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Epistatic effects on carcass composition and meat quality in pigs

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„Fang' an mit dem was notwendig ist,
dann mit dem, was möglich ist,
und plötzlich tust du das Unmögliche."

Franciscus de Assisi

Dedicated to my family

Meiner Familie

Epistatic effects on carcass composition and meat quality in pigs

The analysis of epistasis is not yet a routine, but it has been shown by few studies in livestock animals that interaction effects contribute with considerable proportions to the phenotypic variance. Therefore the objective of this study was to evaluate the importance of epistatic effects in the Bonn Duroc × Pietrain resource population (DuPi) for carcass composition and meat quality traits. This population was investigated so far for single quantitative trait loci (QTL) considering additive, dominance and imprinting effects.

In the first approach, 585 F_2 pigs of DuPi were used to perform a two dimensional QTL scan. All animals were genotyped using 125 genetic markers (microsatellites and SNP) spread across the 18 pig autosomes. Phenotypic information for 26 carcass composition and meat quality traits was available for all F_2 animals. Linkage analysis was performed in a two-step procedure using a maximum likelihood approach implemented in the QxPak program. A number of 56 interacting QTL was observed for 19 different traits. These interacting QTL pairs explained up to 8% of the phenotypic variance. Based on these results a variety of networks among chromosomal regions throughout the porcine genome were identified. Moreover, considering interactions between loci allowed to detect several novel QTL and trait-specific relationships of loci within and across chromosomes.

In a second step the causes of an epistatic QTL pair between *Sus scrofa* chomsosome (SSC) 8 and 15 influencing pH value 1 h *post mortem* in *M. long. dorsi* were investigated. Gene expression data was obtained from loin tissue of 74 F_2 which were selected from 585 animals. Gene expression profiles, genotypes and phenotypes of these pigs were investigated jointly applying three alternative models. Method A considered the phenotypic differences in pH values between groups of pigs with extreme values. Method B was based on differences between the genotype combinations of relevant epistatic QTL pairs between SSC8 and SSC15. Finally, method C was a linear model comprising the epistatic QTL genotypes as fixed effects. Overall method A, B and C revealed 1182, 480 and 1823 differentially expressed or associated genes, respectively. By means of a functional analysis it was possible to set up networks which contained mainly interactions between genes located within the specific regions on SSC8 and SSC15 and allowed a meaningful biological discussion. Expression QTL (eQTL) analyses were performed for functional and positional transcripts in order to assume regulations patterns. This approach showed that combining phenotype, genotype and transcriptome data helped to uncover the involved molecules of observed epistasis.

In conclusion, this study revealed the importance of epistasis for the expression of complex traits. Furthermore, it was possible to uncover potential biological causes of observed epistatic QTL pairs applying different statistical models as well as bioinformatic tools.

Epistatische Effekte auf die Schlachtkörperzusammensetzung und Fleischqualität beim Schwein

Epistasie wird bisher nur selten in Untersuchungen komplexer Merkmale berücksichtigt. Dabei wurde bereits in einer Vielzahl von Studien gezeigt, dass die zu beobachtenden Variationen von quantitativen Merkmalen nicht alleine durch additive Effekte erklärt werden können. Daher war das Ziel dieser Studie, die Bedeutung von epistatischen Effekten auf Schlachtkörper- und Fleischqualitätsmerkmale innerhalb der Bonner Duroc × Piétain Ressourcenpopulation (DuPi) zu untersuchen. Bisherige Studien in der DuPi Population berücksichtigten nur einfache Quantitative Trait Loci (QTL), die additive, Dominanz oder Imprintingeffekte beinhalteten.

In der ersten Analyse wurden 585 Schweine der F_2-Generation verwendet um epistatische QTL Paare zu identifizieren. Diese Tiere sind mit 125 genetischen Markern genotypisiert worden, die sich gleichmäßig über alle 18 Autosomen verteilten. Als phänotypische Informationen wurden 26 verschiedene Schlachtkörper- und Fleischqualitätsmerkmale erfasst. Die Koppelungsanalyse wurde in einer zweistufigen Prozedur innerhalb des Programms Qxpak, basierend auf einem Maximum Likelihood Ansatzes, durchgeführt. Insgesamt konnten 56 interagierende QTL für 19 verschiedene Merkmale beobachtet werden. Für Schlachtkörpermerkmale konnten 17 und für Fleischqualitätsmerkmale 39 epistatische QTL Paare identifiziert werden. Diese interagierenden QTL Paare erklärten bis zu 8% der phänotypischen Varianz. Auf Grundlage dieser Ergebnisse konnten verschiedene Netzwerkstrukturen zwischen den verschiedenen Chromosomensegmenten identifiziert werden. Die Berücksichtigung der Beziehung zwischen zwei Genorten ermöglichte es einige neue QTL zu identifizieren, sowie merkmalsbezogene Beziehungen innerhalb eines Chromosoms und zwischen Chromosomen zu charakterisieren.

In einer zweiten Untersuchung wurde versucht, die biologischen Gründe des epistatischen QTL Paares zwischen den porcinen Chromosomen (SSC) 8 und 15 aufzuklären. Für die Analyse standen die Muskeltranskriptionsprofile von 74 ausgewählten F_2 Tieren der DuPi Population zur Verfügung. Die Interaktion zwischen SSC8 und 15 war assoziiert mit früh *post mortalem* pH Wert im *M. long. dorsi*. Genexpressionsprofile, Genotypen und Phänotypen dieser Tiere wurden mit drei verschiedenen statistischen Ansätzen und Modellen untersucht. Methode A berücksichtigte phänotypische Unterschiede des pH Wertes zwischen zwei Tiergruppen mit extremen Werten, Methode B basierte auf den Unterschieden zwischen den Genotypgruppen des relevanten epistatischen QTL Paares und Methode C berücksichtigte die Genotypen des epistatischen QTL Paares als fixen Effekt innerhalb eines linearen Modells. Insgesamt ließen sich mit Methode A, B und C 1182 und 480 unterschiedlich exprimierte Gene sowie 1823 linear assoziierte Gene identifizieren. Durch funktionale Analysen war es möglich Netzwerke zu erstellen, die nur Gene beinhalteten, die innerhalb der epistatischen Regionen lagen. Die daraus erzielten Ergebnisse erlaubten eine biologisch sinnvolle Diskussion möglicher Kandidatengene der epistatischen Regionen. Des Weiteren wurden Expressions-QTL Analysen durchgeführt um eine Aussage über die Genregulation zu treffen.

Schlussfolgernd konnte gezeigt werden, dass Epistasie eine bedeutende Rolle bei der Ausprägung von komplexen Merkmalen beim Schwein hat. Es war des Weiteren möglich biologische Ursachen beobachteter epistatischer Beziehungen mit Hilfe verschiedener statistischer Methoden zu identifizieren.

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List of abreviations

a	:	additive genetic effects
ACADL	:	*acyl-CoA dehydrogenase, long chain*
ACSL3	:	*acyl-CoA synthetase long-chain family member 3*
ASIP	:	*Agouti-signaling protein*
ATP5A1	:	*ATP synthase, H+ transporting, mitochondrial F1 complex subunit 1*
ATP5B	:	*ATP synthase, H+ transporting, mitochondrial F1 complex*
BFT	:	back fat
CAST	:	*Calpastatin*
cond.	:	conductivity
cont.	:	continued
d	:	dominance
DNA	:	deoxyribonucleic acid
DuPi	:	Duroc × Pietrain resource population
e.g.	:	exempli gratia
EBLC	:	estimated belly lean content
ECLC	:	estimated carcass lean content
eQTL	:	expression quantitative trait loci
ETFDH	:	*electron-transferring flavoprotein dehydrogenase*
FAM114A1	:	*family with sequence similarity 114, member A1*
FDR	:	false discovery rate
FN1	:	*fibronetin*
FS	:	full sibs
GM2A	:	*GM2 activator protein*
HS	:	half sibs
HSA	:	*Homo sapiens* chromosome

IGF-2	:	*insulin-like growth factor 2*
IMF	:	intra muscular fat content
ITGAV	:	*integrin, alpha V*
LRT	:	likelihood ratio test
M. long. dorsi	:	*Musculus longissimus dorsi*
MAS	:	marker assisted selection
Mc1r	:	*melanocortin receptor*
MC4R	:	*melanocortin-4 receptor*
MCMC	:	Markov chain Monte Carlo
MHS	:	malignant hyperthermia syndrome
MSTN	:	*porcine myostatin*
MYBPC1	:	*myosin binding protein C*
MYH6	:	*myosin, heavy chain 6*
MYH7	:	*myosin, heavy chain 7*
n.n.	:	not named
P	:	parental
pH1	:	pH 45 min post mortem in *M. long. dorsi*
PNAS-5	:	*Sus scrofa apoptosis related protein*
PRKAG3	:	*protein kinase, AMP-activated, gamma 3 non-catalytic subunit*
PSE	:	pale soft and exudative
QTL	:	quantitative trait loci
RNA	:	ribonucleic acid
RSE	:	reddish soft and exudative
RYR1	:	*ryanodine receptor 1*
SD	:	standard deviation
SNP	:	single nucleotide polymorphism

SPP1	:	*osteopontin*
SSC	:	*Sus scrofa* chromosome
TGF-β3	:	*transforming growth factor beta-3*
TPM1	:	*alpha-tropomyosin 1*
TPM4	:	*tropomyosin 4*

Chapter 1. General introduction

In livestock species many important economic traits are characterized by a complex (multifactorial) inheritance (Andersson 2007, Andersson and Georges 2004). Several environmental factors and numerous of genes make it hardly possible to clarify even simple biological mechanisms controlling the expression of traits due to incomplete penetrance and phenocopy, genetic heterogeneity, high frequency of a causing allele and other transmission factors (Lander and Schork 1994). Such kind of polygenic foundation might be the norm rather than the exception (Templeton 2000, Wade 2001, Wolf 1997).

Because of many genes are responsible for the expression of a trait, it is surprising that until now many studies revealed numerous of single quantitative trait loci (QTL) to identify promising regions influencing complex traits. As an example, 6432 QTL, representing 594 different traits, have been detected in pigs (Pig QTL db - state: January 2012; Hu *et al.* 2010). However, only a few candidate genes, explaining major proportion of the phenotypic variance, have been identified in pigs underlying these QTL e.g. malignant hyperthermia (RYR1) and glycogen content in skeletal muscle (PRKAG3) (Fujii et al. 1991, Milan et al. 2000).

The identification of the underlying genes and their mutations of a QTL is an analytical challenge because of the inconsistency of QTL across populations, the effect size of a QTL as well as large confidence intervals (Wade 2001, Weller 2001). The detection of a QTL and its effects depends strongly on chosen or designed populations (e.g. commercial population, experimental F_2 or back cross population) and the number of individuals which determines the segregations of the QTL alleles or the recombination frequencies (Mackay *et al.* 2009). Especially QTL with large effects are hardly to find in commercial populations, because these populations underlie selection strategies for particular traits (Georges 2007). Additionally, many QTL located throughout the genome might not be identified through their small effects (Andersson and Georges 2004, Steinmetz et al. 2002). Although many QTL with large and small effects have been identified in commercial and experimental populations, these regions only explain a fraction of the genetic variance and might be overestimated in most of the cases (Georges 2007). Therefore it is necessary to focus the analysis not only on one locus but also to take the relationship between loci into account. The interaction between genes or loci is generally known as epistasis (gene interaction).

Until now epistasis has not been investigated in livestock commonly. In a study from Carlborg et al. (2006) a considerable amount of the phenotypic variation was uncovered by

modeling first-order gene-by-gene interactions in a cross of chicken that were divergently selected for body weight over 41 generations. The detection of these epistatic effects was strongly dependent on selection of the specific chicken line.

Selection, genetic drift and population bottlenecks are all tools of evolution. Many theoretical findings indicated that the presence and absence of epistasis is relevant for many evolutionary processes. Genetic diversity arise and was developed based on the evolutionary definition of the gene (Fenster *et al.* 1997). The genetic divergences between individuals and organisms leaded to population specification, development of sexual reproduction and many other evolutionary phenomena (Phillips 2008). Furthermore it has been shown that mechanisms of epistasis leaded to an improved fitness and adaptations to unfavorable environmental conditions as well as the ability to buffer a phenotype against the effects of mutations (Remold and Lenski 2004, Segre et al. 2005, Whitlock et al. 1995).

1.1. Genetic loci effects

A gene or a locus within the genome can affect a phenotype in several ways. Figure 1 gives an overview about the most common genetic effects. Observed variation in the phenotype based substantially on a polymorphic character of the alleles within several loci. In a single locus case intralocus effects, like additive genetic effects or dominance can be observed. A third mechanism is imprinting where also the origin of an allele is influencing the expression of a trait.

Expanding the model from one single locus to a multilocus case can be performed without any further development by assuming random mating and independent segregation of loci (Falconer and Mackay 1996). In the situation of a pair of loci or even more lead to the appearance of interacting interlocus effects (epistasis). The understanding of epistasis can be divided into two different aspects: statistical and functional epistasis. The following chapters will focus on intralocus effects (chapter 1.2) and interlocus effects (chapter 1.3). Interlocus genetic effects will be described further in the aspects of statistical epistasis (chapter 1.3.1), functional epistasis (chapter 1.3.2) and the relationship between statistical and functional epistasis (chapter 1.3.3). For all interacting phenomena examples of livestock breeding will be given. Furthermore the effects of pleiotropy will be described in the situation of epistasis in chapter 1.4.

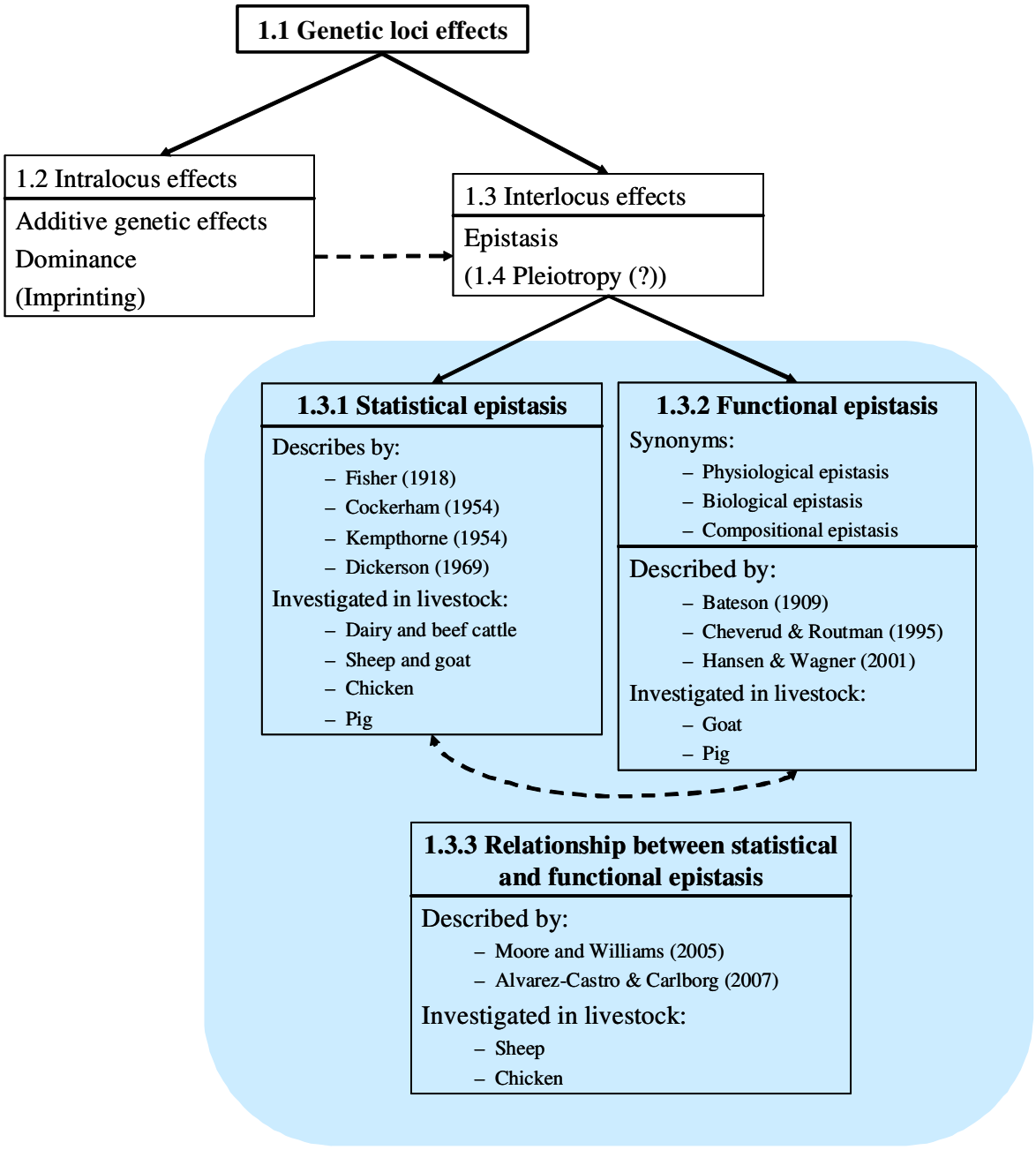

Figure 1: Aspects and definitions of intra- and interlocus genetic effects

1.2. Intralocus effects – additivity, dominance and imprinting

Additive (a) and dominance (d) are the most common effects considered in genetical studies. Additive genetic effects are joint effects of alleles which are summed up by addition (Falconer and Mackay 1996). The homozygote genotypes have an opposite value and the heterozygote genotype intermediates between them (figure 2). One exception is overdominance, where the effect of the heterozygote genotype is larger or smaller than the effect of one of the homozygote genotypes.

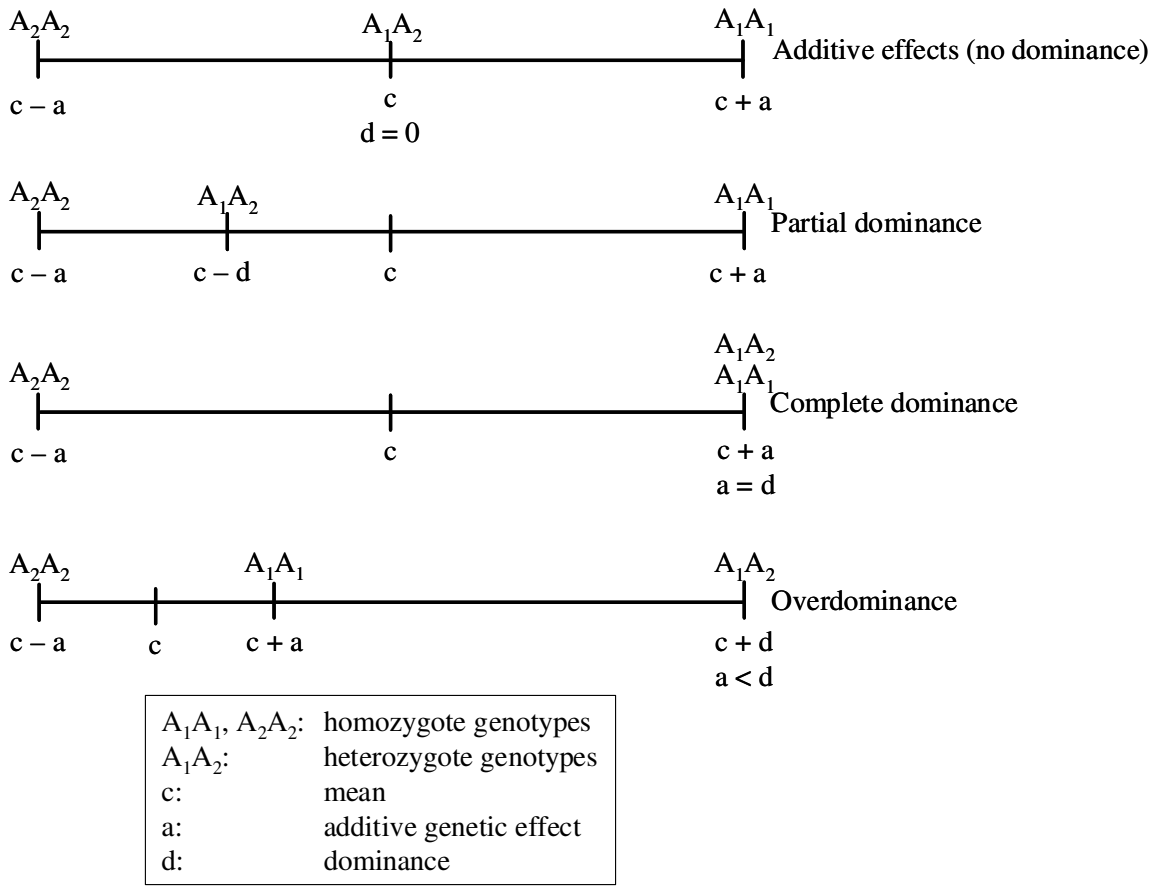

Figure 2: Different pattern of dominance (adapted from Falconer and Mackay (1996))

Dominance can be described as intralocus or within-locus interaction (Falconer and Mackay 1996, Omholt et al. 2000), where the heterozygote genotype results in a different phenotype than the mean of both homozygote genotypes of one loci. Generally it can be differentiated between statistical and physiological dominance (Cheverud and Routman 1995, Falconer and Mackay 1996). Statistical dominance is a population based phenomenon. The effects of dominance are described as the deviation from the single-locus additive effects (Cheverud and Routman 1995, Crow and Kimura 1970). Physiological dominance describes the phenotype of an heterozygote individual and can be characterized by three different forms (Wright 1968): partial dominance, complete dominance and overdomiance (figure 2). Dominance is involved in the expression of complex traits like carcass composition and meat quality in pigs (Andersson-Eklund et al. 1998, de Koning et al. 2001, Liu et al. 2007). Especially overdominance has been discussed in plants to be involved in selection resulting in heterosis and inbreeding depression (Charlesworth and Willis 2009, Li et al. 2008). Regarding gene regulation, dominance is an important factor. Ohmholt et al. (2000) has shown that in the situation of

positive autoregulation, additive gene action is more prevalent, whereas during negative autoregulation dominance is the rule. Dominance is also termed intralocus interaction. This is in many cases not precise enough, because many other phenotype patterns are attributed to dominance and are not influenced by intralocus regulatory mechanisms (Omholt et al. 2000).

A special type of genetic effects is imprinting, also known as parent-of-origin depending effects. Genes or genetic materials are differentially expressed depending whether the information was inherited from the mother or the father (Hall 1997, Walter and Paulsen 2003). Imprinting belongs to the phenomena of epigenetic where beside many different mechanisms DNA methylation is one of the major causes. In general the imprinted copy of a gene is considered to be silent depending on the gender of the parents it belongs to (Bartolomei and Tilghman 1997). Furthermore the distinction between genomic imprinting and maternal effects allows to investigate the mechanisms of the genetic basis and to understand underlying evolutionary processes (Hager et al. 2008, Santure and Spencer 2006). One best investigated imprinted gene in livestock is *insulin-like growth factor 2* (IGF-2). It has been shown in pig that this genomic region is paternally imprinted regarding muscularity and backfat thickness (de Koning et al. 2000, Nezer et al. 1999). The role of imprinted genes within a network and potential epistatic effects have only partially investigated (Stinckens et al. 2009).

1.3. Interlocus effects – epistasis

The first introduction of the term epistasis was given by Bateson (1909). He described epistasis as the divergence between the prediction of segregation ratios based on the action of individual genes and the observed phenotype of a dihybrid cross (Phillips 1998). Fisher (1918) defined epistasis on the basis of a statistical background and described epistasis or ‘epistacy’ as the combination of two Mendelian factors which result in nine genotypes and cannot be clearly explained by biological reasons.

In the last decades the term epistasis has been used by geneticists to discuss different aspects of interlocus gene interaction. Therefore it is necessary to differentiate between statistical epistasis and functional epistasis (Alvarez-Castro and Carlborg 2007, Cheverud and Routman 1995).

