker		cM_{PUP}	$cM_{B\&D}$	cM_{USDA}[1]	HSA	HSA Mb
S0815	M31	0.0			8	88.8
S0826	M04	2.8			8	80.8
S0814	M20	3.1			8	75.9
Sw35		3.8		4		
S0808	M10	5.0	0.0		8	74.1
S0828	M39	6.9	0.0		8	72.0
S0805	M38	8.0			8	70.1
S0804	M11	12.2	6.6		8	59.5
S0217		14.3	14.6	18		
	S0073		14.6			
	Sj412		16.7			
S0824	M14	18.1	16.7		1	167.3
	M53		16.7		1	162.1
	M65		16.7		1	162.0
	M45		17.8		1	162.2
	M57		18.8		1	161.9
S0817	M18	21.4	21.2		1	160.1
S0830	M40	22.1	19.2		1	160.9
	M46		22.9		1	157.6
	M43		25.2		1	155.9
	M66		26.8		1	156.5
	M63		29.1		1	155.4
S0819	M03	26.2	29.1		1	153.7
S0214		26.6		27		
Sw270		27.0	31.3	27		
S0818	M29	29.4	34.2		1	150.0
Sw512		31.3	35.4	29		
	M44		35.4		1	142.8
	M55		35.4		1	148.6
S0809	M36	32.4	36.2		1	147.1
	M59		36.2		1	146.8
	M52		38.6		1	148.5
	M41		39.8		1	146.8
S0811	M15	33.5			1	119.9
Sj551		34.3	43.2			
S0822	M07	36.2	45.6		1	118.3
	M58		47.8		1	118.2
	M48		49.9		1	117.1
S0802	M01	37.6			1	117.0
S0813	M24	37.9	49.9		1	116.8
	M68		49.9		1	115.7
S0812	M06	40.8	49.9		1	115.6

	M69		49.9		1	114.9
	M51		54.5		1	112.3
	M49		58.2		1	111.9
	M60		58.2		1	111.6
Sw524		46.7		47		
	M50		64.1		1	111.7
Sj673		48.0	69.1			
	M47		69.1		1	110.4
S0067		51.2	69.1	51		
	M56		74.6		1	108.0
Sw445		56.4	76.3	54		
S0821	M21	58.6	78.0		1	106.8
Sw58		59.9	80.7	56		
	M70		80.7		1	103.7
	M42		80.7		1	103.4
S0803	M22	61.6			1	103.0
	M67		80.7		1	102.9
S0806	M13	65.1	86.1		1	100.8
	M54		88.8		1	100.9
S0810	M30	69.4	88.8		1	99.2
S0807	M28	77.3			1	95.5
S0097		78.6				
Sw2066		80.8		69		
Sj672		82.5				
S0825	M27	84.0			1	93.1
S0829	M02	85.4			1	93.5
SwR153		90.0		74		
Not linked to SSC4:						
S0820	M37	0.0			1	180.2
S0827	M23	0.2			1	180.1
S0801	M32	9.8			1	174.3
S0831	M17	13.4			1	172.7
S0816	M08	18.9			1	171.8

[1] Marker Sw35 adjusted to 4 cM (original position in USDA-MARC is 56 cM).

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Literature cited:

Fujishima-Kanaya N., D. Toki, K. Suzuki, T. Sawazaki, H. Hiraiwa, M. Lida, T. Hayashi, H. Uenishi, Y. Wada, Y. Ito, and T. Awata. 2003. Development of 50 gene-associated microsatellite markers using BAC clones and the construction of a linkage map of swine chromosome 4. Animal Gen. 34: 135-141.

Green P.K., K. Galls, and S. Crook. 1990. Documentation for CriMap, version 2.4., Washington University School of medicine, St. Louis, MO, USA.

Hamasima N., H. Suzuki, A. Mikawa, K. Suzuki, and T. Awata. 2005. Construction of a comprehensive *Sus scrofa* radiation hybrid (SSRH) map: Porcine Chromosome 4 (SSC4). Poster (P560) abstract Plant & Animal Genomes XIII Conference, San Diego, CA.

Humphray S.J., R.C. Clark, J. Beever, C.E. Scott, B. Plumb, P. Charcon, D. Milan, A. Archibald, L. Schook, and J. Rogers. 2005. An integrated physical map of the porcine genome. Poster (P559) abstract Plant & Animal Genomes XIII Conference, San Diego, CA.

International Human Genome Sequencing Consortium. 2001. Initial sequencing and analysis of the human genome. Nature 409: 860-921.

Jiang Z., and J.J. Michal. 2003. Linking porcine microsatellite markers to known genome regions by identifying their human orthologs. Genome 46: 798-808.

Krause E., L. Morrison, K.M. Reed, and L.J. Alexander. 2002. Radiation hybrid mapping of 273 previously unreported porcine microsatellites. Animal Gen. 33: 468-485.

Li Y.C., A.B. Korol, T. Fahima, A. Beiles, and E. Nevo. 2002. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Mol. Ecol. 11: 2453-2465.

Marra M.A., T.A. Kucaba, N.L. Dietrich, E.D. Green, B. Brownstein, R.K. Wilson, K.M. McDonald, L.W. Hillier, J.D. McPherson, and R.H. Waterston RH. 1997. High throughput fingerprint analysis of large-insert clones. Genome Res. 7: 1072-1084.

Moller M., F. Berg, J. Riquet, D. Pomp, A. Archibald, S. Anderson, K. Feve, Y. Zhang, M. Rothschild, D. Milan, L. Andersson, and C.K. Tuggle. 2004. High-resolution comparative mapping of pig chromosome 4, emphasizing the FAT1 region. Mamm. Genome 15: 717-731.

Mouse Genome Sequencing Consortium. 2002. Initial sequencing and comparative analysis of the mouse genome. Nature 420: 520-562.

Rohrer G.A., L.J. Alexander, J.W. Keele, T.P. Smith, and C.W. Beattie. 1994. A microsatellite linkage map of the porcine genome. Genetics 136: 231-245.

Rohrer G.A., L.J. Alexander, Z. Hu, T.P. Smith, J.W. Keele, and C.W. Beattie. 1996. A comprehensive map of the porcine genome. Genome Res. 6: 371-391.

Rohrer G.A., S.C. Fahrenkrug, D. Nonneman, N. Tao, and W.C. Warren. 2002. Mapping micorsatellite markers identified in porcine EST sequences. Animal Gen. 33: 372-376.

Rozen S., and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. 132: 365-386.

Thiel T., W. Michalek, R.K. Varshney, and A. Graner. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor. Appl. Gen. 106: 411-422.

Van Wijk H.J., D.J.G. Arts, J.O. Matthews, M. Webster, B.J. Ducro, and E.F. Knol. 2005. Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. J Anim. Sci. 83: 324-333.

Van Wijk H.J., B. Dibbits, E.E. Baron, A.D. Brings, B. Harlizius, M.A.M. Groenen, E.F. Knol, and H. Bovenhuis. 2006. Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial cross. J. Anim. Sci. 84: 89-799.

Wernersson R., M.H. Schierup, F.G. Jorgensen, J. Corodkin, F. Panitz, H.H. Staerfeldt, O.F. Christensen, T. Mailund, H. Hornshoj, A. Klein, J. Wang, B. Liu, S. Hu, W. Dong, W. Li, G.K. Wong, J. Yu, J. Wang, C. Bendixen, M. Fredholm, S. Brunak, H. Yang, and L. Bolund. 2005. Pigs in sequence space: a 0.66X coverage pig genome survey based on shotgun sequencing. BMC Genomics 6: 70.

Chapter 7

Fine mapping of a meat color QTL on *Sus scrofa* chromosome 4q and verification of segregation in a commercial purebred line

H.J. van Wijk[*], B. Dibbits[#], J.A.J. Arts[#], B. Harlizius[*], E.F. Knol[*], H. Bovenhuis[#], and M.A.M. Groenen[#]

[*]IPG, Institute for Pig Genetics, P.O. Box 43, 6640 AA, Beuningen, The Netherlands;

[#]Animal Breeding and Genetics Group, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands.

Abstract: Meat color is one of the major meat quality characteristics in commercial pork production. Indirect selection is based on carcass dissections of relatives. Genetic markers for meat color would enable direct selection. On SSC4q, a QTL affecting meat color has been identified to segregate in a commercial crossbred population. The aim of this study was to refine the location of this QTL by constructing a dense linkage map, followed by combined linkage and linkage disequilibrium analyses. Subsequently, the segregation of the QTL was analyzed in the purebred sire line. Forty-one microsatellite markers, including 26 newly developed markers, were genotyped on the crossbred population. The data increased the evidence for the presence of the QTL and contributed to a refined positioning of the QTL to a 21 cM region between markers S0813 and Sw445. Additional analyses based on 22 markers suggest the segregation of the QTL in the purebred sire line, enabling application of the QTL in marker assisted selection or sorting of sires to be sold for commercial production.

Key words: Fine mapping, Linkage disequilibrium, Meat color, Pig, QTL

Introduction

Mapping of quantitative trait loci is the first step towards implementation, identification and characterization of gene(s) underlying quantitative traits of economic interest in livestock. On SSC4q, a QTL affecting meat color has been identified in a commercial crossbred population (Van Wijk et al., 2006a). Further fine mapping of the QTL is necessary to refine the location and thereby facilitating marker assisted selection or positional candidate gene research. Application of marker assisted selection in pig breeding requires that the QTL segregates in one of the purebred selection lines and preferentially information about the effects of the QTL in the end product.

Analysis methods combining linkage and linkage disequilibrium information have been shown to be effective in refining the position of QTL (Meuwissen and Goddard, 2000; Farnir et al., 2002; Meuwissen et al., 2002; Olsen et al., 2004). The accuracy of fine mapping depends on the extent of LD, the number of haplotypes sampled and the marker density. Recent publications report a considerable extent of LD in livestock species, including pig (Farnir et al., 2000; McRae et al.; 2002; Nsengimana et al., 2004; Heifetz et al., 2005), which offers promising perspectives

for combined linkage and linkage disequilibrium mapping with medium dense marker maps. Higher levels of LD, however, may limit the possibilities of high resolution mapping, since LD 'blocks' remain large.

The objectives of the current study were to exploit combined linkage and linkage disequilibrium mapping to refine the location of the previously identified meat color QTL on SSC4q (Van Wijk et al., 2006b) and verification of segregation of this QTL in a related purebred sire line.

Materials and methods

Genetic Material and Phenotypic Measurements

The first population (denoted as PUP) is a previously described paternal half-sib design of a finisher cross between sires of a synthetic Piétrain/Large White halothane free boar line (TOPIGS, The Netherlands) and crossbred sows with unknown pedigree (Van Wijk et al., 2005). The population consisted of 17 paternal half-sib families with a total number of 1,855 animals.

The second population (denoted as B&D) consisted of 37 purebred paternal half-sib families of the above mentioned synthetic Piétrain/Large White sire line with in total 703 offspring originating from 359 sows. Sows were not sampled. Progeny was from consecutive litters of dams mated with different sires resulting in overlapping generations and complex family relations among the individuals. The number of piglets per paternal half-sib family ranged from 10 to 35.

The sires of the PUP and B&D populations (finisher cross and purebred cross) are related. The sires came from the same nucleus population with the PUP sires a few generations before the B&D animals. Therefore, the different families of both populations are expected to represent haplotypes of a common founder which may contribute to fine mapping.

Phenotypes on the PUP population were available from a previous study. Phenotypic measurements on the B&D population were taken as reported in that previous study (Van Wijk et al., 2004). This study aims to fine map a previously identified QTL for Japanese colour score at the loin (Van Wijk et al., 2006a) and analyses are therefore limited to this trait. Japanese colour measurements were taken at the transverse cut surface of the loin (**JCScut**) following the Japanese colour scale (1 to 6, with 1 = pale and 6 = very dark). Estimates of heritability and

common environmental effects with their standard errors were available from the previous study for the PUP population (Van Wijk et al., 2004). Heritability for JCScut on the B&D population was calculated in the same way as described in that previous study.

Genotyping and Linkage Map Construction

Markers were individually amplified by standard PCR protocols. Compatible amplification products were pooled before electrophoresis on a 3730 ABI® sequencer and raw data were analysed with the GeneMapper software (Applied Biosystems, Foster City, CA). A second examiner evaluated all marker genotypes prior to further analysis of the data. Genotypes were checked against pedigree information and treated as missing in case they could not be scored unambiguously. Marker order and map distances, following Kosambi's mapping function, were computed using CriMap 2.4 software (Green et al., 1990). The chrompic option was used to identify unlikely double crossovers.

Quantitative Trait Locus Analysis

Classic linkage analysis (LA) was performed using regression interval analysis nested within half-sib families (Knott et al., 1996; De Koning et al., 1999) for both a one-QTL and a two-QTL model. JCScut measurements on the PUP and B&D population were, in the same way, pre-corrected for systematic effects (sex, combined fixed effect of group, farm and sampling stage (gfp), age and carcass weight) as described in Van Wijk et al. (2006a). Significance thresholds were obtained by performing 10,000 permutations (Churchill and Doerge, 1994). Segregating sire families were identified based on *F*-statistics obtained from individual family analysis. The confidence interval was estimated using bootstrapping as implemented in the web-based version of the QTL Express software (Seaton et al., 2002).

Variance component based linkage analysis (VC LA) and combined linkage and linkage disequilibrium (LDLA) analysis were performed using the method proposed by Meuwissen and Goddard (2000). The method involves the following three steps. 1) Reconstruction of paternally and maternally inherited haplotypes for each of the individuals. SimWalk2 (Sobel and Lange, 1996) was used for reconstructing haplotypes. 2) Calculation of IBD probabilities of pairs of haplotypes for each putative QTL position. Putative QTL positions were set at the midpoint of each marker bracket. This second step results in a series of matrices with IBD probabilities

between all haplotype pairs for each putative QTL position. The matrices with IBD probabilities describe the correlations between the random haplotype effects (Meuwissen and Goddard, 2000). Pedigree information was not used in the calculation of IBD probabilities between the base haplotypes (the parental haplotypes). Step 3 calculates the maximum likelihood estimates of the variance components at each putative QTL position using the appropriate IBD matrix. Calculations were performed using the ASREML package (Gilmour et al., 2000). Phenotypes of the PUP population were analyzed using the following model:

$$\mathbf{Y = Xb + Zu + W_d v_d + W_s v_s + Sc + e} \quad [1]$$

Where **Y** is the vector of phenotypes, **b** is a vector of systematic effects, **u** is a vector of random additive polygenic effects of background loci, $\mathbf{v_x}$ (x = d or s) are vectors of random additive effects due to the maternal ($\mathbf{v_d}$) or paternal ($\mathbf{v_s}$) haplotype, **c** is the vector of random litter effects, and **e** are the random residuals. Separate modeling of maternal and paternal haplotype effects allowed for differences in effect (*i.e.* fitting parent-of-origin and/or breed specific effects). The random effects were assumed normally distributed with mean zero and variances $\mathbf{\sigma^2_u}$, $\mathbf{\sigma^2_{vx}}$, $\mathbf{\sigma^2_c}$ and $\mathbf{\sigma^2_e}$. Matrices **X**, **Z**, $\mathbf{W_x}$ and **S** are known incidence matrices for the effects of **b**, **u**, $\mathbf{v_x}$ and **c** respectively.

