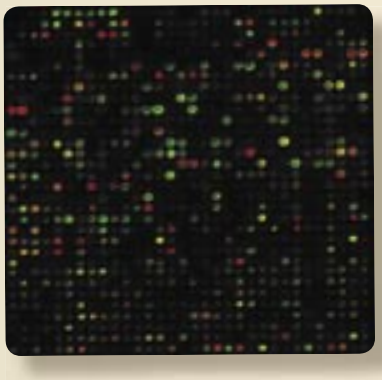
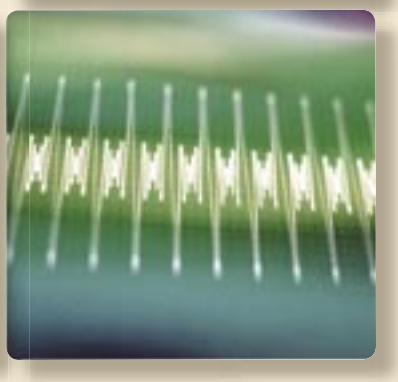
Creating Value From Genomics in the Pork Industry

A Roundtable Discussion





Panel Members Creating Value From

Jack C. M. Dekkers, Ph.D., is a faculty member at Iowa State University, and specializes in animal breeding and genetics. Dr. Dekkers received a B.Sc. and M.Sc. degrees in Animal Science from the Wageningen Agricultural University in The Netherlands, and a Ph.D. degree in Animal Breeding and Genetics from the University of Wisconsin. In 1997, he moved to Iowa State University and currently is a Full Professor in Animal Breeding and Genetics in the Department of Animal Science. His current research focuses on the integration of molecular and quantitative genetics for the genetic improvement of animals, including QTL detection and marker-assisted selection. His main emphasis of application is pigs and poultry.

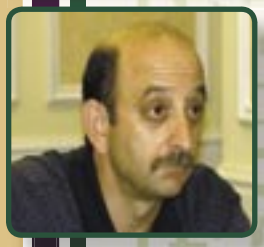
Sue DeNise, Ph.D., is Director of Research and Development for MMI Genomics. Dr. DeNise joined that company three years ago when it was a part of Celera, the company responsible for sequencing the human genome. Dr. DeNise earned her B.S. and M.S. in Animal Science from The Ohio State University, and her Ph.D. in Animal Science from Colorado State University in 1982. Prior to joining MMI, Dr. DeNise was on the faculty at the University of Arizona for almost 20 years.

Rodger Johnson, Ph.D., Professor Animal Science, University of Nebraska. Dr. Johnson provides leadership for a University of Nebraska swine breeding project focusing on genetic improvement of reproductive efficiency and resistance to disease. The project's focus is to identify the major genes and their effects that are responsible for responses in ovulation rate, litter size and uterine capacity observed during 20 generations of selection in the Nebraska lines. Methods to enhance selection responses and potential applications to the industry are also being evaluated. Dr. Johnson also teaches undergraduate and graduate courses in animal breeding, population genetics and quantitative genetics.

Floyd McKeith, Ph.D., University of Illinois. Dr. McKeith is part of the University of Illinois Meat Science and Muscle Biology Department, where he interacts with geneticists and production groups on meat quality issues and projects.

Bill Muir, Ph.D., earned his B.S. in Animal Science in 1971, his M.S. in Genetics from the University of Illinois, and his Ph.D. in Population Genetics in 1977 from Purdue University, where he currently is a professor in the Department of Animal Science. Dr. Muir's primary research is transgenesis applied to fish and methods of Biotechnology Risk Assessment. His quantitative genetics research is in behavioral genomics and group selection theory as applied to poultry and aquatic species.

Albert Paszek, Ph.D., Cargill Corporation, is responsible for animal genomic programs across animal species including cattle, pigs and poultry. Dr. Paszek studied quantitative genetics and genomics at the University of Minnesota, where he helped establish a swine genomic lab where he detected the first significant QTLs for swine genomic traits. Prior to joining Cargill, Dr. Pazcek worked with DeKalb Swine Breeders (now Monsanto Choice Genetics), and was on the faculty at the University of Minnesota where he focused on production data for genomics for discoveries and applications.



