KLEIBE DE MORAES SILVA

META-ANALYSIS OF DATA FROM QUANTITATIVE TRAIT LOCI MAPPING STUDIES ON PIG CHROMOSOME 4

Thesis presented to the Animal Science Graduate Program of the Federal University of Viçosa in partial fulfillment of the requirements for degree of *Doctor Scientiae.*

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Prof. Simone E. F. Guimarães
(Co-Adviser)

Prof. Johan A.M. van Arendonk

Dr. John W.M. Bastiaansen

Dr. Egbert F. Knol

Prof. Paulo Sávio Lopes
(Adviser)

“Great ambition and conquest without contribution is without significance. What will your contribution be?”

Willian Hundert, ***The Emperor’s Club***.

To

My wife Lívia

My parents Ilton and Maura

My brother Dérsio

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BIOGRAPHY

Kleibe de Moraes Silva, son of Ilton Rodrigues da Silva and Maura de Moraes Silva, was born on August 14th, 1978, in Dom Aquino, Mato Grosso State, Brazil.

In 1997, he entered the Federal University of Viçosa, graduating in Animal Science in August 2003.

In 2005, he got his Master's Degree in Genetics and Breeding from Federal University of Viçosa.

In September 2005, he started his PhD course in Animal Science at Federal University of Viçosa. He worked for 12 months at Wageningen University (WUR), The Netherlands, in a Sandwich Program.

In November 2009 submitted himself to the final exams.

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RESUMO

SILVA, Kleibe de Moraes, D.Sc., Universidade Federal de Viçosa, novembro de 2009. **Meta-análise de dados a partir de estudos de mapeamento de locos de características quantitativas no cromossomo 4 de suínos**. Orientador: Paulo Sávio Lopes. Co-Orientadores: Simone Eliza Facioni Guimarães e Robledo de Almeida Torres.

Nas últimas décadas houve um grande aumento no conhecimento do genoma das espécies animais. A identificação de diversos tipos de marcadores e a construção de mapas genéticos possibilitou a realização de vários estudos de ligação. Como resultado, um grande número de estudos em suínos tem sido publicado com QTL envolvidos nas características de crescimento, carcaça, qualidade da carne e reprodutivas. Entre os QTL, vários foram identificados no cromossomo 4. A primeira parte deste estudo apresenta um estudo de ligação no cromossomo 4 suíno para várias características em um cruzamento entre machos da raça naturalizada brasileira Piau e fêmeas de linha comercial (Large White x Landrace x Piétrain). Características relacionadas com crescimento, carcaça e qualidade da carne foram medidas. No total foram identificados 11 QTL ao nível cromossômico e genômico. Os QTL mais significativos foram detectados para característica de carcaça na região *FAT*. Na segunda parte deste estudo, meta-análise foi realizada utilizando diversos QTL presentes em banco de dados disponíveis online, além dos identificados na primeira parte deste trabalho. Apesar de vários estudos relatarem a existência de QTL para uma característica específica no mesmo cromossomo, a posição estimada dos QTL tem variado consideravelmente entre os diferentes estudos. Sendo assim, objetivou-se melhorar as estimativas de posição dos QTL e a redução dos intervalos de confiança. Para isso, meta-análise foi realizada para características individuais, para características correlacionadas com o rendimento de lombo e para inúmeras características as quais puderam ser classificadas em três categorias. Observou-se que a meta-análise melhorou as estimativas de posição e reduziu o intervalo de confiança dos QTL quando comparados com os estudos iniciais. Entretanto, apesar dos ganhos nas

estimativas de posição, a meta-análise por si só não forneceu a resolução suficiente para detecção de genes, mas pode servir como um passo preliminar para selecionar um número menor de genes candidatos presentes nas regiões de QTL para a realização de outros estudos.

ABSTRACT

SILVA, Kleibe de Moraes, D.Sc., Universidade Federal de Viçosa, November, 2009. **Meta-Analysis of Data from Quantitative Trait Loci Mapping Studies on Pig Chromosome 4.** Adviser: Paulo Sávio Lopes. Co-Advisers: Simone Eliza Facioni Guimarães and Robledo de Almeida Torres.

Over the last decades, there has been an enormous increase in knowledge of the genomes of livestock species. Genetic maps of livestock genomes have been applied in several linkage studies. The approach to identify genomic regions that harbor genes affecting the distribution of a measurable trait in a mapping population is termed quantitative trait loci analysis (QTL). As a result, a large number of studies involving QTL analysis has been published on growth, carcass, meat quality and reproduction traits in pigs. Among detected QTL, a large number has been found on chromosome 4 and presents similar results between different QTL studies. The first part of this work presents a chromosome-wide scan for quantitative trait loci in a cross between Piau breed and commercial lines on chromosome 4. Traits related to carcass, growth and quality were measured. In total we identified 11 QTL that reached chromosome and genome-wide significance. The most significant QTL were detected to carcass and a strong candidate gene is the *FAT* region. In the second part of this work, the meta-analysis was performed using data from public datasets available online. Even though multiple independent studies have found QTL for the same trait on the same chromosome, their estimated position and effects show a sometimes large amount of variation among different studies. The meta-analysis was performed for individual traits, for traits correlated to loin yield and for trait sorted into three categories with the aim to improve the power and accuracy in detecting QTL and narrowing the confidence intervals. The meta-analysis approach reduced the number of QTL compared to the number of initial QTL and also reduced the confidence interval compared to confidence interval from individual studies. The meta-analysis approach using published data from independent mapping experiments provided an improvement in the accuracy. However, the gains in accuracy did not by themselves provide gene

level resolution of QTL, but could serve as a preliminary step to select a very small and manageable number of genes for follow up studies.

CHAPTER 1

General Introduction

Many traits of economical importance in livestock species are quantitative. In quantitative traits, both genetic and environmental factors contribute to the variation observed in animal populations. The genetic component of variation has been widely modeled assuming a large number of genes of small effect influenced by the environment (Anderson, 2001). However, according to Lande (1981), some genes may account for a relatively large proportion of the phenotypic variation. The identification of these genes based only on conventional phenotype evaluation is not possible and the development of genetic markers from the 1980s created opportunities for the construction of linkage maps utilized for identifying chromosomal regions controlling those quantitative traits. Such a chromosomal region harboring genes that influence the variation is referred to as quantitative trait loci (QTL) (Gelderman, 1975). Unlike qualitative traits, which the phenotypic values show distinct classes, the quantitative traits are characterized by a continuous variation of phenotypic values.

Advances in molecular biology techniques and the rapid development of DNA markers facilitated the development of comprehensive linkage maps of several livestock species. Genetic markers represent genetic differences between individual organisms or species. Generally, they do not represent the target genes themselves but act as ‘signs’ that are located in close proximity to genes controlling the traits (Collard et al., 2005). Genes and markers that are close together or tightly-linked will be transmitted together from parent to progeny more frequently than genes and markers that are located further apart. The crucial idea is that observed marker genotypes can be used to obtain indirect information on the genotype at a target gene. By using molecular markers it is possible to map the region with effects on the quantitative traits to specific chromosomal segment in the genome by using a mapping population, which maximizes the chance to have such genes segregating. However, the detection of a QTL with desirable power and accuracy depends on the genetic

diversity between the parental strains, heritability of the trait, the number of animals in the population with genotypic and phenotypic information, and the density of informative genetic markers (Kao and Zeng 1997). In general, experimental populations for QTL mapping are comprised of intercrosses of phenotypically divergent founders. In this type of population it is presumed that the genes influencing the trait of interest are fixed enabling the identification of QTL regions that explains the genetic differences between populations. Otherwise, a large population is necessary to detect QTL with small effects, like those present in commercial populations.

Several agricultural species have received great attention because of potential benefits in detecting and identifying QTL and using them in marker-assisted breeding programs. The pig is one of the most important livestock species worldwide and information about genes involved in several traits of economical importance could lead to an improvement in quantity and quality of the meat produced. Since the first successful large QTL analysis in pigs conducted by Andersson et al. (1994), a large number of studies has been published with QTL for growth, carcass, meat quality, reproduction, disease resistance and other traits (Bidanel and Rothschild, 2002).

In many cases, there are several studies where QTL have been identified for the same trait on the same chromosome. Usually, the position of the detected QTL on a region or a chromosome does not coincide among different studies. In some cases, however, the QTL identified for a particular trait are found in the same region and have overlapping confidence interval which could indicate the existence of a common QTL or multiple tightly linked QTL. To find the causative mutations underlying these QTL is a huge challenge because for most detected QTL the identified chromosomal regions are still large and harbor hundreds of genes. Typically, the QTL support interval ranges from 20 to 40 cM. To narrow down these regions to the gene is necessary and investigators often encounter difficulty in obtain better results. Adding markers is helpful but resolution is fundamentally limited by the number of recombination events in the cross population. As a result, only few QTL have been characterized at the gene level and implemented in the pork industry (Dekkers, 2004). The reason for low precise estimates is multicausal, but in general, large variation may occur when a QTL has a small gene effect or when experiments are conducted

using small population size (Guo et al., 2006). In order to increase the power and resolution in QTL detection it is necessary to increase the population size, even though such measure is an expensive proposition.

Although increases the power in detecting QTL, most of time it is not possible to obtain larger population size, mainly because most of the populations used in QTL mapping studies no longer exist. As a result the investigators have to use the data that already exist and an option is to pool the data in a single study and perform the analysis. In the meta-analysis, the results of detected QTL from a variety of mapping studies are combined in a single study and re-analyzed.

Meta-Analysis Approach

Over the past decade, several resource populations have been established to detect QTL for traits of economic importance in pigs using breed crosses and, in some cases, commercial populations. Although each population allows independent identification of QTL, they are often of limited size, which restricts the statistical power and accuracy of QTL identification. It has been suggested that the pooling of results across linkage studies allows for a more precise estimation of QTL position, hence, such approach yields conclusions that are stronger relative to those single studies with low statistical power (Lander and Kruglyak, 1995). The approach to combine results from different studies in a single one is termed meta-analysis and it is recommended in situations where only summary information is available and not the data source.

Meta-analysis, first mentioned by Glass (1976), is a statistical analysis combining results from many sources in a single study. This technique was firstly used by researchers in medical, social and behavioral sciences, and offers considerable potential to extract additional information from the combined results. Meta-analysis can provide a more precise and consensual estimate of the location of a QTL and its effects as compared with any individual study.