For the B&D population the litter effect was omitted from model [1], because it could not be estimated due to the low number of offspring per dam. Furthermore, in the analysis of the B&D population, maternal haplotypes in the offspring were considered as base haplotypes, since genotypes of the sow were not available, and $\mathbf{\sigma^2_{vx}}$ (x = d or s) were assumed equal (Mendelian model).

When base (parental) haplotypes are assumed unrelated only linkage information is exploited, resulting in a variance component based LA (VC LA) model. This in combination with modelling maternal haplotype effects or paternal haplotype effects only allows for a maternal or paternal only VC LA model. The latter resembles the paternal half-sib regression analysis. Ignoring LD in the analysis of the B&D population effectively results in a paternal only VC LA model, because the maternal haplotypes of the offspring were considered as base haplotypes.

A likelihood ratio test (LRT) statistic was obtained as two times the difference of the lnL between the QTL model and a model without a QTL (*i.e.* $LRT=-2(\ln(L_0)-\ln(L_{qtl}))$). The marker bracket with the highest LRT was considered as the most likely QTL position. The nominal

significance level of the LRT was determined from a chi-square distribution with 2 d.f. which is equal to the difference in number of variance components that were fitted in the model with QTL compared to the model without QTL (maternal and paternal haplotype effects were modelled separately). A nominal threshold of 5% was used.

Results

Heritabilities

Heritability estimates, common environmental effect and genetic and phenotypic variances for JCScut estimated based on PUP or B&D are presented in Table 1. The estimated heritability for the two populations differs substantially, as well as the residual variance (σ^2_e). The estimates of the genetic standard deviations (PUP: 0.059^0.5 = 0.24 and B&D: 0.039^0.5 = 0.20) are of the same magnitude.

Linkage Map Construction

On the PUP population, 41 microsatellites were genotyped to build a dense genetic linkage map. These included 26 newly developed microsatellite markers on SSC4q, which were obtained *in silico* from BAC-end sequences aligned to the orthologous human genome region on HSA1 and HSA8 (Van Wijk et al., 2006c). The resulting linkage map spanned 90 cM, with an average marker spacing of 2.2 cM (Table 2, and Van Wijk et al., 2006c), and an average information content of 89%. The sex-average linkage map was used in the QTL analysis.

Table 1. Summary statistics and estimated parameters for Japanese color score measurements at the loin cut surface (JCScut) on both populations; number of animals per trait (n), mean, SD, minimum (Min) and maximum (Max) values, heritabilities (h^2), common environment effects (c^2) with their SE and the genetic (σ^2_a), phenotypic (σ^2_p) and residual variance (σ^2_e).

Population	n	Mean	SD[b]	Min	Max	h^2	SE	c^2	SE	σ^2_a	σ^2_p	σ^2_e
PUP[a]	1797	2.8	0.5	1	5.5	0.22	0.09	0.09	0.02	0.059	0.27	0.12
B&D	691	2.9	0.8	1	6.0	0.08	0.05	-	-	0.039	0.49	0.45

[a] Source, Van Wijk et al., 2005.
[b] Unadjusted data.

The B&D population was genotyped for a shorter genome region harbouring the QTL with a subset of 20 of the 41 markers and an additional two markers: S0073 and Sj412. This marker subset was chosen based on the informativity of the markers in this population. The male genetic linkage map composed of the 22 markers covered 50 cM (Kosambi) with an average marker interval of 2.3 cM. The average information content of the corresponding map was 77%. The obtained linkage map is presented in Table 2. Marker order was similar as in the map based on the PUP population. Genotypes were examined to identify unlikely double crossovers (<0.1% of the genotypes), which were set to missing genotypes.

Table 2. Genetic linkage maps of SSC4q based on PUP and B&D, with marker positions in centi-morgan (cM). New developed markers in italics.

Marker[a]	**PUP**	**B&D**[b]
S0815	0.0	
S0826	2.8	
S0814	3.1	
Sw35	3.8	
S0808	5.0	5.0
S0828	6.9	5.1
S0805	8.0	
S0804	12.2	11.3
S0217	14.3	19.6
S0073		19.6
Sj412		21.4
S0824	18.1	21.4
S0817	21.4	21.4
S0830	22.1	21.4
S0819	26.2	22.1
S0214	26.6	
Sw270	27.0	22.2
S0818	29.4	26.2
Sw512	31.3	
S0809	32.4	26.2
S0811	33.5	
Sj551	34.3	
S0822	36.2	
S0823	36.3	
S0802	37.6	
S0813	37.9	26.5
S0812	40.8	32.0

Sw524	46.7	
Sj673	48.0	40.3
S0067	51.2	40.3
Sw445	56.4	44.7
S0821	58.6	46.4
Sw58	59.9	49.4
S0803	61.6	
S0806	65.1	54.6
S0810	69.4	54.6
S0807	77.3	
S0097	78.6	
Sw2066	80.8	
Sj672	82.5	
S0825	*84.0*	
S0829	*85.4*	
SwR153	90.0	

[a] Characteristics of new markers in Van Wijk et al., 2006c.

[b] First marker (S0808) adjusted to 5 cM to facilitate comparison with positions in PUP map.

QTL Mapping Results

Regression LA: Regression linkage analyses results showed a QTL for JCScut (nominal $P = 7x10^{-5}$, chromosome-wise $P_{chr} = 0.001$). The analysis on this larger data set resulted in increased evidence for the presence of the QTL compared to the previous analyses (nominal $P = 0.001$, Van Wijk et al., 2006b) on part of the data. However, the highest *F*-statistic was found at a different location. In the present analysis, the highest *F*-statistic was found at 19 cM, *i.e.* between the markers S0217 and S0214 (Figure 1), with a 95% bootstrap confidence interval of 88 cM. The *F*-statistic profile remained at a high level over a wide interval along the chromosome possibly due to the presence of a second QTL. In the interval Sw524 and Sw445 the highest *F*-statistic was obtained in previous analysis based on 15 markers (Van Wijk et al., 2006b). A two QTL model did not provide significant evidence for the presence of a second QTL.

Analyses of individual families, performed at the position with the highest *F*-statistic in the overall analysis (*i.e.* at 19 cM), indicated that three out of 17 sires were heterozygous for the QTL affecting JCScut. A similar analyses at the location of the second peak at 50 cM suggested that the same boars were heterozygous, which supports the segregation of a single QTL.

The regression analysis of the B&D data set did not result in significant evidence for the presence of a QTL for JCScut ($P_{chr} = 0.12$) (Figure 2).

Table 3. Variance component QTL mapping results for JCScut, with LRT values, the percentage variance explained by the QTL (σ^2_v) with the SE, polygenic- (h^2), common environment- (c^2) and the total (σ^2_{tot}) and residual (σ^2_e) variance, at the positions where the LRT was at its maximum in between the given marker intervals.

	Analyses	Marker interval	LRT	σ^2_v [d]	SE	h^2	c^2	σ^2_{tot}	σ^2_e
PUP[a]	LA	S0217 – S0819	8.71	0.032	0.019	0.219	0.081	0.263	0.219
		Sj673 – Sw445	*4.39*[c]	*0.022*	*0.016*	*0.229*	*0.079*	*0.262*	*0.220*
	LDLA	*Sj673 – Sw445*	*4.96*[c]	*0.035*	*0.026*	*0.221*	*0.077*	*0.264*	*0.220*
B&D[b]	LA	*Sw445 – S0821*	*3.38*[c]	*0.045*	*0.031*	*0.108*	-	*0.480*	*0.216*
	LDLA	*S0809 – Sj673*	*3.61*[c]	*0.039*	*0.040*	*0.062*	-	*0.508*	*0.407*

[a] 2 degrees of freedom; LRT > 5.99 for $P < 0.05$ and LRT > 9.21 for $P < 0.01$.
[b] 1 degree of freedom; LRT > 3.84 for $P < 0.05$ and LRT > 6.64 for $P < 0.01$.
[c] *Not significant.*
[d] Combined paternal and maternal component.

Variance component LA and LDLA: Table 3 shows the results for the VC LA and LDLA analyses on both populations. Significant LRT values or values close to the threshold are presented. Likelihood ratio test profiles of the different analyses are presented in Figures 1 and 2 for the PUP and B&D population, respectively. Also presented in Table 3 are the total and residual variances as well as the proportion of variance explained by the polygenic-, common environment-, and QTL-effects at the cM positions where the LRT was at its maximum. The estimated proportions of variance due to polygenic- and common environment effects are in good agreement with the values presented in Table 1. Estimated QTL variances ranged from 2.2 to 4.5%, with larger SE for the estimates on the B&D population.

The VC LA analysis resulted in evidence for a QTL affecting JCScut in the PUP population, with a profile that is in good agreement with the *F*-statistic profile of the paternal half-sib regression (PHS) analysis (Figure 1).

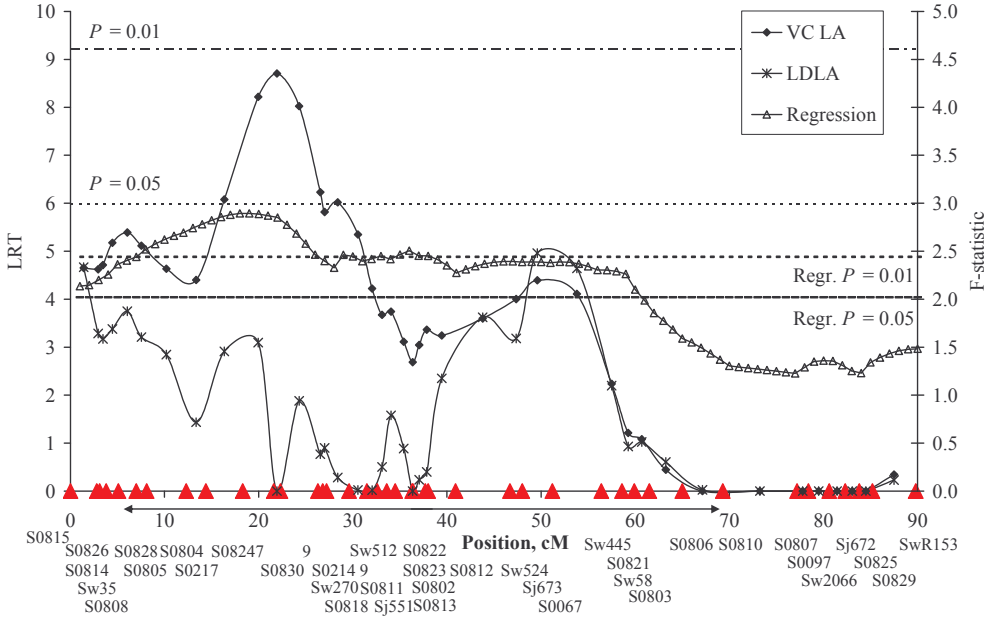

Figure 1. Regression linkage analysis, VC LA and LDLA profiles for JCScut on the PUP population. The regression linkage analysis thresholds are preceded by Regr. and are positioned on the right vertical axis, as well as the regression profile. The arrow indicates the region covered represented in Figure 2.

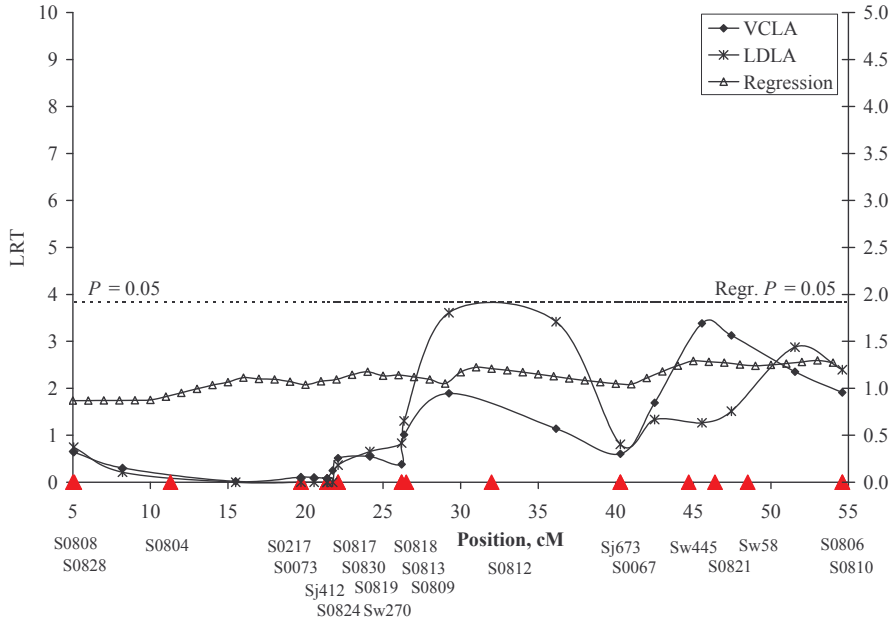

Figure 2. VC LA and LDLA LRT profiles for JCScut on the B&D population.

The highest LRT value was found near marker S0830 at 22 cM. The VC LA analysis showed a profile with two peaks which added to the suggestion of the existence of two QTL on the chromosome. The position of the smaller peak around marker S0067 at 51 cM, corresponds to the position with the highest *F*-statistic of the regression analyses on part of the marker data (15 markers) (Van Wijk et al., 2006b). In that study no effect was found for JCScut in the region around 22 cM.

No significant effects were found in the B&D population, although the highest LRT value of 3.38 approached the threshold of 3.84 (Table 3, and Figure 2). This value was reached near marker Sw445, which is close to the location of the smaller peak obtained on the PUP population.

Utilizing of LD information resulted in different LRT profiles (Figures 1 and 2) as compared to the profiles obtained with the VC LA analysis. Generally, the LRT values were reduced except for the region between markers S0812 and S0067, where the LRT values remains practically unaltered (PUP) or increased (B&D). The high LRT values in the region around 22 cM obtained with the VC LA analyses on the PUP population disappeared completely after inclusion of LD.

The LDLA analysis on both populations did not result in a clearer picture or refined location of the QTL as compared to linkage analyses. Most likely the QTL is located in the ~20 cM interval between markers S0813 and S0067.

