Recent Developments in Genetic Improvement of Pigs

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Summary

Building on a solid foundation of performance testing, quantitative genetics, and statistical estimation of breeding values; recent developments in pig line improvement programs have included the application of both major gene and marker genomics, and an increased focus on genotype by environment interactions and crossbred selection objectives. Additionally, selection objectives have broadened to include meat quality, livability/robustness traits, and commercial breeding companies have also embarked on customized line development programs for various large integrated or highly coordinated pork chains.

Genomic information is increasingly becoming integrated into quantitative genetic practice. Dozens of SNP markers are used in global and custom line improvement programs today. These programs integrate molecular genetics discoveries with quantitative genetic methodologies to increase the accuracy of selection for complex breeding objectives in commercial environments. As the pig genome sequence is elucidated, and genotyping and computational barriers (both financial and theoretical) to genotyping all selection candidates for perhaps 30,000 markers are chipped away at, the radical vision of genomic selection as an alternative to statistical analysis of phenotypic data will receive increasing attention from commercial pig breeders in the years to come.

Introduction

The application of scientific theory to the genetic improvement of pigs has developed in leaps and bounds since Wilhelm Johannsen first coined the term "gene" in 1909, Sewall Wright published the seminal population genetics “Systems of Mating” papers in 1921, and Oswald Avery, Colin MacLeod, and Maclyn McCarty demonstrated that genes were composed of deoxy-ribonucleic acid (DNA) in 1944.

Jay Lush laid out the basic principles and methods of scientific animal breeding in his book “Animal Breeding Plans” first published by the Iowa State College Press in 1937. Lush and his colleague Lanoy Hazel demonstrated the superiority of Index selection over independent culling levels and tandem single trait selection (Hazel and Lush, 1942). The ENIAC (Electronic Numerical Integrator and Computer), built in 1946 and weighing in at 27 tonnes, was the first general purpose digital electronic computer, and in the 1950s Charles Henderson developed his “mixed model equations” that remain the basis for genetic evaluations in all livestock species today (Henderson, 1953).
In the past 15 years we have witnessed phenomenal advances in both information technology and in the application of reproductive technology, technologies that jointly revolutionized the genetic improvement of pigs. Computers capable of solving Henderson’s mixed model equations became available starting in the 1960s. Artificial insemination (AI), which accounted for less than 1% of matings in North America in 1957 and only 5% in 1987, increased to 40% of matings by 1997, and is predicted will represent 87% of matings in 2007. The genetic consequence has been a dramatic reduction in genetic lag between pig genetic nucleus and commercial populations.

The much lauded genomics revolution is now well under way, and leading advocates are predicting that as genotyping costs continue to decrease and the pig genome is sequenced, genome wide genetic marker selection will become not only theoretically possible but practically feasible for use in the genetic improvement of pigs. Like the commercial application of Henderson’s mixed model equations (which took three decades in pigs), it is probably a question of “when”, not “if”.

This review paper focuses on developments in the genetic improvement of pigs that have occurred particularly over the past three to five years. It will not attempt to address possible additional future developments such as improved cryopreservation of pig semen and embryos, semen sexing, in-vitro fertilization, non-surgical embryo transfer, cloning, gene transfer, and juvenile selection techniques, all of which may impact genetic improvement of pigs in the future.

Quantitative Genetics

The basic procedures whereby phenotypic measurements could be combined to optimally select to improve the genotype for a given objective have been available to animal breeders for over 60 years (Hazel and Lush, 1942). The ability to discover and directly manipulate the myriad of genes and their interactions responsible for continuously distributed traits such as growth rate, back fat, meat quality, and disease resistance is a relatively new phenomenon.

Simply inherited qualitative characteristics governed by “major genes” (e.g., PSS, RN, coat color (actually, not that simple), specific E.coli receptors in the gut, etc.) have proved tractable to genomic determinism (see Molecular Genetics below). Quantitative traits controlled by many genes, however, continue to be most amenable to improvement assuming the statistical genetic abstraction of the infinitesimal gene model (i.e., assuming traits to be controlled by an infinite number of genes each alone having an infinitely small contribution). Recent genetic trends for quantitative traits, as illustrated in Table 1, are due if not entirely then almost entirely to selection based upon best linear unbiased prediction (BLUP) of unknown breeding values applying Henderson’s mixed model equations to phenotypic performance data, i.e., statistical or quantitative genetic technology.
Table 1. PIC North American Genetic Nucleus Five Year Genetic Trends  
(Pigs born April 2001 to March 2006, off-tested October 2001 to September 2006)