The meta-analysis approach combines relevant evidence of QTL estimates from many studies in a single one. Meta-analysis adds a degree of precision and significance to the final results, since only assumed true QTL are included in the analysis. This assumption is not always true and some of the

detected QTL may represent a type I error which may reduce the accuracy of the QTL estimates. However, the increasing availability of information can lead to rejection of type I error, thus reducing the false discovery rate (Wacholder et al., 2004). By using meta-analysis it is possible to check whether the detected QTL represent several different QTL close linked or several estimates of the same QTL. One of the great benefits of meta-analysis is to increase the sample size, thus increasing the power in QTL detection. The larger the population, the more accurate is the mapping study and the more likely is the detection of smaller effects QTL (Haley and Andersson, 1997). This is really important, since many genes of small effects are involved in complex traits. In addition, meta-analysis can reduce the confidence interval of the detected QTL. The confidence interval of a QTL is inversely proportional to population size and QTL effects (Visscher et al., 1996; Darvasi and Soller, 1997; Roberts et al, 1999; Bennewitz et al., 2002), so large confidence interval occurs when a QTL has a small gene effect or when small population size is used. In practice, the confidence interval is important since it determines the potential for further experiments to close on QTL, mainly because smaller confidence interval increases the efficiency in selecting candidate genes.

Disadvantages of meta-analyses involve study heterogeneity, which can include differences in marker density, linkage maps, sample sizes, experimental designs, as well as statistical methods used. Environmental effects also make meta-analysis more difficult. Although all studies are not identical, they show some similarities that can be used to connect them. More recently, Etzel and Guerra (2003) developed an approach to overcome the heterogeneity among studies and to refine QTL location. This approach makes possible to combine information from several studies to obtain a consensus linkage map since several linkage maps have been produced to the same species. Such an assessment may be used as a primary study in selecting candidate genes for follow up investigations.

To perform a meta-analysis is necessary that some information needs to be available on published papers. Information such as type of population design, number of animals measured, genetic markers, phenotypic variances and other necessary information from the resource population.

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CHAPTER 2

Mapping of quantitative trait loci on pig chromosome 4

Abstract – A three-generation resource family was developed by using two Piau grand sires and 18 commercial grand dams to detect QTL for growth, carcass and meat quality traits in pigs. A total of 627 F2 progeny from 66 F1 matings was produced. All F2 animals were phenotyped and genotyped for six microsatellite markers covering the porcine chromosome 4. The data were analyzed by multiple regressions developed for the analysis of crosses between outbred lines using the QTL Express software. In total, three genome-wide significant and eight chromosome-wide significant QTL were detected. Each QTL explained from 1.3% to 7.7% of the phenotypic variance of the traits. Gene action for most QTL was additive. For most detected QTL involved economical traits, the Piau alleles had significant unfavorable effects on the phenotypic values. The results show that there are genes on chromosome 4 with a considerable effect on several economical important traits in pigs.

Introduction

Knowledge of the genome and the establishment of genetic maps are fundamental for the isolation and characterization of genes of interest (Rothschild and Plastow, 1999). Genetic markers spread across the animal genome are used for the mapping of regions that influence important traits. An appropriate population design is necessary for this purpose, such as the crossing of two lines that differ in the trait studied. It is assumed that in this type of population alternative alleles are fixed in the two lines. Adequate statistical methods then permit the identification of the position and estimation of the effect of the quantitative trait locus (QTL) on growth, carcass, meat quality and reproductive traits (Bidanel and Rothschild, 2002).

The Brazilian native pig breed Piau is originated from breeds introduced by Portuguese settlers in the XVI century and has also some influence of Dutch and African pig breeds (Vianna, 1985). The main characteristics of these

animals are rusticity, adaptability to poor conditions of management and feeding, and a great resistance to diseases. This pig breed is further characterized by low performance, small litter size, and especially large accumulation of subcutaneous fat (Guimarães and Lopes, 2001).

Pig is one the most extensively studied commercial species. Different QTL have been mapped in several crosses of various breeds, and it is of fundamental interest to know whether similar effects are present in other crosses and, mainly, in commercial populations (Walling et al., 1998). Once the genes and markers have been validated, these data can be used together with traditional methods in marker-assisted selection to improve the efficiency of breeding programs. Another possibility with a potential to improve breeding programs is the introgression of genes of interest from different breeds. The objective of the present study was to map QTL associated with growth, carcass, carcass cut and internal organ traits on swine chromosome 4 in an F2 population obtained by crossing divergent lines.

Material and Methods

The three-generation resource family was developed at the Pig Breeding Farm of the Department of Animal Science, Federal University of Viçosa (UFV), Viçosa, Minas Gerais State, Brazil. A full description of the line cross population, the phenotypes measurements and the DNA extraction procedures can be found elsewhere (Band et al., 2005a and 2006b; Faria et al., 2006; Peixoto et al., 2006).

The animals were genotyped using six microsatellite markers (SW489, S0301, S0001, S0217, S0073 and SW58), whose description and specifications are shown in Table 1. The PCR products were submitted to fragment analysis in an ABI PRISM 310 automatic sequencer (Applied Biosystems) and the amplified fragments were classified using the GENESCAN program (Applied Biosystems). The expected heterozygosity and polymorphic information content (PIC) were calculated with the CERVUS v.3.0 program (Marshall et al., 1998). Multipoint linkage analyses were carried out for males, females and both sexes with the 2.4 version of the CriMap software (Green et al., 1990). Recombination

units were then converted into map distances using the Haldane mapping function. The linkage map was constructed using the "build" option.

Table 1 – Markers used for the mapping of swine chromosome 4.

Primer	Position [1]	Fluorophore	T (ºC)[2]
SW489	0.0	HEX	55
S0301	31.8	FAN	58
S0001	59.1	HEX	50
S0217	102.8	FAN	62
S0073	123.4	HEX	56
SW58	161.9	TET	58

[1] Position in cM; [2] Annealing Temperature in ºC. Reaction condition : Taq polimerase 1 U, dNTPs 0.2 mM, *primers* forward and reverse 0.2 μM each, Tris-HCl pH 8.3 20mM, KCl 50 mM, $MgCl_2$ (2 to 4 mM), genomic DNA 25 ng/μL. The final volume was 20 μL. The PCR fragments were electrophoresed on 8% polyacrylamide gels.

QTL were mapped in the F2 population with the QTL Express program (Seaton et al., 2002) (http://www.qtl.cap.ed.ac.uk) using the average map. The statistical model assumed that the QTL is diallelic, with alternative alleles being fixed in each parental breed (Haley et al., 1994). Genotype ***QQ*** was defined as the homozygous genotype of the naturalized Brazilian Piau breed, with effect ***a,*** genotype ***qq*** was defined as the homozygous genotype of the commercial breed, with effect ***–a***, and ***Qq*** was defined as the heterozygous genotype, with effect ***d***. The probability of each F2 individual showing each of the three QTL genotypes was calculated according to the genotypes of the markers at intervals of 1 cM along the chromosome. The additive fraction of F2 phenotypic variance explained by a QTL was computed in each breed; i.e., $h_Q^2 = a^2 / 2\sigma_y^2$, as described by Pérez-Enciso et al. (2000).

The following statistical model was adopted:

$$y_{ijkl} = S_i + L_j + H_k + (C_{ijkl} - \overline{C})b + c_a a + c_d d + e_{ijkl}$$

where

Y_{ijkl} = phenotype;

S_i= fixed effect of sex i, i = 1, 2;

L_j= fixed effect of batch j, j = 1, 2, 3, 4, 5;

H_k = fixed effect of the halothane genotype k, k = 1 (NN), 2 (Nn);

$(C_{ijkl} - \overline{C})b$ = adjustment for covariables (carcass weight for carcass traits, and cold right side weight for cut traits);

e_{ijkl} = residual errors.

Aditive (c_a) and dominance (c_d) coefficients for a QTL were calculated from the genotypic probabilities under the assumption that two breeds were fixed for alternative alleles as follows:

$$c_a = P(QQ \mid Mi) - P(qq \mid Mi)$$

$$c_d = P(Qq \mid Mi)$$

Where:

$P(QQ \mid Mi)$ = conditional probability that the QTL are homozygous and originated from the Brazilian naturalized breed;

$P(qq \mid Mi)$ = conditional probability that the QTL are homozygous and originated from the commercial breed;

$P(Qq \mid Mi)$ = conditional probability that the QTL are heterozygous.

The previous model was fitted every centimorgan, regressing the phenotypes onto the coefficients c_a and c_d. At each location an F ratio was calculated comparing the model with a QTL to the equivalent model without QTL. Estimates for ***a*** and ***d*** were calculated at the best estimated position with the highest F-ratio. The chromosome-wide significance levels (α = 0.05 or 0.01) were determined by a permutation test using the QTL Express software (Churchill and Doerge, 1994), for a total of 10000 permutations for each trait. Confidence intervals (CI) for QTL location were obtained as suggested by Pérez-Enciso et al. (2000) using the chi-square drop approximation (equivalent to the LOD score drop approximation). An *F*-statistic is equal to χ^2_P/p, approximately, where p is the number of parameters estimated, here two, the additive and dominance effects. The 95% threshold is $\chi^2_{2,\,95}$ = 3.85. Thus, the 95% confidence interval limits were obtained at the chromosome locations where the *F*-statistics decreased 3.85/2 = 1.92 units starting in both directions from the position corresponding to the maximum *F*.

Genome-wide thresholds were obtained applying the Bonferroni correction as described in Knott et al. (1998) and used by Pérez-Enciso et al.

(2000). Suppose that a given value F corresponds to a chromosome significance level Pc, the genome significance level associated is given by PG = 1- (1-Pc)^{19}; 19 is the haploid number of pig chromosomes. This formula assumes lengths and marker spacing in all chromosomes are identical so that results are to be taken only as approximate.