Discussion

The regression analysis on the PUP population provided stronger evidence for the presence of a QTL affecting JCScut compared to previous analyses using data of 15 markers (Van Wijk et al., 2006b). However, the highest *F*-statistic was found at a different position. The regression analysis on the B&D data set only suggested the segregation of the QTL for JCScut ($P_{chr} = 0.12$). However, high *P* values may not be expected on this population because of the limited power (~30%) of this half-sib design due to the small family size. The B&D population was aimed at sampling a higher number of haplotypes and expected to be more appropriate for the LDLA analysis where the families are considered to be related.

Separate modeling of paternal and maternal components revealed that the effects were completely from paternal origin. That was confirmed in the present study and explains the similarity between the profiles obtained from the regression or VC LA analysis. In previous analyses LD did not seem to contribute additional evidence, likely as a consequence of the sparse marker density, which was ~6 cM. In this study the marker density was increased up to an average marker interval of 2.2 and 2.3 cM for the PUP and B&D population, respectively. Nsengimana et al. (2004) calculated the extent of LD in two genomic regions of the pig, including chromosome 4, and predicted that powerful genome-wide association studies must be feasible at marker densities of 5-10 cM. The contribution of LD in the present study, however, was not apparent. This observation may indicate that extent of LD in the PUP population is lower as found by Nsengimana et al. (2004) and marker density was not sufficient for a significant contribution of LD. Also, the markers are not evenly distributed and the QTL coincides with a sparser marker region. Furthermore, the markers were not fully informative, making the effective marker interval larger then 2.2 cM.

Another complicating factor is the PUP population (crossbred) used. The method to calculate IBD between haplotypes assumes a common ancestor for the haplotypes. Given the hybrid origin of the PUP population studied that assumption may not be valid. Uleberg et al. (2005) proposed a method to account for the fact that parents are from different breeds and do not descend from one common base population. Adjusting of IBD probabilities between parental haplotypes of the different breeds to zero had no effect on the results, likely because these estimated IBD probabilities between the haplotypes of the different breeds (paternal origin or maternal origin) with an average of 0.223 were low already. Further, the use of maternal haplotypes is one of the main advantages of LDLA over linkage analysis because information about historical recombinations is mainly carried by the, more abundant, maternal haplotypes (Uleberg et al., 2005). However, low estimated IBD probabilities between haplotypes of paternal and maternal origin results in very limited or no contribution from the maternal side.

LDLA analysis of the B&D does not have this problem as it is a purebred. The B&D population was genotyped in order to confirm the segregation of the QTL in the purebred sire line. Also, additional haplotypes may be sampled that are present in the purebred population. The analyses performed, at best do not reject the existence of a QTL for JCScut in the same chromosomal region.

Clear evidence was found for the segregation of a QTL affecting JCS following regression or variance component linkage analysis. The additional markers did not result in a refined positioning of the QTL. The QTL, however, could not be confirmed with an analysis including LD information. A higher marker density may be required for an apparent contribution of LD to further fine mapping of the QTL and the identification of specific haplotypes representing the different QTL alleles. This conclusion is supported by a limited level of LD. The mean D' across all pairs of markers was 0.298 ±0.136, which was in the range of 0.213 to 0.393 and similar to the average estimate obtained by Nsengimana et al. (2004) for SSC4. Relatively few pair-wise estimates of D', *i.e.* 52, had a value above 0.54 which can be considered as useful LD for mapping purposes. Although no indications can be derived from this study, microsatellite markers may be less powerful than SNP markers to define critical LD regions in combined linkage and linkage disequilibrium analysis approaches. In these approaches IBD coefficients between unrelated founders (base individuals) are estimated based on haplotype similarity (similar microsatellite alleles). The higher mutation rate of microsatellites compared to SNP markers may disturb linkage disequilibrium between markers and QTL.

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Litarature cited:

Churchill G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138: 963-971.

De Koning D.J., L.L. Janss, A.P. Rattink, P.A. van Oers, B.J. de Vries, M.A.M. Groenen, J.J. van der Poel, P.N. de Groot, E.W. Brascamp, and J.A.M. van Arendonk. 1999. Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs (*Sus scrofa*). Genetics 152: 1679-1690.

Farnir F., W. Coppietersen, J.-J. Arranz, P. Berzi, N. Cambisano, B. Crisart, L. Karim, F. Marcq, L. Moreau, M. Mni, C. Nezer, P. Simon, P. Vanmanshoven, D. Wagenaar, and M. Georges. 2000 Extensive genome-wide linkage disequilibrium in cattle. Genome Res. 10: 220–227.

Farnir F., B. Grisart, W. Coppieters, J. Riquet, P. Berzi, N. Cambisano, L. Karim, M. Mni, S. Moisio, P. Simon, D. Wagenaar, J. Vilkki, and M. Georges. 2002. Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. Genetics 161: 275-287.

Gilmour A.R., B.R. Cullis, S.J. Welham, and R. Thompson. 2000. ASReml reference manual. ftp.res.bbsrc.ac.uk/pub/aar.

Green P., K. Falls, and S. Crook. 1990. Documentation for CriMap, Version 2.4. Washington University School of Medicine, St. Louis, MO, USA.

Heifetz E.M., J.E. Fulton, N. O'Sullivan, H. Zhao, J.C.M. Dekkers, and M. Soller. 2005. Extent and consistency across generations of linkage disequilibrium in commercial layer chicken breeding populations. Genetics 171: 1173-1181.

Knott S.A., J.M. Elsen, and C.S. Haley. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theor. Appl. Genet. 93: 71-80.

McRae A.F., J.C. McEwan, K.G. Dodds, T. Wilson, A.M. Crawford, and J. Slate. 2002. Linkage disequilibrium in domestic sheep. Genetics 160: 1113-1122.

Meuwissen T.H.E., and M. E. Goddard. 2000. Fine mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. Genetics 155: 421-430.

Meuwissen T.H.E., A. Karlsen, S. Lien, I. Olsaker, and M.E. Goddard. 2002. Fine mapping of a quantitative trait locus for twinning rate using combined linkage and linkage disequilibrium mapping. Genetics 161: 373-379.

Nsengimana J., P. Baret, C.S. Haley, and P.M. Visscher. 2004. Linkage disequilibrium in the domestic pig. Genetics 166: 1395-1404.

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

Van Wijk H.J., D.J.G. Arts, J.O. Matthews, M. Webster, B.J. Ducro, and E.F. Knol. 2005. Genetic parameters for carcass composition and pork quality estimated in a commercial production chain. J. Anim. Sci. 83: 324-333.

Van Wijk H.J., B. Dibbits, E.E. Baron, A.D. Brings, B. Harlizius, M.A.M. Groenen, E.F. Knol, and H. Bovenhuis. 2006a. Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross. J. Anim. Sci. 84: 789-799.

Van Wijk H.J., H. Buschbell, B. Dibbits, S.C. Liefers, B. Harlizius, H.C.M. Heuven, E.F. Knol, H. Bovenhuis, and M.A.M. Groenen. 2006b. Variance component analysis of QTL for pork carcass composition and meat quality on SSC4 and SSC11. Accepted by J. Anim. Sci.

Van Wijk, H.J., S.C. Liefers, H. Buschbell, B. Dibbits, B. Harlizius, and M.A.M. Groenen. 2006c. *In silico* identification and mapping of microsatellite markers on *Sus scrofa* chromosome 4. Submitted to Animal Biotechnology.

Seaton G., C.S. Haley, S.A. Knott, M. Kearsey, and P.M. Visscher. 2002. QTL Express: mapping quantitative trait loci in simple and complex pedigrees. Bioinformatics 18: 339-340.

Sobel E., and K. Lange. 1996. Descent graphs in pedigree analysis: applications to haplotyping. Location scores, and marker sharing statistics. Am. J. Hum. Genet. 58: 1323-1337.

Uleberg E., I.S. Widerøe, E. Grindflek, J. Szyda, S. Lien, and T.H.E. Meuwissen. 2005. Fine mapping of a QTL for intramuscular fat on porcine chromosome 6 using combined linkage and linkage disequilibrium mapping. J. Anim. Breed. Genet. 122: 1-6.

Chapter 8

General Discussion

General Discussion: This general discussion describes the contribution of the results as presented in this thesis to the field of pig genetics and pig breeding. The chapter is divided in four sections. The first section discusses the findings of the present study in relation to published results on experimental crosses in pure lines. The second section discusses the subject of fine mapping. In the third section implementation of the results will be discussed. The last section describes how the molecular breeding may develop with the availability of the porcine genome sequence and how future pig molecular genetics research may evolve.

QTL mapping in pig

Selection is mainly conducted within closed lines which make the identification of QTL segregating within lines of particular interest. QTL detected in commercial populations can directly be implemented in breeding. A drawback of the use of commercial populations for mapping QTL is that due to selection the genetic and phenotypic variation is smaller than in a cross. Usually, existing half-sib families are used. Statistical power is approximately 8 times lower (assuming a heterozygosity of the QTL = 0.5) when using half-sib families as compared to a line cross (lines fixed at alternative alleles) with the same number of animals. Therefore phenotyping and genotyping of a larger number of animals is required to achieve sufficient power to detect QTL. However, improved techniques make genotyping no longer the limiting factor. With respect to the availability of animals and data, the use of an existing population structure and routinely recorded phenotypes (eventually completed with additionally recorded traits) is an advantage.

Breeding may benefit from knowledge of the genetic factors underlying traits of interest. The development of molecular markers facilitated the structural search for genetic factors underlying quantitative traits of economic importance *i.e.* quantitative or economic trait loci (QTL/ETL). The success of the numerous QTL studies in pig may be measured based on the >1000 QTL that are reported in the pig QTL database (PigQTLDB; Hu et al., 2005). Nevertheless, successful implementation of MAS in pig breeding remains limited to a handful of major genes (RYR (MacLennan, 1990), FUT1 (Meijerink et al., 2000); PRKAG3 (Milan et al., 2000); ESR (Short et al., 1997), MC4R (Kim et al., 2000); IGF2 (Van Laere et al., 2003). Reasons for the limited successes are: 1) Most QTL studies were conducted on experimental crosses between divergent

breeds in particular setup for the identification of QTL underlying the different phenotypes of extreme lines. The identified QTL in these crosses are not necessarily of relevance and/or may not segregate in commercial breeding populations. Whether such QTL are segregating in commercial breeds needs to be verified before they can be utilized (increasing the cost of marker development). 2) The confidence interval for QTL location is generally large as a consequence of the different factors influencing the statistical power of detecting QTL: population size, heritability of the trait, and marker density. Further studies to narrow down the QTL region and/or to identify the underlying gene(s) are required prior to implementation. Fine mapping and in particular positional candidate gene cloning are very demanding tasks in terms of money and labor. The pig genome resources which will make both tasks affordable (markers, sequences, physical map) became available only recently, and additional resources (including complete genome sequence) will become available in the near future.

The genome scan as reported in this thesis is one of the few QTL studies conducted within a commercial crossbred population. Until recently it was uncertain whether QTL could be found within commercial breeds as a consequence of severe selection (Visscher and Haley, 1995). This especially applies to traits in the breeding goal. Very recently two parallel studies by Evans et al. (2003) and Nagamine et al. (2003) evaluated the extent to which QTL segregating in divergent crosses also segregated within commercial lines. Their results demonstrated that some major QTL for growth and fatness, known from experimental crosses, also segregate in commercial lines, and they concluded that they are not fixed. QTL were identified for performance and some quality traits. Evans et al. (2003) obtained 73 significant (nominal 5% level) effects out of 500 trait-by-chromosome combinations. These included 17 QTL at the 1% nominal level and four QTL at the 0.1% nominal level. The QTL detected by Evans et al. (2003) controlled between 6 and 29% (growth and back fat) and 5 and 15% (carcass and meat quality traits) of the phenotypic variance. The average proportion of phenotypic variance (corrected for fixed effects) explained by the QTL were 0.10 for growth, 0.12 for fat, and 0.08 for the quality traits by heritabilities of 0.40, 0.25 and 0.40, respectively. Nagamine et al. (2003) identified 16 significant (nominal 5% level) effects out of 50 trait-by-chromosome combinations, which explained on average 0.12 (growth) and 0.10 (back fat) proportion of phenotypic variance. Effects were found in the range of 0.5 - 0.6 phenotypic standard deviations for growth rate and 0.8 - 1.3 for back fat.

The results of our genome scan were comparable with the findings of Evans et al. (2003) and Nagamine et al. (2003), although no large QTL were identified. The results showed that it is feasible to identify and verify QTL in commercial crossbred populations. The average explained phenotypic variance of the QTL in this study was 0.04 and the average QTL effect was 0.63 trait units (based on paternal half sib regression analysis results). The QTL effect is in the usual range of 0.4 to 0.8 which is found in line cross populations (Hayes and Goddard, 2001). The conclusion of Nagamine et al. (2003) and Evans et al. (2003) can be confirmed *i.e.* segregating QTL are responsible for a considerable part of the phenotypic variance observed in commercial lines. This offers opportunities for marker assisted selection. However, it needs to be mentioned that in contrast to the studies referred to we did not find QTL for growth and fatness traits. Furthermore, it is worth to note that in neither of the studies on commercial populations, including this study, highly significant QTL were identified (genome-wide significance). Genome-wide significanct QTL were found in most reported studies on divergent crosses *i.e.* line cross data. The population used in the work described was one of the largest reported for QTL studies. The power of our experiment was sufficient to detect QTL with large effects, if present, *i.e.* QTL explaining more than 20% of the genetic variance. Given the power of the experiment it is believed that, at the regions covered, QTL with such an effect are not segregating (anymore) in the commercial population studied, or at best they are segregating at very low frequencies. However, with a power of approximately 50 percent we can not exclude that such QTL were missed. Severe selection may have resulted in fixation of favorable, and elimination of deleterious alleles with large effect. This should mainly reflect on traits within the breeding goal. Although our genome scan was targeted to regions harboring QTL for carcass and meat quality traits, on which no direct selection took place until recently, the results obtained support this impression. Until recently daily gain and back fat thickness were the main selection traits for the sire line studied. We did not find QTL for those traits and all sires of the population were scored homozygous for the favorable alleles of the known genes with large effect (RYR1 and IGF2). Another observation which may be the result of severe selection was the relatively low heterozygosity observed in the sire line, which was most prominent for SSC4 (observed heterozygosity = 0.4) which is known for a major growth and back fat QTL (Walling et al., 2000). The relative low marker heterozygosity fits with the observation that most QTL seem to segregate in a limited number of sires *i.e.* often 1 or 2 of the sires in our study. A decreased

heterozygosity reduces experimental power. Halving the heterozygosity of the QTL approximately also halves the power of an experiment. Knowing this information, the power of our genome scan turned out to be lower than anticipated beforehand and was in the order of 20 to 30 percent for QTL explaining more then 20% of the genetic variance. The power for the genome regions that were subject to validation increased to about 40 percent by genotyping all families (Chapters 4 and 5).

