Integration of molecular genetics in selection programs for swine

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1. INTRODUCTION

Selection programs in swine over the past decades have resulted in significant genetic progress in a number of traits of economic importance. These programs have used procedures for identifying genetically superior individuals based on estimates of their breeding values (EBVs). In most cases, the estimates of breeding value have been derived through the analysis of performance records on individuals and their relatives using various genetic evaluation models. These models are based on several assumptions for the genes affecting the traits in absence of information regarding the actual location of the genes on the chromosomes, the number of such genes affecting the traits, number of alleles, etc. In spite of this, considerable advancements have been made in the selection procedures through these quantitative methods and advancements are expected to continue in future.

Advancements in the area of molecular genetics over the past years offer the opportunity to make more genetic progress through information obtained from analysis of DNA samples. DNA can be extracted from very small numbers of cells and many genes can be analyzed simultaneously so that it is possible to generate the data for selection from a few hair roots obtained from a pig. The DNA tests also offer the possibility of direct selection for specific traits. In swine, some candidate genes have been identified that have a major effect on important quantitative traits such as those for meat quality. Examples of such gene are the Halothane gene responsible for Pale Soft Exudative (PSE) pork and the RN gene causing increase in the glycogen content and drip loss resulting into Red Soft Exudative (RSE) pork. It is possible to directly select against these disorders using the tests for these major genes. At the same time there is an opportunity more rapid improvement in meat quality and other economic traits even after elimination of these genes.

One of the approaches that combines molecular and phenotypic information is Marker Assisted Selection (MAS). This approach is often used in conjunction with existing quantitative genetics tools, such as BLUP (Best Linear Unbiased Prediction), in order to improve accuracy and intensity of selection. Some research results suggest that genetic gain may be increased by 9% for a growth trait, 38% for a fertility trait and up to 64% for carcass traits in the first generation (Meuwisen and Goddard, 1996). Webb (1998) reported a significant increase in meat quality through DNA information in a breeding company. In another breeding company Rothschild and Plastow (1999) reported an increase of about 30% in fertility traits through use of the ESR gene. However, the selection responses are different in different populations and are at times contrary to the expectations (Gibson et al.,2002).

There are opportunities as well as challenges associated with the use of molecular genetic information in selection programs. The success of integrating molecular genetics in industry breeding programs depends on the implementation strategies that make effective use the molecular and phenotypic information. There is a need to set priorities by establishing which major genes and genetic markers are the most promising for commercial application and to develop selection and economic models to evaluate the benefits and costs associated with each promising gene or genetic marker before substantial funds are invested in their use. This investigation is an attempt to evaluate the current situation with respect to the molecular research in swine, estimate the economic benefits from the use of this technology and to develop a strategic plan for the benefit of the swine industry.

2. CURRENT STATE OF MOLECUAR RESEARCH IN SWINE

A large number of genes have been identified and mapped through the use of molecular genetic technology over the past years in the main livestock species. In swine, the number of genes mapped is close to 2000. Some of these genes contain the genetic code for a protein and therefore have a functional role in the physiologic affect of the trait. However, a large number of identified genes do not contain genetic code for specific proteins but are associated with the genetic affect of the trait. These non-functional genes are often referred to as genetic markers for the specific traits. The genetic markers are expected to be associated with genes that affect the quantitative trait or the so-called quantitative trait loci or QTL. The genetic markers allow the determination of the marker genotype but not the genotype for the QTL. However, they offer the possibility to select for the QTL based upon the marker information. This is the main basis for the concept of marker assisted selection (MAS). Some of the important genes and genetic markers are listed here.

2.1 Candidate and major genes

A number of candidate and major genes have been identified in swine. A summary of the important genes for industry use is given in Table 1 and a brief description is given herewith.

<u>The Ryanodine receptor (RYR1) mutation</u>

The Ryanodine receptor mutation (RYR1) was also known as the Halothane gene or Malignant hyperthermia gene. Tests for this gene are used to screen commercial pig populations in Canada and other countries. This gene is a classical example of wide spread use of molecular genetics in livestock populations.

The Ryanodine receptors are calcium channels that can be activated by a wide variety of factors such as Ca2+, caffeine, calmodulin, ryanodine and the anaesthetic Halothane. The mutation causes the receptor to be more sensitive to these factors (Meadus 2000). The mutation was found to increase the incidences of porcine stress syndrome (PSS), pale soft exudative pork (PSE), dark firm dry meat (DFD), and back muscle necrosis. Heavy muscled pigs are more likely to carry the gene than leaner pigs. Intensive selections

against backfat will likely result in increased frequency of the halothane gene because of the association of the halothane gene with leanness.