Max Rothschild, Ph.D., received his B.S. in Animal Science and specialization in genetics at the University of California, Davis in 1974, and his M.S. at the University of Wisconsin in Animal Science in 1975. In 1978, he obtained his Ph.D. in animal breeding with minors in statistics and genetics from Cornell University. From 1978 to 1980, he was an assistant professor at the University of Maryland. In 1980, he joined the Department of Animal Science at Iowa State University, and was promoted to associate professor in 1983, professor in 1987 and C.F. Curtiss Distinguished Professor of Agriculture in 1999. From 1993 to present, he has served as the USDA Pig Genome Coordinator. In 2002, he was made co-director of the Center for Integrated Animal Genomics.

The Impact of Marker-Assisted Selection (MAS)

Jack Dekkers, Ph.D.

Genetic improvement of livestock primarily focuses on selection for quantitative traits in outbred populations. To date, most genetic improvement has been achieved through selection on breeding values estimated from phenotype of the individual and/or its relatives. Molecular genetics is now providing tools to enhance rates of genetic improvement by being able to select on quantitative trait loci (QTL), or on linked markers. Sophisticated statistical methods have been developed to estimate the effects of QTL in complex pedigrees. The use of this QTL information in strategies for marker-assisted selection (MAS) has, however, received less attention. I will outline strategies for MAS and the benefits that can be expected from MAS in livestock breeding programs.

Molecular genetics can be used to determine the genotype of individuals at specific genetic loci. Ideally we would like to find the actual genes that affect traits of interest (Figure 1). Often, we are not able to do that, but we can utilize anonymous markers that are linked to QTL and then indirectly select on the genes that affect the trait of interest.

There are two types of linked markers – LD Markers, or linkage disequilibrium markers, and LE Markers or linkage equilibrium markers (Figure 2). There is an important distinction here in terms of finding these markers, how one identifies associations, how to use them and, finally, the ease of using them. LD markers are markers for which the association between the markers and the QTL is consistent across the population, e.g., individuals with marker genotype MM tend to have favorable QTL genotype. And so, in terms of selecting, it's a matter of selecting based on marker genotype and we can select individuals that have the MM genotype across the population.

If we have LE markers, then the association between the marker and the QTL can differ from one family to another. In one family, M is associated with Q, and in another family, it is associated with a small q. That requires one to know the association within each family which makes application of those types of markers more difficult.

When it comes to detection, LE markers are the easiest to detect because they do not require markers that are very close to the QTL. Within a family, there is a lot of association – linkage disequilibrium – and if we look within a family, markers spaced every 10 or 20 centimorgans are sufficient to identify markers that are linked or associated with the QTL. In contrast, LD markers are required to be very closely linked to the QTL to have a consistent association across families. Therefore, when it comes to a genome-wide scan, we are going to need a lot more markers. Alternatively, when using a candidate gene approach, LD markers can be identified by considering markers that are in or near genes that are thought to be involved with the trait. This increases the chance of finding markers that are close to the QTL and with associations that are consistent across the population (LD markers). Recently, with more advanced technologies, we can genotype or identify polymorphic markers very densely across the genome, which opens up the opportunity of using LD markers across the genome. None of these issues exists when we are looking at functional mutations. However, identifying functional mutations is very difficult.

When it comes to ease of use, LE markers are more difficult to use for selection because one has to figure out the association within each family. LD markers and functional mutations are easier to use in selection. Therefore, there is an antagonism between the ease of identifying LE vs. LD markers and the ease of using these respective types of markers.

With regard to traits for application of MAS, we often talk about quantitative traits that we are interested in, but we should not forget that there are many single gene