Results

The distance covered by the linkage map of chromosome 4 using the six markers was 161.9 cM on the sex averaged map. The spacing between adjacent markers ranged from 20 to 43 cM, and the average marker interval was approximately 32 cM. The size of the map agrees with those reported in the literature, ranging from 130.1 cM (USDA-MARC.2; Rohrer et al., 1996) to 165.0 cM (PiGMaP.1; Archibald et al., 1995). No change in the order of the markers was observed, in agreement with other studies (Walling et al., 1998; Gerbens et al., 2000). Table 2 summarizes the characteristic of the microsatellite markers used for the mapping of porcine chromosome 4. The PIC calculated for the parental and F1 generations were low, a finding that might be due to the small number of alleles segregating in this population. However, Botstein et al. (1980) classified markers with a PIC of 0.25 to 0.5 as moderately polymorphic (SW489, S0217 and SW58) and markers with a PIC higher than 0.5 as highly polymorphic (S030, S001 and S0073) and, therefore, both classes are suitable for mapping.

Table 2 – Microsatellite markers used for the mapping of swine chromosome 4.

Marker	Position [1]	PIC [2]	HExp.[3]	HObs.[4]	No. of alleles
SW489	0.0	0.32	0.58	0.68	3
S0301	31.8	0.50	0.52	0.56	3
S0001	59.1	0.50	0.60	0.60	3
S0217	102.8	0.41	0.57	0.73	3
S0073	123.4	0.64	0.71	0.75	6
SW58	161.9	0.42	0.56	0.69	4

[1]Position in cM; [2]Polymorphic Information Content (PIC) were calculated with the CERVUS v.3.0 program; [3,4]The expected (HExp) and the observed (HObs) heterozygosity was obtained with the CERVUS v.3.0 program.

All the QTL that exceeded the threshold for chromosome and genome-wide significance in the analyses are reported. Most of them was identified within an interval of approximately 90 to 135 cM on the chromosome. The additive fraction of F2 phenotypic variance explained by a QTL ranged from 1.3 (HW) to 7.7% (W77). Table 3 shows the maximum likelihood ratios (Fmax) and positions (cM) of the QTL found in the present study and the respective estimates of additive and dominance effects. The chromosome-wide 5% and 1% significance threshold were 4.86 and 6.60, respectively. The 5% and 1% genome-wide statistics were 8.06 and 9.46, respectively.

Table 3 – Summary of the QTL position (cM), maximum likelihood ratios (F_{max}), phenotypic variance in the F2 explained by the QTL (h^2_Q) in percentage and the respective estimates of additive and dominance effects.

Trait	Position[1]	$F_{máx}$ (CI)[2]	(h^2_Q)[3]	a (± SE)[4]	d (± SE)[4]
W42	102	6.03 (80-130)*	5.4	-0.61 (0.18)	0.64 (0.37)
W63	109	5.73 (83-150)*	5.2	-1.07 (0.327)	0.85 (0.64)
W77	112	9.95 (94-139)****	7.7	-1.70 (0.39)	-0.06 (0.75)
W105	103	7.06 (85-129)**	5.3	-2.20 (0.60)	0.18 (1.21)
LEA	107	5.94 (96-138)*	5.2	-1.31 (0.39)	0.03 (0.78)
LUNG	94	6.95 (72-112)**	5.8	-0.03 (0.01)	-0.03 (0.02)
HEART	112	8.81 (85-137)***	5.9	-0.01 (0.01)	-0.01 (0.01)
HW	86	8.49 (71-105)***	1.3	-0.10 (0.05)	-0.30 (0.11)
PSW	91	8.03 (73-116)**	3.3	-0.10 (0.04)	-0.18 (0.08)
RW	158	7.52 (146-161)**	2.1	-0.05 (0.02)	0.09 (0.03)
AF	143	7.66 (129-159)**	3.1	0.04 (0.01)	0.07 (0.03)

*p < 0.05 chromosome-wide significance; **p < 0.01 chromosome-wide significance; ***p < 0.05 genome-wide significance; ****p < 0.01 genome-wide significance; [1] QTL averaged position in centimorgan (cM); [2] Confidence interval in centimorgan (cM); [3] Phenotypic variance explained by a QTL; [4]Standard Error; W42 – weight at 42 days of age (kg); W63 – weight at 63 days of age (kg); W77 – weight at 77 days of age (kg); W105 –weight at 105 days of age (kg); LEA – loin eye area; LUNG – lung weight; HEART –heart weight; HW – skinless and fatless ham weight (kg); PSW – skinless and fatless picnic shoulder weight (Kg) ; RW – Rib weight (kg); AF – abdominal fat (kg).

Four significant QTL were identified for growth traits (Table 3 and Figure 1), being P<0.05 chromosome-wide for W42 and W63, P<0.01 chromosome-wide for W105 and P<0.01 genome-wide for W77. Five QTL were detected for

carcass traits, being P<0.05 chromosome-wide LEA (Table 3 and Figure 2), and P<0.01 chromosome-wide for PSW, RW and AF, and P<00.5 genome-wide for HW (Table 3 and Figure 3). Regarding to internal organs traits, a QTL was observed for HEART (P<0.05 genome-wide) and for LUNG (P<0.01 chromosome-wide) (Table 3 and Figure 3). A more detailed description of all these QTL is provided on Table 3.

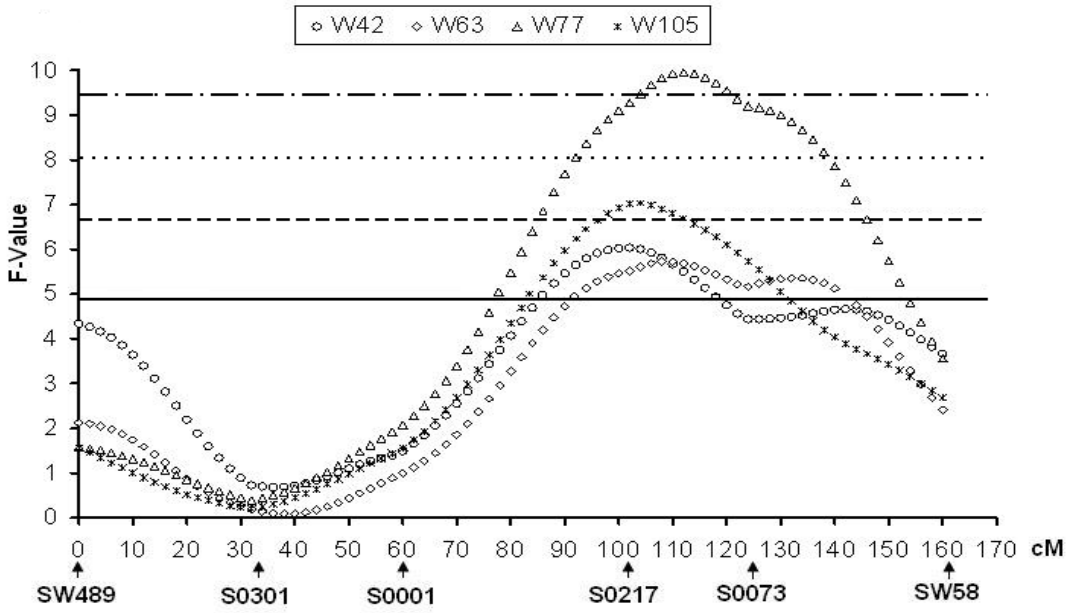

Figure 1 - Estimates of the F ratio for weight at 42 days of age (W42); weight at 63 days of age (W63); weight at 77 days of age (W77); weight at 105 days of age (W105) The horizontal lines indicate the levels of significance chromosome-wide (5% = continuous line, 1% = dashed line) and genome-wide (5% = dotted line and 1% = dot-dashed line).

Discussion

The first genome scan in pigs for QTL used a Wild boar x Large White cross (Anderson et al., 1994) and revealed significant effects of QTL on growth, length of small intestine, average backfat depth and abdominal fat percentage on chromosome 4. QTL for fat deposition and growth located on pig chromosome 4 has also been found in other crosses e.g., Chinese Meishan vs. Large White (Walling et al., 1998; Bidanel et al., 2001), Iberian vs. Landrace (Perez-Enciso et al., 2000; Mercandé et al., 2005) as well as in crosses of commercial populations (Evans et al., 2003; Nagamine et al., 2003).

Furthermore, a joint analysis comprising almost 3000 animals from seven different F2 crosses provided overwhelming statistical support for QTL affecting fatness and growth on SSC4 (Walling et al., 2000). To a certain extent, the results obtained in the present study agree with previous findings. For traits related to growth, four QTL were detected in the region between markers S0217 and S0073 (figure 1). Although the growth measures were obtained in different ages (42, 63, 77 and 105 days), the identified QTL were located in the same region and they may constitute a single gene or a cluster of linked genes each with similar mode of action on growth. Similar results were obtained by Bidanel et al. (2001) that detected QTL for weight at 10, 13, 17 and 22 weeks. It is worth to note that alleles inherited from the commercial breed were associated with heavier pigs (Table 3), demonstrating the contribution of commercial alleles to the development of these traits. These results were expected since Piau breed is characterized by low performance, small litter size, and especially large accumulation of subcutaneous fat (Guimarães and Lopes, 2001).

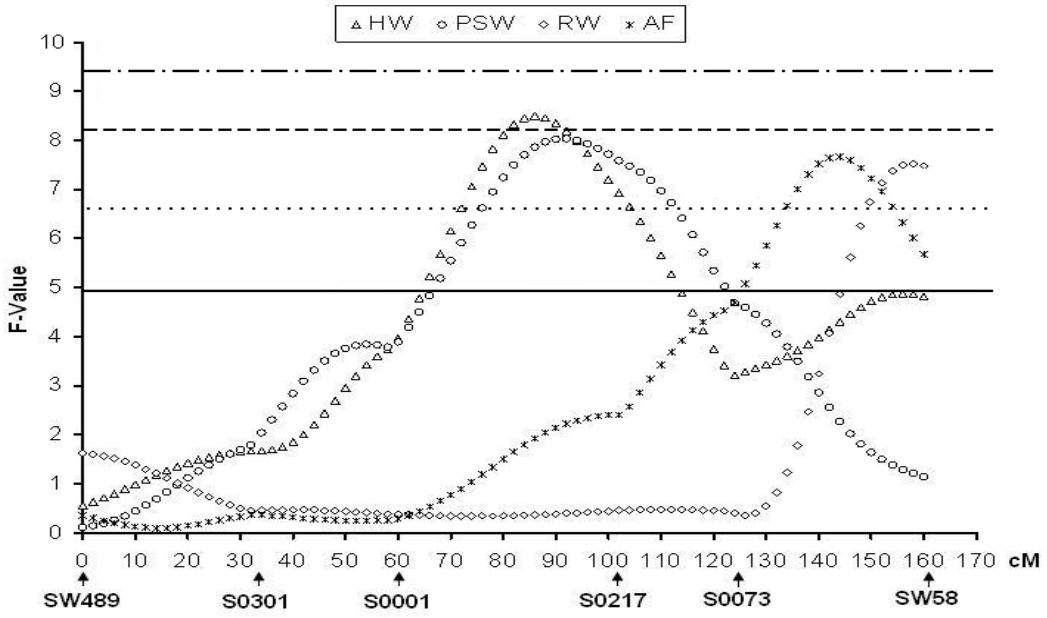

Figure 2 - Estimates of the F ratio for skinless and fatless ham weight (HW), skinless and fatless picnic shoulder weight (PSW), rib weight (RW) and abdominal fat (AF). The horizontal lines indicate the levels of significance chromosome-wide (5% = continuous line, 1% = dashed line) and genome-wide (5% = dotted line and 1% = dot-dashed line).