Halothane gene is a recessive gene. Each pig is homozygous (i.e. possessing a pair of halothane genes), or heterozygous (i.e. possessing one normal gene and one halothane gene) or two normal genes. Homozygous (but not heterozygous) pigs can be identified by their response to the anaesthetic with halothane. Recent developments have produced a gene probe that identifies both the homozygous and heterozygous carriers using only a drop of blood or a single hair.

Table 1. Candidate and major genes in swine

Gene	Chromo-some location	Effect	Source
RYR1 (Halothane)	6	Lean growth and porcine stress syndrome (PSS) and pale soft exudative pork(PSE)	Fuji et al. 1991
RN	15	Increased muscle glycogen leading to higher cooking loss.	Milan et al. 2000
ESR (estrogen receptor)	1	Increased litter size.	Rothschild et al. 1996 Chen et al. 2001
Prolactin receptor (PRLR)	16	Nursing performance and litter size	King et al. 1996 Farmer et al. 2000
Retinol binding protein 4	14	Litter size	Messer et al. 1996
MC4R (Melanocortin-4 receptor)	1	Fatness and feeding behaviour.	Rothschild et al. 2000
IGF2	2	Increases lean muscle mass. Litter size	Nezer et al. 1999 Horak et al. 2001
HFABP/AFABP	6	Increased marbling fat in loin muscle.	Gerbens *et al*. 1997
c-KIT Receptor	8	Blocks coat and skin color.	Andersson et al.1996

The Rendement Napole (RN) gene

The Rendement Napole (RN) gene was first documented by Leroy et al. (1990). Carriers of this dominant gene show lower pH, higher surface and internal reflectance values, lower protein extractability, lower water-holding capacity, lower Napole yield (yield after curing and cooking), and greater cooking loss. The primary cause of these differences is that the mutant allele results in higher stored glycogen content in muscle. A study of the pork products in retail shelves in western Canada showed that more than 25% of the samples had excessively high glycogen (Meadus and MacInnis, 2000). The RN gene has undesirable effects on the processing quality of pork and can lead to estimated industry losses of $14 (Canadian) for each hog car

rying the gene. For example, when manufacturers process ham, the RN gene causes more moisture to be lost during cooking, reducing the weight and the value of the end product. On the positive side, carriers have a lower shear force value, a stronger taste and smell.

A joint study was carried out by the Food Research and Development Centre (FRDC) of Agriculture and Agri-Food Canada, the National Institute for Agricultural Research in France (INRA), and the Canadian Centre for Swine Improvement Inc.(CCSI). The study was done on a sample of 305 boars from artificial insemination centres from all areas of Canada, in order to evaluate the frequency of the gene in the three main breeds used in the country. Since the RN gene has been closely associated with the Hampshire breed, blood samples taken from 89 pigs in the few herds still using this breed in Canada were also analysed for reference (Houde et al. 2002). This showed that the gene probe used in the study was able to detect the high frequency of the RN gene in the Hampshire breed.

The results of this study were very useful for commercial producers that have been using the Canadian Duroc, Yorkshire and Landrace breeds in their production scheme, and for the abattoirs and processors that purchase their pigs. The pigs sampled from these three breeds for the study did not carry the RN gene. Therefore, it is very unlikely the gene is present in the larger population of these breeds. The absence of genes like RN with a negative effect on meat quality and the extensive use of the Duroc breed known for its superior meat quality, are some of the reasons for the excellent reputation of Canadian pork in domestic and international markets

Estrogen Rceptor (ESR) gene

Mutations in the estrogen receptor gene (ESR) can produce considerable phenotypic changes in the mammalian reproductive system. Initial studies of the ESR gene in swine utilized Chinese Meishan swine. Studies using Meishan and Meishan cross discovered variation at the ESR locus in these swine (Rothschild, et al., 1991). The advantageous allele has a positive additive effect on total number born and number born alive in swine. The effect of this allele has been shown to range from 1.25 pigs per litter in Meishan crosses to 0.4-0.6 pigs per litter in Large White (Rothschild et al., 1994; Short et al., 1997). However, Gibson et al. (2002) observed no detectable association of the ESR PvuII mutation with sow productivity in a Meishan x Large White F2 population.

The prolactin receptor (PRLR) gene

In swine, prolactin level has been associated with nursing performance (King et al., 1996) and mammary gland development (Farmer et al., 2000). Polymorphisms in the PRLR gene have been associated with increased litter size in several breeds of swine. In 1998, Vincent et al. reported the discovery of a restriction fragment length polymorphism **(**RFLP**)** within the PRLR gene that was associated with an additive effect of +0.10 to +0.51 pigs born per litter in Large White, Meishan, and Landrace synthetic lines.