Figure 1

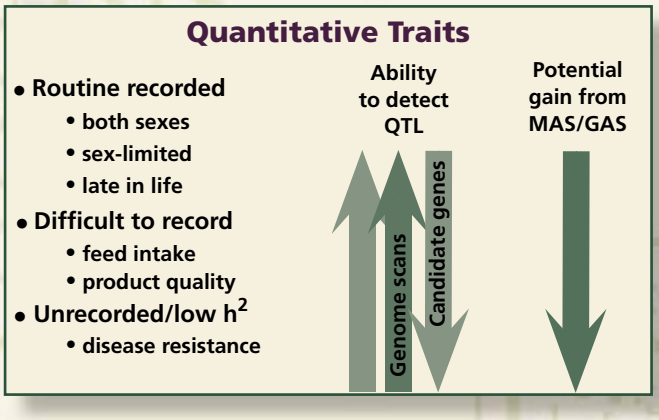


Figure 2

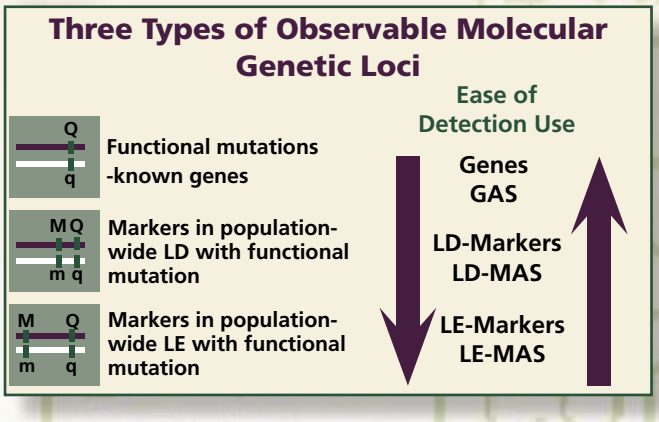


Figure 3

Implementation
LE-MAS vs. LD-MAS vs. GAS

- **Molecular requirements** LE <LD<<GAS
- **Genetic evaluation req's** LE>>LD~>GAS
  - **– Phenotyping** candidate + relatives (LE)
  - **– Genotyping** vs. candidate (LD/GAS)
  - LD/GAS ←→ effects at field level
  - **– Analysis** - MA-BLUP (LE) vs. fixed effect (+prob)
- **Potential genetic gain** LE <LD~<GAS
- **Implementation logistics** LE>>LD~>GAS
- **Marketability** $LE<LD_{HD}<LD_{Cand}\sim<GAS$

traits that are of interest. Identification of the genes behind those traits is actually easier than quantitative trait loci, and also their use may be a little bit more straightforward (Figure 3).

When it comes to the quantitative traits, for traits that are routinely recorded and are highly heritable, we already do a good job of improving them and, therefore, the potential gain from marker-assisted selection for those traits is not going to be very large. Growth rate in pigs would be one example of that. When we move to traits that are difficult to record, like feed intake, product quality, disease resistance, there is going to be potential gain from marker-assisted selection because current selection strategies aren't very effective.

Contrary to that trend of the potential gain from marker-assisted selection, is the ability to detect genes that affect these different types of traits. So the traits for which marker-assisted selection has the highest potential in terms of increasing genetic gain, unfortunately, are the traits for which detection of genes are most difficult.

In terms of QTL detection, many of the initial experiments that have been conducted have used a divergent cross or a cross between two breeds that were quite different for traits of interest. These experiments can be conducted with a limited number of markers because of the extensive disequilibrium that is created in a cross. QTL detection is a cross that can be used for genetic improvement through marker-assisted introgression. While used extensively in plants, introgression is not very feasible within livestock population because of the long generation intervals and because, often, favorable QTL alleles exist in both of the original breeds.

Another possibility for the use of QTL detection in a cross is marker-assisted composite (MAC) selection. To illustrate, if we have the F2 of a cross between two breeds, F2 information can be used to detect QTL and estimate marker effects. This information can be used to select among the F2, with the aim to capture the best alleles from both breeds for different QTL. The same marker effect estimates can be used for selection in subsequent generations. Because there is a lot of linkage disequilibrium in a cross, this can be effective for several generations with a limited number of markers.

The other approach for use of MAS is to look within breeds. This is where most of our interest is because most of our selection is within breeds. For QTL detection within breeds, LE markers have been used primarily, using within family analyses. Such designs do not require many markers across the genome and can lead to LE marker-assisted selection. The difficulty, as I pointed out before, is associations exist within families only which require more phenotypic data and makes selection much more difficult.

With high-density genotyping or candidate gene approaches, we essentially look across the population and identify LD markers that are close enough to the QTL, such that the association between the marker and the QTL is consistent across families without a need to figure the association is within a family.