1.3.1. Statistical epistasis

Statistical epistasis describes a population based phenomenon (Falconer and Mackay 1996, Fisher 1918). This kind of epistasis between alleles is strongly dependent on the allele frequencies within a population. If the allele frequencies have changed, the absence of currently observed epistatic genetic variation does not mean that real gene interaction does not exist in the investigated population (Crow and Kimura 1970). Fisher (1918) was mainly interested in analyzing the correlation between relatives and the response to selection and in the analysis epistasis was included as noise term in an additive genetic model. Moreover, the approach was not extended to estimate all effects of the epistatic components (Hansen and Wagner 2001b).

Cockerham (1954) and Kempthorne (1954) calculated the effects of epistasis including an interaction term into a regression model on allelic effects. In figue 3 a two-locus model is presented where four alleles (two per locus) are considered as the statistical factors.

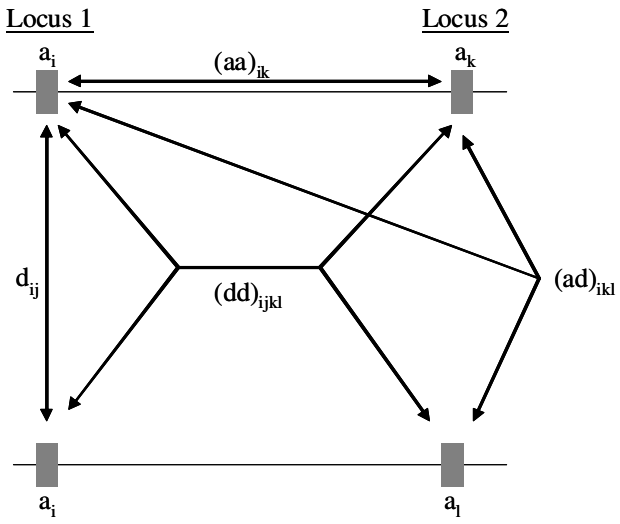

Figure 3: Two-locus gene model from Kempthorne (1957)

Based on this model, additive effects are the sum of the four alleles and dominance appears within one locus as intra-locus interaction. Finally epistasis is presented as additive × additive, additive × dominance and dominance × dominance effects, describing the interaction between alleles of the two loci (Grosshans et al. 1994, Kempthorne 1957). These four component of epistasis have simple interpretations: additive × dominance indicates that the additive effect of locus 1 depends of the genotype of locus 2, whereas the dominance of locus 2 depends on the genotype of locus 1 (Cheverud 2000). Beside the

relationship between a pair of genes also higher dimensional epistasis among three and more genes were examined (Cockerham 1954, Kempthorne 1954). Additional information can be found in chapter 2 (pp. 20), where a regression model comprising epistatic components were applied to data of a porcine resource population.

First investigations of statistical epistasis were related to the estimation of heterosis following the ideas of Dickerson (1969, 1973) and Kinghorn (1980, 1983). Dickerson (1969, 1973) developed a genetic model considering epistasis as loss of favorable genetic interactions within gametes. This kind of recombination (epistatic) loss due to non-allelic interactions did not cover the additive and dominance of these effects (Distl et al. 1990). Kinghorn (1980, 1983) implemented epistasis in a genetic model based on additive × additive interactions, and additionally, heterosis was comprised by dominance effects. Other investigations have shown that in the case of significant epistatic effects, simple dominance models for heterosis cannot always adequately predict the performance of advanced generations of crosses (Bidanel 1993). Therefore, Hill (1982) and Mather and Jinks (1982) suggested to implement all epistatic components, because it allows a more detailed estimation of heterosis and also of epistasis.

1.3.1.1. Statistical epistasis investigated in livestock breeding

Epistasis has been first investigated in livestock animals according to heterosis. Sheridan (1981) reviewed differences in the performance between the F_1 and several following generations in poultry, pigs, dairy cattle, beef cattle and sheep and investigated heterosis under the hypothesis of dominance and “parental epistasis”. The aspects of heterosis and observed differences between F_1-generations, F_2-crosses, backcrosses and rotation-crosses has been mainly discussed related to the loss of recombination (epistatic loss) or as recombination effects (Dickerson 1969, Kinghorn 1980, 1983), because favorable epistatic relationships established in the pure breeds break down through crossing (Kinghorn 1980). Except in some dairy cattle population studies, epistasis has been investigated by means in cross bred animals.

In three different studies in dairy cattle, epistasis was displayed as heritability of additive by additive genetic effects for milk performance (Allaire and Henderson 1965, Fuerst and Solkner 1994) and reproductive traits (Hoeschele 1991). In addition to cross bred cows of Simmental, Fuerst and Soelkner (1994) investigated pure bred cows of Simmental, Swiss

Brown and Braunvieh. The heritablility of additive × additive effects for milk yield, fat and protein percentage and calving interval ranged between 0.04 - 0.37, 0 - 0.02 and 0.03 - 0.07, respectively. This is mostly in accordance with other reported studies where also negative values were reported for fat percentage.

In dual purpose cattle, beef cattle and different cross bred populations estimations of epistatic loss have been performed for calf performance traits (Arthur et al. 1999, Koch et al. 1985, Roso et al. 2005). The estimated effects of epistatic loss were small in all studies and only partially significant. Koch et al. (1985) evaluated epistatic loss effects on weaning gain of Angus × Herford crosses. No significant epistatic effects were detected because only a few numbers of records of cross bred animals were available. Furthermore Arthur et al. (1999) investigated epistasis × environmental interactions in a cross of Brahman and Herford under subtropical and temperate climate conditions in Australia. Significant direct and maternal effects were observed only under subtropical conditions. Hirooka et al. (1997) analyzed carcass traits of pure bred cattle and cross bred animals of dairy, dual purpose and beef cattle. Epistatic loss was evaluated using the model of Dickerson (1969, 1973) as well as the model of Kinghorn (1980, 1983). Significant effects were mainly detected for carcass weight in comparison to fat covering score and fleshiness score depending on the applied model.

An extended model, comprising all epistatic components, was applied by Grosshans et al. (1994) to milk yield performance data of Jersey and German black pied dairy cattle as well as Holstein Friesian three breed crosses. The author clearly showed the importance of considering epistasis in the model to estimate heterosis. Furthermore the additive × additive interaction was most meaningful compared to the other interactions like additive × dominance or dominance × dominance, because the standard error and the accuracy increased with the number of considered components (Grosshans et al. 1994).

The estimations and studies presented so far based on only recorded phenotypic information. Only limited numbers of studies in cattle have analyzed genetic data as well. An epistatic QTL pair was identified for milk fat yield on *Bos taurus* chromosome 6 in a Holstein Friesian population (Freyer et al. 2003). Bardense et al. (2007) studied the epistatic relationships between genes of the calpain family in different beef cattle breeds. A total of seven significant interactions among SNPs have been identified between *calpain 1* and *calpastatin* which had a major impact on meat tenderness.

In sheep epistasis has been described according to differences between cross bred generations or to interpret heterosis (Fogarty et al. 1984, Nitter 1978, Sheridan 1981). First estimations of recombination effects have been given by Rastogi et al. (1982) for lamb growth. The average recombination effects for this trait were small but the proportion regarding to heterosis were 5.7% for average daily gain from birth to weaning and 4.6% for weaning weight in a three-breed cross. Furthermore, individual heterosis was very low or even negative. Boujenane et al. (1991a, 1991b) investigated litter size, ewe productivity and growth performance in a cross of D'man and Sardi breeds. In general, the effects of epistatic loss were negative and not significant, but in accordance to Rastogi et al. (1982) growth performance traits revealed significant epistatic recombination effects on lamb survival. However, no epistatic loss effect was observed in a study related to wool traits in crosses of Merino and Corriedale (Malik and Singh 2006). In goat similar observations have been made for body weights and average daily gain (Mugambi et al. 2007).

Studies in chicken investigating heterosis were mainly performed in order to increase growth performance or egg production using specific selection strategies for back cross systems. Sheridan (1986) observed that heterosis in egg production and total egg mass disappear in the F_2 and backcross generation of White Leghorn and Australorp. The author concluded that one of the causes might be epistasis. Similar observations were made by Omeje and Nwosu (1988) who investigated crosses of Nigerian local and gold link exotic chicken. Body weight heterosis was reduced by 10% in the F_2 or back cross generations compared to the F_1-generation, whereas for egg production parameters heterosis was much higher in one of the backcross generations. This kind of positional effect of one breed is a clear indicator of epistasis and that it contribute to heterosis (Jakubec and Hyanek 1982, Kinghorn 1980).

Interactions between QTL have been investigated by Carlborg et al. (2004a, 2006, 2003) in three different F_2 populations in chicken according to growth performance traits. These studies revealed that epistasis explained up to 34% of the phenotypic variation (Carlborg et al. 2004a) and contributed from 15% to 80% to the genetic variation (Carlborg et al. 2003). In a different approach of a F_2 cross of high and low performing lines generated from a bidirectional long term selection experiment, it was possible to set up an epistatic network, centralizing one region on chromosome 7. Within a single QTL analysis this region only revealed marginal effects on body weight, whereas other interacting regions revealed significantly larger effects on the individual level.

Epistasis in pigs has been considered as recombination effects and as components of heterosis. Identification of recombination effects was performed by Baas et al. (1992a, 1992b) who investigated crosses of Hampshire and Landrace. Significant recombination effects were determined for carcass length and back fat thickness, but not in growth or maternal traits. Different findings have been obtained by Cassady et al. (2002a, 2002b) who analyzed two different data sets comprising different breeds and their crosses of maternal lines and paternal lines, respectively. Recombination effects were determined as single effect as well as net effect with maternal heterosis, because high negative sampling correlations between recombination effect and maternal heterosis have been observed. Significant recombination effects were observed for growth, carcass and reproductive traits, but the findings depended on the particular dataset e.g. more significant recombination effects were detected in the paternal crosses for growth and carcass traits whereas for reproductive traits significant effects were identified in the crosses of the maternal lines (Cassady et al. 2002a, 2002b). A different model comprising epistasis as component of heterosis was performed by Bidanel (1993) in a Large White and Meishan cross. In this study additive × additive and dominance × dominance effects were observed for sow performance during birth and rearing.

Studies containing molecular marker to investigate epistasis are more common in pigs than in other livestock animals (table 1, p. 12). QTL × QTL interactions have been investigated in several traits and populations. In table 1 most of the performed studies are displayed and it can be seen that crosses of different breeds have been used to investigate particular traits. Depending on different traits, numbers of epistatic QTL pairs were detected. Only few studies also declare the proportion of the phenotypic variance explained by the particular QTL pair. Wei et al. (2010a) investigated organ weights and body dimension parameter and observed phenotypic variances through epistasis between 0.7 and 2.1 %. In comparison to other studies these values are really low (table 1). Reasons for this might be the population size, because in small populations interacting QTL regions with larger effects can be detected, whereas a higher number of F_2 animals allows the identification of marginal effects (Wei et al. 2010a, 2010b). In general all 18 autosomes have been investigated according to epistasis, except in the studies of Rodriguez et al. (2005) and Duthie et al. (2010, 2011a, 2011b). Rodriguez et al. (2005) performed an epistatic QTL analysis only between chromosomes that contained previously identified single QTL.

Table 1: Previous analysis for the evidence of QTL x QTL interaction in pigs

Population	No. of F_2 animals	Group of traits	Number of traits	No. of epi. QTL pairs	Var[1]	Reference
Iberian × Landrace	369	Meat quality traits	8	4	n. n.	Olivio et al. (2002)
Iberian × Landrace	321	Growth and carcass traits	19	12	n. n.	Varona et al. (2002)
Chinese Meishan × Dutch pig	1181	Coat color	7	9	n.n.	Hirooka et al. (2002)
Iberian × Meishan	272	Teat number	1	3	n. n.	Rodriguez et al. (2005)[a]
Iberian × Landrace	321	Muscle fiber traits	8	10	n. n.	Estelle et al. (2008)
Duroc × Meishan	166	Fatty acid composition	7	5	n. n.	Uemoto et al. (2009)
Iberian × Meishan	255	Prolificacy traits	2	12	3.1 – 4.0	Noguera el al. (2009)
Pietrain × (Leicoma × (Landrace × Large White))	315	Carcass traits	39	24	5.8 – 10.2	Duthie et al. (2010, 2011a, 2011b)[b]
		Growth and body composition	33	23	5.0 – 8.4	
		Meat quality	6	9	5.7 – 10.9	
White Duroc × Erhualian	1912	Body dimension and organ weights	17	14	0.7 – 2.1	Wei et al. (2010a)

[1] proportion of the phenotypic variation explained by the epistatic components
[a] epistatic QTL analysis based on three chromosomes where previously single QTL were identified
[b] epistatic QTL analysis based on ten autosomes

The investigations of Duthie et al. (2010, 2011a, 2011b) based on a subset of ten autosomes which led to the assumption that probably many epistatic QTL pairs are not yet detected. Furthermore, there was no clear superiority of any of the epistatic effects, but in several studies interaction effects containing dominance seemed to be more prevalent than others (Duthie et al. 2011b, Noguera et al. 2009, Ovilo et al. 2002, Uemoto et al. 2009, Varona et al. 2002)

Investigations to clarify the underling biological reasons for such interactions are, as shown in other livestock species, rare. Fernandez-Rodriguez et al. (2010) explored

candidate genes within the epistatic QTL pair identified by Noguera et al. (2009). It was possible to confirm the epistatic QTL pair using additional genetic marker. However, within an association analysis no significant interaction between identified SNP of the candidate genes was observed.

1.3.2. Functional epistasis

Functional, physiological or biological epistasis is the situation where an allele at one locus mask the effect of another second locus (Cheverud and Routman 1995, Hansen and Wagner 2001b, Moore and Williams 2005). In general, all these designations based on the observation and definition of epistasis was described by Batson (1909) who investigated genes on the level of an organism rather than on the population level (Phillips 1998). On a molecular level such interactions occur at several levels, from the interaction of transcription factors with each other and/or promotor sequence variation to the interaction of an enzyme through a pathway (Moore and Williams 2005, Phillips 2008, Wade 2001).

An example, how to understand the generic phenomena of additivity, dominance and epistasis were given by Omholt et al. (2000) in a simplified model, created for a diploid regulatory interaction structure (figure 4).

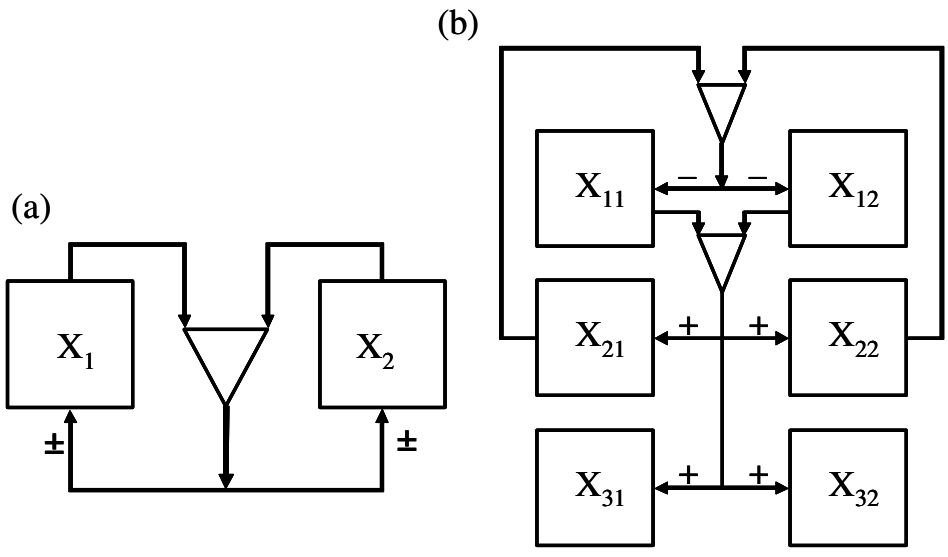

Figure 4: Gene interaction models. (a) intralocus interaction; (b) interlocus interaction – epistasis (adapted from Omholt et al. (2000))

In figure 4a intralocus interaction is displayed. The boxes X_1 and X_2 represents the two alleles of a gene X and the triangle stands for the gene product concentrations of X_1 and X_2 from the allele 1 and 2. The total sum of the transcript concentrations regulated the gene activity by binding to the regulatory regions of X. The regulation can be negative as well as positive. Figure 4b represents the situation of epistasis. The gene product of gene X_2 interacts through a negative feedback loop on gene X_1. X_1 positively regulates the genes X_2 and X_3, so that the total sum of the gene products of X_{21} and X_{22} are regulating the activities of three genes. All loci have to be polymorphic in this model (Omholt et al. 2000).

Hansen and Wagner (2001a) preferred the term functional epistasis, which is defined as an effect of genetic substitution that depends on the genetic background. The functionality of epistasis arises from the functional properties if the gene interactions in determining the expression of a trait (Alvarez-Castro and Carlborg 2007, Hansen and Wagner 2001b). According to Cheverud and Routman (1995) and Hansen and Wagner (2001b) functional epistasis is an extension of physiological epistasis. Based on different genotypic values at one locus, the phenotype depends on the genotype presented at a second locus. Hansen and Wagner (2001b) argued that interactions among genes arise by mechanisms which are not necessarily physiological, because “a nonlinear fitness function leads to functional epistasis for fitness without any necessary interaction among genes affecting the underling character” (Hansen and Wagner 2001b).

Comparable argumentations have been given by Phillips (2008) who stated that the term functional epistasis addresses molecular interaction rather than genetic interactions. For the characterization following the idea of Bateson (1909), epistasis as a consequence of allelic substitution, Phillips (2008) suggested the term ‘compositional epistasis’.

Another term, biological epistasis, has been defined by Moore and Williams (2005) who refer to physical interactions among proteins or molecules that affect the phenotype (Moore 2005, Moore and Williams 2005). This term can be understood as a holistic view on interactions within an organism, where besides the simple interaction between two genes, also the interaction between transcripts, proteins, metabolites and phenotypes are considered. Additionally genetical epistasis, as part of biological epistasis, describes only the interaction among DNA sequence variations which can be seen comparable to functional epistasis (Moore 2005).

However, it is until now not possible to differentiate between the genetical occurring interactions and molecular interactions between transcriptional factors and their mechanisms on the level of an individual (Moore and Williams 2005). Therefore it is necessary to clarify the relationship between statistical and functional epistasis to be able to trace back observed genetic effects and possible consequences of molecular interactions on phenotypic variation. This relationship will be described in chapter 1.3.3 'From statistical to functional epistasis'.

1.3.2.1. Functional epistasis in livestock breeding

In goats, molecular laboratory work has been performed related to coat colors. In two studies it was possible to identify mutations in *melanocortin1 receptor* (Mc1r) and *Agouti-signaling protein* (ASIP) which leaded to different coat color (Fontanesi et al. 2009a, 2009b, Tang et al. 2008).

A different approach was performed by Stinckens et al. (2009). They investigated SNPs and gene expression profiles of porcine *myostatin* (MSTN), *ryanodine receptor* (RYR1), *insulin-like growth factor 2* (IGF-2) and *melanocortin-4 receptor* (MC4R). It was possible to show in different populations that the IGF-2 gene expression level depended on the RYR1 genotype and that the ratio of IGF-2 and MSTN seemed to play a central role in skeletal muscle and heart growth (Stinckens et al. 2009).

1.3.3. From statistical to functional epistasis

Previously the two definitions of epistasis have been described. The relationship between the two theories is important in order to verify biological explanations for interactions as well as potential consequences for observable phenotypes, gene expression patterns and regulatory pathways. Several studies tried to model statistically functional epistasis focused on allele substitution effects (Barton and Turelli 2004, Cheverud and Routman 1995, Hansen and Wagner 2001b), but a direct link between statistical and functional epistasis was only partially realizable. Alvarez-Castro and Carlborg (2007) developed a natural and orthogonal interaction model which allowed transforming functional genetic effects to statistical genetic effects. In order to investigate the relationship of statistical epistasis and functional dependency Gjuvsland et al. (2007) treated gene expression values

as phenotypes. Based on a simulation study several different three-locus motifs of gene regulatory networks were developed and dependencies among them were investigated. The authors were able to show that the observed statistical epistasis which depended on the regulatory structure varied widely if the regulatory structures were affected by genetic polymorphisms (Gjuvsland et al. 2007).

The idea of combining phenotypic, genotypic and gene expression data has been termed "genetical genomics" and helps to characterize different patterns of gene regulation using expression QTL (eQTL) analysis (Jansen and Nap 2001). Detected eQTL can be classified into a locus which is close located to a gene that is being controlled (*cis*-acting) or one or more loci which are located far from the actual gene that is being controlled (*trans*-acting). In the situation of epistasis, these effects have been described as *trans*-regulating effects of more than one loci (Jansen and Nap 2001, Rosa et al. 2006).

A holistic understanding of the different theories has been described by Moore and Williams (2005) related to mendelian traits. In addition to statistical and functional (biological) epistasis the authors implemented genetical epistasis as well. Figure 5 describes the relationship between statistical epistasis and biological epistasis.

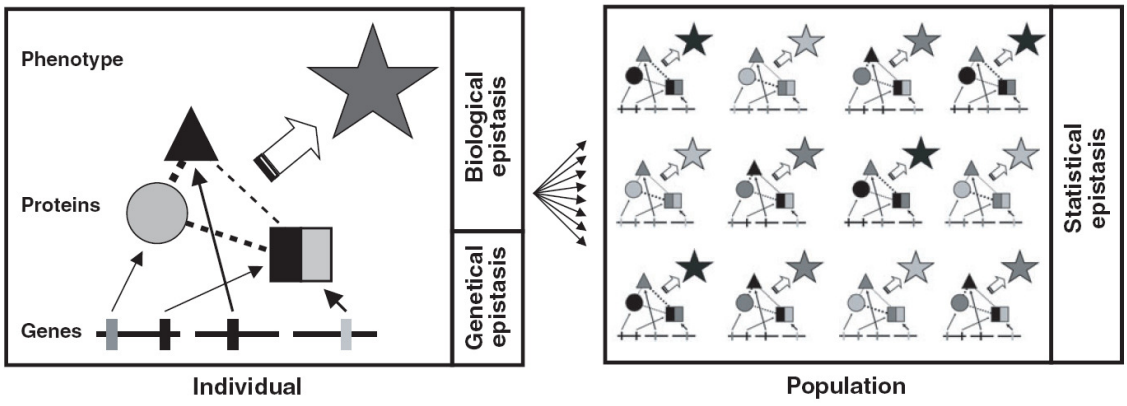

Figure 5: The conceptual relationship between statistical and biological (functional) epistasis, extended by genetical epistasis (Moore and Williams 2005)

In the situation of biological epistasis (figure 5, left side) the genetic information has an influence on the phenotype through a hierarchy of proteins that are involved in biological processes ranging from transcription to physiological homeostasis (Moore and Williams 2005). Genetical epistasis can be understood as an element of biological epistasis and describes a situation where the interaction among DNA variation leads to a particular phenotype in an individual (Moore 2005). Moreover it is often not possible to differentiate

between biological and genetical epistasis. Genetical epistasis links biological and statistical epistasis (figure 5, right side), so that changes of biological epistasis among individuals in a population leads finally to statistical epistasis (Moore 2005, Moore and Williams 2005). In the situation of quantitative traits, genetical epistasis is defined as deviation of additive effects, because masking or repression of one locus by another one lead to a continuous distributed phenotype (Lynch and Walsh 1998).

The integration of different sources of data like phenotypes, genotypes, gene expression profiles leads to approaches of system biology. It allows a holistic view on regulatory networks and pathways which links different biological processes and uncover the causes of epistasis related to gene regulation (Aylor and Zeng 2008, Moore 2005)

Further investigations how to combine statistical and physiological epistasis can be found in chapter 3 (pp. 48), where previous identified epistatic QTL have been combined with whole genome expression data to elucidate the biological causes of epistatic interactions.