The retinol binding protein 4 (RBP4) gene

Messer et al. (1996) mapped the retinol-binding protein 4 (RBP4) gene to porcine Chromosome 14. Retinol-binding protein is a major secretory product of the pig conceptus prior to implantation. The retinol-binding protein 4 (RBP4) gene is a candidate gene for increased litter size in pigs.

Preliminary results with RBP4 as a candidate gene showed an additive gene effect for the favorable allele of 0.52 ± 0.30 pigs per litter in a Large White Hyperprolific line and 0.45 ± 0.43 in the control (Ollivier et al. 1997). The favorable allele was allele 1, and differences between the 11 genotype and the 22 genotype were approximately .50 pigs per litter for TNB and .26 pigs/litter for NBA. The results with 1300 females and 2755 records suggest that RBP4 is associated with some effect on litter size, but the effect is only about half that observed for ESR (Short et al. 1997).

Melanocortin-4 Receptor (MC4R)

Melanocortin-4 receptor **(**MC4R**)** has been found to play a significant role in regulating leptin's effects on food intake and body weight (Seeley et al., 1997; Fan et al., 1997). Kim et al. (2000) demonstrated that a missense mutation in MC4R was associated with backfat thickness, growth, and feed intake in different genetic lines of pigs.

Results with the TaqI MC4R PCR-RFLP (PIC/Iowa State patent pending), developed by Kim et al. (2000), show an allelic frequency of 0.60 for allele 2, which was associated with much fatter animals in the total population. Genotypic frequencies varied within the breeds.

The IGF2 gene

The beneficial effects of growth hormone (GH) or somatotrophin hormone on carcass quality have been known for a long time. The GH does not act directly on muscle cells but is instead an intermediate in a series of hormonal signaling events to increase growth (Meadus, 2000). This includes the PIT1 (pituitary specific transcription factor), GHRH (growth hormone releasing hormone), IGF-1 (the insulin like growth factor-1), and eventually feedback inhibition by somatostatin. Altering any one of these endocrine genes or their respective receptor genes can modify growth.

Insulin Like Growth Factor 2 (IGF-2) is one of the intermediates in the GH endocrine pathway. A G A mutation in exon 2 of IGF2 has been recently found to increase lean yield by ~2.7% in the Pietrain breed of pig which is recognized for its muscularity and leaness (Nezer et al.1999). The combined effect of the RYR1and the IGF2 mutations are believed to account for 50% of the Pietrain versus Large White breed difference in muscularity and leaness (Nezer et al. 1999).

HAFBP/AFABP

Heart fatty acid binding protein-1 **(**HFABP**)** is a member of the fatty acid binding protein family **(**FABP**)**, which is involved in fatty acid transport from the cell membrane to the intracellular sites of fatty acid utilization. HFABP has been considered to be an interesting candidate gene for intramuscular fat percentage and backfat in pigs due to its physiological role. Gerbens et al. (1997) mapped HFABP to pig chromosome 6. The QTL studies have identified intramuscular fat percentage and backfat loci in this region of chromosome 6 (Ovilo et al. 2000; de Koning et al., 1999). Gerbens et al. (1997) reported three polymorphic sites in the porcine HFABP gene (HaeIII, MspI, and HinfI) and conducted an association study to determine the genotype effects on traits in pigs (Gerbens et al., 1999). This study reported intramuscular fat percentage and backfat differences between HFABP genotype groups. Ovilo et al. (2000) also reported differences in genotype groups for the HFABP HaeIII PCR-RFLP.

c-KIT Receptor gene

There is lack of pigment producing melanocytes in the skin in white breeds of pigs such as Yorkshire. In most white pigs, this is due to a mutation of the c-Kit receptor gene known as the Dominant white allele. The Dominant white allele is the result of a gene duplication (Marklund et al. 1998), which disrupts normal melanocyte development in the embryo. This gene is therefore useful in detection of unwanted colour transmission in white breeds. A patented gene test (Andersson et al. 2001) is available that identifies the Dominant white allele by its extra copy of the normal c-Kit receptor sequence (Marklund et al. 1998). However, a variation of the Dominant white allele appears to exist in the commercial white pig population known as Patch. A gene test is required to identify the Patch mutations.

2.1 QTLs for traits of economic importance

A number of QTL mapping experiments have been conducted. These studies cover a large range of traits with varied chromosome regions. Following is a summary of the known QTLS for traits that might be of importance in breeding.

