1 Introduction

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

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

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

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

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

The aim of this work was:

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

Literature review

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

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

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

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

2.1 The pig genome

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

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

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

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

2.2 Genetic markers and genetic maps

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

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

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

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

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

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

Table 2.1: Comparison of different DNA-marker systems

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

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

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

2.3 Strategies for QTL mapping

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

2.3.1. Construction of resource populations

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

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