<table>
<thead>
<tr>
<th>Trait a,b</th>
<th>Annual Genetic Trend, trait units</th>
<th>Annual Economic Value c, C$ / market pig</th>
<th>5 Yr Potential Commercial Genetic Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number born a, pigs / litter</td>
<td>0.19</td>
<td>1.10</td>
<td>0.96</td>
</tr>
<tr>
<td>TNB a, pigs / sow / yr</td>
<td>0.42</td>
<td>NA</td>
<td>2.10</td>
</tr>
<tr>
<td>Stillborn, %TNB</td>
<td>-0.30</td>
<td>0.23</td>
<td>-1.50</td>
</tr>
<tr>
<td>Preweaning survival a, % NBA</td>
<td>0.26</td>
<td>NA</td>
<td>1.31</td>
</tr>
<tr>
<td>Litter weaning wt a, kg</td>
<td>0.44</td>
<td>0.21</td>
<td>2.21</td>
</tr>
<tr>
<td>Teat number a</td>
<td>0.06</td>
<td>NA</td>
<td>0.28</td>
</tr>
<tr>
<td>Leg structure score a</td>
<td>0.18</td>
<td>0.33</td>
<td>0.89</td>
</tr>
<tr>
<td>Sow mortality a, %</td>
<td>-0.28</td>
<td>NA</td>
<td>-1.41</td>
</tr>
<tr>
<td>Days to market b</td>
<td>-1.24</td>
<td>0.74</td>
<td>-6.19</td>
</tr>
<tr>
<td>Average daily feed b, g/d</td>
<td>-1.68</td>
<td>0.04</td>
<td>-8.40</td>
</tr>
<tr>
<td>Feed conversion ratio b</td>
<td>-0.03</td>
<td>NA</td>
<td>-0.13</td>
</tr>
<tr>
<td>Back fat b, mm</td>
<td>-0.08</td>
<td>0.01</td>
<td>-.42</td>
</tr>
<tr>
<td>Loin depth b, mm</td>
<td>0.66</td>
<td>0.51</td>
<td>3.31</td>
</tr>
<tr>
<td>Scrotal hernia, % male pigs</td>
<td>-0.23</td>
<td>NA</td>
<td>-1.16</td>
</tr>
<tr>
<td>Annual value, C$/market pig</td>
<td></td>
<td>C$ 3.18</td>
<td></td>
</tr>
</tbody>
</table>

a Average trend in dam lines comprising the Camborough® sow  
b Average trend in terminal pigs over three sire lines x dam lines comprising the Camborough® sow  
c NA = not applied as economic value credited to another listed trait

Jay Lush outlined the principles and methods for genetic improvement of livestock in 1937. Charles Henderson, a graduate student of Lush’s, went on to develop methods first to estimate the genetic parameters required to apply those principles, and then to best estimate the genetic value of candidates for selection. Henderson’s methods were first incorporated into statistical packages in the 1960s, and became the basis of commercial pig improvement programs with the availability of sufficiently powerful computer hardware, and software programs such as PEST (Groeneveld, 1990; Groeneveld and Kovac, 1990; Kovac and Groeneveld, 1990).

Extensive database development followed (e.g., PIC’s database today tracks approximately 196,000 active sows and includes 8.2 million pig records with pedigrees stretching back 25 generations). Genetic parameter estimates for PIC populations include over 45 traits today, and genetic markers are increasingly being used in conjunction with performance data to improve the accuracy of breeding value estimation.
Computing power has also allowed the practical application of optimal contribution theory to maximize rates of genetic response to selection in closed lines in the medium-long term by controlling the rate of accumulation of inbreeding and associated loss of genetic variance (Newman et al., 2006). Improvements in the quality and quantity of trait EBVs based upon fundamental quantitative genetics principles shows no sign of slowing down in the pig improvement business in the coming years.

Selection Objectives

In an article posted on The PigSite.com in November entitled “What the Commercial Pork Producer Needs To Know About Genetic Improvement”, Dr. Todd See of North Carolina State University stated “the commercial pork producer’s role in genetics is generally to take the end result of a genetic improvement program and manage animals in a manner to maximize expression of their genetic potential”. This indeed has been the classic role of pig breeding until quite recently. Insulate the pure lines in a well managed, high health, genetic nucleus farm and rigorously measure heritable traits of economic importance (growth, feed, backfat) to most accurately select the parents of the next generation. This approach has been very effective both at making genetic improvement in the pure lines (Table 1) and, coupled with increasingly effective dissemination via AI, their commercial crossbred descendents.

