

Swine Genetic Challenges of the Future: One man's thoughts.

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Introduction

Past improvement from selection: Genetic improvement of swine first began with domestication at least 5,000 years ago and perhaps as long as 10,000 years ago. Development of specific strains or so-called breeds occurred as individual small breeders applied the use of inbreeding and selection to fix or improve characteristics that would later help define lines and breeds. Such processes were confined to breeders at the end of the 1800s when Mendel's discoveries initiated the field later to be known as genetics.

While small breeders were certainly the rule in the early 1990s the advent of corn breeding companies and the successful development of inbred lines and crosses paved the way for the development of breeding companies. In the beginning a large number of breeding companies and family breeding operations existed. Market pressures, adoption of modern production technology and business models relying on economies of scale have reduced those numbers greatly and have also reduced the number of individual breeders to a very small share of the marketplace. In the swine breeding business very few companies (less than five) now compete world-wide and in the US the market share of the top three companies' likely makes up 65-70% of the market.

In pigs, primary selection for the past nearly 60 years has been devoted to faster and leaner growth. Such selection has had clear and decisive outcomes. From the 1930s phenotypic measures of back fat have been reduced from 45 mm to 15 -16 mm, with growth rates increasing in some cases by well over 50%. This was achieved while increasing market weights from 80 kg to 125 kg. Feed conversion has also nearly halved with the best commercial herds having efficiency ratios of 2.5 -2.6 kg feed consumed to kg gained over the lifetime of the pig. Even in more recent years genetic improvement has continued with reductions in age to market weight of 0.4days/yr in genetic trend (Chen et al., 2002; 2003). Some industry analysts believe fat levels have now reached a minimum. Unfortunately these changes have been accompanied with reductions in marbling and pH causing meat quality to suffer. In terms of number born alive purebreds from smaller breeders have increased and in some breeding companies genetic rates of improvement of 0.2 pigs per litter per generation appear to be the norm.

The molecular genetics era:

"Every great movement must experience three stages: ridicule, discussion, adoption." John Stuart Mills

Molecular genetic analysis has revolutionized how geneticists examine genetic differences that exist within pigs and poultry. Beginning in about 1991, PiGMaP was formed to develop genetic linkage maps (Archibald et al., 1995). In the first 10 years, efforts were directed toward the

development of useful genetic linkage maps consisting of anonymous genetic markers and a limited number of known genes. In addition, comparative genome maps were developed which have aided greatly in our search for interesting and potentially useful genes in the pig. The coverage on these genetic maps soon became sufficient to allow researchers to search for the causative genes by conducting quantitative trait loci (QTL) linkage analyses. These QTL linkage analyses employ a genome scan where generally F₂ or backcross families were used and genotypes are obtained for many (>100) markers relatively evenly spaced across the genome. Many such experiments in pigs can be reviewed in the Pig QTL database (<http://www.animalgenome.org/QTLdb/pig.html>). In the pig results revealed that there are over 1850 pig QTL in the database from nearly 120 publications representing many different pig traits. Of these QTL most deal with traits such as backfat and meat quality (1400+), production (275+), reproduction (70+) and health (15). These QTL discoveries for both species usually encompassed chromosomal regions of 10-20 cM though on occasion such regions of the chromosomes were much larger.

Candidate gene and comparative positional candidate gene mapping approaches have also been successful in identifying major genes affecting several traits, especially in swine. Candidate gene analyses (Rothschild and Soller, 1997) are undertaken when a gene is chosen based on the physiology of the trait. This is supplemented by comparative gene analysis that allows researchers to find “positional candidate genes” in the regions associated with possible QTL. Significant effects of major genes and candidate genes have also been reported. These include the well known effects of the HAL or RYR1 gene (Fujii et al., 1991) and RN⁻ (Milan et al., 2000) on meat quality. Genetic tests for these are now available and they are used world wide by small breeders and commercial companies alike to remove the deleterious effects. Additionally, the MC4R (melanocortin 4 receptor) gene was found to significantly affect growth rate by 7-9% by influencing feed intake. The MC4R gene maps to chromosome 1 close to a significant QTL. Due to the MC4R effect on feed intake, variation in this gene is also significantly associated with differences of 5-8% in backfat and relates to one QTL for backfat thickness on chromosome 1 (Kim et al., 2000). Another gene, IGF2, was found to significantly affect growth and muscle quantity in pigs and acts as a major gene locus (Van Laere et al., 2003).