1.3.3.1. Approaches in livestock to investigate the link between statistical and functional epistasis

Until now molecular investigations comprising gene-by-gene interaction or epistatic effects of genomic regions are rare in livestock animals. Many times epistasis is used as explanation if QTL could not be detected through masking (Crawford et al. 2006, Gratten et al. 2010). A holistic approach has been performed by Garcia-Gamez (2011) in Merino sheep to study pigmentation. Results of a genome-wide association study were combined with gene expression data and promoter sequence analyses in order to set up regulatory epistatic networks and to identify candidate genes of piebalds in sheep (Garcia-Gamez et al. 2011).

Le Rouzic et al. (2008) extended the epistatic QTL analysis estimating the effects of allele substitution in order to translate the genetic effects into a functional meaning. The authors were able to show in a cross of red jungle fowl and white leghorn that much epistasis detected for single traits resulted from a temporary change of the genetic effects of loci. These loci contribute to interactions with a specific genetic background. Le Rouzic et al. (2008) explained these observations by the different physiological stage of the two chicken breeds and the epistasis appears due to physiological rather than molecular interactions in growth traits.

1.4. The relation between pleiotropy and epistasis

Pleiotropic effects can be observed for loci which are significant associated with more than one trait (Brühl 1912). In more narrow sense pleiotropy is responsible for stable genetic correlations that can be observed between complex traits where a locus affects different traits in the same direction (Cheverud 2001, Falconer and Mackay 1996). Epistasis and pleiotropy have been described as exception of mendalian inheritance, but the impact of these effects is accumulating especially in complex traits where until now functional relationships were not known (Mackay et al. 2009, Tyler et al. 2009). Furthermore the link between epistasis and pleiotropy seems to play an important role related to the genetic architecture of complex traits. In a study by Wolf et al. (2005, 2006) single QTL and epistatic QTL pairs have been investigated regarding pleiotropy in a mouse backcross population. For two different groups of complex traits (skull development and organ weights) they found that pleitropy was less common for loci which were detected by an epistatic model than for single QTL. One explanation might be that epistasis mask or modify the effects of pleiotropy (Wolf et al. 2005, 2006). However, Wolf et al. (2005, 2006) analyzed epistasis only for loci which have been identified previously as single QTL affecting a specific trait, so that no comprehensive statement can be made about the relationship between pleiotropic and epistatic effects.

Studies in *Drosophila melanogaster* revealed that pleiotropic effects can be genetical variable through mutations in genes that share an epistatic relationship (Mackay et al. 2009, Yamamoto et al. 2008). This led to a lack of genetic correlations among all pleiotropic traits, because changes in genes effects on one trait and modify effects of other genes on another traits (Hansen 2006, Hansen and Wagner 2001a, Wagner and Mezey 2000).

Tyler et al. (2009) indicated that there might be a relationship between the number of interactions a gene participates in and the number of phenotypes it modulates. This allows making assumptions about consequences on related phenotypes when epistatic structures change. Therefore network concepts have been described to be suitable to investigate the relationship between genes and phenotypes and to understand the underlying mechanisms and the relationship of epistasis and pleiotropy (Aylor and Zeng 2008, Tyler et al. 2009).

1.5. Scope of the study

The Bonner Duroc × Pietrain resource population (DuPi) is well established investigating quantitative traits. A QTL analysis in 585 F_2 animals of DuPi revealed 58 single QTL for carcass composition and meat quality traits (Liu et al. 2007). Besides additive and dominance effects, also the effect of imprinting was included. Maternal and paternal imprinting effects were detected for back fat thickness in loin ($13/14^{th}$ rib) on SSC2 and for cooking loss on SSC18. In order to examine the effects of a second QTL on the same chromosome a two-QTL model comprising also imprinting effects were applied. In addition the two-QTL model was extended for imprinting effects and revealed a second QTL for back fat thickness.

Furthermore, out of 585 F_2 DuPi pigs, 74 animals were selected based on their phenotype of drip loss and ultimate pH in *M. long. dorsi* as well as on their genotypes on SSC5 and SSC18. On both loci QTL for drip loss were identified (Ponsuksili et al. 2008). For these 74 animals muscle gene expression profiles were recorded using microarray techniques. The relationship between the phenotype and the gene expression profiles was determined using the pearson correlation coefficient. Additionally, based on approaches of 'genetical genomic', expression QTL (eQTL) were detected to characterize genes as *cis*- or *trans*-regulated (Ponsuksili et al. 2008).

Within the study of Liu et al. (2007) additive and dominance effects of QTL across the genome were detected. The relationship between drip loss and gene expression profiles in the investigations of Ponsuksili et al. (2008) were considered as additive effects.

As an extension of these approaches, the aim of this study was to examine epistasis in the DuPi population related to carcass composition and meat quality traits. In the first part (chapter 2, pp. 20), the epistatic effects of interacting QTL pairs were estimated and their proportion to the phenotypic variance was calculated. A second part (chapter 3, pp. 48) was performed to combine previously identified epistatic QTL regions with gene expression profiles in order to uncover the biological causes of one exemplary selected epistatic QTL pair located on SSC8 and SSC15.

Chapter 2. Epistatic QTL pairs associated with meat quality and carcass composition traits in a porcine Duroc × Pietrain population

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2.1. Abstract

Quantitative trait loci (QTL) analyses in pig have revealed numerous individual QTL affecting growth, carcass composition, reproduction and meat quality, indicating a complex genetic architecture. In general, statistical QTL models consider only additive and dominance effects and identification of epistatic effects in livestock is not yet widespread. The aim of this study was to identify and characterize epistatic effects between common and novel QTL regions for carcass composition and meat quality traits in pig. Five hundred and eighty five F_2 pigs from a Duroc x Pietrain resource population were genotyped using 125 genetic markers (microsatellites and SNP) spread over the 18 pig autosomes. Phenotypic information for 26 carcass composition and meat quality traits was available for all F_2 animals. Linkage analysis was performed in a two-step procedure using a maximum likelihood approach implemented in the QxPak program. A number of 56 interacting QTL was observed for different traits, leading to the identification of a variety of networks among chromosomal regions throughout the porcine genome. We distinguished 17 epistatic QTL pairs for carcass composition and 39 for meat quality traits. These interacting QTL pairs explained up to 8% of the phenotypic variance. Our findings demonstrate the significance of epistasis in pigs. We have revealed evidence for epistatic relationships between different chromosomal regions, confirmed known QTL loci and connected regions reported in other studies. Considering interactions between loci allowed us to identify several novel QTL and trait-specific relationships of loci within and across chromosomes.

2.2. Background

Until now, most QTL studies have considered additive and dominance effects and sometimes imprinting effects, but epistatic interactions between two or more loci are commonly ignored. The significance of interactions between different loci in explaining the genetic variability of traits has long been controversial.

Epistatic effects can be clearly defined and verified when a combination of two mutations yields an unexpected phenotype that cannot be explained by the independent effect of each mutation (Roth et al. 2009). For example, Steiner et al. (2007) have demonstrated the effect of gene interactions for a binary expressed trait (coat color), which is influenced by two or

three loci. However, the evaluation of epistasis for complex traits is much more demanding because these traits are influenced by environmental effects and large numbers of polymorphic loci (Phillips 2008). For complex traits, it is useful to analyze the variation in a resource population established for QTL studies, by applying epistatic QTL models.

Most published studies on epistatic effects of interacting QTL have focused on plants and laboratory animals rather than livestock species, which is a paradox since it seems obvious that the variance of a complex trait in livestock animals cannot be explained by additive genetic effects alone (Carlborg and Haley 2004).

In plants, investigations into epistatic effects concern mainly rice hybrids for traits such as grain yield, plant height and heating date (Li et al. 2008, Yu et al. 1997), but epistatic effects have also been identified in maize, oat and *Arabidopsis* (Asíns 2002).

Most epistatic QTL studies related to mammals analyze data from laboratory animals. Brockmann et al. (2000) have shown that in a mouse intercross used to select for body weight and fat accumulation, epistatic effects contributed 33% and 36% of the total phenotypic variation, respectively, whereas epistatic effects contributed only 21% of the variation. Kim et al. (2001) have investigated non-insulin-dependent diabetes in two backcross populations of mice i.e. B6 and CAST crosses. They have detected five interacting QTL in the B6 cross but none in the CAST cross. Shimomura et al. (2001) have detected ten epistatic QTL connected to circadian behavior in mice. Sugiyama et al. (2001) have found six single QTL associated with blood pressure in rats but 36% of this trait's phenotypic variance could be explained by a single two-dimensional epistatic factor. Koller et al. (2008) have examined the mineral density of bones in a reciprocal cross in rats and found epistatic effects between known and novel QTL and between pairs of completely unknown QTL.

In livestock species, epistatic effects have been detected in chicken and swine. In chickens, Carlborg et al. (2003, 2004b) have identified epistatic effects on growth traits, which accounted for up to 80% of the genetic variation. In swine, ten QTL pairs for eight muscle fiber traits in an intercross between Iberian and Landrace breeds (Estelle et al. 2008) and interacting genomic regions for carcass composition traits as well as intramuscular fat content in F_2 crosses between Pietrain and three other commercial lines (Duthie et al. 2010) have been reported. Additional studies have revealed epistatic relationships influencing meat color, fatty acid composition and reproductive traits such as teat number

or litter size (Noguera et al. 2009, Ovilo et al. 2002, Rodriguez et al. 2005, Uemoto et al. 2009).

In this work, we have evaluated the importance of epistatic effects in pig breeding by identifying epistatic QTL effects for carcass composition and meat quality in an F_2 cross composed of commercial pig lines.

2.3. Methods

2.3.1. Animals and analyzed traits

In this study, we used 585 F_2 pigs from 31 full-sib families that were the product of a reciprocal cross of the Duroc and Pietrain (DuPi) breeds. The F_1 generation was the product of crosses between Duroc boars and Pietrain sows and between Pietrain boars and Duroc sows. All animals were kept at the Frankenforst experimental research farm of the Rheinische Friedrich-Wilhelms-University in Bonn. The phenotypes of all the F_2 animals were recorded in a commercial abattoir, according to the rules of German performance stations (ZDS 2003).

Table 2: Mean and standard deviation for carcass composition and meat quality

Traits for carcass composition[1]	**Abbreviation**	**N**[2]	**Mean**	**SD**[3]
Carcass length [cm]	carcass length	585	97.95	2.70
Dressing [%]	dressing	585	76.76	1.93
Backfat shoulder [cm]	BFT-shoulder	585	3.43	0.43
Backfat 13th/14th rib [cm]	BFT-13/14	585	1.64	0.30
Backfat loin [cm]	BFT-loin	585	1.33	0.31
Backfat mean [cm]	BFT-mean	585	2.13	0.31
Backfat thickness above *M. long. dorsi*, 13/14th ribs [cm]	BFT-thickness	585	1.13	0.27
Side fat thickness [cm]	side fat	585	2.72	0.67

[1] Estimated carcass lean content = 59.704-1.744*(loin eye area)-0.147*(fat area)-1.175*(BFT-sh)-0.378*(side BFT)-1.801*(BFT thickness); estimated belly lean content = 65.942+0.145*(loin eye area)-0.479*(fat area)-1.867*(side BFT)-1.819*(BFT-loin); backfat mean = the average of backfat loin, backfat shoulder and backfat 13th /14th rib; dressing: chilled carcass weight relative to live weight at slaughter; fat area [cm²] according to Herbst (1980); [2] N: number of records; [3] SD: standard deviation

Table 2: Mean and standard deviation for carcass composition and meat quality *(cont.)*

Traits for carcass composition[1]	**Abbreviation**	**N**[2]	**Mean**	**SD**[3]
Fat area above the *M. long. dorsi* at 13/14th rib [cm²]	fat area	585	16.27	2.84
Loin eye area at 13/14th rib, *M. long. dorsi* [cm²]	loin eye area	585	51.82	5.37
Ratio of fat to muscle area	Fat muscle ratio	585	0.32	0.06
Estimated carcass lean content, Bonner formula [%]	ECLC	585	58.73	2.42
Estimated belly lean content [%]	EBLC	585	58.16	2.98
Traits for meat quality	**Abbreviation**	**N**[2]	**Mean**	**SD**[3]
pH-value *M. long. dorsi* 45 min p.m.	pH 1h loin	585	6.56	0.20
pH-value *M. long. dorsi* 24h p.m.	pH 24h loin	585	5.51	0.10
pH decline *M. long. dorsi*	pH decline	585	1.05	0.22
pH-value *M. semimembranosus* 24h p.m.	pH 24h ham	585	5.64	0.13
Conductivity *M. long. dorsi* 45 min p.m	cond. 1h loin	585	4.32	0.62
Conductivity *M. long. dorsi* 24h p.m.	cond. 24h loin	585	2.79	0.78
Conductivity *M. semimembranosus* 24h p.m.	cond. 24h ham	585	4.81	2.14
Meat color, opto-value	meat color	585	68.61	5.65
Drip loss [%]	drip loss	342	2.12	0.96
Cooking loss [%]	cooking loss	342	24.87	2.22
Thawing loss [%]	thawing loss	342	8.10	1.98
Warner-Bratzler shear force [kg]	shear force	324	35.27	6.62
Intra muscular fat content [%]	IMF	272	6.99	2.37

[1] Estimated carcass lean content = 59.704-1.744*(loin eye area)-0.147*(fat area)-1.175*(BFT-sh)-0.378*(side BFT)-1.801*(BFT thickness); estimated belly lean content = 65.942+0.145*(loin eye area)-0.479*(fat area)-1.867*(side BFT)-1.819*(BFT-loin); backfat mean = the average of backfat loin, backfat shoulder and backfat 13th /14th rib; dressing: chilled carcass weight relative to live weight at slaughter; fat area [cm²] according to Herbst (1980); [2] N: number of records; [3] SD: standard deviation

In total, 13 traits related to carcass composition and 13 traits related to meat quality were analyzed. Table 2 contains an overview and definitions of all the carcass composition and meat quality traits that were analyzed. Intramuscular fat content (IMF) was determined by the Soxhlet extraction method with petroleum ether (Firth et al. 1985). More detailed

information about the carcass composition and meat quality traits can be found in Liu et al. (2007).

2.3.2. Statistical analyses

One hundred and twenty five microsatellites and six SNP markers were used to genotype animals of the parental (P), F_1 and F_2 generations. Genetic markers were equally spaced on the 18 pig autosomes and covered 89% of these. In comparison to Liu et al. (2007), who analyzed the data with a single QTL model, 18 genetic markers (microsatellites and SNP) were added to the data set. The CRI-MAP 2.4 software was used with the options "build", "twopoint" and "fixed" to recalculate the sex-average linkage map (Green 1992). Additional information regarding the markers, i.e. genetic position (in Kosambi cM), number of identified alleles and polymorphism information content are given in table A1 and figure A1 (see Chapter 7, pp. 94)

To identify significant environmental effects, the data was analyzed by linear models including a relevant fixed effects model (model 0) as in Liu et al. (2007). All the models contained a polygenic effect (u_k), which is distributed as $N(0, A\sigma^2_u)$, where A reflects the numerator relationship matrix and e_{ijk} the residual effect:

$$y_{ijk} = F_i + \beta cov_j + u_k + e_{ijk} \qquad (0)$$

For carcass composition and intramuscular fat content (IMF), the season/year of birth and the sex were included in the model as fixed effects (F) and carcass weight and age at slaughter as covariates (βcov). For traits like pH, conductivity and meat color, factors including sex, slaughter season, carcass weight and age at slaughter were used. Family, sex, carcass weight and age at slaughter were included in the analyses of drip loss, thawing loss, cooking loss and shear force.

Liu et al. (2007) had analyzed the data set by the Haley-Knott regression (Seaton et al. 2002), which was extended in this study for the pH decline and IMF traits.

Interactions between two QTL were detected by the series of model comparisons suggested by Estelle et al. (2008). The statistical analysis can be subdivided into the following two steps, which were performed using the statistical package Qxpak 4.0 (Perez-Enciso and Misztal 2004).

2.3.2.1. Step 1: Preselection of epistatic regions

Additive and dominance effects of individual QTL were excluded from the first step of the analysis. To characterize distinguishable genome regions, all chromosomes were separated into 5 cM intervals because of computational limitations.

$$y_{ijk} = F_i + \beta cov_i + (c_{aa} I_{aa} + c_{ad} I_{ad} + c_{da} I_{da} + c_{dd} I_{dd}) + u_k + e_{ijk} \quad (1)$$

Model 1 includes all the possible genetic interactions between pairs of chromosomal segments (I_{aa}, I_{ad}, I_{da} and I_{dd}) but does not include the main genetic effects themselves. The regression coefficients c_{aa}, c_{ad}, c_{da} and c_{dd} were calculated according to Cockerham's suggestions for epistatic interaction (Cockerham 1954):

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

The definitions of these interaction terms follow the rules of Varona et al. (2002). P_1 and P_2 refer to the probability of a QTL at locations 1 and 2, P(QQ) the probability of the grandparental line (Duroc) being homozygous, P(qq) the probability of the other grandparental line (Pietrain) being homozygous and P(Qq) the probability of being heterozygous. These equations imply unlinked interacting loci (Kao and Zeng 2002). The IBD probabilities were computed by a Markov chain Monte Carlo (MCMC) algorithm with 10000 iterations (Perez-Enciso and Misztal 2004). Model 1 was tested against model 0 with likelihood ratio tests (LRT) to assess the significance of the effects of interacting QTL. Nominal P-values were calculated assuming chi-squared distribution of the LRT with four degrees of freedom. Interacting QTL pairs with a nominal P-value < 0.001 were selected to be further analyzed in step 2.

However, the results of this model comparison cannot be directly used for the detection of epistasis because the two regions might interact solely in an additive way. The exclusion of the main genetic effects and the definition of widely-spaced 5 cM pseudo-loci are justified by the long computing time necessary for this unsaturated genetic model.

In addition to interactions between regions on different chromosomes, intrachromosomal interactions were investigated. To avoid large, overlapping confidence intervals, interacting QTL positions were selected when the genome regions involved were larger

than 30 cM. If the two regions are closer than 30 cM, there is a high risk that an interaction might be observed, which can be explained in reality by a single QTL.

2.3.2.2. Step 2: Calculation of epistasis

Purely epistatic effects were quantified by model 2, which covers all possible genetic main effects and interaction effects. A 1-cM scan was performed within 40 intervals of preselected genome regions identified in step 1.

$$y_{ijk} = F_i + \beta cov_i + (c_{a1}a_1 + c_{d1}d_1) + (c_{a2}a_2 + c_{d2}d_2) + (c_{aa}I_{aa} + c_{ad}I_{ad} + c_{da}I_{da} + c_{dd}I_{dd}) + u_k + e_{ijk} \quad (2)$$

The regression coefficients for the main effects of the two individual QTL were defined as:

$$c_{a1} = P_1(QQ) - P_1(qq)$$
$$c_{d1} = P_1(Qq)$$
$$c_{a2} = P_2(QQ) - P_2(qq)$$
$$c_{d2} = P_2(Qq)$$

Factor “a” in model 2 is defined as the individual additive effect and “c” is the regression coefficient for the differences in probabilities of being homozygous for alleles of the Duroc grandparental line (QQ) and for alleles of the Pietrain line (qq). A positive additive genetic value would indicate that alleles originating from the Duroc line show a greater effect than alleles from the other parental line and vice versa. The dominance effect “d” is described as a deviation of heterozygous animals from the mean of both types of homozygous individuals. In the case of a positive dominance value, an increase in the trait of interest is the result of a heterozygous genotype.

$$y_{ijk} = F_i + \beta cov_i + (c_{a1}a_1 + c_{d1}d_1) + (c_{a2}a_2 + c_{d2}d_2) + u_k + e_{ijk} \quad (3)$$

Finally, the statistical contrast between models 2 and 3 for evidence of epistasis was carried out using an LRT with four degrees of freedom in the numerator.

As discussed in Mercade et al. (2005), permutation techniques cannot be applied here because an infinitesimal genetic value is included. A randomization of the data would destroy the family structure. Nevertheless, it is necessary to prove the reliability of epistatic QTL pairs. For this purpose, a Bonferroni correction assuming statistical independence every 40 cM was used as in Noguera et al. (2009). The genome-wide critical

values of LRT for the significance levels associated with type I errors where $\alpha = 0.05$, 0.01 or 0.001 were 18.00, 20.45 and 26.21, respectively.

To verify the importance of each epistatic interaction effect involved (a×a, a×d, d×a and d×d; a for additive and d for dominance), the simple heuristic method of Estelle et al. (2008) was used. This method judges an epistatic effect as relevant (significant) if the effect size exceeds two residual SD of model 0.

The proportion of the phenotypic variance explained by the genetic components was calculated by the differences between the residual variances of the compared models.

2.4. Results

2.4.1. Step 1: Preselection of QTL pairs

The number of significant QTL pairs identified in step 1 varied from three to 34 for different traits. In general, low numbers were detected for traits that are known to have high measurement errors due to environmental effects (drip loss, cooking loss and thawing loss) or to the error-prone measurement technique (side fat). In this step, all QTL identified as significant in the single-QTL analysis (Liu et al. 2007) were also found to be significant in combination with other QTL in the bi-dimensional analysis of step 1.

The significant QTL regions identified in step 1 are interesting candidates for epistasis, but the results of this scan cannot be used as final proof for such effects because the main and interactive genetic effects are not separated. For a final validation of epistatic effects, a fully saturated model including genetic main effects and interaction effects is needed, which leads directly to step 2.

2.4.2. Step 2: Calculation of epistatic effects

In the final step, the epistatic relationship between two QTL was estimated using model 2. Table 3 gives detailed information on all the significant epistatic QTL pairs according to position, the LR-statistics and the proportion of the phenotypic variance explained by the particular pairs of loci. In general, the number of true epistatic QTL pairs was less than the number of preselected pairs of QTL regions. Fifty-six epistatic QTL pairs were identified across the 18 autosomes for 19 different traits. Intrachromosomal epistatic QTL were

located on porcine chromosomes SSC5 (*Sus scrofa* chromosome 5), 8 and 17 for IMF, fat area and loin eye area, respectively.

Overall, 19 a×a, 11 a×d, 13 d×a and 29 d×d significant interactions were observed. For 16 epistatic QTL pairs, it was not possible to detect any more relevant effects (table 4, pp. 34). Although the general epistatic interaction term was significant for 16 QTL pairs, the effect size of the involved single epistatic effects did not exceed two residual SD (model 2).

The proportion of the phenotypic variance explained by the particular interaction term ranged from 2.5% to 8.5%. The proportion of epistatic variance relative to the entire QTL variance exceeded 50% in most cases (table 3).