Over time competition among pig breeding organizations has resulted in a broadening of technical offerings beyond the core disciplines of genetics and health. Technical services have expanded into areas as diverse as nutrition, reproduction, meat science, and human resource management as the necessity to not only to create healthy breeding stock with increased genetic potential, but to help make sure customers achieve enough of this potential to differentiate products from those of competitors, has become increasingly apparent. Pig breeding organizations have themselves become vested in the management of commercial descendents of their genetics “in a manner to maximize expression of their genetic potential”, as Dr. See has pointed out.

Developments in, particularly, information technology have facilitated pig breeders in tackling the increasingly thorny issue that their largest customers are either not able, or not willing, to move as far as the breeder might like to “manage animals in a manner to maximize expression of their genetic potential”. They manage the animals to maximize their profit, of course, often by minimizing input cost (least cost, not necessarily best cost).

“Genetic potential” does not, in and of itself, win market share for breeding organizations, particularly in consolidating industrial markets. Commercial performance (phenotypic means and variances throughout the value chain), relative to that possible with genetic alternatives, wins and maintains market share. The genetic challenge, then, has been to work cost effectively with the reality of genotype by environment interactions (GxE) to select within the pure lines for commercial, crossbred performance.
Genotype by environment interaction is important when the ranking of animals for selection is affected by the environment in which their commercial progeny perform. As computers, databases, and R&D spend have expanded in recent years, commercial breeders have begun testing the progeny of new GN herd sires not only in the GNs, but also in commercial farms (Perez et al., 2006; Casey et al., 2006). As data have accumulated this has allowed estimation of genetic parameters (heritabilities, genetic correlations) for two sets of traits – growth, for example, in the pure line GN environment, and growth in crossbred commercial environments. These can subsequently be handled in genetic evaluations as correlated traits (Falconer, 1960).

Genetically, growth (and other economically important trait performance) in a crossbred commercial environment is not identical to growth in the purebred GN – i.e., the genetic correlation is not equal to 1.0. Literature estimates for this correlation for growth rate in pigs range from 0.19 to 0.99, while estimates for back fat range from 0.21 to 0.88 (Brandt and Taubert, 1998; Lutaaya et al., 2001). For reproduction traits Nakavisut et al. (2005) reported low to moderate genetic correlations (0.21 to 0.52). Combined crossbred and purebred selection methods have been shown to be superior to pure line selection alone in many situations (Bijma and Arendonk, 1998; Lutaaya, et al., 2002; Perez et al., 2006).

The objective (growth, reproduction, and carcass) traits can now be defined as crossbred performance traits, and the index selection criteria traits as both crossbred (direct) and purebred (indirect, but correlated) performance. This has been implemented by PIC, for instance, since 2005. As the database continues to grow it allows the future opportunity to not simply assume there is one crossbred environment, but to estimate the sensitivity (reaction norm) of a boar’s breeding value across the range of environments measured, which is the focus of ongoing research (Hermesch, 2006; Knap and Wang, 2006).

Selection objectives in commercial pig breeding 15 years ago focused almost exclusively on pure line growth rate, feed efficiency, and backfat. Structural soundness (beyond independent culling) and litter size were added with the adoption of quantitative leg scoring schemes and software allowing BLUP in large commercial breeding populations, both in the early 1990s.

At about the same time, with increasing consolidation and integration in the industry, meat quality began to become an important consideration for pig breeders – first by the elimination of the recessive mutant halothane allele in the period 1991 to 1996, then elimination of the undesirable form of the Rendement Napole (RN, aka acid meat, Hampshire) gene. For the past decade classical estimation of breeding values and index selection for traits such as pH, color, and marbling, based on data collected on GN selection candidate relatives and (essentially) known genetic correlations with other performance traits, in addition to some specific gene (CAST, PRKAG3) polymorphisms, has been used.

Having the basic tools in place, the genetic improvement industry has been able to respond to emerging commercial performance issues – e.g., sow mortality and scrotal...
hernia in the late 1990s and, most recently, pig mortality and GxE interactions. The number of traits with EBVs available for PIC selection indexes today stands at more than 45, and the number of markers being used routinely in EBV calculations has increased to over 75. PIC sire line selection indexes target responses in 12 traits, and dam lines target 17 traits. Those trait EBVs are all influenced by between 2 and 17 markers, depending on the trait. I do not anticipate either the breadth of performance trait measures or the use of genomic information in their evaluation to be decreasing in the future.