Table 2. Identified QTLs for the traits of economic importance

Trait	Variance explained (%)	Chromosome location	Source
Growth	7-12	13	Andersson et al. 1994
	20	1	Paszek et al. 1999
Backfat thickness and Abdominal fat	2-20	2 and 4	Andersson et al. 1994 Nezer et al. 1999 Knott et al. 1998 Jeon et al. 1999
Meat color	10	12	Marlek et al. 2001
	4	17	
Meat PH value	5	5	Marlek et al.2001
	4-6	15	
Intra-muscular fat	14-18	6 and 7	Olivo et al. 2000
Litter size	3	11	Cassady et al. 2001
Ovulation rate	3-4	3, 4, 7, 8 and 9	Bidanel et al. 2001

Growth Traits

QTL affecting growth traits were identified almost on all of the chromosomes. A significant QTL was detected at the end of long arm of chromosome 1 in Large White x Meishan crosses by Paszek et al. (1999), Rohrer (2000) and Bidanel et al. (2001). A second QTL on chromosome 1, which affects growth rate, was located at 60 cM from the first QTL, close to MC4R locus (Bidanel et al. 2001; de Koning et al. 2001; Quintanilla et al. 2002). A QTL for growth accounting for 7% - 12% of the phenotypic variation was found on chromosome 13 (Andersson et al. 1994). This QTL was confirmed later by Knott et al. (1998) and recently by Bidanel et al.(2001) in Meishan x Large White crossbred population. A QTL affecting growth in the center part of chromosome 4 was also detected by Andersson et al. (1994) in a Large White x Wild boar cross and confirmed by Knott et al. (1998) with slightly different position estimate. A QTL for growth and backfat traits in crosses of the Chinese swine has been found on chromosome 7 (Rothschild et al. 1995; Bidanel et al. 1997; Chevalet et al. 1996; Moser et al. 1998; Rohrer and Keele 1998a;Wang et al. 1998). A QTL affecting growth rate was detected on the short arm of chromosome 8 by both de Koning et al. (2001) and Bidanel et al.(2001). However, this QTL affects the growth in the post-weaning stage in the experiment of de Koning et al. (2001) whereas it affects on-test growth rate in the experiment of Bidanel et al. (2001).

Some candidate gene loci also have effects on growth traits. RN locus has a significant effect on average daily gain (Le Roy et al. 2000). Yu et al. (1995) found that PIT1 has significant effect on birth weight that maps in the center of chromosome 13. MC4R locus on chromosome 1 significantly affects the growth rate by influencing feed intake (Kin et al. 2000). MC4R maps to chromosome 1 at the position that is close to the significant QTL found by Bidanel et al. (2001), de Koning et al. (2001) and Quintanilla et al. (2002).

Feed efficiency and feed intake

Rohrer (2002) reported QTLs affecting feed efficiency and feed consumption in the crossbred population of Meishan and Large White. The daily feed intake of Meishan is positively affected by the detected QTL at the end of long arm of Chromosome 1 and negatively influenced by the suggestive QTL on chromosome 5. A chromosome region at the short arm of chromosome 1 has influence on feed efficiency.

Significant effects of RYR1 locus and MC4R locus were reported in the literature (Clutter and Brascamp 1998; Kim et al. 2000).

Backfat thickness

QTLs affecting backfat thickness were reported on most of the porcine chromosomes. QTL regions on chromosomes 1, 4 and 7 were reported to have significant effects on backfat thickness by many studies (e.g. Andersson et al. 1994; Rohrer and Keele 1998; de Koning et al. 1999; Bidanel et al. 2001. Malek et al. 2001). Two QTLs were suggested on chromosome 6 by Bidanel et al. (2001 in a Meishan and Large White cross and by Ovilo et al. (2000) in Landrance x Iberian F2 population. Two QTLs were detected on chromosome 8 by Rohrer (2000) and Bidanel et al. (2001). QTLs were also found on chromosomes 5 (Bidanel et al. 2001), 9 (Rohrer 2000) and 14 (Bidanel et al. 2001), respectively. In the central region of chromosome X, a QTL for backfat thickness with a large effect was also reported (de Koning et al.2001).

The IGF2 locus on chromosome 2 (Nezer et al. 1999; de Koning et al. 2001) has significant effect on backfat thickness. RYR1 and RN loci have significant influences on backfat thickness. Both loci have negative (i.e. favorable) effects on backfat thickness (Clutter and Brascamp 1998; Le Roy et al. 2001).