A QTL located on chromosome 15 was found which had significant effects on ultimate pH in Berkshire x Yorkshire F₂ pigs (Malek et al., 2001b). The QTL explains 4-6% of ultimate pH variance and presents favorable, but partly recessive Berkshire alleles. This muscle glycolytic potential QTL is localized in the same region as the RN locus. The original RN⁻ mutation (Milan et al., 2000) was not present in the population studied. The observed effects were due to three additional mutations inside the gene called PRKAG3 containing the RN locus (Ciobanu et al., 2001). Further study has demonstrated that the three mutations when combined into haplotypes (linked markers) produced differences in pH that may be as high as 0.1 pH unit in all breeds except Berkshires, where the differences may exceed 0.2 units. Unlike the RN⁻ mutation, which is essentially only in Hampshire pigs, these three new mutations are in all breeds, making them extremely important economically. In addition, an investigation revealed that Calpastatin (CAST), mapped under a tenderness QTL, is a specific inhibitor of calpains, a Ca²⁺-activated protease family and considered to be the major cause of initiation of myofibrillar protein degradation. Extensive analysis of the CAST gene revealed several polymorphisms that altered the protein and these had large effect on tenderness (Ciobanu et al., 2004).

Candidate gene analysis for reproduction has also shown considerable merit. Results have clearly demonstrated that the estrogen receptor (ESR) is significantly associated with litter size

(Rothschild et al., 1996). Estimates of allelic effects vary from 1.15 pigs/litter in Meishan synthetics to 0.42 pigs/litter in Large White lines. The ESR marker was incorporated successfully into PIC selection indices for Large White based dam lines, resulting in an increase in the rate of genetic response in its nucleus herds. Iowa State University researchers, working with PIC, demonstrated that the prolactin receptor (PRLR) locus is significantly associated with litter size (Vincent et al., 1998), which was confirmed in two smaller studies. Retinol binding protein 4 (RBP4) was investigated using nearly 2,500 litters and shown to be associated with an increase of about 0.25 pigs per litter (Rothschild et al., 2000). These markers are now available to all breeders and companies.

The existence of a gene responsible for resistance to K88 *E. coli* diarrhea has been known for many years. The gene coding for the K88 *E. coli* receptor in the pig is on chromosome 13 and candidate gene analysis of the region is underway in many labs. Resistance to edema disease caused by F18 *E. coli* has also been reported and was mapped to chromosome 6. The work confirmed that a polymorphism in the FUT1 gene (Meijerink et al., 2000) is probably the causative mutation for adhesion resistant animals in these breeds.

Information at the DNA level can help producers, breeders and veterinarians to select for a specific major mutation such as FUT1 resistance or against negative mutations like the negative Halothane allele or RN allele in the pig. DNA information can also be used to assist in the selection of quantitative traits, called Marker Assisted Selection or MAS (e.g., using ESR B to increase litter size and MC4R to reduce feed intake). Molecular information can increase the accuracy of selection, allow for selection for sex limited traits and allow for selection for traits like meat quality. These approaches have led to a number of genes and markers being used in the swine industry. **Many of these genetic tests were recently made available to the interested public through genotyping service companies. The question remains as to whether all the breeders who need to use the technology are actually using it.**

The genomics era begins:

“The science of today is the technology of tomorrow.” Edward Teller

Sequencing is the unraveling of DNA to better understand the genetic code. It is equivalent to breaking down books into individual sentences and even specific letters in these sentences and words. The letters in the genetic code (A, T, G, and C) are combined into “words” and these words are the genes that control traits or contribute to phenotypes of the animal like rate of growth, level of fat, reproductive performance and disease susceptibility. Knowing the genetic code requires application of modern molecular biology methods to break up the code into smaller pieces to “read” the code.