Table 3: Evidence of epistatic QTL loci for carcass composition and meat quality traits

Carcass composition	**SSC pos.1 (cM)[1]**	**SSC pos. 2 (cM)[1]**	**LR[2]**	**Epist. Var[4]**	**QTL Var[5]**
BFT 13/14 rib	16 (80)	18 (21)	22.9**	3.45	4.59
BFT shoulder	2 (207)	15 (84)	20.8**	3.15	4.56
	9 (57)	10 (151)	19.8**	2.99	3.37
BFT thickness	7 (138)	13 (61)	20.9**	3.27	5.16
Dressing	5 (1)	9 (15)	18.5*	2.82	4.17
ECLC	***2 (135)***	4 (98)	19.0*	2.90	4.53
	2 (125)	7 (1)	19.4*	2.96	5.04
	8 (62)	10 (79)	22.9**	3.49	5.34
Fat area	***6 (112)***	12 (32)	21.0**	3.20	4.13
	6 (73)	13 (11)	19.8*	3.02	5.79
	8 (36)	8 (127)	23.4**	3.55	5.16

SSC *Sus scrofa* chromosome

[1] position in Kosambi cM; in bold presented QTL loci have been detected as single QTL by Liu et al. 2007

[2] LR: 2-log likelihood ratio

[3] three genome-wide significance levels were used: 0.1% significant value (LR = 26.21, nominal $p < 0.0001$,***), 1% significant value (LR = 20.45, nominal $p < 0.0005$,**), 5% suggestive value (LR = 18.00, nominal $p < 0.001$,*)

[4] proportion (%) of phenotypic variance explained by epistasis calculated as the proportion of the residual variances due the epistatic QTL effects on the residual variances excluding the epistatic QTL effects

[5] proportion (%) of phenotypic variance explained by both QTL and their interaction term calculated as the proportion of the residual variances due the QTL effects on the residual variances excluding the QTL effects

Table 3: Evidence of epistatic QTL loci for carcass composition and meat quality traits *(cont.)*

Carcass composition	**SSC pos.1 (cM)[1]**	**SSC pos. 2 (cM)[1]**	**LR[2]**	**Epist. Var[4]**	**QTL Var[5]**
Fat muscle ratio	***2 (125)***	7 (1)	30.4***	5.88	5.88
	8 (62)	10 (80)	21.6**	2.94	2.94
	8 (80)	17 (45)	19.4*	3.03	5.88
Loin eye area	2 (135)	4 (96)	18.8*	2.87	4.86
	8 (58)	10 (70)	24.8**	3.77	6.01
	17 (55)	17 (80)	48.7***	7.26	10.41
Meat quality	**SSC pos.1 (cM)[1]**	**SSC pos.2 (cM)[1]**	**LR[2]**	**Epist. Var[4]**	**QTL Var[5]**
pH 1h loin	2 (156)	18 (9)	18.0*	2.45	3.79
	3 (34)	13 (85)	21.5**	3.14	4.14
	8 (1)	15 (77)	18.1*	2.80	4.14
	12 (45)	16 (1)	26.2***	4.15	4.48
pH 24h loin	3 (16)	11 (39)	21.0**	4.11	4.11
	4 (14)	11 (16)	39.4***	6.85	6.85
	10 (84)	18 (24)	19.6*	2.78	4.11
pH decline loin	3 (13)	6 (41)	21.5**	3.06	4.64
	3 (52)	18 (22)	20.3**	3.05	4.37
	6 (39)	14 (84)	22.5**	3.31	4.37
	8 (6)	**15 (71)**	18.6*	2.78	4.37
	12 (48)	16 (1)	26.8***	4.11	4.37
	15 (61)	17 (29)	19.3*	2.78	4.37

SSC *Sus scrofa* chromosome

[1] position in Kosambi cM; in bold presented QTL loci have been detected as single QTL by Liu et al. 2007

[2] LR: 2-log likelihood ratio

[3] three genome-wide significance levels were used: 0.1% significant value (LR = 26.21, nominal $p < 0.0001$,***), 1% significant value (LR = 20.45, nominal $p < 0.0005$,**), 5% suggestive value (LR = 18.00, nominal $p < 0.001$,*)

[4] proportion (%) of phenotypic variance explained by epistasis calculated as the proportion of the residual variances due the epistatic QTL effects on the residual variances excluding the epistatic QTL effects

[5] proportion (%) of phenotypic variance explained by both QTL and their interaction term calculated as the proportion of the residual variances due the QTL effects on the residual variances excluding the QTL effects

Table 3: Evidence of epistatic QTL loci for carcass composition and meat quality traits *(cont.)*

Meat quality	SSC pos.1 (cM)[1]	SSC pos.2 (cM)[1]	LR[2]	Epist. Var[4]	QTL Var[5]
pH 24h ham	***1 (108)***	5 (126)	26.9***	4.07	12.59
	2 (179)	7 (122)	18.1*	2.27	4.44
	7 (88)	12 (1)	24.5**	3.70	3.70
	10 (84)	18 (23)	23.2**	3.76	5.19
	15 (61)	18 (92)	27.6***	4.51	5.93
Conductivity 1h loin	3 (10)	14 (113)	23.8**	3.62	5.16
Conductivity 24h loin	5 (52)	13 (75)	26.6***	4.04	5.65
	6 (13)	13 (20)	20.5**	3.12	4.77
Conductivity 24h ham	***10 (99)***	13 (30)	18.4*	2.83	4.05
Meat colour	7 (80)	12 (26)	22.4**	3.41	4.06
Cooking loss	1 (97)	16 (63)	21.3**	5.18	6.41
	2 (186)	15 (16)	21.8**	5.27	6.61
	4 (43)	16 (102)	19.6*	4.77	7.03
	5 (4)	18 (82)	22.2**	5.40	7.89
	7 (50)	13 (13)	18.9*	4.59	7.33
	7 (47)	16 (108)	20.5**	4.96	8.48
	7 (40)	17 (60)	24.2**	5.88	8.69
	8 (85)	18 (8)	31.2***	7.50	10.22

SSC *Sus scrofa* chromosome

[1] position in Kosambi cM; in bold presented QTL loci have been detected as single QTL by Liu et al. 2007

[2] LR: 2-log likelihood ratio

[3] three genome-wide significance levels were used: 0.1% significant value (LR = 26.21, nominal $p < 0.0001$,***), 1% significant value (LR = 20.45, nominal $p < 0.0005$,**), 5% suggestive value (LR = 18.00, nominal $p < 0.001$,*)

[4] proportion (%) of phenotypic variance explained by epistasis calculated as the proportion of the residual variances due the epistatic QTL effects on the residual variances excluding the epistatic QTL effects

[5] proportion (%) of phenotypic variance explained by both QTL and their interaction term calculated as the proportion of the residual variances due the QTL effects on the residual variances excluding the QTL effects

Table 3: Evidence of epistatic QTL loci for carcass composition and meat quality traits *(cont.)*

Meat quality	SSC pos.1 (cM)[1]	SSC pos.2 (cM)[1]	LR[2]	Epist. Var[4]	QTL Var[5]
Thawing loss	2 (49)	4 (105)	18.4*	4.48	6.61
	15 (8)	17 (1)	19.1*	4.63	6.52
Shear force	***2 (166)***	7 (87)	19.9*	5.00	9.17
	2 (150)	13 (112)	19.2*	4.83	9.00
	2 (145)	16 (102)	21.8**	5.47	9.74
	8 (84)	8 (111)	18.7*	4.71	6.54
IMF	1 (263)	6 (101)	23.4**	8.23	10.85
	5 (57)	5 (87)	24.2**	8.52	13.34

SSC *Sus scrofa* chromosome
[1] position in Kosambi cM; in bold presented QTL loci have been detected as single QTL by Liu et al. 2007
[2] LR: 2-log likelihood ratio
[3] three genome-wide significance levels were used: 0.1% significant value (LR = 26.21, nominal $p < 0.0001$,***), 1% significant value (LR = 20.45, nominal $p < 0.0005$,**), 5% suggestive value (LR = 18.00, nominal $p < 0.001$,*)
[4] proportion (%) of phenotypic variance explained by epistasis calculated as the proportion of the residual variances due the epistatic QTL effects on the residual variances excluding the epistatic QTL effects
[5] proportion (%) of phenotypic variance explained by both QTL and their interaction term calculated as the proportion of the residual variances due the QTL effects on the residual variances excluding the QTL effects

2.4.2.1. QTL for carcass composition traits

Seventeen epistatic QTL pairs were detected for seven carcass composition traits. These were located on all autosomes except 1, 4, 11 and 14. The epistatic loci were classified into two highly significant ($P < 0.001$), nine significant ($P < 0.01$) and six suggestive ($P < 0.05$) QTL relationships (table 3). Chromosomal loci of interest were located on SSC2, SSC4, SSC7, SSC8 and SSC10, where multiple epistatic QTL pairs were detected (figure 6). Regions located on SSC8 (58 to 62 cM) and SSC10 (70 to 80 cM) showed a significant epistatic interaction for the fat:muscle ratio, the loin eye area and ECLC. The relationship between these two QTL loci explained 3% to 4% of the phenotypic variance of these traits.

Furthermore, high d×d interaction effects were observed for ECLC for one QTL on SSC2 (125 to 135 cM), which interacted with one locus on SSC4 (96 to 98 cM) and another locus on SSC7 (1 cM). Additionally, epistatic QTL pairs were detected for the same loci on SSC2 (135 cM) and SSC4 (96 to 98 cM) related to the loin eye area and also along SSC2 (125 cM) and SSC7 (1 cM) for the fat:muscle ratio. In general, these interacting genomic

areas showed the highest d×d interactions in comparison to other single epistatic effects, except the loci on SSC2 and SSC7, where the d×a interaction was the most prevalent. Two to 6% of the phenotypic variance was explained by the relationships between SSC2 and SSC4 and between SSC2 and SSC7 for these carcass composition traits.

No epistatic effects were identified for carcass length, shoulder BFT, mean BFT, side fat and estimated lean belly content.

Table 4: Impact of epistatic effects for carcass composition and meat quality traits

Carcass composition	SSC pos. 1 (cM)[1]	SSC pos. 2 (cM)[1]	a_1^2	d_1^2	a_2^2	d_2^2	$I_{a\times a}$[3]	$I_{a\times d}$[3]	$I_{d\times a}$[3]	$I_{d\times d}$[3]	SE range[4]
BFT 13/14 rib	16 (80)	18 (21)	-0.01	-0.02	0.09	-0.08	-0.11	-0.07	**-0.18**	0.14	0.03 – 0.04
BFT shoulder	2 (207)	15 (84)	0.08	0.09	0.15	0.04	**0.21**	0.00	**-0.32**	**-0.23**	0.06 – 0.20
	9 (57)	10 (151)	0.02	0.19	0.08	**0.23**	**0.24**	-0.05	-0.10	**-0.38**	0.06 – 0.33
BFT thickness	7 (138)	13 (61)	-0.12	0.11	0.07	-0.04	**-0.14**	**0.27**	-0.02	**-0.21**	0.04 – 0.14
Dressing	5 (1)	9 (15)	-0.23	-0.08	-0.16	-0.51	-0.72	0.12	-0.11	0.32	0.17 – 0.41
ECLC	2 (135)	4 (98)	-0.12	**-7.34**	-1.87	**-7.57**	2.07	-0.99	3.53	**15.47**	0.66 – 6.12
	2 (125)	7 (1)	-0.70	1.35	0.68	0.49	0.83	0.40	-1.76	-1.25	0.28 – 0.86
	8 (62)	10 (79)	-0.33	1.62	-0.60	2.58	0.32	1.75	1.37	-5.20	0.36 – 1.39
Fat area	6 (113)	12 (32)	0.29	0.82	-0.93	0.78	-0.71	-1.22	2.10	-2.00	0.36 – 1.37
	6 (73)	13 (11)	-0.39	0.88	-0.29	1.26	0.33	0.39	1.56	-2.09	0.25 – 0.70
	8 (36)	8 (127)	-0.72	-2.09	0.20	-2.31	0.60	1.65	-1.13	**4.14**	0.42 – 1.14
Fat muscle ratio	2 (125)	7 (1)	0.02	-0.04	-0.02	-0.02	-0.03	-0.01	**0.06**	**0.05**	0.01 – 0.02
	8 (62)	10 (80)	0.01	-0.04	0.02	-0.05	-0.01	-0.04	**-0.05**	**0.12**	0.01 – 0.04
	8 (80)	17 (45)	-0.04	**-0.06**	0.01	-0.03	0.01	**0.06**	-0.03	**0.15**	0.01 – 0.05
Loin eye area	2 (135)	4 (96)	0.27	**-17.51**	-1.47	**-16.54**	4.30	-2.72	3.86	**36.80**	1.28 – 12.07
	8 (58)	10 (70)	-2.21	5.50	-0.54	**6.81**	0.10	7.19	1.97	**-14.98**	0.88 – 2.34
	17 (55)	17 (80)	-3.34	-11.79	6.55	-9.84	**-14.58**	8.62	**-15.32**	4.10	2.78 – 9.85

SSC *Sus scrofa* chromosome

[1] position of QTL in Kosambi cM

[2] estimated additive (a) and dominance (d) effects for individual QTL related to position 1 or position 2

[3] estimated additive × additive ($I_{a\times a}$), additive × dominance ($I_{a\times d}$), dominance × additive ($I_{d\times a}$) and dominance × dominance ($I_{d\times d}$) effects; prevalent epistatic or individual effects which are twice the residual variance of the phenotypic trait are presented in bold

[4] SE standard error ranges for all genetic effects of one epistatic QTL

Table 4: Impact of epistatic effects for carcass composition and meat quality traits *(cont.)*

Meat quality	SSC pos. 1 (cM)[1]	SSC pos. 2 (cM)[1]	a_1^2	d_1^2	a_2^2	d_2^2	$I_{a\times a}$[3]	$I_{a\times d}$[3]	$I_{d\times a}$[3]	$I_{d\times d}$[3]	SE range[4]
pH 1h loin	2 (156)	18 (9)	0.00	**0.06**	0.05	0.03	**-0.11**	0.02	**-0.09**	-0.05	0.02 – 0.07
	3 (34)	13 (85)	**0.06**	**0.09**	**-0.08**	**0.11**	0.03	**-0.09**	**0.20**	**-0.17**	0.03 – .011
	8 (1)	15 (77)	0.00	0.04	0.03	0.04	**0.07**	-0.02	0.01	**-0.11**	0.02 – 0.05
	12 (45)	16 (1)	-0.04	**-0.09**	-0.02	**-0.08**	**-0.12**	0.02	0.02	**0.17**	0.02 – 0.05
pH 24h loin	3 (16)	11 (39)	-0.01	-0.02	-0.01	-0.02	**-0.03**	0.02	**0.03**	**0.06**	0.01 – 0.02
	4 (14)	11 (16)	0.02	**0.05**	**-0.06**	**0.04**	0.01	-0.05	**0.12**	-0.08	0.01 – 0.04
	10 (84)	18 (24)	0.02	0.01	-0.01	-0.02	**-0.04**	**-0.04**	0.00	-0.02	0.01 – 0.02
pH decline loin	3 (13)	6 (41)	-0.01	-0.03	**0.08**	0.00	0.00	**0.07**	**-0.24**	0.06	0.03 – 0.11
	3 (52)	18 (22)	0.03	**-0.12**	0.01	**-0.06**	**-0.09**	-0.01	-0.01	**0.20**	0.02 – 0.07
	6 (39)	14 (84)	**0.11**	**-0.26**	-0.01	**-0.28**	**0.08**	**-0.32**	0.00	**0.57**	0.05 – 0.22
	8 (6)	15 (71)	0.02	**0.09**	0.04	**0.08**	**0.09**	-0.03	0.01	**-0.18**	0.03 – 0.07
	12 (48)	16 (1)	-0.05	-0.10	-0.02	-0.09	-0.13	0.04	0.01	0.18	0.02 – 0.08
	15 (61)	17 (29)	0.10	0.03	0.00	0.01	**-0.14**	**-0.11**	-0.02	-0.04	0.03 – 0.12
pH 24h ham	1 (108)	5 (126)	-0.01	-0.02	**0.05**	**0.03**	0.02	**-0.10**	**-0.09**	0.00	0.02 – 0.05
	2 (179)	7 (122)	0.04	**-0.14**	0.01	**-0.10**	**0.06**	-0.02	-0.02	**0.20**	0.02 – 0.08
	7 (88)	12 (1)	0.01	-0.02	0.01	-0.02	**0.05**	-0.01	0.00	**0.06**	0.01 – 0.03
	10 (84)	18 (23)	0.01	-0.01	0.00	-0.05	**-0.07**	-0.02	0.00	0.03	0.01 – 0.05
	15 (61)	18 (92)	0.02	**0.08**	-0.01	**0.07**	**-0.03**	**-0.07**	**-0.03**	**-0.15**	0.01 – 0.04
Conductivity 1h loin	3 (10)	14 (113)	0.01	-0.34	0.09	0.12	**-0.59**	-0.15	-0.24	**0.39**	0.12 – 0.69

SSC *Sus scrofa* chromosome

[1] position of QTL in Kosambi cM

[2] estimated additive (a) and dominance (d) effects for individual QTL related to position 1 or position 2

[3] estimated additive × additive ($I_{a\times a}$), additive × dominance ($I_{a\times d}$), dominance × additive ($I_{d\times a}$) and dominance × dominance ($I_{d\times d}$) effects; prevalent epistatic or individual effects which are twice the residual variance of the phenotypic trait are presented in bold

[4] SE standard error ranges for all genetic effects of one epistatic QTL

Table 4: Impact of epistatic effects for carcass composition and meat quality traits *(cont.)*

Meat quality	SSC pos. 1 (cM)[1]	SSC pos. 2 (cM)[1]	a_1^2	d_1^2	a_2^2	d_2^2	$I_{a\times a}$[3]	$I_{a\times d}$[3]	$I_{d\times a}$[3]	$I_{d\times d}$[3]	SE range[4]
Conductivity 24h loin	5 (52)	13 (75)	-0.06	**0.99**	0.11	1.61	-0.36	0.11	-0.42	**-2.17**	0.11 – 0.35
	6 (13)	13 (20)	-0.05	0.01	-0.34	0.20	-0.34	-0.15	0.59	0.17	0.11 – 0.39
Conductivity 24h ham	10 (99)	13 (30)	1.05	1.77	-0.39	1.87	-1.37	-2.93	0.41	**-3.31**	0.34 – 2.62
Meat color	7 (80)	12 (26)	1.16	3.95	-1.15	3.93	3.27	-1.98	0.67	**-7.93**	0.77 – 0.03
Cooking loss	1 (97)	16 (63)	-0.69	-0.33	-0.33	0.03	0.95	1.58	1.14	0.36	0.31 – 0.85
	2 (186)	15 (16)	-0.94	-0.38	-0.26	-0.57	-0.85	**2.20**	0.78	1.11	0.36 – 2.20
	4 (43)	16 (102)	-0.37	0.34	0.57	-1.20	1.42	0.71	-0.52	1.48	0.33 – 1.21
	5 (4)	18 (82)	0.73	0.07	0.02	0.28	-0.82	-0.75	0.10	0.03	0.21 – 1.77
	7 (50)	13 (13)	-0.10	1.07	-0.54	0.84	0.68	1.24	0.74	**-2.24**	0.29 – 0.97
	7 (47)	16 (108)	-0.20	0.08	0.67	-0.87	-0.33	1.81	-0.85	0.75	0.26 – 0.76
	7 (40)	17 (60)	-1.55	-1.96	-2.01	**-2.78**	0.76	**3.99**	**4.04**	**3.53**	0.53 – 2.98
	8 (85)	18 (8)	-0.57	0.45	-0.24	0.80	0.66	1.42	-0.32	-2.02	0.23 – 0.62
Thawing loss	2 (49)	4 (105)	-1.18	**-4.01**	0.48	-3.23	**-4.04**	2.73	0.28	**5.07**	0.91 – 11.35
	15 (8)	17 (1)	-0.15	0.07	-0.55	-0.06	-0.37	0.51	1.15	0.93	0.21 – 0.51
Shear force	2 (166)	7 (87)	-0.07	-6.83	-1.44	-4.38	-3.05	4.00	4.82	9.59	1.07 – 4.39
	2 (150)	13 (112)	2.68	-11.46	-0.14	-10.81	2.54	-1.28	2.49	**23.60**	1.23 – 6.34
	2 (145)	16 (102)	1.96	8.43	-2.43	7.31	-0.93	-0.36	6.35	**-16.98**	1.09 – 4.11
	8 (84)	8 (111)	-1.41	1.22	1.55	1.93	-3.37	0.06	-0.80	**-7.77**	1.90 – 3.74

SSC *Sus scrofa* chromosome

[1] position of QTL in Kosambi cM

[2] estimated additive (a) and dominance (d) effects for individual QTL related to position 1 or position 2

[3] estimated additive × additive ($I_{a\times a}$), additive × dominance ($I_{a\times d}$), dominance × additive ($I_{d\times a}$) and dominance × dominance ($I_{d\times d}$) effects; prevalent epistatic or individual effects which are twice the residual variance of the phenotypic trait are presented in bold

[4] SE standard error ranges for all genetic effects of one epistatic QTL

Table 4: Impact of epistatic effects for carcass composition and meat quality traits *(cont.)*

Meat quality	SSC pos. 1 (cM)[1]	SSC pos. 2 (cM)[1]	a_1^2	d_1^2	a_2^2	d_2^2	$I_{a\times a}$[3]	$I_{a\times d}$[3]	$I_{d\times a}$[3]	$I_{d\times d}$[3]	SE range[4]
IMF	1 (263)	6 (101)	-0.35	-0.54	-0.84	-0.65	1.27	-0.12	1.77	0.53	0.33 – 1.00
	5 (57)	5 (87)	-2.74	0.74	2.69	-0.85	0.53	4.01	-4.28	0.82	0.65 – 1.15

SSC *Sus scrofa* chromosome

[1] position of QTL in Kosambi cM

[2] estimated additive (a) and dominance (d) effects for individual QTL related to position 1 or position 2

[3] estimated additive × additive ($I_{a\times a}$), additive × dominance ($I_{a\times d}$), dominance × additive ($I_{d\times a}$) and dominance × dominance ($I_{d\times d}$) effects; prevalent epistatic or individual effects which are twice the residual variance of the phenotypic trait are presented in bold

[4] SE standard error ranges for all genetic effects of one epistatic QTL

2.4.2.2. QTL for meat quality traits

A total of 14 suggestive ($P < 0.05$), 18 significant ($P < 0.01$) and seven highly significant ($P < 0.001$) QTL were identified for all meat quality traits except drip loss (table 3). With regard to the number of epistatic QTL pairs, the cooking loss trait involved eight interacting QTL pairs and the pH decline six, which were the highest numbers of epistatic loci for all meat quality traits.

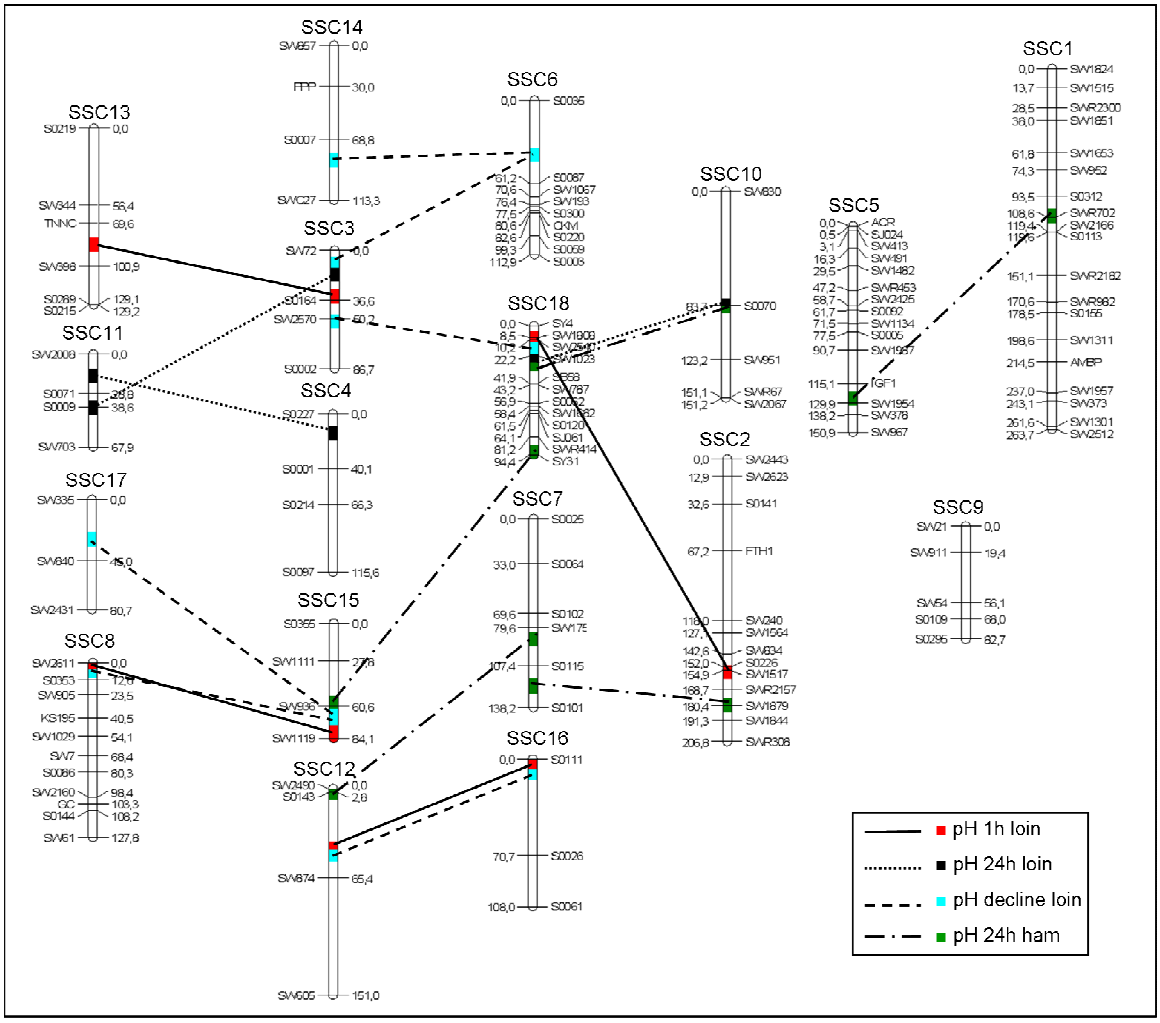

Figure 6: Epistatic QTL network for pH traits

Lines represent the epistatic relationship among two loci; different type of lines displays different traits

Close relationships were found between SSC8 (1 to 6 cM) and SSC15 (71 to 77 cM) and between SSC12 (45 to 48 cM) and SSC16 (1 cM) for pH 1h loin and pH decline (figure 6). For these epistatic effects, a×a and d×d interactions exceeded two SD and were generally more prevalent than a×d or d×a (table 4). The highest explained proportion of the phenotypic variance was 6.85% for an epistatic QTL pair located on SSC4 (14 cM) and

SSC11 (16 cM) related to pH 24h in loin. The proportion of the phenotypic variance of meat quality traits explained by epistasis ranged from 2.27% to 4.51%. For the measurements of conductivity in loin and ham, four epistatic relationships between seven QTL loci were observed.