Meat quality

It is well known that RYR1 and RN loci have major influences on the meat quality as mentioned in section 2.1. In QTL mapping experiments, many chromosome regions were identified to have significant effects on meat quality traits. Significant effects on intramuscular fat content and marbling were detected on chromosome 1 in a Berkshire x Yorkshire crossbred population by Malek et al. (2001), on chromosome 6 in a Landrace x Iberian crossbred population by Ovilo et al. (2000, 2002), Grindflek et al. (2001) and Szyda et al. (2002), on chromosome 7 by Bidanel et al. (2001), and on chromosome X by Harlizius et al. (2000) in Meishan x Large White cross.

Malek et al. (2001) detected a QTL on chromosome 5 and a QTL on chromosome 15 that have significant effects on meat ultimate pH-value. The detected QTL on chromosome 5 explains about 5% phenotypic variance of meat pH-value, and also has significant effect on meat colour. Significant effects on meat colour have also found on chromosome 12 and 17 (Malek et al. 2001). The former explains 10% of the phenotypic colour variance.

Dekkers et al. (2001) summarized the detected QTL positions affecting porcine meat quality traits up to the year 2001 and discussed the marker assisted improvement of meat quality by integrating the molecular information into swine breeding programs.

Female reproductive traits

Many candidate gene loci such as ESR, PRLR and RBP4 have been reported to have significant effects on litter size as mentioned in section 2.1. In contrast, Fewer QTL were detected for litter size in QTL mapping studies. Cassady et al. (2001) reported the significant association of chromosome 11 with the total number of piglets born alive and the association of chromosome 5 and 13 with the number of stillbirths. Several QTLs were reported to have effects on the components of litter size. Bidanel et al. (2001) detected a significant QTL on chromosome 12 affecting embryo survival at 30 days of gestation in a mapping population of Meishan x Large White. Significant QTLs affecting ovulation rate were reported on chromosomes 3, 4, 7, 8 and 9 (Rohrer et al. 1999; Bidanel et al. 2001; Cassady et al. 2001). They explain 3-4% of total variance of ovulation rate in the study of Bidanel et al. (2001). In a recent study, King et al. (2003) utilized a three generation cross in which the founder grandparental animals were purebred Meishan and Large White pigs in a scan for quantitative trait loci. A QTL for the components of litter size and prenatal survival were identified at the distal end of the long arm of chromosome 8.

2.3 Single nucleotide polymorphism (SNP)

Single nucleotide polymorphism (SNP) is a single base change, or variation, that can occur in a DNA sequence. A SNP variation occurs when a single nucleotide, such as an A, replaces one of the other three nucleotides C, G, or T. SNPs can serve as biological markers for pinpointing Mendelian genes such as disease genes or quantitative trait genes of economic importance.

Swine SNPs are increasingly available during recent years. However, only a few of them (Table 4) have been associated with phenotypes of economic importance.

Table 4. Porcine SNPs and their effects

Trait	Gene or chromosome linked to or inclosing SNPs	Source
Semen quality of boars	Heat shock protein 70.2	Huang et al. 2002
Reproduction trait	The long arm of Chromosome 10	Nonneman and Rohrer 2003
Muscle development	Myostatin gene	Jiang et al. 2002
Early conceptus development	Leukemia inhibitory factor	Spotter et al. 2001
Coat colour phenotypes	Melanocortin I receptor gene	Gustafsson et al. 2001

2.4 Expressed Sequence Tags (ESTs) and Microarrays

ESTs

ESTs are short strands of DNA sequence that are generated by sequencing an expressed gene. ESTs provide researchers with a way for discovering genes, for investigating gene expression and regulation, and for constructing genome maps.

ESTs are created by partially sequencing cDNA for a few hundred nucleotides (usually 200 to 500) from either end of the molecule. Sequencing only the beginning portion of the cDNA produces what is called a 5' EST. A 5' EST is obtained from the portion of a transcript that usually codes for a protein. These regions tend to be conserved across species and do not change much within a gene family. Sequencing the ending portion of the cDNA molecule produces what is called a 3' EST. Because these ESTs are generated from the 3' end of a transcript, they are likely to fall within non-coding, or untranslated regions (UTRs), and therefore tend to exhibit less cross-species conservation than do coding sequences.

cDNA (complementary DNA) is DNA that is reverse transcribed from mRNA. Since mRNA is very unstable outside of a cell, scientists use reverse transcriptase to convert it to cDNA. cDNA is a much more stable compound and, importantly, because it was generated from a mRNA in which the introns had been removed, cDNA contains only expressed DNA sequences, or exons.

Microarrays

Microarray is a technology monitoring the combinatory interaction of a set of molecules, such as DNA fragments or proteins, with a predetermined library of molecular probes. The predetermined molecular probes are usually immobilized on a small membrane or glass slide in a regular pattern and hybridized to the set molecules (DNA fragments or proteins). Microarray is called DNA microarray when molecules are DNA. DNA array can be seen as a massively parallel version of Southern blotting and is mostly used in gene expression analysis. By using a DNA array containing many DNA samples, scientists can determine, in a single experiment, the expression levels of hundreds or thousands of genes within a cell by measuring the amount of mRNA bound to each spot on the array.