Fine mapping

The current strategy for complex trait mapping usually includes three stages. A genome scan, followed by fine mapping used as a follow-up to confirm and narrow down the QTL region, followed by SNP genotyping to further saturate the region and discover the candidate gene(s) or causative mutation(s). The rapid increase of the number of QTL identified, however, did not result in the identification of a high number of genes underlying QTL. In mouse, over 2000 QTL were reported while the number of candidate genes that are proposed to underlie QTL is only about 20 (Flint et al., 2005). The exact genetic mechanisms remain therefore poorly understood, although a few successes have been booked (Olofsson et al., 2003; Van Laere et al., 2003; Yalcin et al., 2004; Oliver et al., 2005). The identification of genes underlying QTL has proven to be very difficult and is hampered by limitations in fine mapping. This also hampers the use of QTL in breeding programs by marker assisted selection which requires a much higher mapping resolution than is achieved in most genome scans.

The usual fine mapping approach is genotyping additional animals and markers in the region of interest to increase the genetic information and reduce ‘the noise’ in estimating association. This approach was followed in this thesis in Chapters 4 and 5. Recently, several improved data analysis methods contributing to fine mapping have been proposed (Darvasi, 1998; Riquet et al., 1999; Meuwissen and Goddard, 2000; Meuwissen et al., 2002), in which family-based linkage and population wide linkage disequilibrium information is combined. In Chapters 4 - 6 of this thesis we applied the variance component method using combined linkage and linkage disequilibrium information. This method was successfully used for fine mapping of QTL affecting twinning rate and milk production (Meuwissen et al., 2002; Farnir et al., 2002). The method exploits simultaneously recombination events in the genotyped generations (linkage) as well as historical recombination events (linkage disequilibrium). It is shown that the VC method

is slightly more powerful than the classic regression method (Kolbehdari et al., 2005), although Grapes et al. (2004) reported that the methods are comparable for fine mapping. Advantages are the use of segregation information from both the paternal and maternal side. Effects of the different haplotypes could be estimated using pedigree information, and polygenic effects and QTL effects are estimated simultaneously. The individual haplotype effects and heritabilities provide direct information for marker assisted selection (Fernando and Grossman, 1989; Nagamine et al., 2004). A drawback of the method is the high marker density required, although the availability of an almost unlimited number of markers together with new developments in genotyping methods will make this no longer a limitation. Recent publications have shown that LD in livestock populations may be considerable and extent over large genetic map distances (Farnir et al., 2000; McRae et al., 2002; Nsengimana et al., 2004) with chromosome segments of ~3-10 cM in the pig populations studied by Nsengimana et al. (2004). Based on those findings Nsengimana et al. (2004) suggested that powerful genome-wide association studies should be feasible in commercial pig populations at marker densities of 5-10 cM. Nsengimana et al. (2004) also found that at the population level the global pattern of LD was similar in the different populations studied which include Large White and Landrace pure breeds and synthetic lines of Duroc or Yorkshire and Large White.

Subsequent analysis on the PUP population including the genome scan (Chapter 3), the validation and first phase fine mapping using additional markers and animals (Chapter 4), and the second phase fine mapping (Chapter 7), confirmed the evidence for the presence of a QTL for JCS on SSC4 when applying a paternal half-sib regression analysis (Table 1). However, increasing the number of animals and markers did not result in a more accurate positioning of the QTL. The final data set consisted of 41 markers on SSC4 (Chapter 7), with a 95% bootstrap confidence interval of 88 cM for the QTL. The large confidence interval and the shape of the obtained *F*-statistic or LRT profile suggested the segregation of 2 QTL. However, no evidence could be obtained for the presence of 2 QTL. The expected confidence interval for a QTL with this effect is 76 cM, which is similar to the confidence interval found (Darvasi and Soller, 1997).

The QTL was found by reanalyzing the 15 (Chapter 4) and 41 (Chapter 7) marker data sets using the variance component analysis linkage analysis method (VC LA). Combined linkage and linkage disequilibrium (LDLA) analysis revealed no apparent contribution of LD. This may

indicate that the extent of LD in the PUP material is lower as found by Nsengimana et al. (2004) and therefore marker density is insufficient. In this thesis the combined linkage and linkage disequilibrium analysis method was subsequently applied on marker maps with an average marker spacing of 6.1 and 2.2 cM for SSC4. This raised the question about the level of LD in the material studied. Higher marker densities may be required in case of lower levels of LD.

Table 1. Summary of regression analysis results on subsequent marker data sets on SSC4, with chapter, number of typed families (No. fam.), number of markers (No. markers), average marker interval (cM), information content (IC), chromosome-wise *P* value (P_{chr}), the marker interval with the highest *F*-statistic, and the confidence interval (cM).

Chapter	No. fam.	No. markers	Marker interval	IC	P_{chr}	Marker interval	Confidence interval[a]
3	8	6	19	0.34	0.006	Sw445 - S0097	-
	17	6	16	0.50	0.015	Sw524 – Sw445	-
4	17	15	6.1	0.69	0.007	S0067 - Sw445	-
7	17	41	2.2	0.89	0.001	S0217 - S0214	76/88

[a] Expected/observed 95% confidence interval, with the expected confidence interval calculated using the formula: CI95=530/(n*v), with n = sample size of informative families, and v = proportion of variance explained by the QTL (Darvasi and Soller, 1997).

Information about the LD between markers can be used in order to evaluate the extent of useful LD in a population, although a satisfactory measure of LD between multi-allelic markers with the ability to predict the extent of usable LD for QTL mapping or MAS has not been agreed upon (Zhao et al., 2005). A large extent of LD was observed in dairy cattle, sheep, and pigs using D' as measure of LD (Farnir et al., 2000; McRae et al., 2002; Nsengimana et al., 2004). Pair wise D' was estimated for the 41 markers on SSC4 on different subsets of the data set *i.e.* offspring, maternal haplotypes and paternal haplotypes (Table 2). As may be expected, D' decreased as the distance between loci increased (Figure 1). Nsengimana et al. (2004) showed that extent of LD can be expressed as a function of genetic distance following:

$$D' = rs + (1 - rs)\exp^{(-3d/R)} \quad [1]$$

where, D' = linkage disequilibrium as a fraction of maximum linkage disequilibrium,

rs = residual D' or residual LD (for unlinked loci), which is the component of D' independent of distance,

R = distance at which D' reaches values of rs

d = genetic distance.

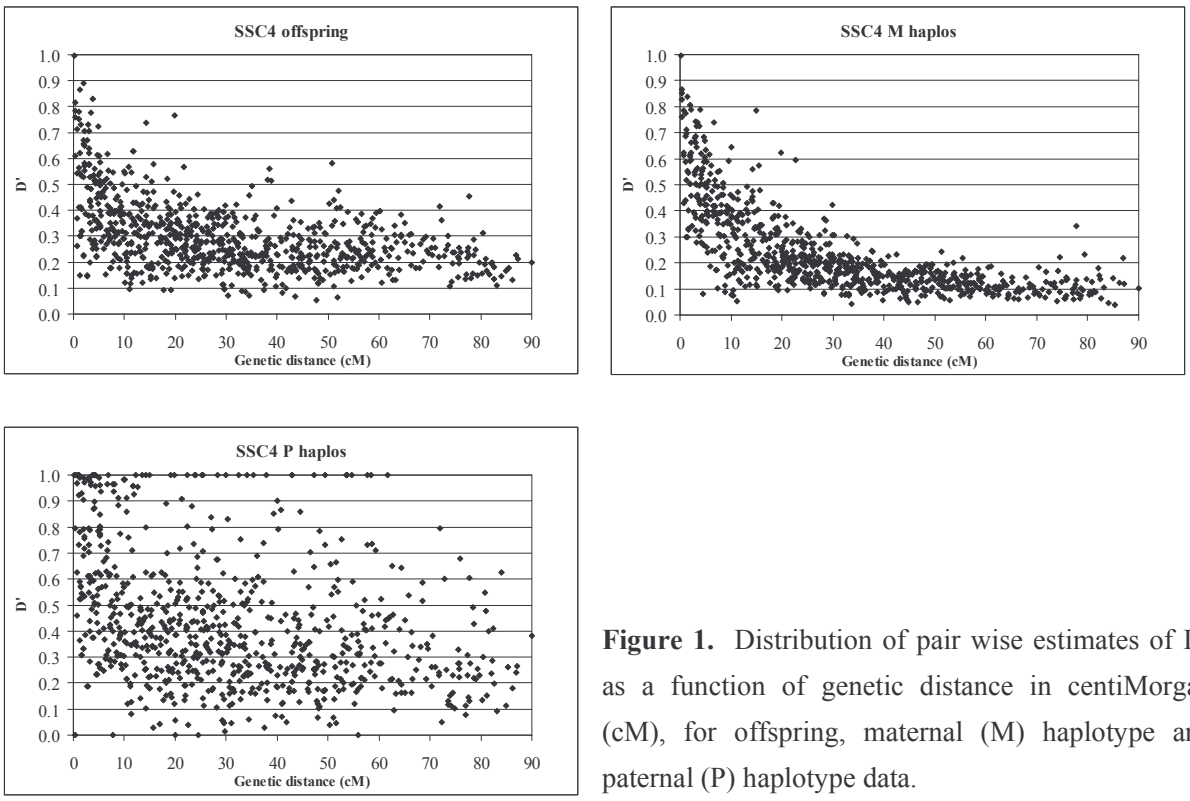

Figure 1. Distribution of pair wise estimates of D' as a function of genetic distance in centiMorgan (cM), for offspring, maternal (M) haplotype and paternal (P) haplotype data.

We applied the exponential function [1] to estimates of D' to estimate parameters rs and R using the NLIN procedure of SAS (SAS Inst. Inc., Cary, NC) (Table 2). Following Nsengimana et al. (2004) and according to function [1], at the distance of R/3, D' is equal to rs + (1 – rs)/*e*. Values of D' larger than the corresponding D' in this formula can be considered as useful LD for mapping purposes. The useful D'-thresholds are presented in Table 2 as well. Nsengimana et al. (2004) reported a range of 0.213 to 0.393 for the mean D' across all pairs of markers with an estimate of 0.290±0.131 for SSC4. The obtained mean D' based on the sire haplotypes is outside the reported range. The extent of LD was comparable to what was found by Nsengimana et al. (2004), who presented values between $0.150 < rs < 0.215$ and $9.6 < R < 21.8$ cM for SSC4.

Table 2. Information regarding pair wise estimates of D' and parameters of the exponential function of the genetic map distance applied to D' per subset of the data, with mean D' across all pairs of markers, residual LD (rs), the range (R) (distance at which D' reaches values of rs), and useful D'-threshold. Standard errors between brackets.

Subset	Mean D'		rs		R		useful D'
Offspring	0.298	(0.136)	0.270	(0.003)	6.7	(0.26)	0.539
Dam haplos	0.239	(0.163)	0.171	(0.003)	13.9	(0.34)	0.476
Sire haplos	0.469	(0.314)	0.404	(0.009)	18.4	(1.56)	0.623

Values of D' > 0.54, 0.48, and 0.62 could be considered as useful LD values for mapping purposes. Relatively few pair-wise estimates of D', *i.e.* 52, 82, and 180 had a value above the useful D'-thresholds. The distribution of these data points over increasing pair wise chromosome distances is presented in Figure 2. The majority of the useful D' data points were found within pair wise chromosome segments of <5 cM for the offspring and maternal haplotype data.

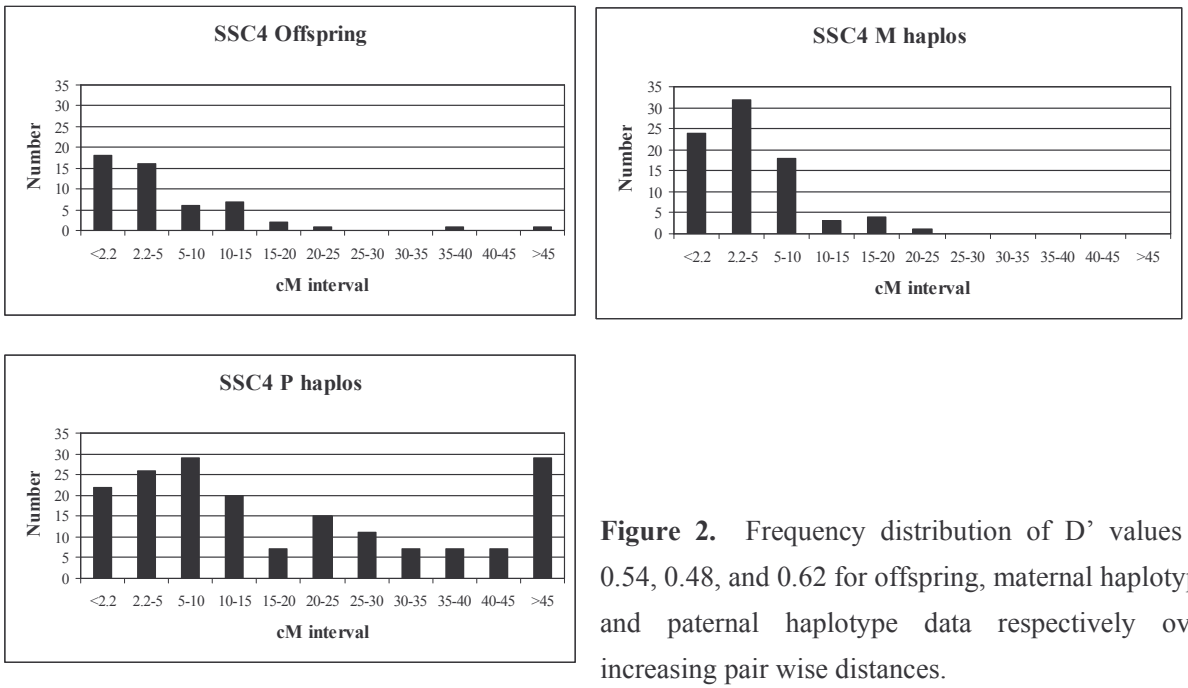

Figure 2. Frequency distribution of D' values > 0.54, 0.48, and 0.62 for offspring, maternal haplotype and paternal haplotype data respectively over increasing pair wise distances.

High D' values over much larger chromosome segments were found within the paternal haplotype data, although a relatively limited number of high D' values within pair wise chromosome distances of 5-10 cM. An increased number of markers is required to generate a higher number of useful LD values within small chromosome segments. This indicates that, based on our data, higher marker densities (<2 cM) are required for LD-based mapping methods.