Favourable (*i.e.* positive) effects on growth are generally, but not systematically associated with favourable (*i.e.* negative) effects on backfat thickness (Bidanel et al., 2001). It is not surprising that evidence of a QTL on chromosome 4 for backfat thickness was not detected in this study. The first QTL reported in pigs was located in chromosome 4 (Andersson et al. 1994) and, despite the high variability of QTL studies, the porcine chromosome 4 QTL affecting fatness and/or growth stands as one of the most repeatable results. It has been confirmed in most if not all successive studies (Knott et al. 1998; Walling et al. 1998; Wang et al. 1998; Paszek et al. 1999; Pérez–Enciso et al. 2000; Walling et al. 2000; Bidanel et al. 2001; De Koning et al. 2001; Milan et al. 2002). The main effect reported initially was on fatness; this locus was named FAT1 after Marklund et al. (1999), but other studies found that it affected mostly growth (De Koning et al. 1999; Rattink et al., 2000). In this study we found highly evidence for QTL affecting growth traits, but only one QTL related to fatness (abdominal fat). The low association may indicate that locus affecting fatness in the Piau breed did not differ from the commercial line, or the study was not large enough to detect additional fat QTL.

QTL for ham weight (HW) and picnic shoulder weight (PSW) (Figure 3) were identified between the S0001 and S0217 markers, at the position 86 and 91 cM, respectively. Both detected QTL had similar effects and the allele inherited from Piau breed was responsible for decrease the phenotypic values. These QTL may represent either a common QTL with pleiotropic effects or represent two or more linked QTL. The QTL for PSW was found in the same region by Mercandé et al. (2005) and interesting result was that Estellé et al. (2006) found a strong association of a single nucleotide polymorphism (SNP) on FABP5 gene in shoulder weight and other traits. Geldermann et al. (2003) observed the presence of QTL for HW and PSW at position 115 cM on SSC4 in a cross between wild boar and Piétrain pigs. The QTL for HW and PSW were responsible for 5.2% and 8.3% of the phenotypic variation. In our study the alleles inherited from the naturalized Brazilian Piau breed were responsible for the decreased the phenotypic values.

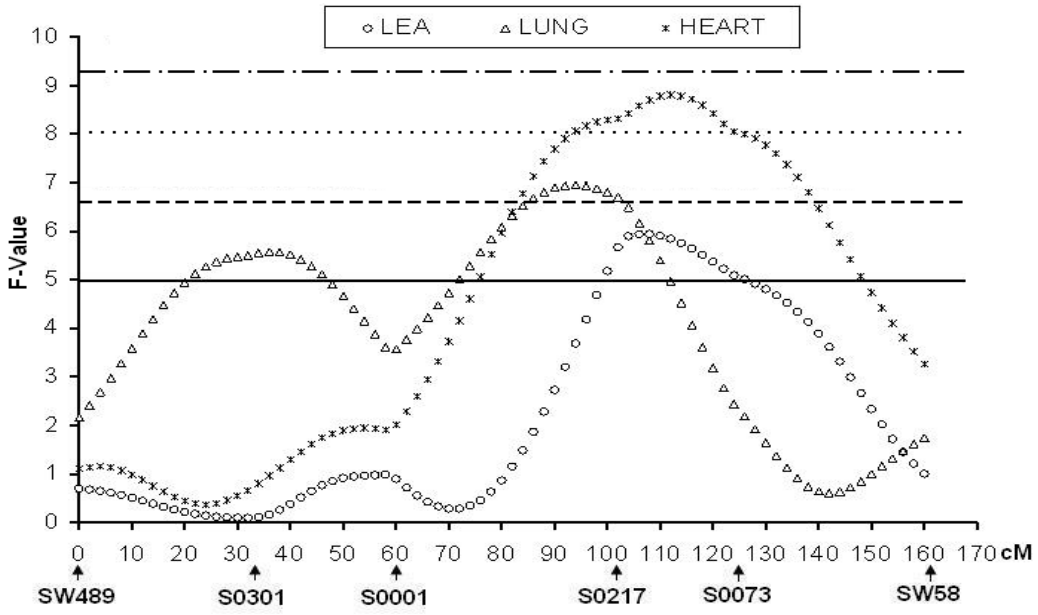

Figure 3 – Estimates of the F ratio for loin eye (LEA), lung weight (LUNG) and heart weight (HEART). The horizontal lines indicate the levels of significance chromosome-wide (5% = continuous line, 1% = dashed line) and genome-wide (5% = dotted line and 1% = dot-dashed line).

A QTL was found to rib weight (RW) in the distal of the chromosome. Only few studies have reported QTL for RW. On chromosome 4, Duthie et al. (2008) identified a QTL when the imprinting effect was taken into consideration. Other results have been found on chromosome 6 (Óvilo et al., 2005) and on chromosome 8 (Vidal et al., 2005). Regarding to abdominal fat (AF), Walling et al. (1994) detected a QTL in the proximal part of the chromosome 4, while in this study the QTL to the same trait was located in the distal part of the chromosome, at the position 143 cM (Figure 2). The estimate of the additive effect for the AF 0.040 ± 0.01 and the QTL found was responsible for 3.1% of the phenotypic variation, demonstrating the contribution of Piau alleles to the phenotypic expression of this trait (Table 3).

QTL for LEA was identified between markers S0217 and S0073 at position 107 cM (Figure 2). The additive effect of LEA was estimated to be -1.31 ± 0.39, demonstrating that allele inherited by the Piau breed was associated with a lower amount of muscling than the allele inherited from the commercial lines. Pérez-Enciso et al. (2000) observed the presence of a QTL affecting loin eye area at position 83 cM on SSC4. The QTL identified by these authors is

close to marker S0214, which is closely related to marker S0217, screened in the present study. A suggestive QTL for eye muscle area in this region reported by Wang et al. (1998) gives additional support to our results. LEA is a trait that has been indirectly selected based on backfat thickness since a reduction in the latter increases the former. However, we did not identified any QTL relate to backfat thickness in this study.

Regarding to internal organ traits, a QTL for HEART was identified at 112 cM, and a QTL for LUNG was identified at 94 cM (Figure 3). Cepica et al. (2003), studying various crosses, found a significant QTL for heart weight on chromosome 4 at position 66 cM in animals originating from a cross between wild European and Chinese Meishan pigs. It is hard to tell if these QTL are the same, since the studies have used different markers, and even the average map may show some differences.

The results of the present study show that most significant QTL are present in common regions on swine chromosome 4, indicating that the same gene (or genes) is controlling traits related to growth and carcass. The main limitation was the poor precision of QTL mapping, with confidence intervals of 20-30 cM, complicating the identification of the causal mutation underlying the QTL effect. Although further analyses and refining of QTL positions are in progress, results of this study are useful in discovering potential QTL regions and leading to further understanding of the role certain regions of the genome have in determining phenotypic differences among potential parents for selection of breeding animals. Our group keeps working on chromosome 4 and on the other pig chromosomes to find more informative markers in such way that a better association can be made to production traits. Further studies on the QTL identified in this research need to be evaluated to determine if they would be useful in commercial swine populations and to identify the genetic and economic impact of these new selection tools on pork industry.

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CHAPTER 3

Meta-Analysis of Results from Quantitative Trait Loci Mapping Studies on Pig Chromosome 4

Abstract - Meta-analysis of results from multiple studies could lead to more precise quantitative trait loci (QTL) position estimates compared to the individual experiments. In this study we performed a meta-analysis of QTL on chromosome 4 in pigs, using data from 25 separate experiments. First, a meta-analysis was performed for individual traits: average daily gain and backfat thickness. Second, a meta-analysis was performed for the QTL of three traits affecting loin yield: loin eye area, carcass length, and loin meat weight. Finally, 78 QTL were selected from 20 traits that could be assigned to one of three broad categories: carcass, fatness, or growth traits. Meta-QTL related to growth and fatness were found in the same region as the *FAT1* gene. For each analysis the number of identified meta-QTL was smaller than the number of initial QTL. In addition, the meta-analysis narrowed down the QTL confidence intervals compared to individual QTL estimates. Results suggest that the meta-analysis is an efficient strategy to estimate the number and positions of QTL using information from multiple populations and experiments. This strategy can be used as a guide for further studies with the aim to select candidate genes related to trait variation.

Introduction

Genome mapping projects have been performed for all main livestock animals over the last two decades. Pigs have been a focus of particular attention because of the potential benefits of detecting and identifying quantitative trait loci (QTL) and using them in breeding programs (Rothschild and Plastow, 2007). The first successful large QTL analysis in pigs was conducted by Andersson et al. (1994) using Wild Boar and Large White in a three-generation cross, and revealed major QTL on chromosome 4 affecting small intestine length, backfat thickness and growth. Since then, a large number

of studies have been published with QTL for growth, carcass, meat quality, reproduction, disease resistance, and other traits (Bidanel and Rothschild, 2002).

Multiple studies have successfully identified QTL for the same trait on the same chromosome (Walling et al., 1998). However, there is variation in the estimated chromosomal positions of QTL detected by individual studies. In addition, QTL identified from individual mapping studies usually have large confidence intervals, such that distinction between one or more tightly linked QTL cannot be made. The confidence interval of a QTL is inversely proportional to population size and QTL effects (Visscher et al., 1996; Darvasi and Soller, 1997; Roberts et al, 1999; Bennewitz et al., 2002), so a large confidence interval occurs when a QTL has a small gene effect or when a small population size is used. In practice, the confidence interval is important because it determines the potential for further experiments to close in on QTL. A smaller confidence interval would increase the efficiency in selecting candidate genes.