Each PIC pure line has its own unique selection objective aimed at emphasizing the trait strengths of that particular line, and also therefore increasing the possible diversity in uncertain end points that may be required under future market conditions. All analyses are multi-trait, and driven by population specific genetic parameter estimates. Indexes are developed beginning with purely economic scenarios, thus as every commercial pig receives ½ of its genes from its father and ½ from its mother, growth and carcass traits are extremely relevant to dam line improvement. However final selection is based on essentially desired gain indexes designed to enhance line strengths and diversity as described above. Between-line genetic variability, exploited via line complementarity (strengths) and heterosis in commercial crosses, is arguably as important in pig breeding as the within-line genetic variability essential for line improvement via selection.

Customized Genetics

As the pork production industry has consolidated and integrated, a number of pork chains in the past five years have identified opportunities to custom improve genetics to best meet their own particular environments, exploit their unique data, and maximize rate of genetic improvement toward their own custom objectives. Some companies and industries have their own genetic improvement programs (e.g., Smithfield Foods in the U.S. http://www.spgenetics.com/history.asp; and the Danish Pig Breeding Program, DanBred http://www.danbred.dk/view.asp?ID=3962); other companies have programs managed by breeding companies like PIC.

In addition to the 10 base lines PIC works with for global product development, we improve 5 genetic lines exclusively for different customers in the U.S. today. As food companies seek to decrease their input costs and create differentiated food products for their domestic and export customers they are increasingly realizing the role genetics has to play in their systems, particularly when they are able to measure and improve objective traits that are not included in mainstream breeding company line development programs.

The majority of meat production chains are, of course, commodity driven businesses (Sosnicki et al., 2003), and pig breeding “companies” increasingly dominate this segment. Niche markets do, however, exist for “story pork” – i.e., pork with various characteristics desired by certain consumers. Examples include certain so-called heritage breeds (e.g., Gloucestershire Old Spot, Large Black, Tamworth, Berkshire (http://www.berkridge.com; http://www.heritagefoodsusa.com)) and other combinations of genetics and production systems (e.g., Premium Standard Farms USDA Process Verified antibiotic free pork http://www.psfarms.com/process_verified_pork.html; Leidy’s Nature’s Tradition,
As global market forces continue the consolidation, intensification, and integration of commodity pork production, the threat to the continued existence of hundreds of pig breeds adapted to specific production environments in different parts of the world also increases (Gibson et al., 2006). Niche markets for specialty products such as the above in North America, and dried cured ham products in Southern Europe, for example, will continue to represent only a small proportion of global pork production, which will adopt more uniform genetics as it strives to increase efficiency and consistency.

The private sector will maintain breeds to fill specific profitable niche market requirements. It remains to be seen to what extent politicians will allocate resources to conservation breeding programs in order to mitigate the risk of losing potentially valuable genetic diversity for adaptation that exists among world pig breeds today. Support for such programs is not made easier by the fact that extremely little, objectively, is known about adaptation characteristics and genetic diversity of the hundreds of pig breeds, many in Asia, existing today.

Molecular Genetics

Application of molecular genetics technology to the pig improvement industry began with discovery of the point mutation responsible for porcine stress syndrome (Fujii et al., 1991), and subsequent commercial availability of a DNA test (HAL-1843™) to determine genotype for the “halothane” gene.

Development of genetic marker technology has created keen anticipation regarding opportunities for marker assisted selection (MAS) in livestock improvement (Meuwissen and Goddard, 1996). However, to date markers have contributed relatively little to the genetic improvement of pigs, with a few notable exceptions. Record rates of improvement in litter size, growth rate, feed efficiency, and carcass composition (Table 1) have been largely achieved through traditional performance testing and applied quantitative genetics, as noted above. Advances in genetic marker technology and decreasing testing cost today, however, have resulted in the ability to include hundreds of marker genotypes in routine genetic evaluations, the impacts of which in MAS will begin to be seen.

Research to map the pig genome began in the early 1990s with creation of the international PiGMaP project (Archibald et al., 1995). Initial pig genomic breakthroughs included DNA tests for deleterious forms of “major genes” such as the “halothane” gene and the RN (Rendement Napole) “acid meat” gene (Milan et al., 2000). A number of research centers around the world established “reference families” using genetically diverse breeds to study gene segregation in F2 populations and determine the probability
of chromosomal locations of quantitative trait loci (QTL), i.e., genes (loci) affecting various quantitative traits (Bidanel and Rothschild, 2002).