Linkage disequilibrium mapping exploits LD between genetic markers and functional genetic variants underlying the traits. An interesting debate is which types of markers should be employed for LD based mapping methods. The relationship between haplotypes based on stable markers (SNPs) and potentially less stable markers (markers with higher mutation rate, e.g. microsatellites) have been investigated (Tishkoff et al., 1996, 2000; Kidd et al., 1998; Burgner et al., 2003) with different results. Patterns of LD between SNPs and microsatellite markers varied considerable between loci and although some results point to strong LD between microsatellite alleles and SNPs, the majority of the SNP haploytpes were accompanied by several microsatellite alleles, reflecting eroded LD between microsatellite alleles and SNP haplotypes. The lack of LD in the population described may be the result of the use of microsatellite markers and the estimated extent of LD may be an under representation of the real value of LD in the population. The use of stable markers seems therefore be preferred in LD mapping.

As mentioned in Chapter 7, another complication factor was the crossbred data used. Given the fact that parents are from different breeds paternal and maternal haplotypes may not originate from a common population, which is one of the assumptions underlying the LDLA method described by Meuwissen and Goddard (2000). Haplotypes originating from different populations which have gone through a different 'history' might therefore have different LD. The method assumes one uniform population. Uleberg et al. (2005) accounted for that by adjusting the IBD probabilities between parental haplotypes of the different breeds. Average IBD probabilities were calculated between pairs of haplotypes originating from the sires only (paternal haplotypes (P - P)), sows only (Maternal haplotypes (M – M)), hybrid haplotype pairs (P – M) and all pairs of haplotypes (P & M). Results were plotted along the chromosome (Figure 3).

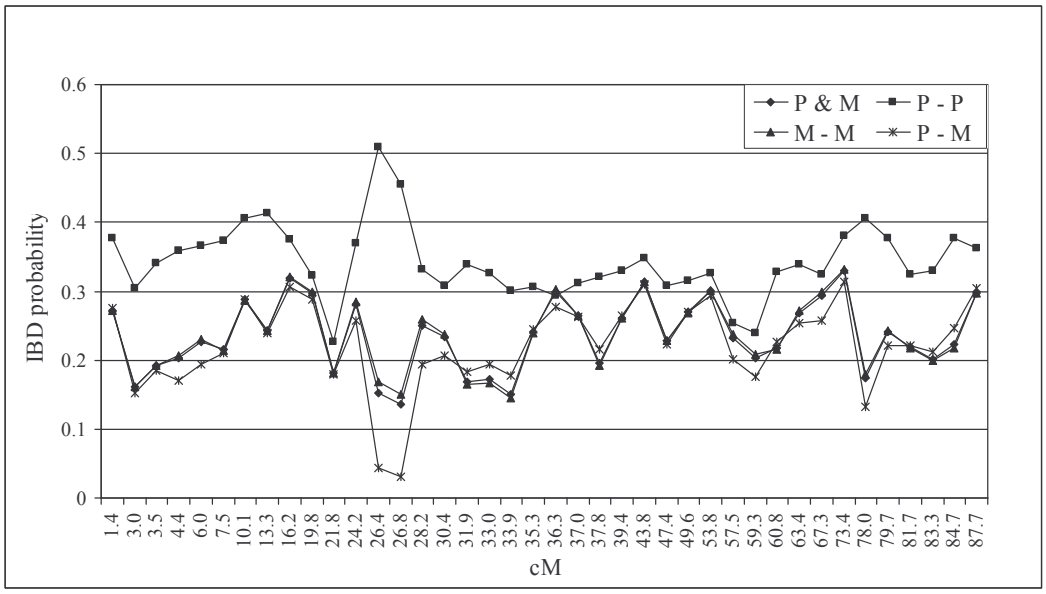

Figure 3. Averages of calculated IBD probabilities plotted along SSC4.

The average IBD probabilities between hybrid (P-M) haplotype pairs along SSC4 are within the range of 0.031 to 0.314 with an average of 0.223. Adjusting of IBD probabilities estimated between maternal and paternal haplotypes (P-M or hybrid haplotype pairs) to zero, following Uleberg et al. (2005), did not lead to other results, which may be explained by the already low IBD probabilities between the haplotypes of the different breeds.

The calculation of IBD probabilities between haplotype pairs following Meuwissen and Goddard (2001) may be optimized for crossbred populations. One suggestion is the use of breed specific IBS probabilities in the calculation of IBD probabilities between sire or sow haplotype pairs. Other improvements for analysis methods of commercial crossbred populations may be derived from admixture linkage disequilibrium mapping methods exploiting different allele frequencies in the parental populations. Methods should also allow for mistyping.

Traditional selection and MAS

Combining traditional breeding data with molecular data generated insights into the (genetic) factors affecting complex traits, including carcass and meat quality traits. QTL have been

identified, which will facilitate breeding for improved carcass and meat quality. The knowledge can also be applied for sorting of parents of crossbreds and/or development of genetic material with improved uniformity or specific for tailor-made (market specific) production. Nevertheless, successful implementation of QTL in pig breeding is limited at present. The question remains on 'how to use QTL information in animal breeding efficiently'? It is generally known that MAS can be effective for traits with low heritability, traits which are difficult to measure, late in life, or on relatives only (Meuwissen and Van Arendonk, 1992; Meuwissen and Goddard, 1996).

The selection response per year is a function of selection intensity, selection accuracy, genetic variance, and generation interval. Applications of MAS will mainly affect accuracy of selection and/or generation interval and intensity of selection for traits where a limited number of phenotypic records can be obtained.

As a reaction to market demands current pig selection indexes include carcass and meat quality traits, besides the classic selection traits such as litter size, survival, feed intake, gain and back fat. The main problem with carcass and meat quality traits is the lack of own performance records, affecting the accuracy of selection. Ultrasound measurements were introduced successfully to estimate muscle quantity and were a good alternative for carcass dissections. Such an alternative does not exist for meat quality measurements, which still requires slaughtering of relatives or progeny. Selection trends for meat quality traits are therefore small and not always positive (Sellier, 1998; Sonesson et al., 1998). The use of markers may have a beneficial effect on the response of selection for those traits.

In order to get a rough idea of the application of results as presented in this thesis, predicted selection response was calculated for some alternative scenarios. Selection response was calculated, using the program SelAction (Rutten et al., 2002), for a closed sire nucleus population. The alternative scenarios, with selection on ham and loin muscles, JCS and water retention:

- Basic scenario, selection on muscle quantity based on large scale ultrasound measurements,
- Dissection scenario, carcass dissection of commercial half-sibs and one full sib per litter,
- Biopsy scenario, a theoretical scenario with accurate measurements for JCS and water retention, based on a biopsy of the selection candidates,

- JCS MAS scenario, using a marker for a QTL affecting JCS similar as identified in this study, h^2=0.22, variance QTL (marker) =0.032.

The following parameters were used:

- No. of animals per year 30 sires and 300 sows
- phenotypic variance (σ^2_p) 0.27
- common environmental variance (σ^2_c) 0.09
- genetic variance (σ^2_a) 0.059

The progress per generation is presented in Table 3.

Table 3. Progress per year for the different simulated selection scenarios.

	Scenario			
Trait	**Basic**	**Dissection**	**Biopsy**	**JCS MAS**
Ham, g	270	250	260	230
Loin, g	190	180	180	170
JCS	0.01	0.05	0.06	0.09
Drip loss, %	0.04	-0.09	-0.17	0.04
€	1.46	1.86	2.13	1.80
Relative	100%	119%	137%	115%

The results showed that progress in carcass traits is relatively easy through unilateral selection based on muscle quantity (basic scenario). Genetic gain in meat quality traits requires more sophisticated (and expensive) trait recording protocols. The highest genetic response was, not surprisingly, obtained with the theoretical Biopsy scenario in which accurate own performance measurements of the quality traits were assumed. The accuracy of selection is lower in the Dissection and JCS MAS scenarios, but the benefits of both selection scenarios are substantial and of similar magnitude. These latter two scenarios are both realistic and differ in the allocation of costs towards phenotyping or genotyping. The advantage of the marker scenario may increase with an increased association between marker and trait, by typing a larger number of animals and a decrease of generation interval. The long term gain may be decreasing with an increasing frequency of the favorable QTL allele. However, maintenance and the ability to make use of genetic variation should be the scope of MAS, and not necessarily fixation of alleles.

This brings us to the other aspect that became important over the past years, *i.e.* tailor-made (marker-specific) and uniform production of pork in the retail chain. The demands differ per market and above all 'uniform cuts', in size and appearance, are of major concern for the industry. Marker information allows sorting of sires based on the different QTL alleles, reducing the variation in their offspring and differentiating the average trait value. Color affects product attractiveness and is therefore one of the major quality characteristics. The average Japanese color score of the studied population was 2.76 (±0.52). Premium prices are paid for carcasses with a JCS of three or higher. Forty-four percent of the individuals scored below this cut-off value, from which the majority, 33 % of the population, had a score of 2.5. A limited increase of the average JCS results in a higher percentage of animals within the premium price class. A selection response of 0.09 per year (JCS MAS, Table 3) results in an increase of 5.7 % of the animals with a score of three or higher (Figure 4).

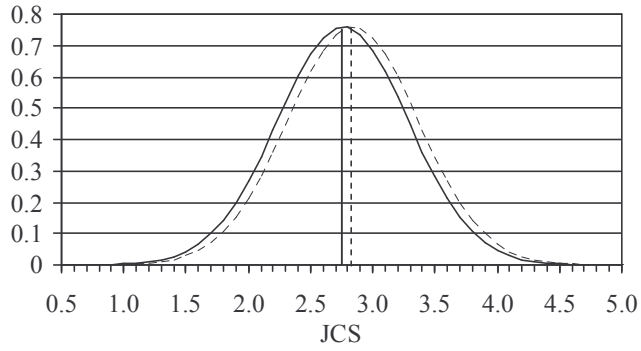

Figure 4. Visualized increase of the average JCS based on a yearly response of 0.09 (a normal distribution was assumed).

Optimal benefit of MAS may require reconsideration of classic breeding design. Possible alternative options greatly differ in the number of animals that need to be genotyped. The number of animals to genotype decreases with an increasing time point in the life cycle at which MAS will be applied, and consequently economic impact and the expected effect on genetic gain will differ substantially. Optimization of pig breeding schemes may be subject of further study. The diagram in Figure 5 represents a nucleus breeding scheme for a sire line. In the figure different numbers are presented indicating some of the points in the breeding scheme where

MAS can be applied. The different points may be a starting point for research towards the most optimal and cost effective marker assisted pig breeding scheme for improvement of meat quality.

1 – Top down or first stage marker assisted selection. Genotyping of all offspring, or the litters that are segregating for the QTL based on the parent genotypes, followed by pre-selection of animals entering the field test based on the marker information. MAS in this stage can reduce the number of animals tested or increase the efficiency of the test capacity by testing only those individuals with favorable genotypes. Also, (in case of a low allele frequency of the favorable QTL allele) more offspring can be produced than required for testing, followed by selection of progeny based on markers prior to testing. Selection preceding trait recording results in increased selection intensity.

2 – Field test coupled marker assisted selection. Genotyping of all individuals that entered the field test. MAS at this time point saves genotyping but allows the continuous assessment of phenotypic and genotypic associations based on a maximum of individuals on which trait records are obtained. Marker data on relatives increase the accuracy of selection compared to current non-MAS strategies in which only phenotypic data of relatives become available.

3 – Marker assisted selection on tested individuals. Genotyping of individuals that passed the field test, which is approximately 20% of the animals, and selection of replacement animals based on both phenotypic and marker information. Sorting of tested premium boars based on phenotypic and genotypic information is still feasible in this scenario.

4 – Genotyping of the top ~100 candidate nucleus replacement individuals and/or the premium boars for delivering of tailor made genetics.

5 – Genotyping of individuals routed to a sophisticated field test to allow assessment of phenotypic and genotypic associations for the traits recorded. Crossbreds are tested to ensure that selection of purebreds based on MAS results in improved crossbred pigs. It also allows to study possible interaction among QTL of different lines.

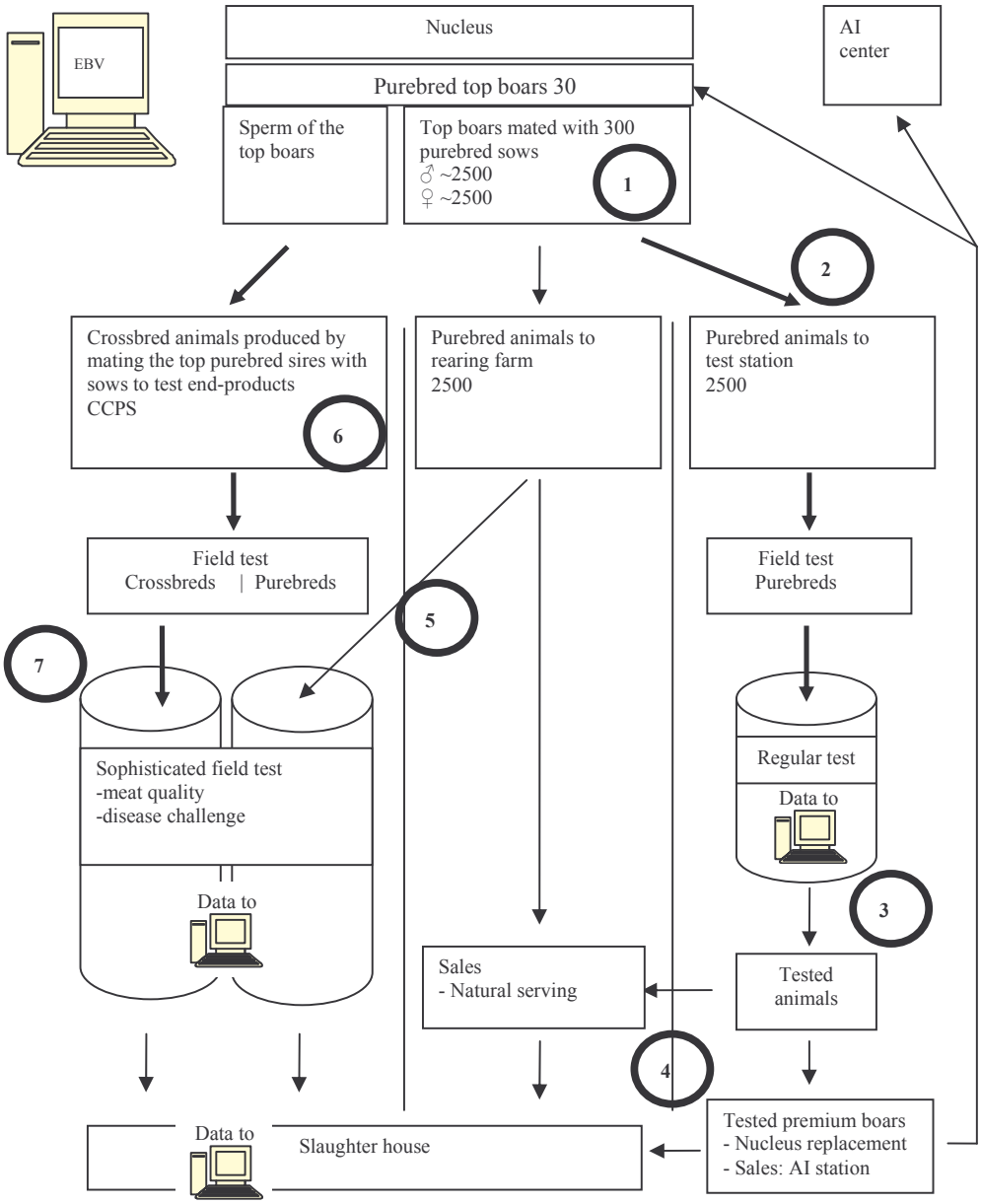

Figure 5. Schematic representation of a closed nucleus sire breeding scheme in which is indicated (numbered circles) were MAS can be applied.