Results from independent pig studies are available online in a QTL database (http://www.animalgenome.org/QTLdb/pig.html, Hu et al., 2005). In pigs, the highest density of QTL has been found on chromosome 4 (Rothschild et al, 2007). Pig chromosome 4 presents a large number of similar results among various QTL studies (Andersson et., 1994; Knott et al., 1998; Milan et al., 1998; Moser et al., 1998; Walling et al., 1998; Wang et al., 1998; de Koning et al., 1999; Marklund et al., 1999; Gelderman et al., 2003; Cepica et al., 2003). It would be useful to determine from all of these results whether QTL detected in independent experiments and located in the same region of a particular chromosome are multiple discoveries of the position of a single QTL or reflect the involvement of more than one QTL among different populations.

Meta-analysis, first mentioned by Glass (1976), is a statistical analysis combining results from different sources in a single study. This technique was first used by researchers in medical, social, and behavioral sciences, and offers considerable potential to extract additional information from the combined results. Combining results from different experiments and populations could also provide an idea of how many different QTL exist for a specific trait, and could narrow down their chromosomal locations (Lander and Kruglyak, 1995).

Reports of QTL studies in pigs have been based on an individual population or, in a few cases, on a combined analysis using raw data from a small number of studies (Walling et al., 2000; Kim et al., 2005; Guo, et al., 2008). To our knowledge, this is the first report on a meta-analysis in pigs using QTL results from separately reported studies. Some studies have reported results from meta-analysis in plants (Chardon et al., 2004; Wang et al, 2006; Guo et al., 2006). In the present study, the aim was to investigate the benefits of using meta-analysis to identify QTL underlying economic traits in pigs. We also aimed to refine the estimated positions of underlying QTL on chromosome 4 and narrow down their confidence intervals. Chromosome 4 was chosen because data from a large number of independent studies was available.

Materials and Methods

MetaQTL program: The purpose of the meta-QTL analysis is to evaluate, for a given trait, the degree of congruency of the QTL detected in independent mapping experiments. The MetaQTL program was developed by Veyrieras et al. (2007) (available on http://www.bioinformatics.org/mqtl). The program estimates the number and positions of the underlying QTL that best explain the observed distribution of QTL positions in the mapping experiments. The program uses a general database containing an experimental table, trait ontology table, and marker dictionary table, plus two sub-databases: the genetic linkage map database and the QTL database. The method assumes that each experiment was performed on an independent population sample. Recombination is modeled to be independent in each marker interval. The genetic maps are assumed to be connected when there are at least two common markers between them. The MetaQTL program builds a consensus genetic marker map that takes into account the statistical properties of genetic distance estimates using a Weighted Least Squares (WLS) strategy. The individual QTL locations are then projected onto the consensus map.

To identify the number of underlying QTL, the program uses a clustering algorithm based on a Gaussian mixture model as suggested by Goffinet and Gerber (2000). This approach provides decision rules based on the Akaike criterion to suggest the number of underlying QTL that best fit the results on a

given chromosome. It also groups QTL detected in independent experiments into sets that correspond to the same QTL and provides an estimation of the underlying QTL positions.

To perform the meta-analysis, the MetaQTL program requires the following information: population type (F_2, backcross, etc.), population size, QTL position, QTL confidence interval or R^2 (proportion of variance explained by each QTL), and QTL additive effects. When the confidence interval for the QTL position was not available, a 95% confidence interval was estimated by the MetaQTL program with the approach proposed by Darvasi and Soller (1997), as C.I. = $530/NxR^2$, where R^2 is the proportion of variance explained by each QTL and N is the size of the population.

Data: Data from identified QTL on SSC4 were received from PigQTLdb (http://www.animalgenome.org/QTLdb/pig.html, Hu et al., 2005) and from three additional populations (van Wijk et al., 2006; Slawinska et al., 2008; Silva et al., 2008). From the PigQTLdb, all QTL on chromosome 4 were used for which information was complete. From the additional populations, all QTL on chromosome 4 were used that were reported as significant ($P<0.05$).

First, the potential of meta-analysis was explored by choosing traits which have a large number of QTL in the dataset. These traits were backfat thickness and average daily gain. Second, three traits for which pleiotropic QTL effects could be expected were chosen for analysis. The chosen traits were loin eye area, carcass length, and loin meat weight. The three traits were analyzed individually, and combined into one single analysis, as well. Third, all QTL were used that could be assigned to one of the three categories of carcass, fatness, and growth, and meta-analyses were performed on QTL in each of these three categories.

Genetic map: The MetaQTL program uses different genetic maps that share some common markers to build a consensus map. The map locations for the QTL obtained from PigQTLdb were already interpolated to form a single map for each chromosome (Hu et al. 2005) using the comparative framework of ArkDB (Hu et al. 2001). Our study used the same genetic map file of chromosome 4 for all experiments from PigQTLdb. Here we define the QTL

analysis in a single population as an “experiment”. For the additional populations we used their own genetic map, which was built using the CRIMAP program (Green et al., 1990); details can be found elsewhere (van Wijk et al., 2006; Slawinska et al., 2008; Silva et al., 2008).

In order to study the congruency between the individual QTL, the MetaQTL program projects the meta-QTL onto a reference map. In this case we used a reference map on SSC4 that was obtained from the website (http://www.marc.usda.gov/genome/swine/swine.html), and contains 87 markers on a map size of 131 cM.

Results

At the time of analysis, the PigQTLdb contained 1831 QTL covering all 18 pig autosomes and the X and Y chromosomes. QTL were obtained from 113 publications and represented 316 different traits. There were 234 QTL on SSC4 for a large variety of traits. Most QTL were identified in F2 populations between commercial breeds and exotic breeds, such as Chinese Meishan, Wild Boar, and Iberian pigs. There are also a few results from commercial breed crosses, as well as some use of other population designs, such as backcrosses and half-sib populations. For this study, results were collected from 25 experiments from which all required data was available to perform the meta-analysis. The source of each population, its size, type of cross, and selected traits for each experiment are summarized in Table 1.

Meta-analysis of backfat thickness and average daily gain – Meta-analyses for backfat thickness and average daily gain were performed using results from seven and nine experiments, respectively. Two meta-QTL were found underlying each trait on chromosome 4 (Figure 1). For backfat thickness, two distant meta-QTL were found: backfat thickness meta-QTL1 at 79 cM and backfat thickness meta-QTL2 at 118 cM. For average daily gain, the two meta-QTL were found closer together: average daily gain meta-QTL1 at 74 cM and another meta-QTL, average daily meta-QTL2, at 99 cM. The number of meta-QTL was much smaller than the number of initial QTL (Table 2).

Table 1 - Summary of selected studies to perform the meta-analysis for traits related to fat, carcass, and growth.

QTL studies	Parents	Type of population	Population size	Traits
Bidanel	Large white x Meishan	F2	1103	ADG
Cepica1	Meishan x Piétrain	F2	316	BF10thRIB, BFWT, CCWT ,CRCL ,DRESS ,HAMWT, LEA, LNW
Cepica2	Wild Boar x Piétrain	F2	315	CRCL, DRESS, FATP, HAMWT, LRIBBF, LEA, LEAN, LNWT, S
Cepica3	Wild Boar x Meishan	F2	335	ADG, CCWT, CRCL, ENDWT, FATP, HAMWT, LEA, LNWT, SHO
Murani	Berlin x Dutch	F2	438	BFT, ADG, CRCL, LEA
Edwards1	Duroc x Piétrain	F2	510	BF10thRIB, LEA, LEAN
Edwards2	Duroc x Piétrain	F2	510	ENDWT
[1]Gelderman1	***Meishan x Piétrain***	***F2***	***316***	***BFWT, CRCL, CCWT, HAMWT, LNWT***
[1]Gelderman2	***Wild Boar x Piétrain***	***F2***	***315***	***BFWT, DRESS, SHOMTWT***
[1]Gelderman3	***Wild Boar x Meishan***	***F2***	***335***	***LNWT***
Silva	Piau x Commercial Line[2]	F2	617	AF, HAMWT, LEA, SHOMTWT, WT
Knott	Wild Boar x Large White	F2	199	AF, ADG, BFT
De Koning	Meishan x Dutch	F2	350	IMF
Andersson	Wild Boar x Large White	F2	200	ADG
Malek	Berkshire x Yorkshire	F2	525	ADG, CCWT, LUMBF, LRIBBF, LEA
Marklund	Wild Boar x Meishan	BC	85	AF, ADG, CRCL
Milan	Meishan x Large White	F2	488	BFWT, LRIBBF
Pazek	Meishan x Yorkshire	F2	298	BIRTHWT
Perez	Iberian x Landrace	F2	250	BFT, BFWT, CCWT, LEA
Rattink	Meishan x Dutch	F2	619	IMF
Sanshez	Large White x Meishan	F2	1103	ADG, BFWT, BIRTHWT, LUMBF
Slawinska	Piétrain/Large White x Commercial[3]	F1	306	BFT, HAMWT
Su1	Large White x Meishan	F2	81	BFT, FATP
Su2	Large White x Meishan	F2	140	IMF
Varona	Iberian x landrace	F2	577	BFT, CRCL, LRIBBF, LEA
Walling	Meishan x Large White	F2	390	ADG, BFT
Wijk	Piétrain/Large White x Commercial[3]	F1	1855	HAMWT
Wimmers	Berlin x Dutch	F2	438	BFT, CRCL, LEA

ADG – average daily gain; AF – abdominal fat; BF10thRib – fat depth at about 10th rib, BFT – backfat thickness; BIRTHWT – birth weight; BFWT – backfat weight; CCWT – carcass weight; CRCL – carcass length; DRESSING – dressing percentage; ENDWT – body weight at slaughter; FATP – fat percentage; HAMWT – ham weight; IMF – Intramuscular fat; LEA - loin eye area; LEAN – lean meat percentage; LNWT – loin and neck meat weight; LRIBBF - last-rib back fat; LUMBF – last lumbar backfat; SHOMTWT – shoulder meat weight; WT– body weight. [1]These studies were not used for meta-analysis because they represent the same populations as Cepica1, Cepica2, and Cepica3. [2]Synthetic Landrace/Large White/Piétrain sows line. [3]Commercial crossbred sows.