Within the group of meat quality traits examined, 16 epistatic relationships among loci were identified (table 3). For cooking loss, a locus on SSC7 (40 to 50 cM) showed a×d, d×a and d×d interactions with regions on SSC13 (13 cM), SSC16 (108 cM) and SSC17 (60 cM). Additionally, a relationship was identified between the epistatic QTL on SSC16 (102 cM) and one locus on SSC4 (43 cM), but none of the epistatic effects exceeded two SD. The identified loci on SSC4 and SSC7 in combination had no significant effect on cooking loss. In addition, the epistatic locus on SSC16 (102 to 106 cM) did not only affect cooking loss. Influences on shear force were also detectable within an interaction between SSC2 (145 cM) and SSC16 (102 cM). The highest explained proportion of the phenotypic variance was 8.2% for IMF between SSC1 (263 cM) and SSC6 (101 cM) and 8.5% for an intrachromosomal epistatic QTL pair on SSC5.

2.5. Discussion

Most QTL studies in pigs involve additive and dominance effects but epistasis is often ignored. To our knowledge, seven studies using epistatic models in pigs have been published (Duthie et al. 2010, Estelle et al. 2008, Noguera et al. 2009, Ovilo et al. 2002, Rodriguez et al. 2005, Uemoto et al. 2009, Varona et al. 2002). In general, the use of epistatic models makes it possible to identify QTL, which interact with other QTL not only in an additive way but also via a×a, a×d, d×a and d×d interactions. In comparison to single- or double-QTL analyses, the main benefit of including epistatic QTL effects is the detection of novel QTL that affect a quantitative trait through epistatic interactions with another locus (Carlborg and Haley 2004). The identification of a considerable number of novel QTL in our study underlines this advantage. However, analyzing epistatic effects between two loci is computationally demanding because all pairwise combinations must be investigated (Duthie et al. 2010, Estelle et al. 2008). In addition, the use of microsatellite information renders the distinction between two loci on the same or different chromosomes approximate.

In this study, 56 epistatic QTL pairs involving 104 interacting QTL positions were identified across all the autosomes for porcine carcass composition and meat quality traits. As shown in tables 3 and table A2 (see Chapter 7, pp. 100), 12 of these epistatic QTL positions were detected both in the single-QTL analysis of Liu et al. (2007, 2008) and as novel epistatic QTL in our study. Six regions were related to carcass composition and six to meat quality traits. It can be assumed that these epistatic QTL play an important role in the expression of these phenotypes.

In regard to carcass composition (ECLC and fat muscle ratio), one epistatic QTL position located on SSC2 (125 to 135 cM) interacts with two other QTL regions on SSC4 (98 cM) and SSC7 (1 cM), respectively. This SSC2 locus was previously reported by Liu et al. (2007) as a single QTL and by Lee et al. (2003a), who analyzed a Meishan×Pietrain cross. The same position was also detected for the loin eye area trait by Estelle et al. (2005).

The epistatic relationships between SSC2 (125 to 135 cM) and regions on SSC4 (98 cM) and SSC7 (1 cM) explain 2.9% of the phenotypic variance for ECLC. The corresponding entire QTL variances (sum of epistatic and individual QTL variances) at these positions are 4.5% and 5% respectively, for the interactions between SSC2 (135 cM) and SSC4 (98 cM) and SSC2 (125 cM) and SSC7 (1 cM). It can be assumed that the 2% difference between epistatic and entire QTL variances is due to the individual QTL effect of the locus on SSC2, which was reported by Liu et al. (2007). It follows from this that the effects of the individual QTL loci on SSC4 and SSC7 are presumably small and difficult to detect in a single-QTL analysis. *Calpastatin* (CAST) and *tropomyosin* (TPM4) located on SSC2 between 125 and 135 cM are potential candidate genes for ECLC (Ernst et al. 1998, Fridolfsson et al. 1997b). The locus on SSC4 (98 cM) is related to backfat and loin eye area traits (Knott et al. 1998, Malek et al. 2001, Perez-Enciso et al. 2000) and carries the candidate gene *transforming growth factor beta-3* (TGF-β3) (Johnson et al. 1995). In conclusion, all three genes play roles in skeletal, muscle and tissue development. The locus on SSC2 (125 cM) is also influenced by a region on SSC7 (1 cM) where Ponsuksili et al. (2005) have identified a QTL for several backfat traits in a Duroc × Berlin Miniature pig F_2 cross.

Additionally, we observed an interacting QTL pair between SSC8 (58 to 62 cM) and SSC10 (70 to 80 cM) that influences the loin eye area, ECLC and fat:muscle ratio traits. The involvement of the SSC8 locus had already been detected by a single-QTL analysis of these three traits (Liu et al. 2007). For the fat:muscle ratio, the proportion of phenotypic

variance was completely explained by epistatic effects. There was a 2% difference between epistatic variance and the sum of epistatic and individual QTL variances for the ECLC and loin eye area traits. Considering the single QTL variances presented by Liu et al. (2007), we conclude that the SSC8 locus (58 to 62 cM) has important single QTL and epistatic QTL effects, whereas the SSC10 locus (70 to 80 cM) has only epistatic effects. This assumption is partially contradicted by Thomsen et al. (2004), who has reported a single QTL at the same position on SSC10 that only affects the loin eye area trait.

In regard to the fat area trait, a region on the p arm of SSC6 (73 cM) interacts with SSC13 (11 cM), and a region on the q arm of SSC6 (113 cM) interacts with SSC12 (32 cM). The locus on the p arm of SSC6 has been previously detected by Liu et al. (2008) and the locus on the q arm by Mohrmann et al. (2006) in a resource family of Pietrain and crossbred dams (created from Large White, Landrace and Leicoma breeds). *Leptin receptor* (LEPR), which is involved in neonatal growth and development (Attig et al. 2008), is a candidate gene for the region on the SSC6 q arm.

A significant epistatic relationship was detected between SSC16 (80 cM) and SSC18 (21 cM) for BFT-13/14 rib. As shown by the QTL variance ratios in table 3, this effect between both positions is mainly epistatic. However, Liu et al. (2007) had identified the QTL region on SSC16 not for BFT-13/14 rib but for other backfat traits in the DuPi population. The locus on SSC18 was detected in the DuPi population by Edwards et al. (2008) and in a cross of Berkshire and Yorkshire breeds (Thomsen et al. 2004). Both studies included imprinting effects in the single-QTL models. Although Liu et al. (2007) had applied a similar imprinting model, they did not identify an effect on SSC18 for backfat traits.

In this study, BFT thickness is influenced by an epistatic QTL pair on SSC7 (138 cM) and SSC13 (61 cM). The QTL position on SSC7 has not been identified as a single QTL in our population but it has already been reported in two studies (Ponsuksili et al. 2005, Rohrer and Keele 1998a). Ponsuksili et al. (2005) have shown that the region surrounding the locus on SSC7 is involved in the hepatic metabolic pathway.

Five epistatic QTL pairs involving ten loci were identified for pH 24h in ham. Three QTL, located on SSC1 (108 cM), SSC2 (179 cM) and SSC15 (61 cM), have been previously detected by Liu et al. (2007) in a single-QTL analysis and the QTL on SSC1 (108 cM) was shown to interact with a region on SSC5 (126 cM). Twelve percent of the phenotypic

variance has been explained by this QTL pair, with 4% going back to the epistatic term and 8% to the single QTL on SSC1 reported by Liu et al. (2007). In addition to the work of Liu et al. (2007), we analyzed the IMF and pH decline traits with a single-QTL model. No single QTL was found for IMF, whereas SSC15 (69 cM), which is comparable to the position detected for pH 24h mentioned above, and SSC1 (119 cM) were identified for pH decline.

Furthermore, all these regions have been shown to carry several candidate genes involved in muscle development, composition and metabolism (Jennen et al. 2007), e.g., *alpha-tropomyosin* (TPM1) and *ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1* (ATP5A1) related to the region on SSC1; and *myosin binding protein C* (MYBPC1) and *ATP synthase, H+ transporting, mitochondrial F1 complex* (ATP5B) related to SSC5 (Davoli et al. 2002, Wu et al. 2004).

A position on SSC2 (145 to 166 cM) related to shear force is significant for individual and epistatic QTL effects (Liu et al. 2007) and has been identified in a Berkshire×Duroc intercross (Meyers et al. 2007). This region interacts with loci on SSC7, SSC13 and SSC16. The SSC7 and SSC13 loci have been described as single QTL in other studies (de Koning et al. 2001, Edwards et al. 2008, Harmegnies et al. 2006). A particularly large number of candidate genes has been identified for the epistatic relationship between SSC2 (166 cM) and SSC7 (87 cM). The SSC2 locus contains genes such as *tropomyosin-4* (TMP4) and *GM2 activator protein* (GM2A) (Fridolfsson et al. 1997a, Pinton et al. 2000), whereas SSC7 carries the *myosin, heavy chain 6* (MYH6) and *myosin, heavy chain 7* (MYH7) genes (Pinton et al. 2000). The biological functions of these genes are primarily related to muscle composition.

Until now, we have only discussed epistatic QTL pairs with at least one locus previously detected as a single QTL in the DuPi population analyzed by Liu et al. (2007). We have identified many other epistatic loci that do not have a corresponding result in the single-QTL analysis. Of the 104 QTL positions involved in the 56 epistatic QTL, 12 have been reported by Liu et al. (2007) and are detected by our single-QTL analysis, 30 have been reported in the literature and 62 are presumably novel positions. In general, the effects of these QTL pairs can be explained by purely epistatic effects, in which the single QTL of each involved position is of minor importance. The significance of the epistatic effects can be inferred from the difference between the epistastic variance and the sum of epistatic and individual QTL variances, which is frequently close to zero (table 3). Similar results have

been reported by Duthie et al. (2010), who also detect novel QTL based on an epistatic QTL analysis. Although many QTL have been reported in the literature (table 5), we did not detect any single QTL for the IMF trait.

Table 5: Reported QTL in the literature around similar locations as the QTL identified in the present study

Carcass composition	**SSC (position cM)[1]**	**Flanking marker**	**Reference[2]**
BFT 13/14 rib	18 (21)	SW2540 – SW1023	Edwards et al. (2008), Thomsen et al. (2004)
BFT shoulder	10 (151)	SW2067	Guo et al. (2008)
	15 (84)	SW1119	Duthie et al. (2008)
BFT thickness	7(138)	S0101	Ponsuksili et al. (2005), Rohrer and Keele (1998a)
ECLC	2 (135)	SW1564 – SW834	Lee et al. (2003b), Liu et al. (2007)
	8 (62)	SW1029 – SW7	Liu et al. (2007)
Fat area	6 (112)	S0003	Liu et al. (2008), Mohrmann et al. (2006)
Fat muscle ratio	2 (125)	SW240 – SW1564	Lee et al. (2003a), Liu et al. (2007)
	8 (62)	SW1029 – SW7	Liu et al. (2007)
Loin eye area	2 (135)	SW1564 – SW834	Estelle et al. (2005)
	4 (96)	S0214 – S0097	Malek et al. (2001)
	8 (58)	SW1029 – SW7	Edwards et al. (2008), Liu et al. (2007), Rohrer and Keele (1998b)
	10 (70)	SW830 – S0070	Thomsen et al. (2004)
	17 (55)	SW840 – SW2431	Rohrer et al. (2006)
Meat quality	**SSC (position cM)[1]**	**Flanking marker**	**Reference[2]**
pH decline loin	3 (52)	SW2570 – S0002	Edwards et al. (2008)
	6 (39)	S0035 – S0087	Duan et al. (2009)
	15 (61)	SW936 – SW1119	Duan et al. (2009)

SSC *Sus scrofa* chromosome, [1] position of the QTL in cM, [2] references of other studies reporting QTL in similar regions of the specific chromosome

Table 5: Reported QTL in the literature around similar locations as the QTL identified in the present study *(cont.)*

Meat quality	SSC (position cM)[1]	Flanking marker	Reference[2]
pH 24h loin	3 (16)	SW27 – S0164	Ovilo et al. (2002)
	4 (14)	S0227 – S0001	de Koning et al. (2001)
	10 (84)	S0070 – SW951	Evans et al. (2003)
	11(39)	S0071 – S0009	de Koning et al. (2001)
	18 (24)	SW1023 – SB58	Harmegnis et al. (2006)
pH 24 ham	1 (108)	S0312 – SW2166	Beeckmann et al. (2003), Liu et al. (2007), Sanchez et al. (2006)
	2 (179)	SWR2157 – SW1879	Estelle et al. (2005), Liu et al. (2007)
	5 (126)	IGF1 – SW1954	Duan et al. (2009), Ramos et al. (2009)
	10 (84)	S0070 – SW951	Evans et al. (2003)
	15 (61)	SW936 – SW1119	Liu et al. (2008)
	18 (23)	SW1023 – SB58	Harmegnis et al. (2006)
Conductivity. 24h loin	5 (52)	SWR453 – SW2425	Srikanchai et al. (2009)
	13 (75)	TNNC – SW398	Geldermann et al. (2003), Yue et al. (2003)
Conductivity 24h ham	10 (99)	S0070 – SW951	Liu et al. (2008)
Meat color	7 (80)	SW175 – S0115	Ovilo et al. (2002)
Cooking loss	7 (45)	S0025 – S0064	de Koning et al. (2001)
	13 (13)	S0219 – SW344	Kim et al. (2005)
	15 (16)	S0355 – SW1111	Rohrer et al. (2006)
Shear force	2 (150)	SW834 – S0226	Liu et al. (2007), Meyers et al. (2007)
	7 (87)	SW175 – S0115	Edwards et al. (2008), Harmegnis et al. (2006)
	13 (112)	SW398 – S0289	de Koning et al. (2001)

SSC *Sus scrofa* chromosome, [1] position of the QTL in cM, [2] references of other studies reporting QTL in similar regions of the specific chromosome

Table 5: Reported QTL in the literature around similar locations as the QTL identified in the present study *(cont.)*

Meat quality	SSC (position cM)[1]	Flanking marker	Reference[2]
IMF	1(263)	SW2512	Beeckmann et al. (2003), Duthie et al. (2010), Edwards et al. (2008)
	5 (87)	S0005 – SW1987	Ma et al. (2009)
	6 (101)	S0059 – S0003	de Koning et al. (1999)

SSC *Sus scrofa* chromosome, [1] position of the QTL in cM, [2] references of other studies reporting QTL in similar regions of the specific chromosome

Of particular relevance to this trait are the two epistatic QTL studies of Ovilio et al. (2002) and Duthie et al. (2010), which have revealed two epistatic QTL pairs related to loci on SSC1 and SSC4 and on SSC6 and SSC9. Here we identified four epistatic QTL loci on SSC1 (263 cM), SSC5 (87 cM) and SSC6 (101 cM). The QTL region detected on SSC1 was comparable to the identified epistatic QTL locus described by Duthie et al. (2010) and to the individual QTL in other studies on this trait (Beeckmann et al. 2003, Edwards et al. 2008). In other single-QTL studies, loci on SSC5 (87 cM) and SSC6 (101 cM) have been identified as influencing IMF (de Koning et al. 1999, Ma et al. 2009).

Significant epistatic relationships can be observed between QTL positions on SSC7, SSC13 and SSC16, which mainly influence the expression of cooking loss and shear force. A QTL locus on SSC7 (40 to 50 cM) for cooking loss has been reported by de Koning et al. (2001) in an F_2 cross of Meishan and commercial Dutch pigs and this region carries the MHC genes, which are potential candidate genes (Smith et al. 1995). Other single-QTL analyses have revealed epistatic loci on SSC13 (13 cM) and SSC16 (108 cM) (Kim et al. 2005, Liu et al. 2008). The epistatic QTL position on SSC16 (102 to 108 cM) also interacts with loci on SSC4 (43 cM, cooking loss) and SSC2 (145 to 160 cM, shear force). Though a novel QTL, SSC16 may play an important role in tenderness traits.

Three epistatic QTL pairs not yet mentioned are involved in the expression of loin pH 24h. All the QTL positions involved have been reported in the literature and are relevant for meat quality (de Koning et al. 2001, Evans et al. 2003, Harmegnies et al. 2006, Ovilo et al. 2002). Moreover, four QTL pairs involving eight epistatic QTL loci are relevant for loin pH 1h. Although all the positions for this trait have not been published yet, many other loci are well known. The high number of epistatic interactions shows the complexity of

postmortem metabolic processes in meat, which need further clarification (Carlborg et al. 2004a). As an example of this complexity, figure 6 depicts all the epistatic loci for pH traits. Most QTL pairs have an impact on more than one trait, and the number of QTL positions that epistatically influence a single trait ranges from three to eight. Pleiotropy and co-regulation are important factors of genetic control to compensate for up- and down-regulation of correlated traits by gene interactions (Brockmann et al. 2000, Wolf et al. 2006).

Epistasis appears to be an important contributor to genetic variation in carcass composition and meat quality traits. Subdividing epistatic effects into the structural types (a×a, a×d, d×a and d×d) allows a deeper insight into the genetic mechanisms behind the expression of these phenotypes. As shown in table 4, all types of structural epistasis can be found across all traits. Often, more than one component is significant, indicating complex genetic structures, particularly for meat quality traits. On average, d×d interactions are the most prevalent. Twenty-nine pairs exhibit d×d, 19 a×a, 11 a×d and 13 d×a epistatic effects. Moreover, the importance of dominance becomes more obvious by summing up the three epistatic effects (a×d, d×a and d×d) that comprise dominance. With respect to all traits, we observed this composite effect for 33 of 40 cases, which makes it more important than a×a effects. Epistatic dominance contributes to heterosis, and it has been widely shown that heterosis plays an important role in the genetics of carcass composition and meat quality (Sellier and Monin 1994).

For seven QTL pairs, a×a effects were more prevalent in the expression of traits (e.g., epistasis among SSC3 and SSC14 for conductivity 1h loin) than were other interaction effects containing dominance. According to Carlborg and Haley (2004), a×a effects are indicators of co-adaptive epistasis and occur when the homozygous alleles of the two loci that originate from the same parental line show enhanced performance. This type of gene interaction is particularly interesting, since the loci have no significant individual effects (Carlborg and Haley 2004). This might be the reason why some of our novel epistatic QTL positions have not been found in a single-QTL analysis. Selection strategies among the parental lines might lead to fixation of different alleles at the relevant loci, regulating the expression of a specific phenotype in a way that makes statistical epistasis unapparent in either population (Noguera et al. 2009).

2.6. Conclusions

In the present study, a bi-dimensional scan identified a large number of epistatic QTL pairs involved in the expression of carcass composition and meat quality traits. These results show that the genetic architecture of carcass composition and meat quality is mainly composed of a complex network of interacting genes rather than of the sum of individual QTL effects. Combining epistatic QTL experiments with subsequent gene expression profiling can be a promising strategy to clarify the underlying biological processes of muscle development and metabolism.

Chapter 3. Selective transcriptional profiling considering epistatic QTL genotype pairs in pig

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3.1. Abstract

Based on a previous survey, the aim of our study was to identify potential candidate genes on porcine chromosome (SSC) 8 and 15 for muscle pH in pigs. In order to analyse performance of F_2 animals of a Duroc × Pietrain cross, epistatic QTL and gene expression profiling approaches were combined. Three alternative statistical methods were used to investigate the expression profiles for pH in loin, recorded 1 h post morten. Method A considers the phenotypic differences in pH values between groups of pigs with extreme values. Method B was based on differences between the genotype combinations of relevant epistatic QTL pairs between SSC8 and SSC15. Finally, method C was a linear model comprising the epistatic QTL genotypes as fixed effects. Overall method A, B and C revealed 1182, 480 and 1823 differentially expressed or associated genes, respectively. By means of a functional analysis it was possible to set up networks which contained mainly interactions between genes located within the specific regions on SSC8 and SSC15 and allowed a meaningful biological discussion. Expression QTL (eQTL) analysis was performed for functional and positional transcripts using a simple regression model. It revealed that the highest number of eQTL was detected for transcripts selected by method B through additive effects. The previously identified epistatic QTL positions were included as genetic background effects into the model so that four eQTL were additionally detected. This approach showed that combining phenotype, genotype and transcriptome data helps to uncover the involved molecules of observed epistasis.

3.2. Introduction

Technological and sensorial parameters are important key factors in pork processing. Particular pH, measured at different time points after slaughtering, is highly influencing the water holding capacity, tenderness and flavor of pork meat. It is known that pH traits have a mixture of polygenic and quantitative trait loci (QTL), comprising large effects (Sellier 1998). Until now many QTL and potential candidate genes e.g. RYR1, PRAG3 have been identified related to meat quality traits (Fujii et al. 1991, Hu et al. 2010, Milan et al. 2000).

Furthermore, expression QTL (eQTL) studies were used to dissect the genetical background of meat quality traits and helped to identify potential candidate genes (Jansen and Nap 2001, Lobjois et al. 2008). QTL and eQTL analysis considering additive and dominance effects of a single locus have been successfully performed and revealed several

promising regions and candidate genes related to meat quality traits in a Duroc × Pietrain population (Liu et al. 2007, Ponsuksili et al. 2008). Epistasis, as a more complex genetic effects, have been considered in investigations in pigs (Duthie et al. 2010, Ovilo et al. 2002). Grosse-Brinkhaus et al. (2010) revealed that a considerable proportion of the genetic variance could be explained by the interaction of epistatic QTL pairs in the Duroc × Pietrain population. Particular the analysis of the functional relationship among two loci is promising to reconstruct genetic pathways that are involved in complex trait regulation (Carlborg and Haley 2004).

It has been shown in yeast, *Drosophila melanogaster* and mouse strains that epistasis plays an important role related to the variation in gene expression (Brem et al. 2002, Chesler et al. 2005, Wittkopp et al. 2008). Until now, whole transcriptional profiling related to eQTL analysis considering epistasis has not yet been performed for livestock species. Detected eQTL can be classified into a locus which is close located to a gene that is being controlled (cis-acting) or one or more loci which are located far from the actual gene that is being controlled (trans-acting) (Jansen and Nap 2001). Epistasis has been described in such a situation as trans-acting effect of a gene that is controlled by more than one factor (Bueno Filho et al. 2006, Jansen and Nap 2001).