Microarray can also be applied to genotyping. Instead of measuring mRNA, it measures DNA. SNP array is a prospective technology for gene identification and gene mapping.

2.5 Prospects for gene mapping studies

During the last decade, swine gene mapping studies have achieved considerable progress and a large number of QTL regions have been identified. Some of these QTLs have been

mapped with a high-level of significance or mutually confirmed by different experiments. However, many others have low mapping resolution and require further studies to confirm. QTL mapping so far has been generally based on the maps of microsatellite markers. The recent advances in lab technologies and marker techniques will certainly provide the chance to accelerate the progress of gene mapping and lead to more exact estimations of gene positions and effects.

SNP represents a very promising technology for gene mapping research, and therefore, will also be useful for improving the accuracy of breeding value prediction of livestock animals due to the reasons as follows:

- SNPs are abundant and stable compared to other types of DNA markers. SNP frequency ranged from one in 200 to one in 400 base pairs in the swine genome. A high- density SNP map allows estimating QTL positions and effects more exactly since SNPs can be seen as a type of markers that are much closer linked to QTLs and genes. Furthermore, SNPs can also be targeted within porcine genes and QTLs. The gene segregation can, therefore, be traced by SNPs directly.

- SNP markers are suitable for use in high throughput genotyping systems. Because of the use of Microarray technology, rapid screening of thousands of SNPs becomes feasible. The targeted price can be as low as $0.25 per SNP score (http://www.mergen-ltd.com/snp.htm).

Microarray technology seems to be very promising for large-scale gene mapping studies and for bridging the gap of knowledge from a QTL detected to the genes included in the QTL region. Wayne and Mclntyre (2002, PNAS 99:14903-14906) applied Affymetrix microarray to identify the genes of *Drosophila menlanogaster* and showed that the use of microarray technology allows an efficient, objective and quantitative evaluation of genes included in QTL regions.

Porcine gene discovery can also be enhanced by the increasing availability of porcine sequence and location of expressed sequence. The comparison of sequence data from EST production projects, especially if the libraries used were constructed using tissues from different individuals, can be a good source to find SNPs (Vignal et al. 2002). The SNPs found by this way are located in the coding region of a gene and have a direct association with phenotype. In addition, comparison of porcine ESTs with sequence from other species (especially human) is a valuable way to do comparative mapping of porcine genes.

3. POTENTIAL BENEFITS AND LIMITATIONS

Genetic improvement programs have typically two important components: Selection and mating systems. The genetic progress due to selection depends upon the intensity of selection, genetic variability, accuracy of genetic evaluation and generation interval. The genetic progress at the nucleus herds can be used in appropriate mating systems to achieve maximum economic returns in the commercial production systems. The potential

benefits and limitations of molecular markers can be described under the following points.

Benefits

Selection intensity The intensity of selection in swine breeding programs is mainly determined by the number of pigs tested and the number required to maintain the herd size. The costs associated with testing and test capacity usually limit the number of animals that can be tested, leading to lower selection intensity and the rate of genetic progress. However, multistage selection through molecular genetics can increase the selection differential (Xu and Muir 1992, Muir and Xu. 1992). If genetic markers linked to important QTLs have been identified, then it is possible to do a first stage selection at a young age on a large population, then only test those in the later stage which pass the first culling. Another example where multistage selection will be advantageous is lost selection intensity on males. Due to cost constraints, usually only one or two boars are tested from each litter especially in dam lines. By use of molecular genetics it would be possible to choose among litter mates at birth or weaning in the first stage, then do the final selection at the time of probing. In both cases this added selection differential would be lost without molecular genetics and represents lost opportunity (Muir 1997b, 1999).

Accuracy: The use of molecular genetics for sex limited traits (litter size), or traits which cannot be measured on either sex, is perhaps one of the most compelling reasons to use molecular genetics (Lande and Thompson, 1990). For traits which cannot be measured directly in either sex, such as disease resistance, meat quality, and animal well-being, quantitative genetic techniques would require a sib or progeny test which would be costly and/ or increase the generation interval.

Improvement of animal well-being could greatly benefit from molecular genetics. Selection to improve animal well-being is difficult and requires either direct measurement for traits related to well-being (Faure et al. 2002) or indirect measurement using group selection (Muir, 2002). Markers linked to QTL's which improve well being, while at least not compromising productivity, would allow genotypic selection of hard to measure phenotypes.