QTL regions discovered, however, were often very large (20-40 centiMorgan (cM) long; ~20-40 mega base pairs (Mb) of DNA), and could contain 200-400 positional candidate genes (Andersson and Georges, 2004; Schook et al., 2005). They therefore had limited or no application in genetic improvement programs as marker-QTL relationships would not hold up on a population basis, but had to be established on a within-family basis.

An alternative approach was to look for markers associated with candidate genes (Rothschild and Soller, 1997; Rothschild and Plastow, 1999). By focusing on markers for genes thought likely to be causative for variation in traits of interest, based often on comparisons with other mapped mammalian genomes, a number of useful causative mutations and linked marker polymorphisms for reproduction (e.g., ESR, PRLR, RBP4, FSHB), feed intake and growth (e.g., MC4R), body composition (e.g., IGF2), coat color (e.g., KIT, MC1R), meat quality (e.g., HAL, RN, PRKAG3, CAST) and disease resistance (FUT1) were discovered and became applied in pig breeding (Plastow et al., 2003; Andersson and Georges, 2004; Dekkers, 2004; Rothschild, 2004a).

The candidate gene approach is limited, however, in that it works with only a small part of the genome. Development of “gene chip” (microarray) technology and decreasing cost per marker genotype have made use of high-density marker genotyping to detect QTL feasible in the past few years. The objective of high density genotyping is that, by having markers spaced at approximately 1cM intervals throughout the genome, there will be markers in linkage disequilibrium (LD) with all the QTL which will be practical to implement for improvement using population-wide LD-MAS.

Developments related to the use of genetic markers in livestock improvement have by no means been restricted to the field of molecular genetics. Much statistical genetics work has been conducted on methods for linkage mapping of QTL in livestock (Meuwissen and Goddard, 2004: Lee et al., 2005; Boitard et al., 2006; Dekkers et al., 2006; Tier, 2006; Zhao et al., 2006). The breeding industry has also added bioinformatics specialists to genetic improvement teams comprised of quantitative, statistical, and molecular geneticists as the complexity of managing and analyzing biological data rapidly increase.

Understanding the complexity of the pig genome – which consists of some 2 to 3 billion (2 to 3 x 10^9) base-pairs (pairs of nucleotides, the “letters” of the DNA code), is 2 to 3 thousand centi-Morgans (linkage map units) in length, and comprises 25 to 30 thousand genes, is of course far from trivial. Also, it is not the intent of practical pig breeders to necessarily understand the specific genes and biological pathways involved as much as to maximize the chances of having useful markers, in the quantitative sense, in linkage disequilibrium with significant quantitative trait genes throughout the pig genome. The “black box” of statistical breeding value estimation may be becoming a little greyer, but not much!
From the first “halothane” gene testing in 1991 to incorporating 75 single nucleotide polymorphism (SNP) gene test markers into daily EBV calculations has taken PIC 15 years. Integrating molecular genetics with traditional selection on phenotypes to improve accuracy and response to multi-trait index selection has been a consistent vision for quantitative geneticists (Lande and Thompson, 1990). There are relatively few major genes and simply inherited characteristics of economic importance in pig breeding. Integrating markers into the quantitative genetic program has occurred as a consequence of both the number of available markers and the decreasing cost per data point of genotyping (but not absolute cost, as millions of genotypes are required annually).

Once relevant DNA markers have been identified and selected in the research phase they are added into a genotyping panel for which hundreds of pigs from diverse genetic lines are routinely tested. Having the markers in these panels allows identification of all pleiotropic effects on economically important traits in addition to the typically limited number of traits targeted in the discovery research, a critical step in validation where the breeding program is designed to simultaneously improve multiple correlated traits. In addition, the “effects” of linked (to QTL) markers are frequently population specific and subject to change over time due to recombination, and therefore have to be continuously re-estimated, much as is the case for polygenic effects.

As more markers are discovered and validated, individual marker effects become less and less important. Whereas initially patent protection was sought for many individual markers (Rothschild and Newman, 2002), their increasing number and line-trait specificity has seen a more recent shift towards intellectual property protection via trade secret as opposed to the patenting route.

**Sequencing the Pig Genome**

Sequencing of genomes is a basic research endeavor. The first draft human genome sequence was published in 2001 (Venter et al.), the mouse genome sequence in 2002 (Mouse Genome Sequencing Consortium), the chicken in 2004 (International Chicken Genome Sequencing Consortium). A draft bovine genome sequence was also released in 2004 (http://www.genome.gov/12512874).