Although a meta-QTL analysis using QTL data from pigs was not found in the literature, the results were in agreement with results reported from combined analysis. Guo et al. (2008) carried out a whole genome combined analysis using raw data from two F2 crosses between Large White and Chinese Meishan pigs. On chromosome 4, they found three QTL: one QTL for fat at mid-back (MBF), one for body weight at the end of the test (ETW), and one for teat number (TN). The QTL for MBF was found at 80 cM (CI, 23-121 cM) and the QTL for ETW was found at 61 cM (CI, 29.5-112 cM). Both meta-QTL for average daily gain found in this study (meta-QTL1 and meta-QTL2) were located within the confidence interval of the ETW QTL from the combined analysis. However, only one meta-QTL for backfat thickness (backfat thickness meta-QTL1) was found within the confidence interval of the BFT QTL. TN was not analyzed in this study.

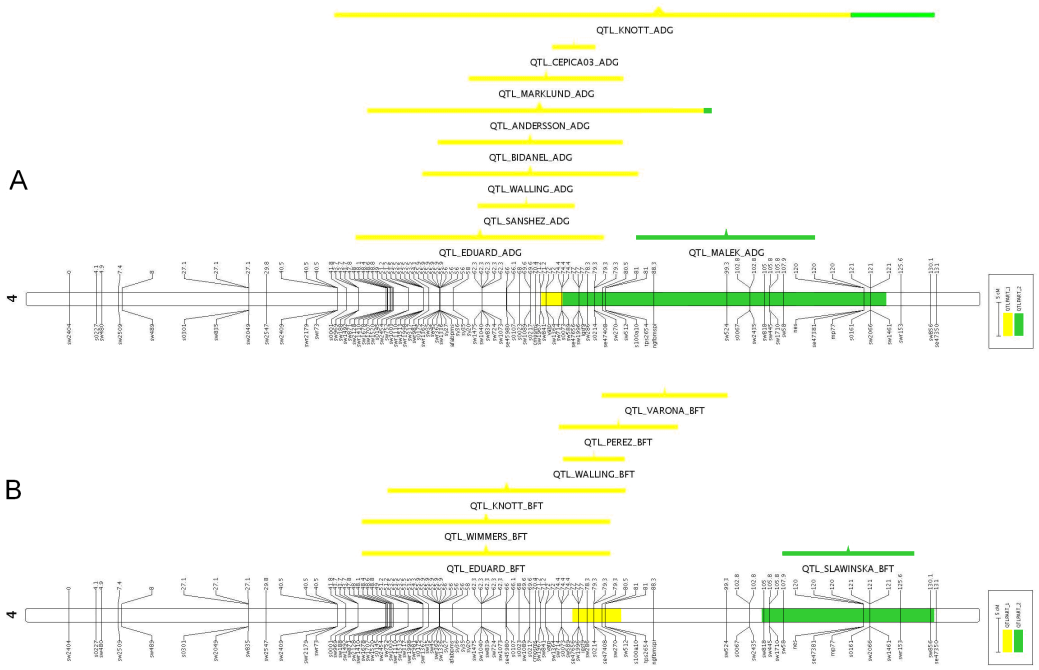

Figure 1 – Visualization of the QTL meta-analysis result on pig SSC4 for individual traits: average daily gain – ADG (A) backfat thickness – BFT (B). Each colored bar represents an individual QTL and its length represents the confidence interval. The individual QTL positions are depicted at their most probable position (triangle). Each meta-QTL and its confidence interval are indicated on SSC4 by colored segments.

In another combined analysis, Walling et al. (2000) combined data from six different F2 populations between commercial breeds and exotic breeds, such as Meishan and Wild boar. A combined analysis was performed for three traits: birth weight (BWT), growth rate from birth to the end of test (GRE), and subcutaneous fat depth at the end of test (FAT). Five out of the six populations that were used by Walling et al. (2000) were also included in our study. Walling et al. (2000) found one QTL for BWT at 85 cM (CI not provided by authors), one QTL for GRE at 81 cM (CI, 63-97 cM) and one QTL for FAT at 86 cM (CI not provided by authors). In that study, the QTL for BWT and GRE were identified at nearby positions and may represent a common QTL. It is worth noting that average daily gain meta-QTL1, the QTL for ETW found by Guo et al. (2008), and the QTL for GRE found by Walling et al. (2000) are all located in the same region on pig chromosome 4 with overlapping confidence intervals for QTL position.

Table 2 – Total number of individual QTL used in the meta-analysis for each trait, and number of meta-QTL found for each trait.

ADG			BFT		
Total of QTL[1]	Meta-QTL[2]	%[3]	Total of QTL[1]	Meta-QTL[2]	%[3]
9	2	77.8	7	2	71.0

[1]Total number of QTL used to perform the meta-analysis. [2]Number of Meta-QTL observed in the meta-analysis. [3]Percentage decrease comparing the total number of individual QTL in each category to the total of meta-QTL.

Meta-analysis of loin traits – Three traits that are related to loin yield were chosen to perform meta-analysis: loin eye area, carcass length, and loin meat weight. These traits were analyzed individually and also pooled into a single analysis (Figure 2). The most commercially relevant trait is loin weight. However, observations are generally more easily available for loin eye area and carcass length. We performed the meta-analysis for loin eye area and carcass length separately from the meta-analysis for loin weight, assuming that loin weight is a mathematical function of loin eye area and carcass length. By using meta-analysis, the separate QTL for loin eye area (10 QTL), carcass length (seven QTL), and loin meat weight (three QTL) revealed two, two, and three

meta-QTL, respectively. The combined analysis of all 20 QTL across the three traits revealed four meta-QTL. The QTL found for loin eye area and for carcass length fit nicely with the three meta-QTL found for loin weight. Combining all data splits the middle group of QTL into two meta-QTL, while a single meta-QTL was concluded from analysis on loin weight alone (Figure 2, Table 3). If animal total weight gain is considered as skeletal gain and increase in muscling and fat reserves, then these same QTL positions could be expected in the analysis of gain in Figure 3. The results from analysis of gain match quite nicely. We found a QTL for muscling (loin eye area) at 75.07 (Table 3), which is found in the analysis of gain at 73.7 (Table 6), and a QTL for skeletal development at 89.02 (Table 3), which relates to a QTL at 86.4 for gain (Table 6).

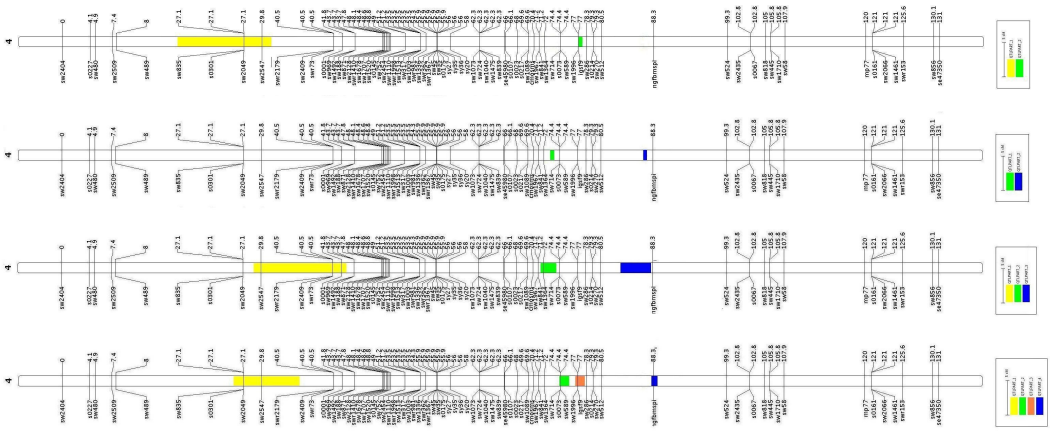

Figure 2 - Visualization of the QTL meta-analysis result on pig SSC4 for each trait (from top to bottom): loin eye area – LEA, carcass length (CRCL), loin weight (LNWT), and all traits pooled (ALL). Each colored bar represents an individual meta-QTL and its length represents the confidence interval.

Meta-analysis of carcass, fatness and growth categories – In the final analysis, QTL were selected for 20 traits that could be sorted into three categories: fatness, growth, and carcass. These three categories were chosen for meta-analysis because they are the most frequently represented categories among the QTL identified on SSC4. Traits were sorted into categories according to the definition on PigQTLdb (Hu et al., 2005). A total of 78 QTL

were selected, for which all the required information was available (Table 4). The remaining QTL (156) were omitted from the analysis either because their complete information was not available, or because they did not fit into any of the three categories.

Table 3 - Number, position, and length of confidence interval for the meta-QTL for each individual trait and pooled traits.

LEA			CRCL			LNWT			ALL (LEA+LNWT+CRCL)		
QTL[1]	Position[2]	CI95%[3]	QTL[1]	Position[2]	CI95%[3]	QTL[1]	Position[2]	CI95%[3]	QTL[1]	Position[2]	CI95%[3]
1	24.04	14.09	1	73.42	2.20	1	35.61	13.42	1	30.57	9.71
2	77.34	0.52	2	87.01	0.48	2	72.71	2.33	2	75.07	1.57
						3	85.75	2.81	3	77.43	1.75
									4	89.02	0.39

[1]Consecutive number assigned to meta-QTL by the MetaQTL Program. [2]Meta-QTL position in centimorgans (cM). [3]Confidence Interval length of the meta-QTL in centimorgans (cM).

The 78 QTL positions are spread out over most of the chromosome, with somewhat higher QTL density towards the middle of the chromosome (Figure 3). By using meta-analysis, the initial QTL for carcass (36 QTL), fatness (27 QTL), and growth (15 QTL) categories revealed five, five, and four meta-QTL, respectively (Table 4). The number of meta-QTL was large compared to the individual trait meta-analysis, but still much smaller than the total number of independent QTL in each category (Table 5). Two meta-QTL observed for carcass (meta-QTL2 and meta-QTL3) were also observed in the same position for fatness (meta-QTL3 and meta-QTL4). One meta-QTL region was common among all three trait categories. The carcass meta-QTL3, fatness meta-QTL4, and growth meta-QTL2 are positioned in the same region with the confidence interval between them overlapping from 75.2 cM to 76.1 cM.