Sequencing of the pig genome began in part when a Danish-Chinese project was initiated several years ago. This project produced limited genome coverage. To have a usable sequence a 6X copy is required. This effort was resumed in 2006 by the US, UK and other countries with additional sequencing coverage being obtained from foreign contributions. This new effort is progressing nicely. Daily updates can be accessed at www.animalgenome.org/pigs/genomesequence/. Other information about the sequencing project can also be seen at web sites hosted by the Sanger Institute and the University of Illinois (www.piggenome.org/sequence.php). Sequencing is over 70% completed and expected to be completed by 2009. Funding to sequence the pig genome is an international effort provided by

the USDA, National Pork Board, Iowa Pork Producers Association, University of Illinois, Iowa State University, North Carolina Pork Council, North Carolina State University, the Wellcome Trust Sanger Institute, and a number of research institutions from around the world including China, Denmark, France, Japan, Korea, Scotland and the U.K.

Sequencing is but the first step. Further gene and trait discovery will be made possible by development of new tools like the SNP (Single Nucleotide Polymorphism) chip. These chips are designed to genotype animals for 50,000 SNPs or more at one time for a cost of less than \$150 per animal. Such SNP genotyping will allow for “whole genome association trials” and discovery of many significant associations. The pig SNP chip has been recently produced and publications on their initial use are expected in 2009.

Once found these SNP associations are determined they then can be used to perform genomic selection. Genomic selection (Meuwissen et al., 2001) can be described as prediction of an animal’s breeding value using 1000s of SNP associations. Such predictions offer many opportunities to advance genetic improvement. First and foremost they offer a real look at the relatedness of animals on a DNA basis instead of predicting it on average from pedigrees. Secondly genomic selection would allow the ability to predict trait values for animals which have not been measured. For example we could predict breeding values for litter size on boars with out ever progeny testing. Finally, for some traits that have been difficult and (or) expensive to measure on the live animal, like meat quality or disease resistance, such associations will likely give breeders real opportunities to make noticeable genetic improvement. Such opportunities with genomic selection should make it possible to develop specialized lines for niche markets and unusual environmental conditions. **The key question will be who can and will adopt these technologies and the opportunities they spin off?**

One man’s assumptions:

To forecast the challenges of the future requires a number of useful and correct assumptions. Some of these assumptions/predictions are listed below.

Food and Environment:

1. People will continue to eat meat produced by animals as long as it is nutritious, relatively inexpensive and healthy.
2. People will not support livestock production that is environmentally unsound or negative from an animal welfare standpoint.
3. People might eat “fake” meat if cheap, healthy and would mean animals will not be killed.

Swine Breeding:

4. Breeding companies or family breeders can only compete if they produce a superior product that can be sold at a reasonable price.
5. Competition between companies and small breeders will intensify and will be based on a combination of technology implementation, service and healthiness of their pigs.
6. There will be fewer breeders, family or company, in the future.
7. Some breeds will decrease in importance and will be maintained only as hobby breeds.

Technology:

8. Technology will increase the power and ability to produce better genetics and better products.
9. Understanding technology will require better training and better integration.
10. Collection of better and more useful phenotypes will speed genetic progress.
11. Some parties will reject technology in favor of tradition methods and doom their long term financial stability.

Challenges and opportunities missed.

“You can't expect to meet the challenges of today with yesterday's tools and expect to be in business tomorrow” Unknown source

The following examples serve as indicators about genomics discoveries, challenges and breeder acceptance and usage.