6 & 7 – Genotyping of sows (6) and crossbreds (7). Crossbreds are tested to ensure that selection of purebreds is based on MAS results in improved crossbred pigs. It also allows studying possible interaction among QTL of different lines in the hybrid sow and the final product.

MAS in an early stage in the nucleus breeding scheme achieves a continuous stream of phenotyped and genotyped individuals allowing for an increasing accuracy of genotype and trait associations and continuous identification of new QTL. Also, as selection proceeds, phenotypic and genotypic associations will change, and the gradual increase of data allows a regular re-evaluation of the associations. Furthermore, more accurate genetic effects of the QTL could be obtained based on information of subsequent generations (Lande and Thompson, 1990).
A continuous stream of genotypes and phenotypes requires both genotyping and phenotyping of future generations. The benefits of MAS are then limited for traits which can effectively be selected based on phenotype. Maximal benefits of MAS can be obtained once associations are known and phenotyping can be omitted for one to several generations. However, a continuous data stream contributes to a more accurate positioning of QTL. Together with genome information this may result in intellectual property, *i.e.* regarding (candidate) genes and/or the understanding of the biological mechanisms, which eventually could be protected by patents.

Directions for future research; gene detection or genomic selection

The identification of genes and QTL affecting traits of relevance for the pig breeding industry has led to the first applications of MAS, although primarily through gene-assisted selection (GAS) using so called direct markers *i.e.* functional mutations from which only a few are known, and through a lesser extent using markers (that form a haplotype) which are in population wide linkage disequilibrium with the functional mutation (LD markers; Dekkers, 2004). A third type of markers as defined by Dekkers (2004) is LE markers, *i.e.* markers that are in population wide linkage equilibrium with the functional mutation in outbred populations. QTL studies usually result in LE markers. The linkage phase between LE markers and the QTL can differ from family to family, making this type of markers difficult to use in MAS. This explains the difficult and time consuming approaches to fine map QTL and/or candidate gene analysis resulting in LD markers or ultimately the functional mutations underlying QTL. The successes up to now are limited but genome resources facilitating these approaches are in continuous

development. This includes sequencing of the pig genome which was started in early 2006 (Schook et al., 2005). The first draft genome sequence, which is expected to be finished early 2008, and the subsequent identification of SNPs brings new tools which will further boost the identification of haplotypes or genes underlying QTL.

Several studies have shown an increase in accuracy of selection and selection response by inclusion of molecular marker data into breeding value estimation (Fernando and Grossman, 1989; Lande and Thompson, 1990; Meuwissen and Goddard, 1996; Dekkers and Van Arendonk, 1998; Dekkers, 1999; Goddard and Hayes, 2002). Most studies assumed one or a limited number of QTL with known and often intermediate to large effects. As long as the genetic variation explained by several markers or haplotypes remains limited compared to the polygenic effect, selection on phenotype can not be discarded. Once many more QTL or genes become available for selection, an important decision in MAS programs will be how many QTL or genes are going to be followed by markers, and for which traits. This will be determinative for the proportion of trait variance explained by the QTL, which is an important factor determining the accuracy of MAS (Lande and Thompson, 1990). Quantitative traits are influenced by numerous loci with small effects (infinitesimal model), which makes it difficult to identify all QTL. Once identified, this also makes it necessary to follow a number of loci in MAS schemes which together explain a significant part of the variance for a trait. Marker assisted selection for several traits then requires following several tens of regions across the genome.

For long it was recognized that quantitative and molecular genetics should be integrated in order to obtain the maximum improvement (Lande and Thompson, 1990). Meuwissen et al. (2001) proposed ‘genomic selection’ as an approach to explain all genetic variation using genome wide dense marker maps and simultaneous estimation of the effects of all marker intervals on the different traits. High numbers of SNP markers and high throughput genotyping technologies will make this approach feasible. The approach estimates the effects on the quantitative traits of each putative QTL (chromosome segment) simultaneously in a Bayesian framework assuming an exponential distribution of QTL effect sizes, followed by the use of all QTL affecting the traits of interest in marker assisted breeding value estimation weighted relative to the effect and importance of the trait. When performed on subsequent generations, QTL detection, fine mapping and MAS, either both gene detection and genomic selection, becomes a simultaneous and continuous process. The approach seems promising and although technically

feasible, future research may be dedicated to concerns dedicated to implementation of 'genomic selection' in industry settings, which includes:
- development of specific SNP sets informative in the different commercial lines,
- implementation of high throughput and more cost efficient genotyping technologies,
- determination of the optimum marker density (will depend on the extent of LD and the minor allele frequency (MAF)),
- study of breeding programs to determine the optimum number of animals that needs to be phenotyped and genotyped,
- development of improved software tools for SNP data analysis and improved algorithms for analyzing genome scans and a more efficient simultaneous fitting of the many effects. Improved and more efficient algorithms are required for simultaneously fitting the many effects, in particular when applied to the large pedigrees common in breeding with usually substantial amount of missing data.

However, 'genomic selection' does not need to be implemented genome-wide at once, but may gradually be shaped by inclusion of an increasing number of QTL regions (markers) in a MAS program.

The primary goal of QTL mapping studies has been to dissect the genetic basis of complex traits using genetic methods. Expression profiling is a second approach to identify genes associated with a trait of interest. Both approaches have been brought together in what is called 'genetical genomics' (Jansen and Nap, 2001). In this approach, expression levels are considered as quantitative phenotypes in genetic linkage analysis, and genetic variants with effect on expression levels are sought. An effect indicates that marker segregation is associated with different expression levels, a so called expression QTL or eQTL. These eQTLs can lead to candidate genes for downstream analysis and be linked with QTL information of measured phenotypes and information of genes in biological pathways may contribute to an increased understanding of the biology of the phenotypes (Li and Burmeister, 2005) which fundamentally change breeding moving from a "black-box" to a "glass-box" approach. An additional benefit of the approach may be the dissection of the phenotype ('traditional trait') into a series of (functionally related) 'lower-level traits' which are closer to the underlying genes, and which can be measured more accurately and eventually in a non destructive manner. As whether you are

able to look inside and see what is happening as the pathways underlying phenotypes of interest are in action. When following the integrative genetic approach, expression levels of hundreds of genes can be evaluated in parallel as highly heritable molecular quantitative traits. With that the 'genetical genomics' approach may contribute to what can be considered as the most limiting factor in genetic research, *i.e.* understanding the phenotypic traits. Although challenging, in a futuristic view genomic selection and (genetical) genomics approaches would be integrated into a genomics-assisted evaluation process complementing the conventional breeding and evaluation process.

Literature cited:

Burgner D., K. Rockett, H. Ackerman, J. Hull, S. Usen, M. Pinder, and D.P. Kwiatkowski. 2003. Haplotypic relationship between SNP and microsatellite markers at the NOS2A locus in two populations. Genes Immun. 4: 506-514.

Darvasi A., and M. Soller. 1997. A simple method to calculate resolving power and confidence interval of QTL map location. Behav. Genet. 27: 125-132.

Darvasi A. 1998. Experimental strategies for the genetic dissection of complex traits in animal models. Nat. Genet. 18: 19-24.

Dekkers J.C.M., and J.A.M. van Arendonk. 1998. Optimizing selection for quantitative traits with information on an identified locus in outbred populations. Genet. Res. 71: 257-275.

Dekkers J.C.M. 1999. Breeding in the 21st century: Application of molecular technology. Proc. Assoc. Adv. Anim. Breed. Genet. 13: 1-16.

Dekkers J.C.M. 2004. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. J. Anim. Sci. 82: E313-328.

Farnir F., W. Coppieters, J.-J. Arranz, P. Berzi, N. Cambisano, B. Crisart, L. Karim, F. Marcq, L. Moreau, M. Mni, C. Nezer, P. Simon, P. Vanmanshoven, D. Wagenaar, and M. Georges. 2000 Extensive genome-wide linkage disequilibrium in cattle. Genome Res. 10: 220–227.

Farnir F., B. Grisart, W. Coppieters, J. Riquet, P. Berzi, N. Cambisano, L. Karim, M. Mni, S. Moisio, P. Simon, D. Wagenaar, J. Vilkki, and M. Georges. 2002. Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. Genetics 161: 275-287.

Fernando R.L., and M. Grossman. 1989. Marker assisted selection using best linear unbiased prediction. Genet. Sel. Evol. 21: 467-477.

Flint J., W. Valdar, S. Shifman, and R. Mott. 2005. Strategies for mapping and cloning quantitative trait genes in rodents. Nat. Rev. Gen. 6: 271-285.

Goddard M.E., and B.J. Hayes. 2002. Proc. 7th World Congr. Genet. Appl. Livest. Prod., Montpellier, France.

Grapes L., J.C. Dekkers, M.F. Rothschild, and R.L. Fernando. 2004. Comparing linkage disequilibrium-based methods for fine mapping quantitative trait loci. Genetics 166: 1561-1570.

Hayes B., and M.E. Goddard. 2001. The distribution of the effects of genes affecting quantitative traits in livestock. Genet. Sel. Evol. 33: 209-229.

Jansen R.C., and J-P. Nap. 2001. Genetical genomics: the added value from segregation. Trends Genet. 17: 388-391.

Kidd K.K., B. Morar, C.M. Castiglione, H. Zhao, A.J. Pakstis, W.C. Speed, B. Bonne-Tamir, R.B. Lu, D. Goldman, C. Lee, Y.S. Nam, D.K. Grandy, T. Jenkins, and J.R. Kidd. 1998. A global survey of haplotype frequencies and linkage disequilibrium at the DRD2 locus. Hum. Genet. 103: 211-227.

Kim K.S., N. Larsen, T. Short, G. Plastow, and M.F. Rothschild. 2000. A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. Mamm. Genome 11: 131-135.

Kolbehdari D., G.B. Jansen, L.R. Schaeffer, and B.O. Allen. 2005. Power of QTL detection by either fixed or random models in half-sib designs. Genet. Sel. Evol. 37: 601-614.

Lande R., and R. Thompson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124: 743-756.

Li J., and M. Burmeister. 2005. Genetical genomics: combining genetics with gene expression analysis. Hum. Mol. Genet. 14: R163-R169.

MacLennan D.H., C. Duff, F. Zorzato, J. Fujii, M. Phillips, R.G. Korneluk, W. Frodis, B.A. Britt, and R.G. Worton. 1990. Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. Nature 343: 559-561.

McRae A.F., J.C. McEwan, K.G. Dodds, T. Wilson, A.M. Crawford, and J. Slate. 2002. Linkage disequilibrium in domestic sheep. Genetics 160: 1113-1122.

Meijerink E., A. Neuenschwander, R. Fries, A. Dinter, H.U. Bertschinger, G. Stranzinger, and P. Vogeli. 2000. A DNA polymorphism influencing alpha(1,2)fucosyltransferase activity of the pig FUT1 enzyme determines susceptibility of small intestinal epithelium to Escherichia coli F18 adhesion. Immunogenetics 52: 129-136.

Meuwissen T.H.E., and J.A.M. van Arendonk. 1992. Potential improvements in rate of genetic gain from marker assisted selection in dairy cattle breeding schemes. J. Dairy Sci. 75: 1651-1659.

Meuwissen T.H.E., and M.E. Goddard. 1996. The use of marker haplotypes in animal breeding schemes. Genet. Sel. Evol. 28: 161-176.

Meuwissen T.H.E., and M.E. Goddard. 2000. Fine scale mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. Genetics 155: 421-430.

Meuwissen T.H.E., B.J. Hayes, and M.E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157: 1819-1829.

Meuwissen T.H.E., A. Karlsen, S. Lien, I. Olsaker, and M.E. Goddard. 2002. Fine mapping of a quantitative trait locus for twinning rate using combined linkage and linkage disequilibrium mapping. Genetics 161: 373-379.

Milan D., J.T. Jeon, C. Looft, V. Amarger, A. Robic, M. Thelander, C. Rogel-Gaillard, S. Paul, N. Ianuccelli, L. Rask. H. Ronne, K. Lundstrom, N. Reinsch, J. Gellin, E. Kalm, P.L. Roy, P. Chardon, and L. Andersson. 2000. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science 288: 1248-1251.

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

Nagamine Y., P.M. Visscher, and C.S. Haley. 2004. QTL detection and allelic effects for growth and fat traits in outbred pig populations. Genet. Sel. Evol. 36: 83-96.

Nsengimana J., P. Baret, C.S. Haley, and P.M. Visscher. 2004. Linkage disequilibrium in the domestic pig. Genetics 166: 1395-1404.

Oliver F., J.K. Christians, X. Liu, S. Rhind, V. Verma, C. Davison, S.D.M. Brown, P. Denny, and P.D. Keightley. 2005 Regulatory variation at glypican-3 underlies a major growth QTL in mice. Plos Biology 3(5): 872-877.

Olofsson P., J. Holmberg, J. Tordsson, S Lu, B. Akerstrom, and R. Holmdahl. 2003 Positional identification of Ncf1 as a gene that regulates arthritis severity in rats. Nat. Genet. 33: 25–32.

Riquet J., W. Coppieters, N. Cambisano, J.-J. Arranz, P. Berzi, S.K. Davis, B. Grisart, F. Farnir, L. Karim, M. Mni, P. Simon, J.F. Taylor, P. Vanmanshoven, D. Wagenaar, J.E. Womack, and M. Georges. 1999. Fine mapping of quantitative trait loci by identity by descent in outbred populations: application to milk production in dairy cattle. Proc. Natl. Acad. Sci. 96: 9252-9257.

Rutten M.J., P. Bijma, J.A. Wooliams, and J.A.M. van Arendonk. 2002. SelAction: software to predict selection response and rate of inbreeding in livestock breeding programs. J. Hered. 93: 456-458.

Schook L.B., J.E. Beever, J. Rogers, S. Humphray, A. Archibald, P. Chardon, D. Milan, G. Rohrer, and K. Eversole. 2005. Swine Genome Sequencing Consortium (SGSC): a strategic roadmap for sequencing the pig genome. Comp. Funct. Genom. 6: 251-255.