As can be observed on Table 6, the new confidence intervals for meta-positions were narrowed down in comparison to the initial QTL. This reduction of the confidence interval should facilitate the selection of relevant candidate genes.

Table 4- Assignment of traits to categories used in meta-analysis with the number of individual QTL per trait.

Carcass category		Fat category		Growth category	
Trait	QTL	Trait	QTL	Trait	QTL
HAMWT	5	AF	3	ADG	9
LEA	10	BF10thRIB	2	BIRTHWT	2
LEAN	2	BFT	2	ENDWT	2
LNWT	3	BFWT	4	WT	2
SHOMTWT	3	FATP	2	-	-
CCWT	4	IMF	3	-	-
CRCL	7	LRIBBF	5	-	-
DRESSING	2	LUMBF	2	-	-

ADG – average daily gain; AF – abdominal fat; BF10thRib – fat depth at about 10th rib, BFT – backfat thickness; BIRTHWT – birth weight; BFWT – backfat weight; CCWT – carcass weight; CRCL – carcass length; DRESSING – dressing percentage; ENDWT – body weight at slaughter; FATP – fat percentage; HAMWT – ham weight; IMF – Intramuscular fat; LEA – loin eye area LEAN – lean meat percentage; LNWT – loin and neck meat weight; LRIBBF - last-rib back fat; LUMBF – last lumbar backfat; SHOMTWT – shoulder meat weight; WT– body weight.

Discussion

The identification of a gene containing the causative mutation underlying a QTL has been and still is a great challenge. A few genes with large effects have been identified and used by the pork industry (Dekkers, 2004; Van der Steen et al., 2005). Because many genes can be involved in a complex trait, the probability of identifying them from individual smaller studies is low, primarily because of modest QTL effects. When QTL are detected, the precision of QTL location is generally poor. Using results from independent studies in a meta-analysis is a way to increase the precision of QTL detection when the positions of the underlying QTL are effectively the same across different crosses.

In this study we have undertaken a meta-analysis of results derived from 25 separate studies and we focused on pig chromosome 4, for which there is substantial prior evidence for the presence of QTL (Andersson et., 1994; Knott et al., 1998; Milan et al., 1998; Moser et al., 1998; Walling et al., 1998; Wang et al., 1998; de Koning et al., 1999; Marklund et al., 1999; Gelderman et al., 2003;

Table 5 – Total number of QTL used in the meta-analysis for each category and number of meta-QTL found for each category.

Carcass category			Fatness Category			Growth Category		
Total of QTL[1]	Meta-QTL[2]	%[3]	Total of QTL[1]	Meta-QTL[2]	%[3]	Total of QTL[1]	Meta-QTL[2]	%[3]
36	5	86.1	27	5	81.5	15	4	73.3

[1]Total number of QTL used to perform the meta-analysis. [2]Number of meta-QTL observed in the meta-analysis. [3]Percentage decrease comparing the total number of individual QTL in each category to the total of meta-QTL.

Table 6 – Position and confidence interval length for meta-QTL and smallest individual QTL confidence interval length contributing to each meta-QTL, organized by category.

Carcass Category				Fatness Category				Growth Category			
QT	Positio	*m*CI	*i*CI	QT	Positio	*m*CI	*i*CI	QT	Positio	*m*CI	*i*CI
1	32.1	7.5	13.	1	13.9	16.	16.	1	23.3	18.	19.7
2	72.9	1.4	7.3	2	36.9	8.6	13.	2	73.7	7.1	6.6
3	75.5	1.2	2.4	3	69.7	5.5	7.3	3	86.4	7.5	7.3
4	87.5	1.7	1.8	4	76.0	1.6	1.8	4	119.2	18.	18.
5	113.3	3.3	20.0	5	103.2	2.2	15.2	-	-	-	-

[1]Number of meta-QTL obtained from the MetaQTL Program. [2]Meta-QTL position in centimorgans (cM). [3]Confidence Interval length of the meta-QTL in centimorgans (cM). [4]The smallest QTL's individual Confidence Interval (CI) contributing to the meta-QTL.

Cepica et al., 2003). In general, QTL confidence intervals were narrowed substantially by the application of meta-analysis, even when compared with the smallest confidence interval of the initial QTL (Table 6). However, in the growth QTL category, the confidence intervals of meta-QTL2 and meta-QTL3 were slightly larger than the most precise individual QTL. In all cases, the meta-QTL confidence intervals were smaller than the average confidence interval for each category. The confidence interval for the map location of QTL is a very important quantity for geneticists. A large confidence interval is impractical for both breeding and molecular biology purposes, since the width of the confidence interval of a given QTL map location will determine the chromosomal area that needs to be traced in selection and the potential group of candidate genes that must be considered. The QTL confidence intervals in the present study for the separate QTL experiments ranged from 5 to 80 cM, where even 5 cM in chromosome length may correspond to many candidate genes. Positional candidate genes contributing to quantitative traits can be found by comparative analysis of QTL locations based on their confidence intervals. This methodology has already been used successfully in pigs to identify genes underlying QTL with large effects, such as KIT (Johansson Moller et al., 1996), PRKAG3 (Milan et al. 2000, Ciobanu et al., 2001) and IGF2 (Van Laere et al. 2003). This study is the first to present a meta-analysis of QTL in pigs that combines information in a formal manner.

Combined analysis using raw data to improve the power of detecting QTL and mapping precision has been suggested (Allison and Heo, 1998), and a limited number of studies have reported results from such analyses in pigs (Walling et al., 2000; Kim et al., 2005; Guo, et al., 2008). A combined analysis requires the use of individual animal data, which can be difficult to obtain. Each of the combined analyses used a much smaller number of studies than the 25 we were able to use for meta-analysis. In contrast, datasets required for meta-analysis are relatively easily available. Furthermore, a study by Olkin and Sampson (1998) showed that meta-analysis of summary statistics can be as powerful as analysis of the combined data.

Pig chromosome 4 was chosen for our study because a large number of QTL were detected. The QTL found for fatness and growth (Andersson et al., 1994) were confirmed by analyses of subsequent backcross generations of the

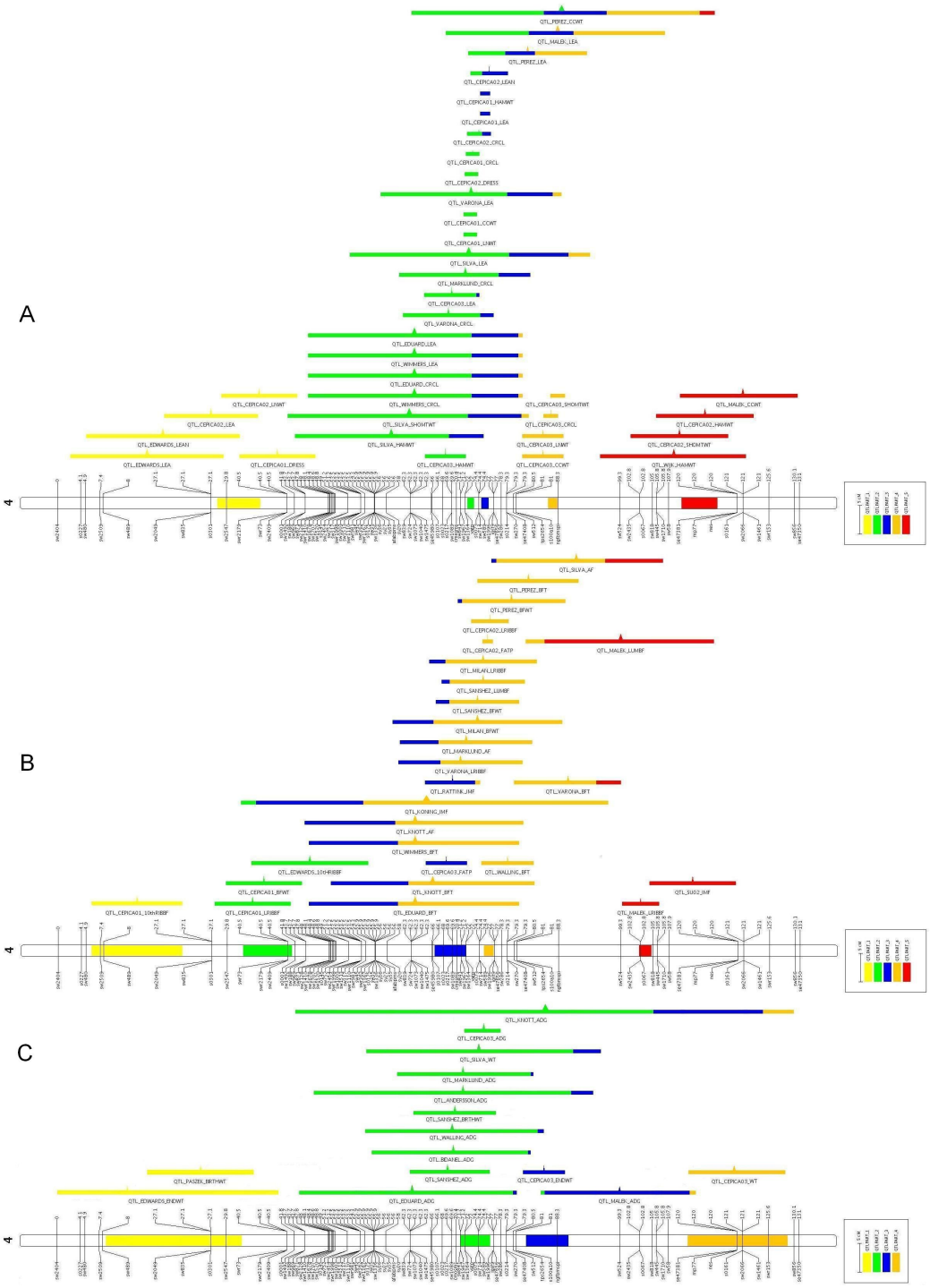

Figure 3 – Visualization of the QTL meta-analysis result on pig SSC4 for each category: Carcass (A), Fatness (B), and Growth (C). Each bar represents an individual QTL and its length represents the confidence interval. The individual QTL positions are depicted at their most probable position (triangle). The meta-QTL and confidence interval are indicated on SSC4 by colored segments.