A previous study by Große-Brinkhaus et al. (2010) showed many epistatic QTL pairs for carcass composition and meat quality traits in pigs. A single epistatic QTL pair among the porcine chromosomes 8 (SSC8) and 15 (SSC15) related to early pH in loin has been chosen to be investigated further and to explain underlying biological mechanisms. Moreover these QTL regions are functionally promising since they have been also detected previously by a single QTL analysis (Liu et al. 2007).

The aim of this study was to combine epistatic QTL and gene expression profiling approaches in order to elucidate biological causes of the identified interaction. Furthermore eQTL analysis was performed to investigate interacting background effects of potential candidate genes.

3.3. Material and methods

3.3.1. Animals and experimental design

Phenotypes and genetic data of reciprocal Duroc × Pietrain cross (DuPi) was used. This porcine resource population has been already described in detail by Liu et al. (2007). In brief, all animals were kept and performance tested at the Frankenforst experimental research farm of the Rheinische Friedrich-Wilhelms-University in Bonn according to the rules of German performance stations (ZDS 2003). Carcass composition and meat quality traits of 585 F_2 animals were recorded in a commercial abattoir. PH-values have been measured at 45 min and at 24 h post mortem in loin, *M. long. dorsi*, between the 13th and 14th ribs using the star-series equipment (Rudolf Matthaeus Company, Germany). pH decline was calculated as difference of these two measurements. In order to obtain muscle gene expression data, 74 F_2 animals were selected based on their drip loss phenotype.

3.3.2. RNA isolation and microarray preparation

Tissue samples were taken between the 13th and 14th ribs from the center of *M. long. dorsi* and snap frozen. Total RNA was isolated using TRI Reagents (Sigma, Taufkirchen, Germany) and used for target preparation for microarray hybridization. According to Affymetrix (Affymetrix, UK) protocols 5 µg of total RNA was used to prepare antisense biotinylated RNA based on the Affymetrix One cycle synthesis and labeling kit. The quality of hybridization was assessed in all samples following the manufacturer's recommendations. Data of the processed Porcine Genome Array provided by Affymetrix were analyzed with Affymetrix GCOS 1.1.1 software using global scaling to a target signal of 500. More detailed information are given in the study of Ponsuksili et al. (2008). The microarray data related to all samples were deposited in the Gene Expression Omnibus public repository (GEO accession number: GSE10204). Annotation and localization of probe-sets was based on assembly Sscrofa9 (April 2011). In order to identify positional candidates related to the epistatic QTL pair, 14 microsatellites on SSC8 and SSC15 were used to set up a genetic map, which were linked to the linkage map of our QTL study. Eight microsatellites could not be localized based on their sequence on Scrofa9. Therefore close located markers identified by USDA-MARC database (Rohrer et al. 1996) were used to map an average position.

3.3.3. Statistical microarray processing

All analysis were performed with the statistical software R (version 2.10.1) and related packages from Bioconductor. Generally, all microarrays were background corrected and normalized with the GeneChip Robust Multiarray Analysis (GCRMA) algorithm (Irizarry et al. 2003). This algorithm uses sequence information within a model for the background correction of the raw intensity values followed by a quantile normalization (Workman et al. 2002). In comparison to other normalization methods like MAS, RMA or PerfectMatch it has been shown that this method is superior in accuracy and precision (Wu and Irizarry 2004). Differential expression and associated expression of individual genes was performed with linear modeling and empirical Bayes methods as implemented in the R package “Linear Models for Microarray Analysis” (LIMMA) (Smyth 2004).

3.3.4. Selective transcriptional profiling

Three alternative statistical methods were used to analyze the expression profiles related to the early pH value recorded 1 hour post mortem (pH1) in loin and an identified epistatic QTL pair located on SSC8 and SSC15.

Method A considered the distribution of pH1 records. Among the 74 DuPi selected by Ponsuksili et al. (2008), two extreme groups of five discordant sib pairs were chosen. A linear contrast was used to test the gene expression differences of the current extreme phenotype groups. This frequently used method was realized to show the general differences between a low and high early pH in muscles.

A second approach (Method B) was focused on a phenotypic within-genotype selection. For each animal the probability of line of origin (Duroc or Pietrain) was estimated for the epistatic positions on SSC8 (1 cM) and SSC15 (77 cM) by means of the Markov chain Monte Carlo (MCMC) algorithm of Qxpak (Cardoso et al. 2008, Perez-Enciso and Misztal 2004). According to the highest probability, all F_2 pigs were assigned to the breed specific genotypes. The P and Q allele on SSC8 and SSC15 were assigned to the Duroc breed (PP, QQ), whereas allele p and q traced back to the Pietrain breed (pp, qq). The genotypes Pp and pP as well as Qq and qQ were treated as identical genotypes, resulting in 9 different genetic group combinations. A linear model was used to identify significantly different genotype combinations:

$$y_{ijklm} = gen_j + sea_k + \beta_a(age_{ijklm}) + \beta_s(sw_{ijklm}) + SSC8_l + SSC15_m + (SSC8_l \times SSC15_m) + e_{ijklm} \quad \text{Eq [1]}$$

where y is the phenotype of pH1 of the i^{th} F_2 offspring. Along with the effect of the genotype, the model comprises the fixed effect of slaughter season (sea), gender (gen), age (age) and weight (sw) at slaughtering as covariables (β_a and β_s). Differences between all genotype combinations were tested by multiple mean comparisons (Tukey Test, $P < 0.05$). Significant differences were observed between QQpp:QqPP, QqPp:QqPP and QQpp:QQPP ($p < 0.05$) (figure 7). Genotypes involved in these contrasts were chosen for further analysis of the gene expression profiles.

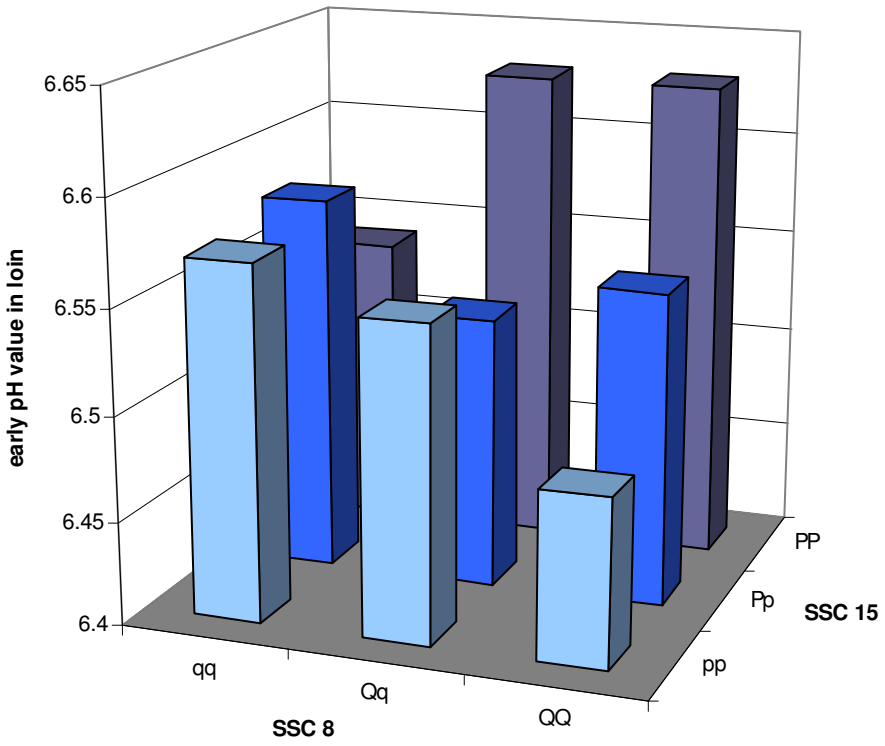


Figure 7: Average early pH values in *M. long. dorsi* of different genotype combinations between an epistatic QTL pair located on SSC8 and SSC15.

Genotypes were obtained from a full epistatic genome scan for meat quality and carcass composition traits using Qxpak (Grosse-Brinkhaus et al. 2010).

Corresponding microarray data were available from pigs which belong to the groups QqPp (N = 21), QqPP (N = 4), QQpp (N = 7), QQPP (N = 2) (table 6). Differences between the

divergent groups were validated by linear contrasts of gene expression profiles. In order to reduce within group variation, group QqPp was splitted into 3 sub groups, which contain only fullsibs QqPp FS (N = 4), half-sibs QqPp HS, (N = 6) or a combination of full- and halfsibs QqPp HS, FS (N = 9). All other genotype groups (QqPP, QQpp) are characterized by a mixed full- and halfsib structure. Additionally, to avoid unbalanced contrasts, number of samples within each group was restricted to a maximum of 4. Surplus samples were removed at random. Because only two microarrays were available which contained the genotype QQPP, linear contrasts were not performed. The results of all linear contrasts were combined to one data set for further pathway and network analysis.

Table 6: Distribution of genotype combinations among the different data sets and the average early pH value in loin.

Genotypes[1]	SSC8	qq	Qq	QQ	qq	Qq	QQ	qq	Qq	QQ
	SSC15	pp	pp	pp	Pp	Pp	Pp	PP	PP	PP
N[2]	585	36	92	45	77	173	86	39	72	28
	74	6	10	7	7	21	9	5	4	2
early pH values[3]		6.57	6.55	6.48	6.58	6.53	6.55	6.54	6.63	6.63

[1]The genotypes were derived from the line of origin probability taken from the MCMC algorithm of Qxpak for the specific positions on SSC8 (1 cM – QQ, Qq, qq) and SSC15 (77 cM – PP, Pp, pp), the QTL combinations were assigned to the highest value of the probability for all 585 animals. The genotypes Pp and pP as well as Qq and qQ were treated as identical genotypes, resulting in 9 different groups. [2]N = number of animals in total and per genotype combination. [3]average early pH in loin for the group of 585 animals

The last analysis (Method C) considered all 74 animals and the expression profiles of the transcripts (exp) were analyzed with the following model:

$$\begin{aligned} exp_{ijk\,ln\,m} = {} & gen_j + sea_k + fam_n + \beta_a(age_{ijklm}) + \beta_s(sw_{ijklm}) \\ & + SSC8_k + SSC15_l + (SSC8_k \times SSC15_l) + e_{ijk\,ln\,mn} \end{aligned} \qquad \text{Eq [2]}$$

The expression values were corrected for the same effects as Eq [1] including the genotype information of the loci on SSC8 and SSC15. Additionally, the effect of full-sib family (fam) was included to avoid family stratification (Kraft et al. 2003). Significant association was observed when following thresholds were fulfilled: p-value < 0.05 and false discovery rate (FDR) < 0.3.

3.3.5. Pathway and network analysis

The lists of significant differentially expressed transcripts according to method A and B or significantly associated transcripts according to method C were evaluated using Ingenuity Pathway Analysis software (IPA 2008, www.ingenuity.com). The analysis of RNA expression data was performed taken into account known biological response, functional categories and regulatory networks as well as other higher-order response pathways. The modules of IPA identified biological functions that were most significant. For all analyses, Fisher's exact test was used to calculate a *p*-value which expresses the probability that each biological function assigned to that data set was due to chance alone.

The online tool STRING-8 was applied to identify the relationship among molecules which transcripts were located on SSC8 or SSC15. STRING-8 is based on large databases of known and predicted protein interactions and contain direct (physical) and indirect (functional) interactions, derived from four sources: high-throughput experimental repositories, conserved expression, previous knowledge and, computational prediction methods (Jensen et al. 2009). To concentrate this analysis on the specific regions of the epistatic QTL pairs, the data was filtered by the homologous human chromosomal regions according to the information of comparative maps (Fridolfsson et al. 1997b). Transcripts were included, if they have been located on *Homo sapiens* (HSA) chromosome HSA2 or HSA4 which contain the ortholog regions according to SSC8 and SSC15. For the analysis with STRING, the official homolog human gene names corresponding to the particular porcine probe sets have been used as reported by Tsai et al. (2006).

3.3.6. Expression QTL analysis

For the eQTL analysis, 125 microsatellites and six SNP markers were available for all animals of the parental (P), F_1 and F_2 generations as described by Grosse-Brinkhaus et al. (2010). Genetic markers were equally distributed on the 18 pig autosomes and covered 89% of these. The detection of eQTL has been already performed by Ponsuksili et al. (2008) for 11,457 probe-sets of this data set related to drip loss. Due to the additional genetic marker located on SSC8, the analysis was extended for transcripts which were located on SSC8 or SSC15.

In order to analyze transcripts within the region of the previously identified epistatic QTL region, the average genetic position of these QTL were determined. Corresponding

confidence intervals of the QTL locations were calculated using the Likelihood drop method (Lander and Botstein 1989, Noguera et al. 2009). Confidence intervals for epistatic QTL on SSC8 (1 cM) and SSC15 (77 cM) were calculated as 0 to 36 cM and 53 to 84 cM, respectively. The genetic positions were determined using closely located genetic markers, so that transcripts located with in an area of 0 to 42 mb on SSC8 and 98 to 133 mb on SSC15 were used for the eQTL analysis.

The eQTL detection was performed using the F_2-option of GridQTL. Additive and dominance effects were evaluated within 1 cM intervals using a regression approach (Seaton et al. 2006). The eQTL model comprises the fixed effect of slaughter season, family and gender, age and weight at slaughtering as covariables. These effects have been chosen according to the QTL studies performed in meat quality traits and gene expression profiles (Liu et al. 2007, Ponsuksili et al. 2008). Chromosome-wide significance levels were estimated by permutation tests using 1000 permutations (Churchill and Doerge 1994). Lander and Kruglyak (1995) proposed a suggestive threshold which approximately corresponds to a 5% chromosome-wide significance level. To test for epistatic influences of the QTL loci detected previously (Grosse-Brinkhaus et al. 2010), the epistatic QTL positions on SSC8 (1 cM) and SSC15 (77 cM) were included into the model as background genetic effects, successively.

3.4. Results

As has been described above three alternative methods (A, B and C) were used, in order to identify biological causes of the epistatic relationship between SSC8 and SSC15. All applied methods revealed different numbers of candidate genes (table 7). Method A is a standard approach to investigate differences among extreme phenotype groups. For each group, five discordant halfsibs were selected based on pH1-phenotypes. These two groups showed a distinct difference in pH1 of 0.58 (6.88±0.05 vs 6.30±0.12, mean ± SD, ($p \leq 0.00001$)). Across the whole genome 1419 significant differentially expressed transcripts between both pH-groups comprising 1182 genes have been identified. Regarding the group of low pH values, 651 genes were down regulated, whereas 531 were up regulated. The observed FDRs ranged from <0.0001 to 0.24 which is reasonable for such kind of microarray taking into account the relatively relaxed p-value (< 0.05) (Wimmers et al.

2010). Moreover within this group of 1182 differential expressed genes, 35 porcine orthologe genes were localized on SSC8 and 47 on SSC15.

Whereas method A only depends on phenotype differences, method B and C considers genotypes of the epistatic QTL pair between SSC8 and SSC15.

Table 7: Differentially expressed or associated genes of the different applied Methods

Method	diff. expressed[1]	no. of genes[2]	up regulated		down regulated		SSC8[3]	SSC15[3]
A	1419	1182	531		651		35	47
B	515	480	353		127		12	24
Method	sign. associated[1]	no. of genes[2]	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.001$	$p \leq 0.0001$	SSC8[3]	SSC15[3]
C	2073	1823	704	791	260	68	42	55

[1]Number of differentially expressed or significant associated transcripts. [2]Number of genes, derived from the differentially expressed or significant associated transcripts based on the gene annotation. [3]Number of genes located on *Sus scrofa* chromosome (SSC) 8 or 15

According to the results of method B, 515 differential expressed transcripts which belong to 480 genes. Regarding genotypes with low pH1 groups, 353 transcripts were up regulated and 127 were down regulated. Furthermore, among the genes received applying method B, 12 genes were found to be located on SSC8 and 24 on SSC15.

In method C all array were investigated using a linear model. Moreover, this method depends on the frequencies of the genotype combinations. The frequency of the observations within each genotype combination is given in table 6 (p. 54). The proportion of observations within the whole dataset (N=585) and within the subset (N=74) is almost the same. Based on fixed model factors genotype combinations 2073 transcripts belonging to 1823 genes were significantly associated. Among these genes 68 were highly significant associated ($P<0.0001$) with the epistatic genotypes. Moreover, 42 and 55 porcine orthologe genes were located on SSC8 and SSC15, respectively.

According to the underlying statistical methods differentially expressed or associated genes are termed gene set A, B and C, respectively. All three gene sets had 24 genes in common,

including only four genes which were located on SSC15. This was not surprising because this result can be explained by the different applied methods.

3.4.1. Functional analysis of genes located within the epistatic regions

Based on the significant results of all applied statistical methods, Ingenuity Pathway Knowledge Base (IPA) was used to verify the biological importance and to elaborate the functional annotation of the particular gene sets as well as their impact to canonical pathways.

40, 22, and 56 different significant canonical pathways were identified for the gene sets of these methods, respectively. Figure 8 contains eight selected pathways where at least the gene sets of two of three applied methods were enriched. Caveolar-mediated Endocytosis Signaling pathway was detected by the results of all three methods. Method A was predominating in protein ubiquitination pathway and butanoate metabolism. Calcium signaling was detected for genes of method B and less enriched for genes of method A. The results of method C related to the association to an epistatic QTL pair, revealed mainly significant enriched pathways regarding signaling: Integrin signaling, actin cytoskeleton signaling and ILK signaling. Additionally, mitochondrial dysfunction was only significantly identified through the genes of method C.

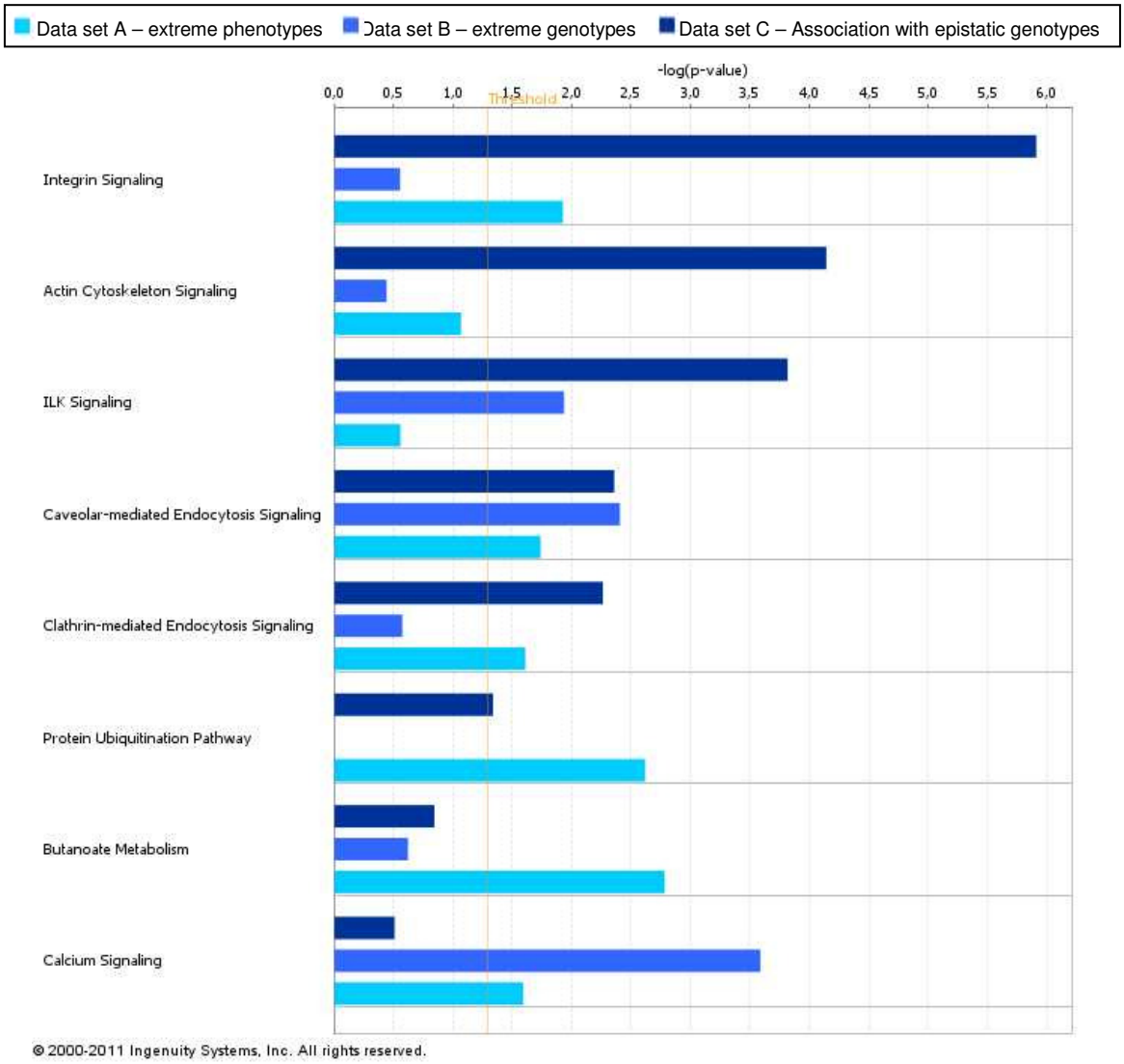
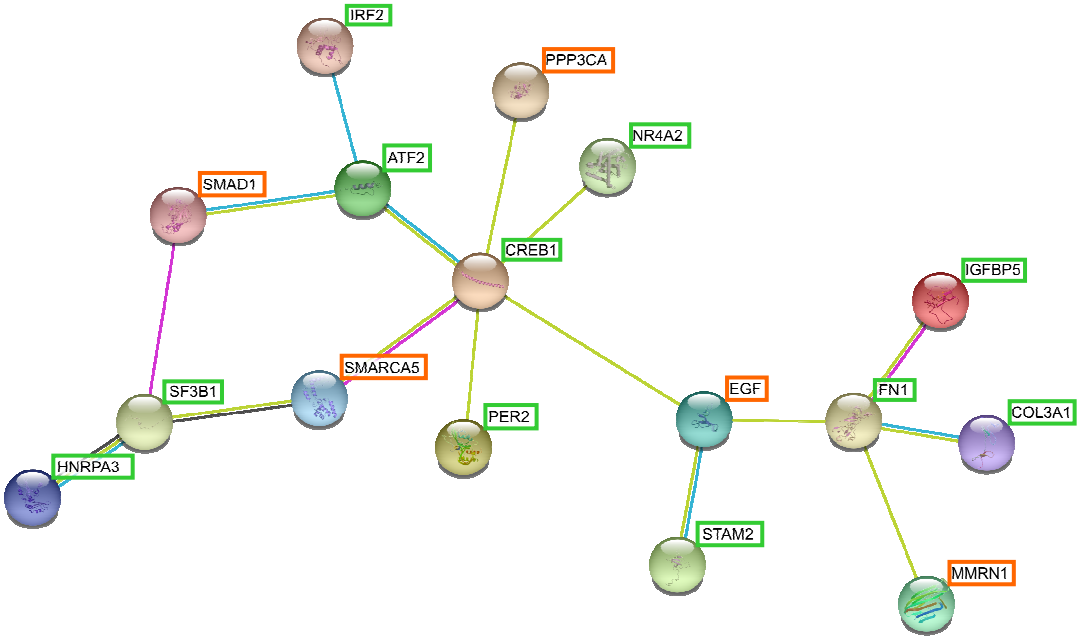


Figure 8: Global canonical pathway analysis

Comparison of three data sets (Data set A: extreme phenotypes, data set B: extreme genotypes, data set C: association with epistatic genotypes). The data sets were analyzed using the Ingenuity Pathways Analysis Software. The significance is expressed as a p value calculated by a Fisher's exact test.