Sellier, P. 1998. Genetics of Meat and Carcass Traits. Pages 463-510 in Rothschild, M.F., and A. Ruvinsky, eds, The Genetics of the Pig, CAB International, New York.

Short T.H., M.F. Rothschild, O.I. Southwood, D.G. McLaren, A. de Vries, H. van der Steen, G.R. Eckardt, C.K. Tuggle, J. Helm, D.A. Vaske, A.J. Mileham, and G.S. Plastow. 1997. Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. J. Anim. Sci. 75: 3138-3142.

Sonesson A.K., K.H. de Greef, and T.H.E. Meuwissen. 1998. Genetic parameters and trends of meat quality, carcass composition and performance traits in two selected lines of large white pigs. Livest. Prod. Sci. 57: 23-32.

The International HapMap Consortium. 2005. A haplotype map of the human genome. Nature 437: 1299-1320.

Tishkoff S.A., E. Dietzsch, W. Speed, A.J. Pakstis, J.R. Kidd, K. Cheung, B. Bonne-Tamir, A.S. Santachiara-Benerecetti, P. Moral, and M. Krings. 1996. Global patterns of linkage disequilibrium at the CD4 locus and modern human origins. Science 271: 1380-1387.

Tishkoff S.A., A.J. Pakstis, M. Stoneking, J.R. Kidd, G. Destro-Bisol, A. Sanjantila, R.B. Lu, A.S. Deinard, G. Sirugo, T. Jenkins, K.K. Kidd, and A.G. Clark. 2000. Short tandem-repeat polymorphism/alu haplotype variation at the PLAT locus: implications for modern human origins. Am. J. Hum. Genet. 67: 901-925.

Van Laere A.S., M. Nguyen., M. Braunschweig, C. Nezer, C. Collette, L. Moreau, A.L. Archibald, C.S. Haley, N. Buys, M. Tally, G. Andersson, M. Georges, and L. Andersson. 2003 A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature 425: 832–836.

Visscher P.M., and C.S. Haley. 1995. Utilizing genetic markers in pig breeding programmes. Anim. Breed. Abstr. 63: 803-811.

Walling G.A., P.M. Visscher, L. Andersson, M.F. Rothschild, L. Wang, G. Moser, M.A.M. Groenen, J-P. Bindanel, S. Cepica, A.L. Archibald, H. Geldermann, D.J. de Koning, D. Milan, and C.S. Haley. 2000. Combined analyses of data from quantitative trait loci mapping studies: chromosome 4 effects on porcine growth and fatness. Genetics 155: 1369-1378.

Williams J.L. 2005. The use of marker-assisted selection in animal breeding and biotechnology. Rev. sci. tech. Off. int. Epiz. 24(1): 379-391.

Yalcin B., S.A. Willis-Owen, J. Fullerton, A. Meesaq, R.M. Deacon, J.N. Rawlins R.R. Copley, A.P. Morris, J. Flint, and R. Mott. 2004 Genetic dissection of a behavioral quantitative trait locus shows that Rgs2 modulates anxiety in mice. Nat. Genet. 36: 1197–1202.

Zhao H., D. Nettleton, M. Soller, and J.C.M. Dekkers. 2005. Evaluation of linkage disequilibrium measures between multi-allelic markers as predictors of linakge disequilibrium between markers and QTL. Genet. Res. 86: 77-87.

Summary

Consumers interest in quality of food resulted in increased relevance of carcass and meat quality for the pork and pig breeding industry. Breeding goals are therefore much more directed to quality. Genetic improvement of quality requires estimates of genetic parameters. Also, genomic information can increase selection efficiency, in particular for traits with low heritability or for traits which cannot be measured on the selection candidate itself. Consequently, the underlying genetic architecture of quantitative traits, including carcass and meat quality characteristics, has become subject of numerous research papers during the past 15 years. Most of the QTL mapping projects were undertaken on divergent crosses. However, results obtained on breed crosses need to be confirmed on commercial lines prior to implementation. The aim of this thesis was identification and fine mapping of carcass composition and meat quality QTL within a commercial finishing cross.

The project was initiated by generating a finisher population. Twenty sires of a synthetic Piétrain/Large White halothane-free boar line were mated with 239 sows of a commercial sow cross with unknown pedigree generating 1,855 pigs. Pigs were raised under commercial finisher conditions and extensively phenotyped at slaughter for a series of growth, carcass composition, and meat quality traits.

In Chapter 2 genetic parameters were estimated. Heritability estimates for carcass composition traits varied from 0.29 to 0.51. Heritability estimates for the meat quality traits were lower and ranged from 0.08 to 0.28, with low estimates (average 0.10) for the water-holding capacity traits (purge, drip, and pH), and higher values for the color traits measured objectively (Minolta measurements, average 0.17) or subjectively (Japanese color score scale; average 0.25). Correlations of average daily gain with subprimals (on average -0.29) and with most meat quality characteristics (on average 0.70 in absolute value) were unfavorable, indicating that selection for higher growth may have a declining effect on weight and quality of the loin and ham. High correlations were found between most meat quality traits. Also, the meat quality traits showed favorable relationships with the primal and subprimal weights of the ham and loin. The estimated correlations indicated that selection towards increased carcass value by increasing the weights of ham and loin and improving quality will be feasible.

Chapter 3 reports the results of QTL analysis performed on the population. Half of the population (8 paternal half-sib families with 715 animals and their parents) was typed for 73 microsatellite markers covering 14 chromosomal regions, representing approximately half of the genome. The covered genome regions were selected based on literature reporting the presence of QTL affecting carcass composition and meat quality traits. Chapter 1 reports about the findings of the literature search and presents the number of reported QTL per chromosome and traits identified with linkage analysis using half-sib regression analysis. Thirty-two QTL affecting 20 traits were identified at the 5% chromosome-wise threshold. Some related traits showed effects at a similar chromosomal position, so the number of independent QTL is estimated at 26. Among these, four QTL were significant at the 1% chromosome-wise level; a QTL for Japanese color score on SSC4, a QTL affecting loin weight and loin muscle depth on SSC11, a QTL for shrinkage of the carcass on SSC12, and a QTL for initial pH on SSC13. The findings of the QTL scan suggested that QTL with large effect (explaining >30-35% of the phenotypic variance) did not segregate within the sire line examined, at least not for the regions considered.

Following the QTL scan and in order to confirm the results obtained on the first half of population, the remaining part of the population (9 half-sib families) was genotyped for the regions on SSC4 and SSC11 (Chapter 4), and SSC2, SSC13, and SSC14 (Chapter 5). Additional markers were added to the regions on SSC4 and SSC11, increasing the information content considerable. This two-step genotyping approach was followed to save genotyping. A variance component QTL mapping method was implemented for the analysis of the increased data sets. The method allows modeling of maternal and paternal haplotype effects, allowing for a better characterization of QTL. The analysis resulted in the identification of additional QTL on the 5 chromosome regions. However, not all results obtained on the first half of the population could be confirmed on the whole pedigree. Increased evidence for the QTL affecting Japanese color score on SSC4 was found. Furthermore, QTL affecting loin muscle mass were found on SSC2 and SSC14, as well as QTL affecting different quality traits (Minolta L*, a*, b*, ultimate pH, and Japanese color score) on SSC2. The findings on SSC2 and SSC4 made those two regions the most promising candidates for further research. Also, the results on both chromosomes agreed with published QTL studies involving both chromosomes.

For further fine mapping of the Japanese colour score QTL on SSC4, additional markers were developed. New markers were identified by *in silico* mining of publicly available sequence

data (Chapter 6). Porcine sequences were predicted within the region on SSC4 using a BLAST search against the homologous region in the human genome based on pig-human comparative map information. Approximately 300 potential new microsatellites were identified predicted in the region of interest on SSC4, corresponding to one microsatellite every ~40 Kbp. This frequency is similar to the frequency found in human and mice. The results showed that the approach is very efficient for targeted development of new markers for specific genome regions of interest.

Chapter 7 reports on fine mapping of the QTL and verification of segregation of the QTL in the purebred sire line. Hereto, a denser linkage map was constructed. A selection of 30 newly developed markers was typed on the crossbred population. Twenty-six markers mapped to SSC4, bringing the total number of markers typed on this chromosome up to 41, with an average marker spacing of 2.2 cM. For validation, the purebred sire population consisting of 37 paternal half-sib families and a total of 703 offspring was genotyped for a subset (20) of the 41 markers and an additional 2 markers. Regression and variance component linkage analysis on the 41 marker data set resulted in increased evidence for the presence of the QTL, although the most likely position differed between both analyses. The obtained profiles were in agreement with each other and suggested the presence of two QTL. However, a two QTL model did not provide significant evidence for the presence of a second QTL.

The VC LA analysis of the 22 marker data set on the purebred population only suggested the segregation of the QTL with values that approached the significance threshold. A combined linkage and linkage disequilibrium (LDLA) analysis of the 41 and 22 marker data sets did not result in a more accurate positioning of the QTL. The obtained LDLA LRT profiles on both populations approached the threshold, with the highest LRT values in the same region. Although the LRT values were not significant, most likely the QTL is located in this ~20 cM region interval between markers S0813 and S0067. In contrast to the expectation, LD information contributed hardly to a refined positioning of the QTL. The extent of LD in the population was calculated and lower as anticipated based on the literature. A higher marker density seems to be required for an apparent contribution of LD. The fact that parents were from a different breed may interfere with the underlying assumption that haplotypes originate from a common ancestor, although no evidence could be found for that.

Samenvatting

De groeiende interesse van consumenten in de kwaliteit van hun voedsel heeft geleid tot toenemende interesse in karkas- en vleeskwaliteit bij de slachterijen en de varkens fokkerij. Kwaliteit van het eindproduct is een onderdeel geworden van het fokkerij doel. Genetische verbetering van kwaliteit vereist het meten ervan, en vereist ook informatie over erfelijkheidsgraden en correlaties. Informatie over het genoom en meer specifiek de genen kan ook bijdragen aan de effectiviteit van selectie, vooral waar het gaat om eigenschappen met een lage erfelijkheidsgraad of eigenschappen die niet aan de selectie kandidaten zelf gemeten kan worden. Sinds de laatste 15 jaar wordt dan ook veel onderzoek verricht naar de genetische basis van kwantitatieve kenmerken, waaronder ook karkas- en vleeskwaliteit kenmerken. Veel van die studies richtte zich op het identificeren van genoom regio's waar genen lijken te liggen die invloed hebben op bepaalde kenmerken, zogenaamde quantitative trait loci (QTL). De meeste van de QTL karteringsprojecten werden gebaseerd op kruisingen tussen verschillende rassen. De resultaten verkregen op zulke kruisingen zijn niet altijd onmiddellijk toepasbaar op commerciële lijnen, en dienen dan ook geverifieerd te worden voorafgaand aan eventuele toepassing. Het voornaamste doel van het werk onderliggende dit proefschrift was het identificeren en fijn karteren van QTL voor verschillende karkas- en vleeskwaliteit kenmerken op basis van een commerciële kruising. Resultaten verkregen op commercieel materiaal zouden dan eventueel direct toegepast kunnen worden in de fokkerij van de betreffende commerciële lijn.

Hiertoe werd een kruising gemaakt tussen 20 beren van een Piétrain/Yorkshire berenlijn en 239 commerciële kruisingszeugen. Dit leverde een populatie op van 1855 vleesvarkens, welke uitgebreid werden gefenotypeerd voor een serie groei, karkas- en vleeskwaliteit kenmerken.

Op basis van de fenotypes werden genetische parameters geschat. Dit staat beschreven in hoofdstuk 2. De geschatte erfelijkheidsgraden voor de verschillende karkas compositie eigenschappen varieerde van 0.29 tot 0.51. De erfelijkheidsgraden voor de verschillende vleeskwaliteit kenmerken waren lager en varieerde van 0.08 tot 0.28, met lage schattingen (gemiddeld 0.10) voor kenmerken gerelateerd aan het water bindend vermogen van het vlees (pH, drip verlies, uitlekgewicht), en hogere schattingen voor de objectief gemeten kleur kenmerken (Minolta metingen; gemiddeld 0.17) of subjectief gemeten kleur (Japanse kleur schaal; gemiddeld 0.25). De geschatte correlaties tussen gemiddelde groei per dag en ham of

karbonade streng gewicht en tussen gemiddelde groei per dag en de meeste gemeten kwaliteit kenmerken waren vrijwel allemaal ongunstig. Dit betekent dat selectie op harde groei een nadelig effect zal hebben op het gewicht en de kwaliteit van de ham en karbonade streng. De correlaties tussen de vleeskwaliteit kenmerken onderling waren hoog. De correlaties tussen de vleeskwaliteit kenmerken en de ham en karbonade streng gewichten waren gunstig. De geschatte correlaties geven aan dat selectie gericht op een hogere karkas waarde mogelijk moet zijn middels een toename van het gewicht als ook de kwaliteit van de meest waardevolle delen van het karkas, *i.e.* de ham en de karbonade streng.

In hoofdstuk 3 worden de resultaten beschreven van een analyse gericht op de identificatie van QTL, een zogenaamde QTL kartering. Hiertoe werd de helft van de populatie (8 families met 715 dieren en hun ouders) gegenotypeerd met 73 microsatelliet merkers. De merkers bestreken 14 verschillende chromosomale regio's, welke gezamenlijk ongeveer de helft van het totale genoom vertegenwoordigde. De 14 genoom regio's werden geselecteerd op basis van literatuur gegevens en zouden QTL bevatten die van invloed zijn op karkas- of vleeskwaliteit kenmerken. In hoofdstuk 1 word het literatuur onderzoek samengevat onder andere in de vorm van een tabel waarin het aantal QTL per chromosoom en per kenmerk staat gerapporteerd. De koppelingsanalyse werd uitgevoerd middels een regressie analyse. Er werden 32 QTL gevonden voor 20 verschillende kenmerken bij een 5% chromosoom specifiek significantie niveau. Voor enkele aan elkaar gerelateerde kenmerken werd een QTL gevonden op dezelfde chromosoom positie. Het daadwerkelijke aantal verschillende QTL dat is gevonden is dan ook lager dan 32 en wordt geschat op 26. Onder de QTL waren er vier die significant bleken bij een 1% chromosoom specifiek significantie niveau; een QTL voor Japanse kleur schaal op chromosoom 4 (SSC4), een QTL op SSC11 met effect op karbonade streng gewicht en de dikte van de karbonade spier, een QTL voor krimp van het karkas op SSC12 en een QTL voor pH gemeten op 24 uur op SSC13. De resultaten van de QTL kartering wijzen erop dat QTL met grote effecten niet uitsplitsen binnen de onderzochte beren lijn, althans niet op de chromosoom regio's die zijn bekeken.