Example 1. In 1980 the “market share” of large breeding companies selling breeding stock in the U.S. was 25-30% and the market share of small breeders was 70-75%. Today, market share of the largest company is approaching 50% and the top three companies hold nearly 75% market share. Small family farm breeders hold less than 10% of market share. Likely reasons for the shift include application of genetics and other technologies, large scale production supply and demand, and superior products for some traits.

Example 2. Use of genetic markers in the market place is variable. Use of single gene markers HAL and RN were relatively swiftly adopted in some breeds. Large scale marker use was confined to one or two breeding companies. Likely reasons were due to contractual relations with universities based on directed funding. Failure of individual breeds to fund large scale projects limited initial use. Lack of large scale collection of phenotypes limited usefulness of breed information for discovery.

Example 3. A number of genetic markers were re-released to US and Canadian breeders at reasonable testing prices in 2007. These include markers for leanness, meat quality, feed efficiency and litter size and have been tested in over 10,000 animals under many herd conditions. Even after years of complaints of failure to have access, breeders have proven to be slow to adopt technology and even insist on retesting. Likely reasons include failure to understand the technology, other cost issues including feed, and failure to see the value to their marketplace.

Example 4. The Berkshire breed is “reborn” due to demand for improved meat quality. The Duroc breed also receives increased emphasis based on “general” characterization for meat quality. Selection emphasis is limited to quantitative genetic aspects only. To date the Berkshires have failed to use genetic markers to verify “true” Berkshire colors and have failed to use markers to rid themselves of new meat quality recessives. Likely reasons include political issues and failure to grasp expected outcomes.

Example 5. The National Pork Board (NPB) pushes public funding of sequence information and SNP discoveries. Funding allows getting USDA funds and sequence is available to all researchers. Initial outcome is positive with new tools available to all researchers. Likely reason is funding is being provided by USDA, NPB and others for public discovery.

Example 6. Development of 50,000 SNP chip advances quickly and will be delivered in late 2008. This allows SNP-trait association trials with collected phenotypes. All major universities and breeding companies have bought chips to speed discoveries. Breeding companies are poised to use discoveries immediately. Not clear if breeders will adopt discoveries given most do not use markers.

"Challenges are many, opportunities few" Unknown source

Predictions for the future are easy in generalized terms. Gene discoveries will be made over the next 5-10 years that will revolutionize our ability to create better pigs. Using first and second generation sequencing and resulting SNP chips we will be selecting on 1000s of genes. The largest challenges will be deciding which traits to select for and how much emphasis to place on individual traits. Challenges can also be relative easily predicted.

- 1) Who will pay for future discoveries? Industry, government??
- 2) Who will collect the difficult to measure and expensive phenotypes?
- 3) Who will develop useful statistical tools to use genomic results?
- 4) Which breeders or breeding companies will be ready to take advantage of these discoveries?

Producers must ask the difficult questions. How can they help increase funding in the public sector (NPB or state and federal grants)? Are they positioned to first understand the new genetics and genomics information and second to use it effectively? Are there genetic systems in which they can use this information more effectively to improve pig production? Do they have niche markets they wish to fill or new products to produce? Team work and partnerships with the right seed stock breeders or breeding companies and university research faculty are likely to be keys in transforming this public information from a useful resource to a real payoff. Only then will producers, companies and geneticists help members of the pig industry really bring home the bacon.

Conclusions

"Those who cannot learn from history are doomed to repeat it." George Santayana

Genetic improvement in the 20th century in pigs was considerable. Sequencing efforts are moving along at reasonable speed. Results of these efforts are already being used to help select markers for improved production and reproduction traits in pigs. The recent availability of SNP chips and the advent of genomic selection will help to revolutionize livestock breeding. Breeders, producers and consumers will all benefit from these advances in genomics and the

resulting genetic improvement. This will require adoption of technology, advanced training and the ability to remove the shackles of past tradition and worn out ideas.

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