Availability of the porcine genome sequence will speed up QTL detection, and create the possibility of generating gene chip arrays for porcine QTL expression analysis that are highly informative for traits of economic importance and result in powerful tools with which to estimate breeding values for these traits. Nonetheless, under even the most optimistic scenarios, understanding the genetic basis of phenotypic diversity in quantitative traits to allow more deterministic manipulation of metabolic pathways than
currently achieved using statistics and performance data is a long way in the future. With that level of understanding it will probably be possible to grow “pork” without the messy intermediary of the pig.

Andersson and Georges (2004) estimated a marker density of at least 10 markers per cM would be required for screen for pig QTLs, corresponding to ~30,000 markers for a genome wide scan. Cost of SNP genotyping or sequencing will in the near future prohibit practical application of such high density methods in pig breeding.

While various researchers have claimed that “we absolutely have to understand the genes and biology involved” (e.g., Rothschild, 2004b), in practice this is not required for successful application of MAS. The largest QTL effects detected in livestock have been observed to be accounted for by numerous loosely linked QTL, and detecting and evaluating mutations in regulatory, non-coding, regions of the genome is still rudimentary – but will improve greatly with availability of the porcine sequence in the future (Andersson and Georges, 2004). Adding to the complexity of elucidating the underlying biology are the as yet largely unknown but frequently significant impacts of epistatic effects on quantitative trait genetics, for instance QTL frequently have different effects in different genetic backgrounds (Noguera et al., 2006).

Research continues to discover new genetic markers for all traits of economic importance, but a particular focus today is on traits associated with robustness – pig preweaning survival, nursery mortality, grow-finish mortality, and post farm gate losses. Many of the genes investigated and discoveries made to date have been the result of comparative genomics. Sequencing of the porcine genome will be an important milestone in the development of enabling technology in all areas of research involving the biology of the pig.

**Genetic Markers for Decreased Susceptibility to Pig Diseases**

Perhaps the most alluring target for the use of markers in livestock genetic improvement has been resistance to clinical and sub-clinical disease. Studies of various immunological traits have demonstrated they possess additive genetic variation (Henryon et al., 2006a), and some have been shown to respond to selection in the pig (Mallard et al., 1998). It has been more challenging, however, to find a genetic relationship between immunological traits and resistance to disease (Henryon et al., 2006b).

Genetic marker technologies to identify markers relevant to genetic improvement in disease resistance have developed over time from initial candidate gene studies, many of which candidates were putative immunological trait pathway genes, to whole genome scans and gene expression analyses. A key PIC objective is the development of practical tools to help select pigs that perform better in commercial environments. This has been tackled by using samples obtained from crossbred commercial genotypes under specific health and management environments. This makes the marker discovery work more readily applicable than research with non-commercial (but genetically diverse) reference
families, or with pure lines in high health nucleus farms not experiencing the health and management challenges faced commercially.

Particular focus has been on studying genes that affect specific diseases such as the Porcine Reproductive and Respiratory Syndrome virus (PRRSV), *Haemophilus parasuis* and Salmonellosis. Results to date indicate that multiple host (pig) genes influence susceptibility to these diseases, which is lowly heritable (PIC, unpublished data). In contrast, for example, F18 *E. coli* resistance is monogenic, i.e., governed by a single gene (FUT1) with a major effect (complete resistance to edema disease). Implementation of DNA markers in the breeding program to influence more complex diseases increasingly requires a quantitative approach as selection includes multiple genes, as discussed above.

In the last two years, more than 700 SNPs targeting candidate genes associated with health traits have been discovered in the PIC program. Results of some specific research trials are detailed below.

*Intracellular bacteria*

Some of the earliest health genetics work PIC conducted involved a resource population of pigs challenged experimentally with *Salmonella choleraesuis*. Differences in susceptibility to Salmonellosis were reported (Van Diemen *et al.*, 2002), and subsequently candidate gene SNPs were found to be significantly associated with 10-fold bacterial count differences in liver and spleen samples. SNPs in the NRAMP1, BPI and NFKB1 were among those markers found to be linked to susceptibility in this population (Shi *et al.*, 2003; Tuggle *et al.*, 2003; Zhao *et al.*, 2005).