Volgend op de QTL kartering en ter validatie van de verkregen resultaten op de eerste helft van de populatie werd ook het tweede deel van de populatie (9 families) gegenotypeerd maar dan alleen voor de meest belovende chromosoom regio's; SSC2, SSC4, SSC11, SSC13 en SSC14. Deze benadering, om in twee achtereenvolgende stappen te genotyperen werd gekozen om kosten uit te sparen. Op SSC4 en SSC11 werden tevens additionele merkers geplaatst, wat de

hoeveelheid informatie op beide regio's ten goede kwam. De analyse van de grotere dataset voor SSC4 en SSC11 staat beschreven in hoofdstuk 4. Hoofdstuk 5 beschrijft vervolgens de analyse van de data op de gehele populatie voor de regio's SSC2, SSC13 en SSC14. Voor de analyse werd gebruik gemaakt van een nieuwe methode, de variantie componenten methode. De methode maakt het mogelijk zowel haplotypen effecten van vaders als moeders kant te modeleren, wat bijdraagt aan een betere karakterisering van QTL. De analyse resulteerde in additionele QTL in de 5 regio's, alhoewel niet alle resultaten verkregen op de eerste helft van de populatie bevestigd konden worden op data op de gehele populatie. Er werd additioneel bewijs gevonden voor de QTL met effect op het Japanse kleur schaal kenmerk op SSC4. Verder werden op SSC2 en SSC14 QTL gevonden voor het kenmerk karbonade streng gewicht. Op SSC2 werden ook QTL gevonden voor enkele vlees kwaliteit kenmerken (Minolta L*, a*, b*, pH gemeten op 48 uur en Japanse kleur schaal). De QTL gevonden op SSC2 en SSC4 maakt van deze twee regio's de meest belovende voor vervolg onderzoek. De resultaten op deze twee regio's komen ook overeen met gepubliceerde informatie.

Het vervolg was gericht op het nauwkeurig in kaart brengen van de QTL met effect op Japanse kleur schaal op SSC4, zogenaamd fijn karteren. Voor het fijn karteren waren additionele merkers nodig. Nieuwe merkers werden geïdentificeerd middels een computer programma geschreven in Perl. Dit staat beschreven in hoofdstuk 6. Het computer programma doorzoekt publiek beschikbare sequentie data op de aanwezigheid van potentiële microsatelliet merkers, zogenaamd *in silico* mining. De sequenties met een dergelijk potentiële merker werden vervolgens middels een zogenaamde BLAST zoekactie vergeleken met de sequentie van het menselijke genoom. Aan de hand van genen die gekarteerd zijn op zowel het genoom van varken als het genoom van de mens, zogenaamde vergelijkende genoom informatie, kan voorspeld worden waar de potentiële nieuwe microsatelliet merkers liggen op het varkensgenoom. Ongeveer 300 potentiële nieuwe merkers werden voorspeld in de chromosoom regio op SSC4. Dit correspondeerd met één microsatelliet elke ~40 Kbp. Deze frequentie komt overeen met de frequentie in mens en muis. De resultaten laten zien dat de gevolgde benadering een zeer efficiënte manier kan zijn om heel gericht nieuwe merkers te vinden voor bepaalde chromosoom regio's.

Hoofdstuk 7 beschrijft het fijn karteren van de QTL op SSC4, en de verificatie van uitsplitsing van de QTL in de zuivere beren lijn. Hiertoe werd de populatie gegenotypeerd met

een selectie van 31 nieuwe merkers om zodoende een koppelingskaart te krijgen met meer merkers. Van de 31 nieuwe merkers bleken er 26 daadwerkelijk op SSC4 te liggen. Het totale aantal merkers gegenotypeerd op deze genoom regio komt daarmee op 41. De merkers hebben een onderlinge afstand van gemiddeld 2.2 centiMorgan (cM). Analyse van de data middels een regressie analyse en variantie componenten analyse resulteerde in toegenomen bewijs voor de aanwezigheid van de QTL. De verkregen profielen vertoonde veel gelijkenis alhoewel de meest waarschijnlijke positie van de QTL enigszins verschilde tussen de twee alternatieve analyse methoden. De profielen duiden ook op de aanwezigheid van twee QTL op SSC4 van invloed op het Japanse kleur schaal kenmerk. Echter, de analyse van een twee QTL model leverde geen significant bewijs op voor de aanwezigheid van twee QTL.

Voor validatie van de QTL in de zuivere beren lijn was een populatie beschikbaar bestaande uit 37 vaderlijke nakomelinggroepen met een totaal van 703 nakomelingen. Deze populatie werd gegenotypeerd voor een subset van 20 van de 41 merkers en 2 additionele merkers. De analyse van de data, middels de variantie componenten methode, leverde geen significant bewijs op voor het segregeren van de QTL. De verkregen likelihood ratio test waarde waren wel bijna significant en de resultaten suggereren dan ook dat de QTL segregeert in de zuivere beren lijn. De variantie componenten methode het ook mogelijk om koppelingsinformatie en linkage disequilibrium (LD) informatie te combineren, zogenaamde LDLA analyse. Het combineren van de beide soorten van informatie kan bijdragen aan de fijnkartering. Een LDLA analyse van de 41 (kruisingspopulatie) en 22 (zuivere lijnspopulatie) merker datasets resulteerde echter nauwelijks in een nauwkeurigere positionering van de QTL. De verkregen likelihood ratio test profielen op beide populaties benaderde het significantie niveau met de hoogste likelihood ratio test waarden in dezelfde regio. Ondanks dat de waarden niet significant waren is de meest waarschijnlijke gelegen in het 20 cM gebied tussen de merkers S0813 en S0067. In tegenstelling tot wat verwacht werd droeg de LD informatie nauwelijks bij aan een betere positionering van de QTL. De mate van LD in de populatie werd berekend en lager bevonden dan kon worden verwacht op basis van de literatuur. Een verhoogde merker dichtheid lijkt nodig om te komen tot een duidelijke bijdrage van LD in een LDLA analyse. Eén van de aannames onderliggende de analyse is dat haplotypen afstammen van dezelfde gemeenschappelijke voorouder. Het feit dat de ouders afstammen van verschillende lijnen kan interfereren met deze aanname alhoewel daar geen bewijs voor gevonden werd.

Nawoord

Tegen de tijd dat je aan het schrijven van het nawoord toekomt is er veel werk verricht, en niet alleen door mijzelf. Dit proefschrift is het resultaat van de vele inspanningen van de afgelopen jaren in het kader van de projecten "Nationaal project bioinformatica voor nutsdieren" en "PUP – Predictable Uniform Pork".

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Als werknemer van IPG heb ik mijn werk grotendeels uitgevoerd bij de Leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit in het kader van een nauwe en zeer prettige samenwerking tussen IPG en de Leerstoelgroep. Het project "Nationaal project bioinformatica voor nutsdieren" was een door Senter gefinancierd project o.a. in samenwerking met Nutreco, Holland Genetics en Plant Research International. Ook de inbreng vanuit deze partners in het project heeft mijn horizon helpen te verbreden.

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Rik

Curriculum Vitae

Henricus Johannes (Rik) van Wijk werd op 29 maart 1969 geboren te Dordrecht. Hij groeide op temidden van plant en dier op een boerderij in Oud-Alblas (Z-H). Na het behalen van het MAVO diploma aan het Prins Bernhard College te Papendrecht begon hij in 1985 aan de laboratorium opleiding botanie op het Van Leeuwenhoek Instituut te Delft. Na een succesvolle afsluiting in 1989 begon hij aan een vervolgopleiding biotechnologie aan Hogenschool Larenstein te Wageningen. Na het eerste jaar van deze vervolgopleiding werd in 1990 de overstap gemaakt naar de Landbouw Universiteit te Wageningen (LUW) voor de voltijdsstudie plantenveredeling. Een eerste afstudeervak werd gedaan bij het biotechnologie bedrijf Plant Genetic Systems te Gent in België, alwaar hij zijn vuurdoop in de moleculaire biologie kreeg. Met de veredelingspraktijk werd vervolgens kennis gemaakt gedurende zijn stage bij het veredelingsbedrijf Zelder B.V. te Ottersum. In een tweede afstudeervak bij Keygene N.V. te Wageningen ontwikkelde zich de liefde voor de moleculaire genetica. Direct na zijn afstudeervak in september 1995 ging hij aan het werk bij Keygene om vervolgens, een maand later in november 1995, af te studeren in de plantenveredeling.

In januari 1996 verruilde hij zijn werk als onderzoeker binnen de R&D groep voor de functie van project leider binnen de marker assisted breeding (MAB) groep van Keygene. Tot april 2002 heeft hij daar met veel plezier gewerkt en mede vormgegeven aan de ontwikkeling van de moleculaire merker services binnen Keygene. Een nieuwe uitdaging werd gevonden bij het IPG - Institute for Pig Genetics B.V. te Beuningen alwaar hij in mei 2002 aan de slag ging als moleculair geneticus op de projecten “Nationaal project bioinformatica voor nutsdieren” en “PUP – Predictable Uniform Pork”. Het waren de werkzaamheden in het kader van deze projecten die geleidt hebben tot dit proefschrift. De projecten werden in nauwe samenwerking met en grotendeels ook op de leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit en Research Centrum (WUR) uitgevoerd. In maart 2006 werd een postdoc positie aanvaard in deze groep, alwaar hij mede vorm geeft aan het ‘imprinting onderzoek’ in het kader van het project “Detection and utilization of (non-)Mendelian genes in commercial pig populations”.

Curriculum Vitae

Henricus Johannes (Rik) van Wijk was born on March 29, 1969 in Dordrecht, The Netherlands. He grew up between plants and animals on a farm in Oud-Alblas. After secondary education in 1985 he started a training botany at the laboratory school 'Van Leeuwenhoek Institute' in Delft, The Netherlands. After completion in 1989 he started in continuation of a training biotechnology on the laboratory school 'Larenstein' in Wageningen. After the successful first year he made the transfer to the Wageningen Agricultural University (WAU) in 1990 for a study plant breeding. For a major in molecular biology he went to Plant Genetic Systems, a biotechnology company in Gent, Belgium. A stage plant breeding (small grains, maize and grass) was done at Zelder B.V., Ottersum, The Netherlands. Research for a major in molecular genetics was conducted at the biotechnology company Keygene N.V., Wageningen, The Netherlands. During this period the love for molecular genetics developed. In September 1995, directly following his major molecular genetics he was appointed as researcher at the R&D department of Keygene. A month later, in November 1995 he graduated.

In January 1996 he moved within Keygene and started as project manager within the marker assisted breeding (MAB) group. He contributed to the development of the molecular marker services group. He worked there with a lot of pleasure until April 2002. He started a new challenge in May 2002 at IPG - Institute for Pig Genetics B.V., Beuningen, The Netherlands. Appointed as molecular geneticist he worked on the projects "National bioinformatics project for farm animals" and "PUP – Predictable Uniform Pork". The work done in the framework of both projects has resulted in this thesis. Both projects were executed in close collaboration with and partly at the Breeding and Genetics group of Wageningen University and Research Center (WUR). He accepted a post-doc position within that group in March 2006 were he continued with research in the framework of the research program "Detection and utilization of (non-)Mendelian genes in commercial pig populations".

Van Wijk H.J., H. Buschbell, B. Dibbits, S.C. Liefers, B. Harlizius, H.C.M. Heuven, E.F. Knol, H. Bovenhuis, and M.A.M. Groenen (Accepted by J.Anim.Sci.) Fine mapping of QTL for pork carcass composition and meat quality on SSC4 and SSC11 using variance components.

Van Wijk H.J., S.C. Liefers, B. Dibbits, B. Harlizius, and M.A.M. Groenen (submitted to Animal Biotechnology) Sequencing porcine EST and BAC-end sequences to identify SNP markers for SSC4q

Van Wijk H.J., B. Dibbits, E.E. Baron, A.D. Brings, B. Harlizius, M.A.M. Groenen, E.F. Knol, and H. Bovenhuis (2006) Identification of quantitative trait loci for carcass composition and meat quality traits in a commercial finishing cross. J. Anim. Sci. 84: 789 - 799.

Van Wijk H.J., D.J.G. Arts, J.O. Matthews, M. Webster, B.J. Ducro, and E.F. Knol (2005) Genetic parameters for pork quality and carcass composition estimated in a commercial crossbred environment. J. Anim. Sci. 83: 324-333.

Harlizius B., **H.J. van Wijk**, and J.W.M. Merks (2004) Genomics for food safety and sustainable animal production. Journal of Biotechnology, Vol. 113/1-3: 33-42.

Heuven H.C.M., **H.J. van Wijk,** and J.A.M. van Arendonk (2003) Combining traditional breeding and genomics to improve pork quality. Outlook on Agriculture Vol.32, no.4: 235-239

Jeuken M., **R. van Wijk**, J. Peleman, and P. Lindhout (2001) An integrated interspecific AFLP map of lettuce (*Lactuca*) based on two *L. sativa* x *L. saligna* F2 populations. Theor. Appl. Genet. 103: 638-647.

Ajmone Marsan P., C. Gorni, A. Chitto, R. Redaelli, **R. van Wijk**, P. Stam, and M. Motto (2001) Identification of QTLs for grain yield and grain-related traits of maize (*Zea mays* L.) using an AFLP map, different testers, and cofactor analysis. Theor. Appl. Genet. 102: 230-243.

Ben Chaim A., I. Paran, K.C. Grube, M. Jahn, **R. van Wijk**, and J. Peleman (2001) QTL mapping of fruit-related traits in pepper (*Capsicum annuum*). Theor. Appl. Genet. 102: 1016-1028.

Johnson W.C., L.E. Jackson, O. Ochoa, **R. van Wijk**, J. Peleman, D.A. St. Clair, and R.W. Michelmore (2000) Lettuce, a shallow-rooted crop, and *Lactuca serriola*, its wild progenitor, differ at QTL determining root architecture and deep soil water exploitation. Theor. Appl. Genet. 101: 1066-1073.

Ajmone Marsan P., P. Castiglioni, M. Ferrarini, **R. van Wijk**, and M. Motto (1999) Polymorphism, distribution, and segregation of *Eco*RI and *Pst*I based AFLP markers in a molecular linkage map in maize. Maize Genetics Cooperation newsletter 73: 13.

Castiglioni P., P. Ajmone-Marsan, **R. van Wijk**, M. Motto (1999) AFLP markers in a molecular linkage map of maize: codominant scoring and linkage group distribution. Theor. Appl. Genet. 99: 425-431.

Livingstone K.D., V.K. Lackney, J.R. Blauth, **R. van Wijk**, M. Kyle Jahn (1999) Genome Mapping in *Capsicum* and the Evolution of Genome Structure in the Solanaceae. Genetics 152: 1183-1202.

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