Initial Genetic Variation: Long term response to selection for any trait is dependent on polymorphic loci which influence the trait. Quantitative and molecular genetics is limited to changing frequencies of existing alleles. Many alleles are lost in the selection process due to random genetic drift. Random genetic drift is a consequence of finite populations and is proportional to the rate of inbreeding. Beneficial alleles lost during the selection process can be found in wild ancestral populations. A beneficial use of molecular genetics is to search for alleles in wild ancestors of domesticated species which have become lost (Muir 1994). In every instance where this technique was used, new alleles that outperformed the elite parents by as much as 20% were found (Tanksly 1997).

While it would be possible to cross such populations with domesticated lines, and start selecting from the new synthetic line, the frequency of undesirable alleles would also be dramatically increased and require long term selection to restore the population to high productivity. A second advantage of molecular genetics is introgression. Introgression

has traditionally been used when genes must be quickly and economically introduced into poultry populations. However, undesirable genes in the donor genome must be excluded as far as possible. Theoretically, DNA-based markers can enhance the efficiency of introgression (Groen & Timmermans 1992; Hospital et al. 1992). Ideal introgression would employ equally spaced markers in the host genome and tightly linked flanking markers for the donor gene. The gene of interest could then be introgressed with the highest recovery of the host genome (Dekkers and Hospital, 2002).

Mating System: Optimal long term response to selection is achieved by minimizing loss of favorable alleles, which occurs as a result of random genetic drift and associated inbreeding depression, while maximizing frequency of desirable alleles (Robertson 1960, 1961). On the one hand selection increases frequency of favorable alleles and opposes loss of alleles through drift. On the other hand, increasing selection intensity also reduces the effective population size which increases rate of loss of favorable alleles. Similarly, for a given selection intensity, selection programs which increase the accuracy of selection, such as Best Linear Unbiased Prediction (BLUP), reduces the effective population size because relatives tended to be selected. Thus, selection programs which optimize short vs. long term response are usually not the same (Muir 1997a, 2000, Quinton 2002).

Molecular tools can help in this regard by limiting the rate of inbreeding. Van Arendonk et al. (1998) and Van der Beek (1996) suggested using mixed semen and determining the sire from parentage testing based on information from genetic markers. As a result, a factorial mating design can be implemented which leads to a higher selection response without increasing the rate of inbreeding.

Limitations

Several problems have been observed with implementation of MAS, some of these are most likely due to genotype-environment interactions, negative epistasis between QTL or epistasis between QTL and the genetic background. Also, QTLs that were detected by crossing divergent lines identify QTL that differ between breeds, have limited direct application for within breed improvement (Dekkers and Hospital, 2002).

There are four overriding factors which limit the application of this technology 1) biological, 2) theoretical, 3) statistical, and 4) economics.

Biological: One of the main limitations of MAS in within family selection is the large number of offspring needed from each mating. Van Arendonk *et al*. (1994) concluded that the fraction of the within-family variance that can be explained by markers is of critical importance. To predict 10% of the within-family variance in grand-offspring, informative genotypes on 500 daughters of both grand-sires are needed. In this case, a 20% increase in annual genetic gain is expected (Meuwissen and Van Arendonk,1992). In contrast, only 2%-4% increase is expected in swine due to smaller family size.

Theoretical: Use of MAS, either for introgression or recurrent selection programs, diverts some selection pressure away from traits of economic importance. Thus in introgression the benefit of the target gene must be greater than that which could be achieved by

regular selection over the same time period (Dekkers and Hospital, 2002). In recurrent selection programs, monetary resources devoted to MAS could also be allocated to phenotypic selection programs. Alternatively, conventional selection programs can also be enhanced by increasing the number of individuals tested and/or the effective population size. This would allow either the selection intensity to increase or the rate of inbreeding to decrease, thus increasing both short and long term responses (Muir 1997a, 2000).

Use of Marker Assisted Selection (MAS) in any form requires linkage disequilibrium, either at the family or population level. In the case of a randomly mating population, different individuals will tend to be in equilibrium with QTL alleles segregating in proportion to the relative frequencies of the alleles. Alternative marker genotypes will include both positive and negative alleles at any linked QTL, and the mean quantitative value of the alternate marker genotypes will not differ even when a linked segregating QTL is present in the population. If the population under consideration has been selected for many generations without crossing to divergent lines or breeds, it is unreasonable to assume linkage disequilibrium between markers and QTL unless they are very close together (Visscher and Haley, 1998).