*PRRSV*

Infections with PRRSV have been estimated to cost the global swine industry $3 to 10 per affected pig. PRRSV represents a risk of disruption to gene dissemination, affects the reproductive and growing production stages, and prevention, treatment and control are not adequate for most of the industry. Associations between 60 SNPs and litter traits of sows affected with PRRSV were evaluated. The DNA markers tested targeted known viral receptors, immune genes, viral replication genes, genes differentially expressed in PRRSV infected macrophages, and other candidates. The tissues used for genotyping originated from 1,400 sows from two farms affected by PRRSV in 2001 and previously negative to the virus. The majority of the animals were exposed to the virus and one of the farms conducted a planned exposure program where all adult animals in the herd were intentionally exposed to the virus. Association analysis revealed multiple markers associated with reproductive traits (Galina-Pantoja *et al.*, 2006). Results from a group of selected PRRSV markers associated with litter size born alive in one of the farms are shown in Table 2. As an example, results indicate that sows with a 22 genotype for SNP 1 had no significant differences to sows with an 11 genotype before PRRSV. However, during the PRRSV outbreak, sows with an 11 genotype had on average 1.54 pigs born alive more for each copy they carried of the favorable allele 1.
Table 2. DNA markers associated with pigs born alive (BA) in a farm before and during a PRRSV outbreak.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Mean BA before PRRS</th>
<th>Mean BA during PRRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP1</td>
<td>11</td>
<td>10.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>9.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.51&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>10.10</td>
<td>4.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Additive Effect</td>
<td>NS</td>
<td>-1.54*</td>
</tr>
<tr>
<td>SNP2</td>
<td>11</td>
<td>11.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>12</td>
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<td>6.75&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>6.64&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Additive Effect</td>
<td>-0.70*</td>
<td>-1.52*</td>
</tr>
<tr>
<td>SNP3</td>
<td>11</td>
<td>9.51</td>
<td>8.22&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>12</td>
<td>9.25</td>
<td>7.38</td>
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<td>9.45</td>
<td>6.45&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Additive Effect</td>
<td>NS</td>
<td>-0.88*</td>
</tr>
</tbody>
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* = P<.05, <sup>ab</sup> within an column = P <.10, NS = non-significant, P>.10

**Glässer’s disease.**

Multiple gene markers are associated with host responses to *H. parasuis* infection, the cause of Glässer’s disease, which is characterized by polyserositis, arthritis and depending on the strain, pneumonia. Glässer’s disease is associated with significant mortality in some farms, often in large multi-site systems where pigs are weaned very young and commingled with other infected pigs when passive immunity is decreasing. To identify differences in susceptibility, an experimental infection challenge was used in pigs with the same genetic background. Pigs showed differences in their susceptibility to Glässer’s disease (Blanco et al., 2006), and a microarray was used to identify genes differentially expressed. SNPs in those differentially expressed genes were discovered (Galina-Pantoja et al., 2005) and evaluated in unrelated samples from field cases of polyserositis and pneumonia associated with *H. parasuis*. In addition, a scan using 1,400 anonymous SNPs distributed across the pig genome was used to find SNP alleles more common in pigs that died with polyserositis or pneumonia vs healthy controls exposed to the same environment. Both approaches identified multiple SNPs significantly associated with susceptibility to Glässer’s disease.

**Practical implications**

Today, many of the markers described above are incorporated in routine PIC genotyping to validate their effects in multiple lines and across different traits, in addition to continuing to monitor associations within additional commercial pig population health data sets. Once fully validated, markers are implemented in the PIC line improvement program. These markers, either alone (e.g., FUT2, the gene marker associated with resistance to edema disease, has been included as a selection criteria in line improvement since 2005), or increasingly in combination with each other and pure and crossbred pedigreed performance test data, show promise in providing a tool to reduce the impact
of disease when used for genetic selection. Marker assisted breeding value estimation of traits associated with disease susceptibility and mortality is particularly attractive as a practical tool for the very reason that these traits have low heritabilities and are difficult to measure, and hence relatively intractable to traditional quantitative methods.

Today, efforts aim to identify gene markers for robust growth and survival in commercial environments and reduced susceptibility to Porcine Circovirus type 2 Associated Disease (PCV2AD) using molecular tools. Each gene marker incorporated into PIC’s global genotyping panel is intended to be used, once validated, to increase the accuracy of the estimated breeding values (EBVs) for pigs tested in the nucleus herds.

Genomic Selection

The promise of “genomic selection” involving thousands of markers is tantalizing, but far from reality today in the pig. Haley and Visscher (1998) and Meuwissen et al. (2001) postulated that with the availability of dense marker maps, genomes could be considered as many thousand small segments the effects of which could be estimated and summed to arrive at trait breeding values estimated solely based on “marker” genotype (and pedigree). Hayes et al. (2006) estimated that to obtain results similar to Meuwissen et al.'s simulations would require 10 markers per centi-Morgan, or approximately 30,000 markers in total, in cattle.