In the next step, the data sets of method A-C were analyzed using text mining online tool STRING-8. STRING-8 in comparison to IPA enables to analyze small sets of genes and an assumption about a concrete functional relationship between these two chromosomal regions. This advantage leads finally to the relevant genes that underlie the epistatic QTL pair. Based on analogous human information it was possible to set up three different networks (figure 9).

Network A:



Network B:

Network C:

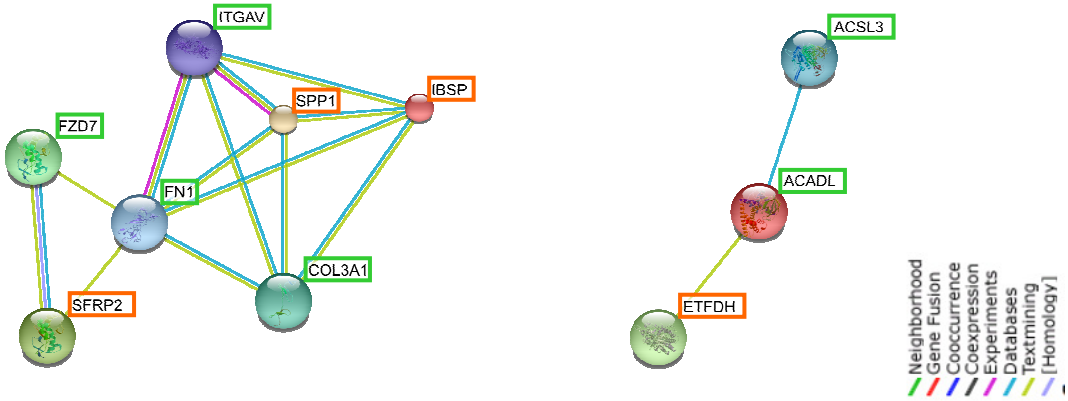


Figure 9: Evidence of biological interactions between the porcine chromosome 8 and 15.

The network A, B and C corresponds to the data set A, B and C, respectively. Genes located on SSC8 are surrounded by a orange box and genes located on SSC15 with a green one. The networks were obtained using the online tool STRING

Networks were only retrained when an interaction among molecules could be observed and the positions of transcripts of involved molecules were located on SSC8 or SSC15. These protein-networks contained three to 16 molecules. The majority of the interacting proteins were based on text mining and databases of known and predicted protein interactions. Moreover, it was also possible to observe relationships among proteins that traced back on experiments, co-occurrence and co-expression.

3.4.2. The eQTL analysis

The eQTL analysis was concentrated on genes which were located in the intervals of the epistatic QTL on SSC8 and SSC15. Additional genes were selected based on functional interactions due to network analysis (table A3, see chapter 7, pp. 101), so that for the eQTL studies 18 positional and 23 functional candidate genes were finally used. Furthermore, three genes had two different probe sets so that both were used for analysis.

In total seven eQTL were identified on SSC8 and six on SSC15 (figure 10, table 4A see chapter 7, p. 104).

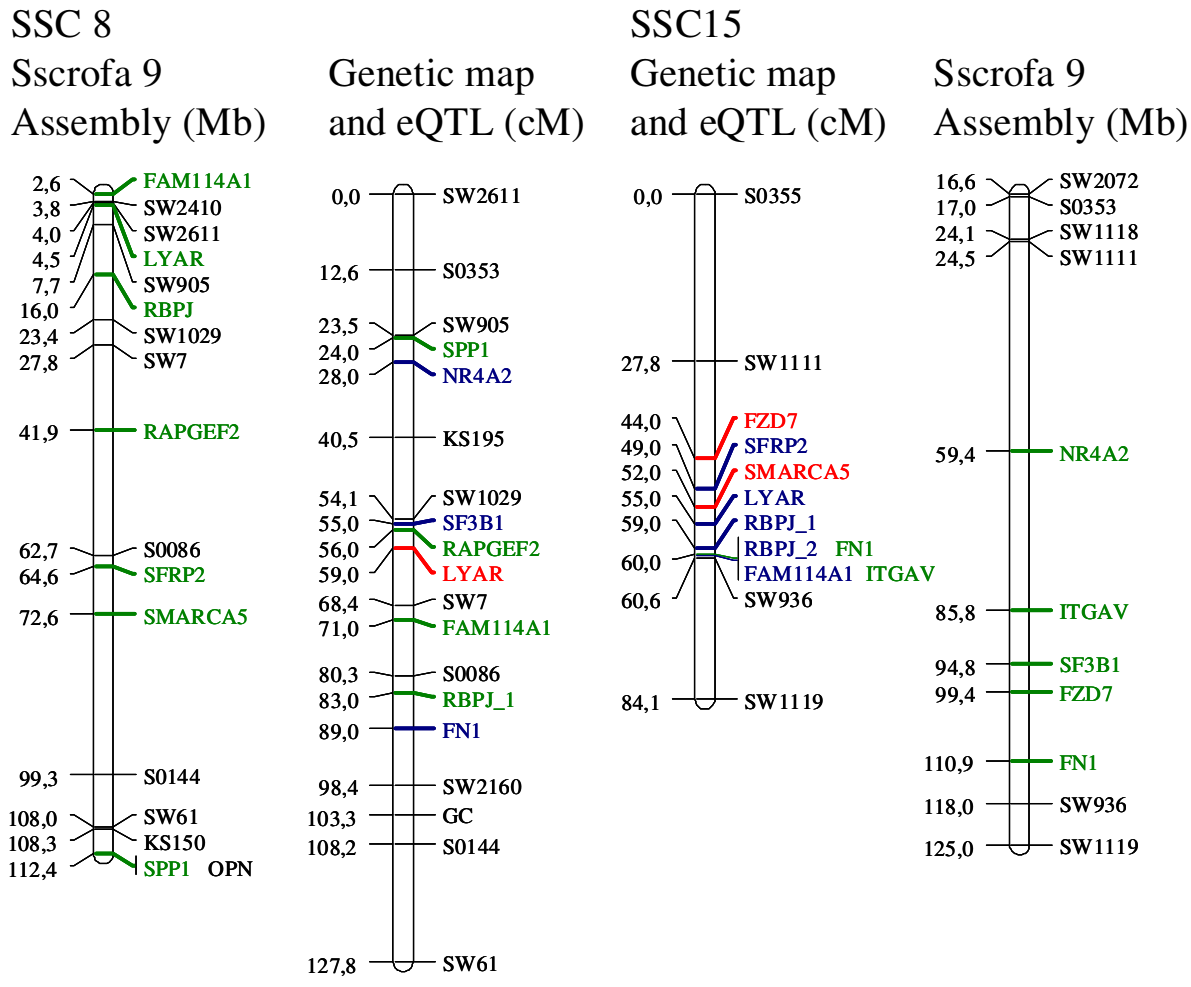


Figure 10: Position of eQTL of probe sets that were obtained by the various applied methodologies.

Left and right outside: physical map with position of the microsatellite marker (black) and genes (green) represented by the probesets Mb according to the Sscrofa9 genome sequence. Middle: sex-average linkage maps of SSC8 and SSC15 with the position of the microsatellites (black) and eQTL corresponding probe sets (cM). eQTL of transcriptes on the same chromosome like the corresponding gene (green), eQTL of transcriptes on different chromosome like the corresponding gene (blue) and eQTL of transcripts because of epistasis as background genetic effect (red)

The genes, *fibronectin* (FN1) and *integrin alpha V* (ITGAV) seemed to be cis-regulated. Within the previously detected epistatic QTL region on SSC8 only two eQTL were identified, whereas for the position on SSC15 nine eQTL were detected through different methods. Only FN1 showed an interaction with SSC8, but the observed eQTL was not located within the epistatic region. Furthermore one novel eQTL was detected on SSC8 by including the epistatic position on SSC15 into the model. This was also observed for two eQTL on SSC15 where the epistatic location observed on SSC8 was included as background genetic effect.

Comparing the outcome of the different applied methods, the highest number of eQTL (six) were observed for method B, although this method revealed the lowest number of candidates according to the number of differentially expressed genes. Transcripts of method A allowed to identify four eQTL and performed better than method C (two eQTL), where the most transcripts for this analysis step were available.

3.5. Discussion

Grosse-Brinkhaus et al. (2010) have detected in their study epistatic QTL pairs. Based on these results different approaches were used to analyze expression profiles to explore the genetic causes of epistatic relationships between SSC8 and SSC15 influencing pH traits. Method A considered the differences between extreme phenotypes (extreme pH values). This approach is a standard method to identify genes which were generally differentially regulated according to the difference between the phenotypes. Several studies in pigs applied this method in order to identify differential expressed genes according to the extreme phenotypes (Canovas et al. 2010, Grindflek et al. 2010, Ponsuksili et al. 2008). In the present study only a few numbers of the identified genes were located within the epistatic QTL regions of SSC8 and SSC15. The linear relationship between the detected genes and the phenotypes might prevent the detection of epistatic interaction. It can be assumed that the gentoypes of the animals within the groups is mainly homozygote, therefore mainly additive effects among the genes will be observed (Bueno Filho et al. 2006). Generally, this method was less suitable to investigate the causes of epistatic QTL pairs because the relationship between two or more loci was not considered in the model.

In order to identify genes which are controlled by the epistatic QTL regions, method B was applied. Method B allowed to investigate the impact of specific gene regions (SSC8 and

SSC15) on the transcriptomes. Bueno Filho et al. (2006) developed a design to estimate *trans*-acting epistatic effects. In this simulation study only 10 two-color slides were used to investigate the various groups and effects. In order to consider the effect of the specific genotypes on the phenotype (pH1) only significant genotype combinations were compared following the concept of selective transcriptional profiling (Jin et al. 2004, Wang and Nettleton 2006). Furthermore the number of animals per group was limited to four because of the number of available animals per genotype class. Moreover, in this experiment it was not possible to control the phenotypic variance within the specific genotype completely. It can be assumed that specifically selected animals for each genotype combination would lead to more precise results. The number of observed differential expressed genes was smaller compared to the results of method A. The consideration of the genotypes of the QTL in SSC8 and SSC15 allowed to identify more unique genes according to the epistatic interaction.

In contrast to the other methods, method C considered all microarrays within a linear model which increases the power to clarify the causes of the epistasis between the QTL in SSC8 and SSC15. The consideration of gene interaction within the analysis of transcriptional profiles is novel in livestock animals. However, it can be expected that this method revealed the highest numbers of genes within the epistatic QTL regions. However, the risk to detect possible artifacts was increased, because pleiotropic effects of the QTL genotypes might also affect other traits. Pleiotropy in relation to gene interaction was described in maize by Schadt et al. (2003). These authors observed interactions among genes related to ear leaf tissue. Investigation according to epistasis have been mainly reported in yeast and drosophila, (Brem et al. 2002, Gibson et al. 2004, Storey et al. 2005).

Currently, the specific biochemical mechanisms of the pH change and the consequences for the muscle *post mortem* conversion to meat are not fully understood. The complex genetic structure of such quantitative traits requires the investigation of epistasis to uncover the various biological processes.

The received genes of the particular applied methods were further investigated using pathway and network analysis. These approaches allowed to draw a conclusion of the molecular function of interacting epistatic QTL regions. In general, all enriched canonical pathways were related to signaling processes. Results of method A revealed pathways which have been discussed in previous studies focused on drip loss, like integrin signaling, actin cytoskeleton or the protein ubiquitination pathway (Ponsuksili et al. 2008). The

calcium signaling pathway was significantly enriched in the data set of method B. Calcium plays a central role in the muscle metabolism. A high post mortal calcium concentration causes a rapid contraction and in consequence of an accelerated muscle metabolism a rapid pH (Huff-Lonergan and Lonergan 2005). The integrin signaling pathway was most prominent for genes obtained by method C. The rate of early pH decline is strong correlated with water holding capacity, because the degradation of membrane proteins is associated with the increase of drip loss (Zhang et al. 2006).

The functional analyses of entire gene sets provide valuable information of biological relevance, but concrete characterizations of the interaction itself is not given. Therefore the network analysis of the online software STRING has been used. The network identified for the data sets of method A and B (figure 9, p. 60) revealed mainly biological function related to extracellular matrix region and gene regulatory mechanisms. Although method C obtained the highest number of genes, only one functional interaction between two genes could be observed. Here, cellular processes within the mitochondria lumen and its matrix were enriched. The dysfunction of *acyl-CoA synthetase long-chain family member 3* (ACSL3), *acyl-CoA dehydrogenase, long chain* (ACADL) and *electron-transferring-flavoprotein dehydrogenase* (ETFDH) leads to several disorders like mitochondrial dysfunction in human (Illig et al. 2010).

In pigs, mitochondrial dysfunctions have already been described by Eikelenboom and van den Bergh (1973) and based on the mitochondria respiratory rate in muscle. This dysfunction was associated with the RYR1 locus (Fujii et al. 1991) which was manifested in the Pietrain breed. Werner et al. (2010) repeated this experiment with breeds of Duroc, Piertrain and their crosses and differentiated between MHS (Malignant Hyperthermia Syndrome) positive and negative animals. They observed no differences between the breeds during the analysis of mitochondrial respiratory rate, but during chilling in negative MHS pigs the mitochondria respiratory activity was higher extended than in the other animals.

Despite the identification of the RYR1 loci that is involved in PSE meat, there is still variation within pH1 and the pH decline from 1 to 24h which leads to an abnormal meat quality like RSE (reddish soft and exudative). It can be assumed that one reason might be the described mutations identified in human and could have a similar effect in the metabolic processes in pigs. Moreover, it has been shown that high muscle pigs with a high

lean meat content usually are associated with worsened meat quality parameters (Tholen et al. 2005) which leads to differences in fatty acid metabolism.

An eQTL analysis was performed in order to investigate whether a gene was regulated by one of the epistatic QTL locus. Therefore, genes located on SSC8 or SSC15 were selected based on their location within the interval of the epistatic QTL regions or if they belong to a functional interaction. Epistasis has been in the context related to gene expression as *trans*-regulating factor (Bueno Filho et al. 2006, Rosa et al. 2006). In the present study the epistatic relationship was analyzed including the second loci as background genetic effect into the model, because for the investigation of epistatic eQTL, a sufficient large number of individuals and gene expression records are necessary (Carlborg and Haley 2004). It has been described by Jin et al. (2004) that detecting a major QTL with an additive genetic effect in a F_2 population has the most power in a sample that has a 1:1 ratio of individual homozygous for a QTL locus. However, a random sample, being in Hardy-Weinberg-equilibrium, would reveal a ratio of 1:2:1 where heterozygote individuals appear twice. From this follows, that this would require up to twice the sample size in order to investigate non-additive effects with the same power as additive relationships. Method A and B were focused on genes associated with extreme phenotype or genotype groups. It can be assumed that interactions among these genes are mainly additive and thus it was not surprising that most of the transcripts chosen by method A and B revealed more significant eQTL than transcripts of method C. In order to analyze epistatic effects among eQTL it would be necessary to increase the sample size threefold or even more to perform an epistatic eQTL analysis (Carlborg and Haley 2004).

Nevertheless it was possible to detect eQTL that support the relationship between genes on SSC8 and SSC15. Although *fibronectin* (FN1) is not located on SSC8, it was possible to observe a *trans*-regulated eQTL. However, the eQTL of FN1 on SSC8 was not located within the epistatic QTL area so that further investigations are necessary to clarify the relationship of FN1 and the various regions on SSC8. Another eQTL was observed for *osteopontin* (SPP1) on SSC8. Based on the functional analysis of STRING, it was possible to observe an interaction between SPP1 and FN1. This interaction is based on co-expression within the extracellular matrix because both are known substrates of transglutaminase (Beninati et al. 1994, Prince et al. 1991). The eQTL of *Sus scrofa apoptosis-related protein* (PNAS-5) and *family with sequence similarity 114, member A1* (FAM114A1) were found on SSC15 in accordance with the epistatic interacting region.

Moreover both genes have been physically mapped on SSC8 and seem to be regulated by a region on SSC15 based on this study. Especially the eQTL related to PNAS-5 have been only detected while taking the epistatic position into the model. In contradiction, *trans*-regulation of a gene describes the situation where the regulation of a gene by another transcription factor is due to the additive effect (Wayne et al. 2004).

3.6. Conclusion

In the present study, previously identified epistatic QTL pairs were combined with muscle transcriptome profiles to investigate the biological causes of the interaction. The comparison of the alternative methods revealed different numbers of candidate genes characterizing the epistatic QTL regions of SSC8 and SSC15. Furthermore, pathway and network analysis revealed common as well as different biological functions for the particular gene sets. However, particularly the investigation of the epistatic patterns of eQTL was limited by number of analyzed animals. Different databases supported to find reasonable biological explanation for the epistatic interaction among SSC8 and SSC15 affecting the pH-value in pork. A complete epistatic analysis of gene expression profiles with an increased number of individuals will be the next step to investigate the relevance of epistasis on the gene expression level in more details.

Chapter 4. General discussion and conclusion

Herein the impact of epistasis and potential biological causes of observed epistatic QTL pairs were investigated in a Duroc × Pietrain population. It was possible to show that in epistatic QTL screening of 585 F_2 animals a substantial proportion of the phenotypic variance was coved by two-way interactions. This is in accordance with other studies in pigs (compare table 1, p. 12), but it was also possible to detect epistatic QTL pairs with small interacting effects (< 3% of the phenotypic variance is explained by an interacting QTL pair). One reason might be the population size of 585 animals. It has been shown by Wei et al. (2010a) that large populations (500 - 1000 animals) allow to identify QTL with marginal effects. In general, other studies investigated populations with an average size of 300 animals. However, QTL mapping in small populations confounds the effects of QTL with statistical artifacts caused by sampling. Therefore the genetics of complex traits should be studied in large populations (Beavis 1994). In addition, single QTL detection mainly reveals loci with moderate to large effects (Hayes and Goddard 2001), whereas the analysis of epistatic QTL allows to identify loci with minor or even no individual main effects (Montooth et al. 2003).

The effects of the epistatic QTL in the present study were estimated following the model of Cockerham (1954). Kao and Zeng (2002) have shown that Cockerham's model performs more appropriate than other models for studying epistasis in F_2 or backcross populations. However, the estimation of epistatic effects including pairwise interactions of all loci is still computer and time demanding. This is fact especially when the number of parameters is bigger than the number of observations, as it can be observed in high throughput data like SNP-arrays. In the recent years Bayesian methods became popular that allow to model more parameter and effects than the number of observations (Xu 2003). Wittenburg et al. (2011) extended a fast Bayesian method including dominance as well as epistatic effects. This method is computational feasible, but an inherent bias of variance components estimation was observed (Wittenburg et al. 2011). Therefore Gianola et al. (2010) suggested machine learning techniques, combined with parametric models, which might discover hidden patterns of gene by gene interactions and allows further to investigate higher dimensional interactions.

The implementation of genetic information into breeding value prediction and therefore into animal breeding was performed with marker assisted selection techniques (MAS). MAS allowed to improve traits which were influenced by a few number of major genes like RYR1 or PRKAG3. It can be clearly seen that this might insufficient for quantitative

traits where a high number of genes contributes to the complex genetic architecture (Beavis 1994, Dekkers 2004). In contrast, genomic selection approaches allow to implement a large number of SNP into the model for estimating of genomic breeding values (Goddard and Hayes 2007). However, until now the prediction of the total genetic values considers mainly additively acting marker allele effects (Meuwissen et al. 2001). Hu et al. (2011) applied genomic selection techniques to estimate genomic values for somatic embryo number in a cross of soybeans. In addition, it was shown that a successful implementation of epistatic effects leaded to an increased squared coefficient of determination. However, the genetic background in plants is based on recombinant inbred lines which makes it difficult to apply this approach directly to livestock species.

In livestock breeding the performance of a population is improved for a comprehensive multiple-trait breeding goal. Therefore, the genetic relationship between different traits plays an important role especially when common genes have contrary effects on genes which have a favorable effect of these traits as well. A high positive or negative genetic covariance among two traits is founded on a common genetic background (Wolf et al. 2006). Pleiotropy in relation to epistasis has been described in mouse and *Drosophila melanogaster* (Wolf et al. 2006, Yamamoto et al. 2008). It has been described by Tyler et al. (2009) that a locus which is pleiotropic to several traits also participates in a high number of interactions. In the present study several epistatic loci were detected in locations of previously detected single QTL of other traits (Liu et al. 2007). Comparing overlapping single QTL and epistatic QTL region of this study, it was possible to set up complex networks that support the assumption of Tyler et al. (2009). Furthermore, Tyler et al. (2009) showed that pleiotropy and epistasis were elementary characteristics in biological network influencing several complex human diseases. One example in pig might be the RYR1 locus which influences carcass composition as well as meat quality traits. Stinckens et al. (2009) identified that RYR1 had a significant effect of the gene expression level of *insulin-like growth factor* (IGF-2). IGF-2 is known to be a key player gene in muscle growth and development as well as carcass characteristics in pig (Van Laere et al. 2003).

In general, studies which try to uncover biological causes of occurring epistatic effects are rare in pig. Fernandez-Rodriguez et al. (2010) validated previously detected epistatic QTL regions affecting litter size by applying fine mapping techniques. Two candidate genes were chosen according to their localization and their biological relevance. The researchers were able to show that the haplotypes of one gene seemed to be a causative mutation of

one epistatic QTL. In our study, genetic, genomic and phenotypic data were analyzed jointly to identify potential candidate genes. Especially the identification of relevant candidate genes is important in order to clarify the biological causes of an observed interaction as well as to uncover the different mechanisms of epistasis. Furthermore, in both present studies the reliability of the detected epistatic QTL and potential underlying genes was proven applying different statistical criteria, bioinformatic tools and comparisons to literature.

In a two dimensional QTL scan as well as during the analysis of microarray data, the statistical problem of multiple testing occurs, because a large set of statistical interference is fitted at the same time. In order to control the problems of multiple testing, in a two dimensional scan p-values were corrected using a Bonferroni correction. Other authors suggest permutation techniques in order to derive genome-wide thresholds so that false positives are avoided (Stich et al. 2007, Storey et al. 2005, Wei et al. 2010b, Yang et al. 2007). Wei et al. (2010b) performed a regression method of Haley and Knott (1992) and performed finally a F ratio test statistic for the specific model comparisons. In our study permutations techniques could not be applied because of the infinitesimal genetic value within the model, so that a randomization would break the family structure of the data (Mercade et al. 2005).

In order to proof the confidence of detected epistatic QTL, Carlborg and Haley (2004) propose a stepwise procedure using many independent external sources of data like publications or gene databases. In the recent analysis it was possible to compare most of the identified epistatic QTL with previously identified single QTL or relevant candidate genes. A comparable approach was suggested by Hayes and Goddard (2001) who performed a meta-analysis to increase the power of a QTL study and to discriminate between false positive and true QTL which allows an improved estimation of QTL effects. However, until now only few epistatic QTL studies in comparision to single QTL studies were performed in pig so that it is hardly possible to realize a meta-analysis for epistatic QTL.

In the situation of large datasets like microarray data an efficient control of type I error is indispensable, because multiple comparison involve an overall decision which is based on multiple inference (Benjamini and Hochberg 1995). Therefore in chapter 3 (pp. 48) a false discovery rate (FDR) was calculated following Benjamini and Hochberg (1995). This FDR method has been widely applied to genomewide studies (Storey and Tibshirani 2003).

Pathway and network analysis allowed to proof the biological meaning of the results using external information. In general a validation of the experiment is necessary to investigate the transferability to other pig breeds.

Further work might be the extension of the epistatic model by imprinting effects. In a study of Wolf and Cheverud (2009) who investigated body weight in two divergent mouse lines, two epistatic QTL pairs containing imprinting effects were detected. It was possible to show that imprinting patterns were controlled by a genetic background which might modulate the paternal or maternal origin (Wolf and Cheverud 2009). Liu et al. (2007) identified several imprinting QTL in the DuPi population, so that it is promising to extent the epistatic QTL model by imprinting effects.