Swedish population, which designated this QTL as *FAT1* (Marklund et al., 1999). The meta-analysis also identified a QTL overlapping with the *FAT1* location. The confidence intervals of backfat thickness meta-QTL1 and average daily gain meta-QTL1 cover the region of estimated QTL positions for *FAT1*. The results of Marklund et al. (1999) were included in this study, but many other populations were also selected for meta-analysis. Therefore, even though the data resulting in the assignment of *FAT1* comprises only a minor proportion of our total dataset, the suggested meta-QTL for backfat thickness and average daily gain were still positioned in the region of *FAT1*.

Combining correlated traits in single analysis could be expected to result in additional power for identifying QTL. However, partially correlated traits are not always affected in the same way or by the same QTL. In our study we tested meta-analyses of single traits and two different combinations of correlated traits. The combination of correlated traits appears useful for specific sets of traits, as shown by the results for loin weight and its component traits. The results from combining traits into one meta-analysis were difficult to interpret in the other examples. For the three large trait categories (fatness, growth and carcass), pooling many lowly correlated traits in a single category may have resulted in a reduced but still large set of underlying QTL that are spread along most parts of the chromosome. It can therefore be difficult to decide which traits to pool together and how to interpret the outcomes.

In conclusion, meta-analysis using information from independent sources provides greater power to estimate the positions and narrow down the confidence intervals of QTL underlying quantitative traits. Compared to individual studies, the meta-analysis approach of using published data from independent mapping experiments provides an improved starting point for follow-up work. The outcome of meta-QTL analyses is expected to greatly improve the success rates of further investigations of the candidate genes within the consensus QTL regions.

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CHAPTER 4

General Discussion

Substantial advances have been made over the past decades through the application of molecular genetics in the identification of genes and chromosomal regions that affect traits of importance in livestock production. Basically two approaches have been applied: QTL analysis and candidate gene approach (Rothschild & Soller, 1997). In QTL analysis, markers are used to perform a whole genome scan and statistical analysis is applied to provide evidence that a certain genomic region is segregating together with a specific phenotype. To do this a detailed marker map is required. QTL analysis is especially suitable for detecting genes with a large effect, since it is difficult to reach statistical significance levels for genes that only modestly contribute to the phenotype. In the candidate gene approach the focus is on mutations in genes with an expected impact on the studied phenotype to detect association between the gene and the phenotype. In practice, often a combination of both strategies is used, since QTL mapping reveals chromosomal regions that have an impact on the trait and subsequently functional candidate genes are selected from this region and associated to phenotypic variation.

Due to multiple studies in farm animals, especially in pigs, a large number of QTL has been identified by QTL analysis either within linecross populations or in commercial populations. In linecross, a tree-generation population is generally used to detect QTL between divergent breeds, whereas for commercial lines a half-sib paternal family structure is used. Many of these QTL have been identified in the same region on the chromosome to a particular trait and they might represent either several estimates to single QTL or multiple tightly linked QTL. Therefore, the exactly position of the QTL in the genome has been hard to find, mainly because the limited precision of QTL location among independent studies and because numerous QTL contribute to the phenotype. Since complex traits are controlled by many genes with low individual effect on the feature, the localization of QTL mutations might be complicate to identify.

In the meta-analysis, the results of detected QTL from variety of mapping studies are combined in a single study and re-analyzed. One of the greatest

benefits of meta-analysis is to increase the sample size which increases the power in detecting QTL. By using meta-analysis it is possible to check whether the detected QTL represent several different QTL closely linked or several estimates of the same QTL. The combination of results across studies can provide a more precise and consensual estimate of the location of a QTL as compared with any single study. In addition, meta-analysis of all available published results can also be used to improve the QTL confidence interval and may provide further insights into the genetic determinants of complex phenotypes.

In the chapter 2, we performed QTL mapping on pig chromosome 4 to several traits using a F2 cross between two outbred breeds. Some of the identified QTL presented large confidence intervals, but were found in the same region as several other QTL from independent published studies. In the chapter 3, we performed a meta-analysis approach using 25 independent studies on pig chromosome 4 for individual traits, correlated traits and for traits assigned in three major categories. The primary aim was to produce a more accurate estimate of the location for a common QTL than it is possible using only a single study. It is interesting to note that most of the QTL detected on chromosome 4 by QTL analysis were consistent with the results obtained in the meta-analysis approach. All QTL related to growth in the QTL analysis were identified in the region between the S0217 and S073 markers and may represent a single QTL with pleotropic effects on growth. In the meta-analysis using only results from several QTL to ADG or using all QTL related to growth assigned in one category, the meta-QTL were also found between the markers mentioned above. Similar results were also observed for the QTL identified for loin eye area, abdominal fat and those related to carcass traits for which the results from QTL analysis are in agreement with results from meta-analysis. By using the meta-analysis it was possible to show that the confidence interval for consensus positions was smaller than those corresponding to QTL detected by QTL analysis. However, even though the meta-analysis has narrowed down the QTL confidence interval and has reduced the number of QTL underlying the phenotypic variation, the gains in accuracy were modest to provide a gene level resolution. The results, nevertheless, could serve as a preliminary step to select

a small and manageable number of positional candidate genes to follow up studies.

If meta-QTL analysis is limited to simple combination of results from multiple studies, the combined analysis uses the raw data from multiple studies and a new analysis is performed. To carry out the later approach, it is important to have access to the original data as well as the specific parameters used to perform the data analysis. Combined analysis may increase the precision on QTL location and may allow some cross-validation of results (Walling et al., 2000; Kim et al., 2005; Guo, et al., 2008). An increase in population size provides gains in statistical power, estimates of gene effects and confidence intervals of the QTL locations (Beavis, 1998). This approach has the advantage that it can identify small QTL effects, since the larger the population, the more likely the detection of QTL with smaller effects (Haley & Andersson, 1997). In addition, extra information is useful to identify false positive QTL.

Other considerable advantage of the combined analysis is that several parental lines are used. In any single cross of two strains, we are limited to discovering only loci that show allelic variation between those strains. By looking at multiple crosses, we can sample more allelic variation, and thus we have an opportunity to detect additional loci that can be implicated in the economic trait. A QTL analysis of multibreed experiments offers clear advantages over classical two-breed crosses; among them is the increased power and a more comprehensive coverage of the total genetic variability in the species. In principle, multibreed crosses should allow the uncovering of a much larger fraction of the total genetic variation than two parental crosses (Pérez-Encizo et al., 2005).

In practice, however, there is a number of problems to be solved before combined analysis can be performed. Ideally, all QTL mapping studies should use the same genetic marker sets, animals should be reared under similar conditions and use the same population design. These conditions are not possible most of the time. Furthermore, researchers may be reluctant to provide full access to the original data. Walling et al. (2000) used the combined analysis in seven independent divergent line crosses between a Western commercial breed and either the Meishan or European Wild Boar to detect QTL for birth weight, backfat, and growth rate on chromosome 4. According to them, there

was substantial increase in the power to detect QTL and in the ability to reduce the confidence interval with the extra information. However, despite the benefits, the results did not provide the definitive explanation to the effects presented on porcine chromosome 4. As meta-analysis, the combined analysis could be used as a tool to select candidate genes in follow up studies.

The identification of the actual gene and the causative mutation comprising a QTL has been a challenge for several reasons. Causative mutations for quantitative traits are hard to find and difficult to prove. A major hurdle is poor precision of the localization of QTL. Even after fine mapping of a QTL to a relatively small segment, the progression from QTL to gene remains a daunting task. For this reason, further studies to narrow down the QTL region and to identify the underlying genes are required. Fine mapping in the relevant region by adding genetic markers and increasing the marker density is an extremely valuable tool, although the resolution is fundamentally limited by the number of recombination events in the cross population. Therefore, fine mapping will only be favourable when the population size is increased. Although fine mapping can be applied to get closer to gene, the association between a linked marker and the causative mutation might be disrupted by a recombination event and therefore association between the marker and the phenotype has to be monitored in new generations.

Currently, the sequencing of several livestock genome has provided the opportunity to identify a large number of sites in which a single base-pair varies from individual to individual. Single nucleotide polymorphisms (SNPs) are now becoming the markers of choice and their high availability across the genome open up possibilities to include genome-wide marker information in prediction of total breeding values. In the genome-wide selection the estimated breeding value uses the information from all QTL at once and allows researchers to check essential traits of an animal even before it is born or without having to measure traits. Compared to traditional breeding practice, genomic information yields a considerable increase in selection responses for juvenile animals (Meuwissen et al. 2001) and can reduce the costs of a breeding program up to 90% (Schaeffer 2006). The underlying assumption of genomic selection is that haplotypes at some loci are in linkage disequilibrium (LD) with QTL alleles that affect the traits that are subject to selection. The idea of genomic selection is to

omit the significance testing, and simply estimate the effects of all genes or chromosomal positions simultaneously (Meuwissen et al., 2001).

Different ways to derive haplotypes for linked marker alleles, and the relationship between haplotypes inside a locus, have been described. One method is to consider each different marker allele at a single locus to be a different haplotype, considering no relationships between different haplotypes and thus estimating breeding values directly for the marker alleles. A second method is to construct haplotypes for two alleles or more at adjacent markers, assuming a zero relation between haplotypes at the same locus (Meuwissen et al. 2001). A third method is to construct haplotypes using two or more surrounding marker alleles and derive identical-by-descent (IBD) probabilities between the different haplotypes at the same locus (Meuwissen and Goddard 2001). A potential drawback of genome-wide selection may be the existence of interactions or epistatic effects between QTL. In addition to genomic selection, a number of other strategies for gene discovery need to be integrated to understand the genetic architecture of complex traits, namely, genomics (analysis of the transcriptome), proteomics (protein based gene expression analysis) and metabolomics (analysis of metabolic pathways).

Finally, once the causative mutation is identified, a further step is normally required for practical use of this variation in selection and breeding programs. The application of marker assisted selection has the potential to increase the rate of genetic gain especially if traditional selection is less effective. Thus, it will be easy to select animals with the favorable allele for the next generations and hence to fix the mutation in populations where it is present. In addition, it will also aid the introgression of the mutant allele in pig breeds where it is absent.

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