One should not expect to find loci with large effects on a trait in a population which has undergone long term selection for that trait (Lin *et al*., 1992). Simulations show that the probability is very low of finding genes with major effects for traits that have undergone phenotypic selection for several generations (Sehested and Mao, 1992). With phenotypic selection, genes with large effects reach fixation sooner than those with small effects, and after several generations of selection, the likelihood of finding genes with major effects is very low. Traits which show substantial genetic variation after several generations of selection are likely to be determined by a large number of loci each with trivial effects.

However, linkage, mutation, genetic correlations, and non-additive genetic effects such as over-dominance can keep genes segregating in populations. Unfortunately maintenance of polymorphism's through a genetic correlation implies a negative pleiotropic effect on fitness or other traits of selection (Lin *et al*. 1992, Muir, 1994). Also, deleterious alleles may be maintained in a population by heterozygous advantage (Lin *et al*., 1992). In either case, superiority cannot be fixed, because of low fitness in the former case and by definition in the latter (Muir, 1994). Thus, if genes with large effects are found to be segregating in a highly selected population with a moderate heritability, chances are that selecting on the gene would have a negative effect on overall productivity.

There is an abundance of evidence indicating that perhaps epistasis is the norm and additivity is a statistical artifact. From studies published for agronomic traits in plants, Mayo and Franklin(1998) concluded that it is becoming increasingly clear, that epistasis among loci with large effects on a quantitative trait are common and biologically, additivity is rare. The implications of epistasis for both detecting and using QTL in breeding programs are obvious. For example, if interactions exist, the effects of individual QTL cannot be meaningfully isolated and such QTL will not contribute as predicted to selection response.

Non-additivity would also affect expression of a gene in different backgrounds. For example Rothschild *et al.* (1994) found a positive effect on a estrogen receptor allele on litter size but the size of the effect was dependent on the genetic background of the pigs (Nielsen and Sorensen,1998).

Statistical*:* The effect of the QTL must be established empirically on the basis of statistical associations between markers and phenotype, and hence suffer from the same limitations as quantitative genetic selection. Thus, although combined selection is most effective with traits of low heritability or traits difficult to measure (Lande and Thompson, 1990), the ability to detect QTL also requires phenotypic data and is similarly limited in such cases. Thus, the "greatest opportunities for MAS might exist for traits with moderate rather than low heritability" (Dekkers and Hospital, 2002)

Economics: Integration of present and new genetic approaches to problems requires consideration of only one factor: economics. Breeders will use any approach which increases their overall profitability. Profitability is determined by the difference in income generated by sales less cost of development. Sales are mainly determined by the genetic superiority of stocks. Geneticists in academia are usually only concerned with the first aspect of profitability (genetic superiority), and often neglect the equally important consideration of costs to implement the program (Muir, 1994).

Colleau (1998) assessed the superiority of MAS over conventional schemes in dairy cattle selection nuclei by deterministic prediction of genetic gains for a given overall investment, in a situation where a single QTL was involved in addition to polygenes. This superiority was moderate (less than 5%) and depended on the heritability and the typing costs. An inferiority of MAS was observed for a moderate heritability (0.25), when the cost of typing 20 calves exceeded that of obtaining a calf from embryo transfer. When no typing costs occurred, the percentage of MAS superiority was generally small. It became relatively substantial (around 5%) for the lowest heritability and for the highest proportion of genetic variance explained by the QTL. Thus Visscher and Haley (1998) conclude that it may only be feasible to use microsatellite markers to select on limited areas of the genome (e.g. to introgress a QTL from one breed to another) but using many such markers at the same time in a breeding program may be impossible until costs are reduced.

Dekkers and Hospital (2002) state that MAS will be most cost effective when molecular costs are less than that of phenotypic observations, such as genotype building and population wide LD, or the ability to select early. But with combined selection, costs are a greater issue because molecular information is in addition to, rather than in place of, phenotypic information, in which case the cost:benefit of MAS may not be more effective than simple phenotypic selection. Arthur and Albers (2003) agree with this conclusion "As the economic value of individual chickens is relatively low, DNA based genotyping of individual breeding candidates must be done at low cost per bird. Therefore commercial application of genotyping at the DNA level will largely be through direct genotyping for critical genes and not through Marker Assisted Selection approaches *per se* that are being designed for larger species."

4. CONCLUDING REMARKS

While there are clearly several potential advantages to use of molecular genetics in swine breeding, there are considerable limitations. The current challenge is to determine where the benefits of MAS can be utilized without exposure to the risks. There are two clear cases were the downside is minimal: 1) utilization of lost selection intensity in multistage selection programs (selection among males before castration), and 2) selection on traits more expensive or difficult to measure on the phenotype than the genotype (disease resistance, animal well-being, and meat quality)

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