Additionally, the analysis of eQTL should be extended by the components of the epistatic effects. Therefore, higher numbers of gene expression records are necessary in order to perform a two dimensional genome scan. This might allow comparing epistatic networks among classical phenotypes and transcriptional profiles and would help to clarify the genetic architecture of complex traits.

In general, the analysis of epistasis is profitable to clarify the genetic background of complex traits. It was possible by the present studies that epistasis play an important role for the expression of carcass composition and meat quality traits in the Du × Pi population.

Chapter 5. Summary

The genetic architecture of many quantitative traits is characterized by complex structures. Until now investigations of such complex traits are mainly focused on single genes or genomic regions. Genetic effects were considered as additive, dominance or imprinting effects. The analysis of epistasis is not yet a routine, but it has been shown by few studies in livestock animals that interaction effects contribute with considerable proportions to the phenotypic variance.

Therefore two studies were performed to evaluate the importance of epistatic effects in the Bonn Duroc × Pietrain resource population for carcass composition and meat quality traits. This population was investigated so far for single QTL containing additive, dominance and imprinting effects. The aim of this thesis was to detect epistatic QTL pairs for carcass composition and meat quality traits. The importance of epistasis was determined by the proportion of the phenotypic variance. In a second step the biological causes of an observed epistatic QTL pair were investigated.

Overall 585 five F_2 pigs from DuPi were genotyped using 125 genetic markers (microsatellites and SNP) spread over the 18 pig autosomes. Phenotypic information for 26 carcass composition and meat quality traits was available for all F_2 animals. Linkage analysis was performed in a two-step procedure using a maximum likelihood approach implemented in the QxPak program. A number of 56 interacting QTL was observed for 19 different traits. Based on these results a variety of networks among chromosomal regions throughout the porcine genome were identified. We distinguished 17 epistatic QTL pairs for carcass composition and 39 for meat quality traits. These interacting QTL pairs explained up to 8% of the phenotypic variance. Beside inter-chromosomal epistatsis, it was possible to detect three intrachromosal epistatic QTL pairs. These findings demonstrate the importance of epistasis in pigs. The study revealed evidence for epistatic relationships between different chromosomal regions, confirmed known QTL loci and connected regions reported in other studies. Moreover, considering interactions between loci allowed to identify several novel QTL and trait-specific relationships of loci within and across chromosomes.

In a second step the causes of an epistatic QTL pair between SSC8 and SSC15 influencing pH value 1 h post mortem in loin were investigated. Gene expression data was obtained from loin tissue of 74 F_2 which were selected from 585 DuPi animals. Gene expression profiles, genotypes and phenotypes of these animals were investigated jointly applying three alternative models. Method A considered the phenotypic differences in pH values

between groups of pigs with extreme values. Method B was based on differences between the genotype combinations of relevant epistatic QTL pairs between SSC8 and SSC15. Finally, method C was a linear model comprising the epistatic QTL genotypes as fixed effects. Overall method A, B and C revealed 1182, 480 and 1823 differentially expressed or associated genes, respectively. All three methods have 24 genes in common of which four were located on SSC15. By means of a functional analysis it was possible to set up networks which contained mainly interactions between genes located within the specific regions on SSC8 and SSC15 and allowed a meaningful biological discussion. eQTL analyses were performed for functional and positional transcripts using a simple regression model. It revealed that the highest number of eQTL was detected for transcripts selected by method B through additive effects. The previously identified epistatic QTL positions were included as genetic background effects into the model so that four eQTL were detected additionally. This approach showed that combining phenotype, genotype and transcriptome data helps to uncover the involved molecules of observed epistasis.

In conclusion, this study revealed the importance of epistatsis for the expression of complex traits. Furthermore, it was possible to uncover biological causes of observed epistatic QTL pairs applying different statistical models.

Chapter 6. References

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Chapter 7. Appendix

Table A1: Genetic markers used in this study

SSC	Locus[1]	Position[2]	Allele	PIC[3]
1	SW1824	0.0	4	0.64
	SW1515	13.7	6	0.74
	SWR2300	28.5	4	0.57
	SW1851	38.0	4	0.67
	SW1653*	61.8	6	0.37
	SW952*	74.3	2	0.00
	S0312	93.5	5	0.78
	SWR702*	108.6	5	0.12
	SW2166	119.4	5	0.69
	S0113	119.6	2	0.04
	SWR2182*	151.1	4	0.17
	SWR982*	170.6	4	0.14
	S0155	178.5	4	0.98
	SW1311*	198.6	5	0.45
	AMBP*	214.5	2	0.00
	SW1957	237.0	5	0.54
	SW373	243.1	4	0.22
	SW1301	261.6	6	0.90
	SW2512	263.7	5	0.50
2	SW2443	0.0	4	0.43
	SW2623	12.9	5	0.52
	S0141	32.6	4	0.32
	FTH1*	67.2	2	0.00
	SW240	118.0	7	0.75
	SW1564	127.1	2	0.04
	SW834	142.6	8	0.89
	S0226	152.0	6	0.94

SSC *Sus scrofa* chromosome

[1] additional included genetic markers are marked with * in comparison to the study of Liu et al. (2007)

[2] position on genetic map in Kosambi cM

[3] polymorphic information content

Table A1: Genetic markers used in this study *(cont.)*

SSC	Locus[1]	Position[2]	Allele	PIC[3]
2	SW1517	154.9	6	0.86
	SWR2157	168.7	6	0.77
	SW1879	180.4	4	0.47
	SW1844	191.3	3	0.77
	SWR308	206.8	7	0.78
3	SW72	0.0	5	0.85
	S0164	36.6	9	0.93
	SW2570	50.2	5	0.30
	S0002	86.7	5	0.54
4	S0227	0.0	2	0.41
	S0001	40.1	4	0.90
	S0214	66.3	6	0.72
	S0097	115.6	5	0.42
5	ACR	0.0	7	0.63
	SJ024*	0.5	6	0.36
	SW314	3.1	4	0.55
	SW491	16.3	1	0.00
	SW1482	29.5	4	0.90
	SWR453	47.2	3	0.58
	SW2425*	58.7	6	0.31
	S0092	61.7	6	0.88
	SW1134	71.5	2	0.05
	S0005	77.5	9	0.84
	SW1987	90.7	4	0.34
	IGF1	115.1	5	0.20
	SW1954	129.9	3	0.41
	SW378	138.2	2	0.01

SSC *Sus scrofa* chromosome

[1] additional included genetic markers are marked with * in comparison to the study of Liu et al. (2007)

[2] position on genetic map in Kosambi cM

[3] polymorphic information content

Table A1: Genetic markers used in this study *(cont.)*

SSC	Locus[1]	Position[2]	Allele	PIC[3]
5	SW967	150.9	5	0.57
6	S0035	0.0	5	0.71
	S0087	61.2	4	0.81
	SW1067	70.6	5	0.98
	SW193	76.4	2	0.49
	S0300	77.5	3	0.64
	CKM*	80.6	2	0.00
	S0220	82.6	4	0.70
	S0059	99.3	5	0.50
	S0003	112.9	5	0.90
7	S0025	0.0	5	1.00
	S0064	33.0	6	0.75
	S0102	69.6	7	1.05
	SW175	79.6	4	0.95
	S0115	107.4	6	0.71
	S0101	138.2	4	0.83
8	SW2611	0.0	5	0.33
	S0353*	12.6	4	0.21
	SW905*	23.5	4	0.16
	KS195*	40.5	6	0.37
	SW1029*	54.1	2	0.01
	SW7*	68.4	5	0.38
	S0086	80.3	5	0.99
	SW2160*	98.4	3	0.28
	GC*	103.3	2	0.00
	S0144	108.2	3	0.32
	SW61	127.8	9	0.93

SSC *Sus scrofa* chromosome

[1] additional included genetic markers are marked with * in comparison to the study of Liu et al. (2007)

[2] position on genetic map in Kosambi cM

[3] polymorphic information content

Table A1: Genetic markers used in this study *(cont.)*

SSC	Locus[1]	Position[2]	Allele	PIC[3]
9	SW21	0.0	3	0.89
	SW911	19.4	5	0.57
	SW54	56.1	4	0.63
	S0109	68.0	2	0.25
	S0295	82.7	3	0.29
10	SW830	0.0	4	0.66
	S0070	83.7	7	1.00
	SW951	123.2	4	0.41
	SWR67	151.1	2	0.51
	SW2067	151.2	5	0.16
11	SW2008	0.0	4	0.72
	S0071	28.8	5	0.92
	S0009	38.6	4	0.62
	SW703	67.9	3	0.69
12	SW2490	0.0	6	0.98
	S0143	2.8	3	0.09
	SW874	65.4	5	0.67
	SW605	151.0	3	0.38
13	S0219	0.0	3	0.50
	SW344	56.4	5	0.48
	TNNC*	69.6	2	0.00
	SW398	100.9	5	0.96
	S0289	129.1	5	0.60
	S0215	129.2	2	0.01

SSC *Sus scrofa* chromosome

[1] additional included genetic markers are marked with * in comparison to the study of Liu et al. (2007)

[2] position on genetic map in Kosambi cM

[3] polymorphic information content

Table A1: Genetic markers used in this study *(cont.)*

SSC	Locus[1]	Position[2]	Allele	PIC[3]
14	SW857	0.0	5	0.70
	PPP*	30.0	2	0.00
	S0007	68.8	8	0.91
	SWC27	113.3	4	0.31
15	S0355	0.0	5	0.84
	SW1111	27.8	7	0.91
	SW936	60.6	5	0.76
	SW1119	84.1	5	0.69
16	S0111	0.0	6	0.84
	S0026	70.7	3	0.59
	S0061	108.0	4	0.65
17	SW335	0.0	3	0.71
	SW840	45.0	2	0.13
	SW2431	80.7	3	0.54
18	SY4*	0.0	4	0.46
	SW1808	8.5	5	0.58
	SW2540*	10.2	4	0.28
	SW1023	22.2	5	0.73
	SB58*	41.9	4	0.30
	SW787	43.2	5	0.94
	S0062	56.9	3	0.25
	SW1682*	58.4	4	0.35
	S0120*	61.5	4	0.35
	SJ061*	64.1	4	0.17
	SWR414*	81.2	4	0.97
	SY31*	94.4	3	0.07

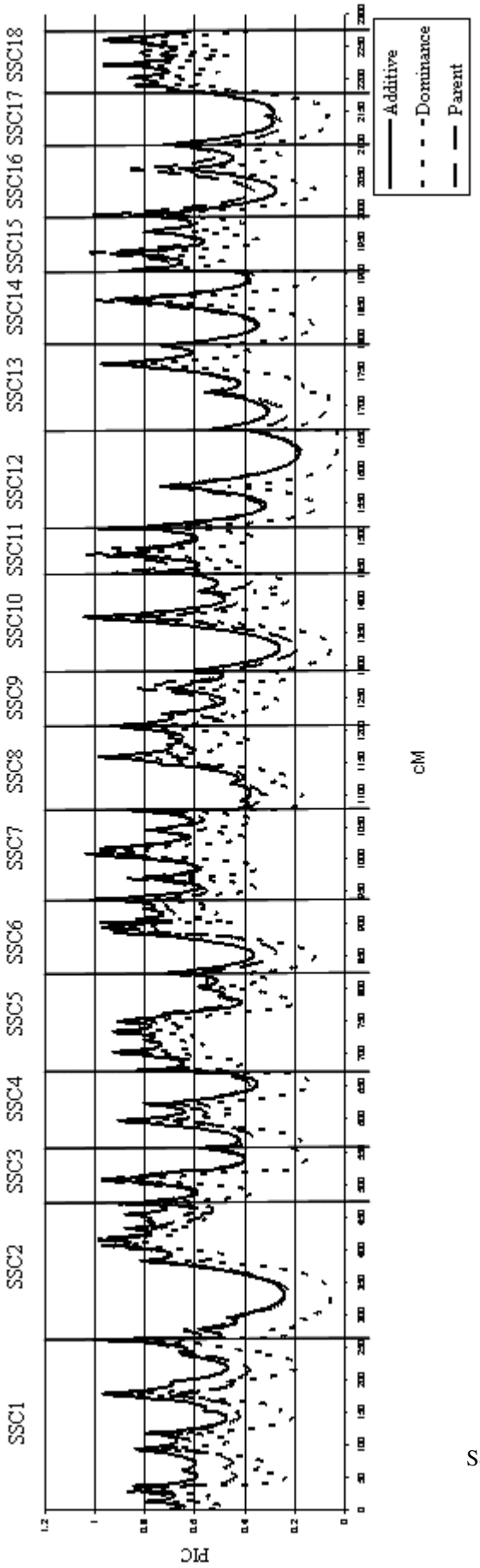
SSC *Sus scrofa* chromosome

[1] additional included genetic markers are marked with * in comparison to the study of Liu et al. (2007)

[2] position on genetic map in Kosambi cM

[3] polymorphic information content

Figure A1: PIC-plot of genetic markers used in this study



SSC: *Sus scrofa* chromosome

Table A2: Relevant single QTL identified in the study of Liu et al. (2007, 2008) for carcass composition and meat quality traits

SSC[1]	Trait[2]	F-ratio[3]	Pos.[4]	Flanking markers	Add.[5]	Dom.[5]	SE[6]	Vari.[7]
1	pH 24 h ham	24.66***	55.2	S0312-S0113	0.05	-0.02	0.01	9.08
2	pH 24 h ham	7.46*	61.8	SW1564-S0226	-0.02	-0.02	0.01	2.94
2	shear force	6.53*	65.5	SW834-S0226	-1.82	-0.51	0.62	4.52
2	fat muscle ratio	8.48**	54.6	SW2443-SWR308	-0.01	0	0.00	2.9
2	ECLC	9.67**	55.2	SW2623-SWR308	0.63	0.22	0.20	3.31
6	fat area[8]		35	S0035-S0087				6.60
8	loin eye area	9.49**	86.5	SW2611-S0144	-1.24	-0.87	0.42	3.23
8	fat muscle ratio	6.24*	86	S0086-S0144	0.01	0.01	0.01	2.15
8	ECLC	7.22*	86	S0086-S0144	-0.53	-0.42	0.21	2.5
10	cond. 24 h ham[8]		156	S0070-SW951				2.25
15	pH 24 h ham	5.86*	52.5	SW1111-SW1119	0.03	0.01	0.01	2.32
15	**pH dec loin[2]**	**5.09***	**69**	**SW936-SW1119**	**-0.05**	**-0.01**	**0.02**	**1.37**

[1] SSC *Sus scrofa* chromosme
[2] line in bold: extended results for pH decline
[3] three significant levels were used: 5% chromosome wide significant level, i.e. suggestive level (*); 5% genome-wide significant level (F = 8.02 **); and 1% genome-wide significant level (F=9.76 ***)
[4] position in Kosambi cM
[5] add: additive effects, dom: dominance effects
[6] the average of the standard error (SE) for additive and dominance effects
[7] proportion of phenotypic variance explained by a QTL as a percentage of the residual variance in the F_2 population
[8] these QTL were identified by Liu et al. 2008 using a combined line cross and half-sib analysis; therefore F-statistic and genetic values are missing

Table A3: Gene and transcripts located on SSC8 and SSC15 used for eQTL analysis

Sus scofa chromosome 8

Gene description	**Associated gene name**	**Sscrofa9 assembly (Mb)[1]**	**Probe set id[2]**	**method[3]**	**cate-gory[4]**
Sus scrofa apoptosis-related protein mRNA	PNAS – 5 (LYAR)	4.51	Ssc.10536.1.S1_at	M1	pos
PARK2 co-regulated-like	PACRGL	12.07	Ssc.8104.1.A1_at	M3	pos
recombination signal binding protein for immunoglobulin kappa J region	RBPJ	16.04	Ssc.29086.2.S1_a_at/ Ssc.29086.3.S1_a_at	M3	pos
TBC1 domain family, member 19	TBC1D19	16.26	Ssc.26814.1.S1_at	M3	pos
family with sequence similarity 114, member A1	FAM114A1	25.75	Ssc.19323.1.S1_at/ Ssc.19323.2.S1_at	M3	pos
COMM domain containing protein 8	COMMD8	32.05	Ssc.10419.1.A1_at	M1	pos
OCIA domain containing 1	OCIAD1	33.24	Ssc.6249.2.S1_at	M3	pos
Kelch-like protein 2 (Actin-binding protein Mayven).	KLHL2	37.85	Ssc.5842.1.A1_at	M3	pos
electron-transferring-flavoprotein dehydrogenase	ETFDH	41.19	Ssc.6919.1.A1_at	M3	pos/ func
Rap guanine nucleotide exchange factor (GEF) 2	RAPGEF2	41.88	Ssc.10907.1.A1_at	M1	pos
Secreted frizzled-related protein 2 Precursor	SFRP2	64.59	Ssc.3232.1.S1_at	M2	func
SMAD family member 1	SMAD1	71.57	Ssc.11757.1.S1_at	M1	funct
SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	SMARCA5	72.65	Ssc.15021.1.S1_at	M1	funct
Pro-epidermal growth factor precursor	EGF	96.47	Ssc.9392.2.S1_at/ Ssc.9392.3.A1_at	M1	funct

[1]Localization of probe sets based on assembly Sscrofa9 (April 2011).
[2]Probe set identifier of the porcine genome array of AffyMetrix.
[3]The method which has been applied to identify this transcript as candidate.
[4]Selection of the probe set through position or functional interaction

Table A3: Gene and transcripts located on SSC8 and SSC15 used for eQTL analysis *(cont.)*

Sus scofa chromosome 8

Gene description	**Associated gene name**	**Sscrofa9 assembly (Mb)[1]**	**Probe set id[2]**	**method[3]**	**cate-gory[4]**
protein phosphatase 3, catalytic subunit, alpha isozyme	PPP3CA	102.53	Ssc.6764.2.S1_at	M1	funct
multimerin 1	MMRN1	110.92	Ssc.14368.1.A1_at	M1	funct
secreted phosphoprotein 1	SPP1	112.40	Ssc.101.1.S1_at	M2	funct
integrin-binding sialoprotein	IBSP	112.72	Ssc.237.1.A1_at	M2	funct

Sus scofa chromosome 15

Gene description	**Associated gene name**	**Sscrofa9 assembly (Mb)[1]**	**Probe set id[2]**	**method[3]**	**cate-gory[4]**
interferon regulatory factor 2	IRF2	42.61	Ssc.19537.1.S1_at	M1	funct
nuclear receptor subfamily 4, group A, member 2	NR4A2	59.41	Ssc.4643.1.A1_at	M1	funct
activating transcription factor 2	ATF2	75.90	Ssc.8318.1.A1_at	M1	funct
heterogeneous nuclear ribonucleoprotein A3	HNRPA3	77.80	Ssc.25162.1.S1_at	M1	funct
integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	ITGAV	85.83	Ssc.6737.2.A1_at	M2	funct
collagen, type III, alpha 1	COL3A1	88.06	Ssc.11302.1.S2_at	M1/M2	funct
splicing factor 3b, subunit 1, 155kDa	SF3B1	94.82	Ssc.12295.1.A1_at	M1/M2/M3	funct
CDC-like kinase 1	CLK1	98.24	Ssc.10998.1.A1_at	M1/M2/M3	pos
NADH-ubiquinone oxidoreductase B12 subunit	NDUFB3	98.44	Ssc.20297.1.S1_at	M1	pos
cAMP responsive element binding protein 1	CREB1	103.67	Ssc.8827.1.A1_at	M1	pos/ funct

[1]Localization of probe sets based on assembly Sscrofa9 (April 2011).
[2]Probe set identifier of the porcine genome array of AffyMetrix.
[3]The method which has been applied to identify this transcript as candidate.
[4]Selection of the probe set through position or functional interaction

Table A3: Gene and transcripts located on SSC8 and SSC15 used for eQTL analysis *(cont.)*

Sus scofa chromosome 15

Gene description	**Associate gene name**	**Sscrofa9 assembly (Mb)[1]**	**Probe set id[2]**	**method[3]**	**cate-gory[4]**
acyl-CoA dehydrogenase, long chain	ACADL	106.12	Ssc.14530.1.S1_at	M3	pos/ func
tubulin, alpha 4a	TUBA4A	106.34	Ssc.4873.1.S1_at	M3	pos
fibronectin 1	FN1	110.94	Ssc.16743.1.S1_at	M1/M2	pos/ func
insulin-like growth factor binding protein 5	IGFBP5	112.02	Ssc.15800.1.S1_at	M1/M3	pos/ func
similar to zinc finger protein 142 (clone pHZ-49)	LOC 100152736	113.80	Ssc.5522.1.S1_at	M3	pos
similar to alpha-tubulin isotype M-alpha-6	LOC 100151951	114.29	Ssc.29036.1.S1_at	M3	pos
acyl-CoA synthetase long-chain family member 3	ACSL3	114.83	Ssc.6654.1.A1_at	M3	pos/ funct
mitochondrial fission factor	MFF	121.25	Ssc.6814.1.A1_at	M3	pos
nucleolin	NCL	124.48	Ssc.2695.2.S1_a_at	M2	pos
COP9 signalosome subunit 8 isoform 1	COPS8	129.30	Ssc.6380.1.S1_at	M3	pos
period homolog 2 (Drosophila)	PER2	130.27	Ssc.19174.1.A1_at	M1	pos/ funct
nebulin (NEB), mRNA	NEB	133.85	Ssc.20198.1.S1_at	M3	pos
signal transducing adaptor molecule (SH3 domain and ITAM motif) 2	STAM2	134.20	Ssc.13391.1.S1_at	M1	pos/ funct

[1]Localization of probe sets based on assembly Sscrofa9 (April 2011).
[2]Probe set identifier of the porcine genome array of AffyMetrix.
[3]The method which has been applied to identify this transcript as candidate.
[4]Selection of the probe set through position or functional interaction

Table A4: Results of single eQTL analysis

SSC[1]	Probe set id	Associated gene name	Position[2] (cM)	F[3]	Likelihood ratio	LOD-score	Category[4]
8	Ssc.101.1.S1_at	SPP1	24	6.55*	10.81	2.346	funct
8	Ssc.4643.1.A1_at	NR4A2	28	11.1**	16.49	3.58	funct
8	Ssc.12295.1.A1_at	SF3B1	55	11.03**	16.41	3.562	pos
8	Ssc.10907.1.A1_at	RAPGEF2	56	6.7**	11.01	2.392	pos
8	Ssc.19323.1.S1_at	FAM114A1	71	7.05*	11.49	2.494	pos
8	Ssc.29086.2.S1_a_at	RBPJ	83	7.45*	12.02	2.61	funct
8	Ssc.8843.1.A1_at	FN1	89	7.28*	11.79	2.561	funct
15	Ssc.3232.1.S1_at	SFRP2	49	7.03*	11.47	2.491	pos
15	Ssc.29086.2.S1_a_at	RBPJ	59	7.63*	12.26	2.662	pos
15	Ssc.29086.3.S1_a_at	RBPJ	60	8.14*	12.92	2.806	pos
15	Ssc.19323.1.S1_at	FAM114A1	60	8.49**	13.36	2.902	funct
15	Ssc.8843.1.A1_at	FN1	60	7.26*	11.78	2.557	funct
15	Ssc.6737.2.A1_at	ITGAV	60	11.94**	17.42	3.782	pos/funct
Epistatic position of SSC15 (77 cM) considered as genetic background effect in the model							
8	Ssc.10907.1.A1_at	RAPGEF2	53	8.49**	13.17	2.86	pos
8	Ssc.10536.1.S1_at	PNAS - 5 (LYAR)	59	8.21*	12.82	2.784	pos
Epistatic position of SSC8 (1 cM) considered as genetic background effect in the model							
15	Ssc.9253.1.A1_at	FZD7	44	6.06*	10.01	2.174	funct
15	Ssc.15021.1.S1_at	SMARCA5	52	6.61*	10.76	2.336	funct

[1] *Sus scrofa* chromosome (SSC),
[2] position in Kosambi cM,
[3] *: $p < 0.05$, **: $p < 0.01$,
[4] Selection of the probe set through position (pos) or functional (funct) interaction