It has been 12 years since the first commercial DNA “chips” (microarrays capable of measuring gene expression) were manufactured. Today, microarrays and associated equipment represent a $700 million market dominated by human medicine (Anon, 2006). Genetical genomics (expression QTL) research has been conducted in livestock species, and resulted in useful marker discovery in pigs (e.g., http://www.pathochipproject.com; http://www.qualityporkgenes.com), however in the near future livestock microarrays are unlikely to prove important outside of research because they are too expensive to use in routine evaluations. Kadarmideen et al. (2006) cited the price of a “good quality” Affymetrix GeneChip® at $400, and about $100 for a spotted array with less features.

Not everyone, however, would agree. Recently, Dutch breeding companies announced they were beginning “the revolutionary implementation of the application of QTLs in breeding: Genomic Selection”. Hendrix Genetics claim they are applying genomic selection using 3,000 markers in broilers, while Euribrid (Nutreco) announced they are ready with a custom 20,000 DNA marker chip to start testing some broilers born in November, 2006: “the first time such a large set of DNA markers has been used in commercial animal breeding” (www.thepigsite.com/swinenews/12912/first-use-of-commercial-genomic-selection).

Affymetrix have offered a 10,000 marker bovine chip since 2005, and the dairy cooperative research center in Australia announced in October 2006 that they were “the first in the world” to link 15,000 genetic markers with 37 dairy production traits. According to the press release, these markers are now being evaluated in the field (http://www.biomelbourne.org/fileadmin/Events_Calendar_downloads/2006/December/D
airy_CRC_-_Australia_takes_world_dairy_genetics_lead_28_O.pdf), but as Hayes et al. (2006) pointed out, first results suggest marker density is insufficient to take directly to linkage disequilibrium marker assisted selection (LD-MAS). Thus while Schaeffer (2006) estimated that the cost of proving dairy bulls in Canada might be reduced by 92% and rate of genetic improvement increased by a factor of two by using genomic selection, the gap between theory and practical application today remains significant in all livestock species.

Conclusions

Genetic improvement of pigs has been greatly enhanced by developments in statistical genetics, information technology, application of AI, and most recently genomics. Steady, cumulative improvement by within line selection based on quantitative genetic theory, and the use of specialized sire and dam lines in crossbreeding systems, continue to be predominant technologies in pig improvement.

The tools used by pig breeders have become increasingly sophisticated in recent years, and require a wide range of technical specialists working in a disciplined, well coordinated fashion to implement in an industry that requires updated EBVs on a daily basis. Nevertheless, genetic improvement of pigs continues to be underwritten by the collection of accurate phenotypic data on pedigreed pigs in multiple environments and at all stages of the life cycle.

Increasing use of markers to aid in selection for lowly heritable, hard to measure traits such as those associated with disease resistance and robustness is promising. Marker assisted BLUP is already implemented for many sow productivity, growth, efficiency, and meat quality traits. Gene effects need to be better evaluated in commercial crossbred populations, however, which will also open the opportunity to use markers to capitalize on non-additive effects in line cross products (Dekkers and Chakraborty, 2004; Thaller et al., 2006).

Research investments will continue to map QTL in the pig. Marker verification, effect estimation, and integration into the classic BLUP methodology is limited only by available relevant data, genotyping costs, and computational challenges associated with including these data into BLUP algorithms. The “black box” quantitative genetics model will endure long into the 21st century, despite (or perhaps due to) rapidly increasing understanding of the biology underlying selection response.

There is great excitement and enthusiasm, particularly in academic circles, that genomics and bioinformatics will revolutionize animal breeding to the point where we will be able to select based upon detailed marker genotypes alone (genomic selection). For the foreseeable future, however, commercial pig breeders will need to continue to performance test for the heritable traits of economic importance they wish to improve, and measure all such traits to constantly track pleiotropic effects that may be expected to change as the underlying genetic architecture changes.
Despite significant genetic improvement of production traits as a result of four decades of intense selection in commercial pig lines, there are no indications that genetic variability is declining within these large populations. The resources to apply expensive developing technology, which is increasingly proprietary and consolidated in the hands of a relatively few global breeding organizations, are being made available where value is created for both the customer and, consequently, the breeding organization.

Future competitive position of pig breeding organizations will continue to depend on the diversity and strength of their base germplasm, their selection strategies, and their ability to implement genetic improvement programs effectively to create product value differentiation, rather than on the technologies they use per se. The access to high quality data, germplasm, computers, technocrats, and organizational commitment to constantly upgrading implementation of genetic improvement best practices will ensure the continued rapid genetic improvement of pigs for food.

References