We do that by identifying three selection strategies by using molecular data in conjunction with the regular phenotypic data. We typically only identify some of the QTL vs. the whole genome-wide approach where the majority of the QTL may be identified or may be marked. We have genotypic data from the marker data and we still have the phenotypic data, and, in most cases, we want to select on the combination of those two. We can do that by using two-stage selection, where we first select the individuals that have the favorable markers, and then select those that have the estimated breeding value based on phenotype.

With regard to the use of markers for within-breed selection, three selection strategies can be identified for the use of molecular data in conjunction with regular phenotypic data. Considering that we are yet unable to identify all genes that control a trait, we want to select on a combination of molecular and phenotypic information. One approach is by using two-stage selection where we first select the individuals that have the favorable markers, and then select those that have the estimated breeding value based on phenotype. A better approach is to use index selection. In both of these approaches, however, a tradeoff is made between selecting on markers and selecting on polygenes.

The best situation for marker-assisted selection is for preselection at a young age. At a young age, limited phenotypic information is available and we currently have to make random choices on which animals move into the breeding program. However, if we have QTL information, we could select individuals that have the favorable QTL genotypes and move those into the testing program. Depending on the effect of the QTL, we can expect to get an increase in the genetic level of the pigs that enter the testing program.

When it comes to deciding how to implement marker-assisted selection, it is very important to keep in mind, "what are your breeding or business goals?" From an academic standpoint, we talk about the benefits of marker-assisted selection as the impact on genetic gain. While that is certainly important, producers and breeding companies must look at it in regards to market share and, perhaps, marker differentiation of the product (Figure 4).

To illustrate with an example from dairy sire selection (Figure 5), preselection of 50% of young bulls for entry into the progeny test can increase the genetic level of progeny-tested bulls by up to 12%. The increase of genetic gain here is about 12%. The increase of market share in terms of the number of bulls that enter into the top 10% is, however, as high as 35%, and the increase of the percentage of bulls that enter into the top 1% is as high as 50%. The impact on market share can be different, and in this case, can be quite a bit larger than the impact on genetic gain. From a business perspective, those are the kinds of things that we need to focus on.

Figure 4

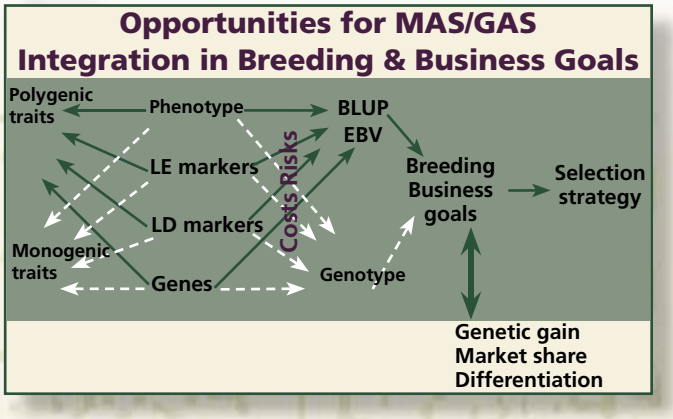
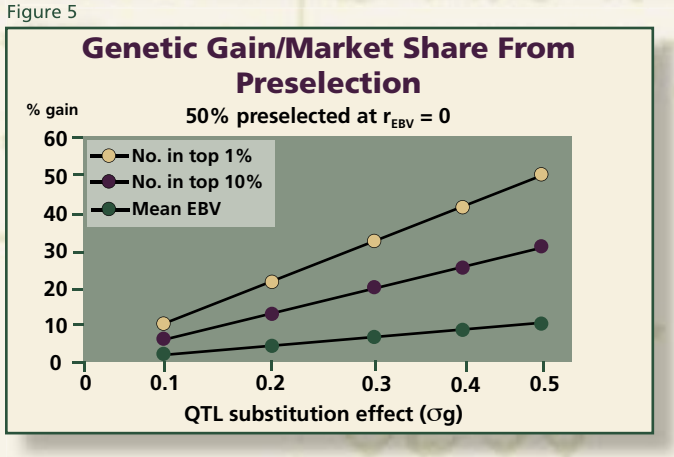


Figure 5



Q Which of these methods will be the most successful in the swine industry?

Dr. Dekkers: Understanding the fact that we don't actually need to find the genes for breeding purposes, even though it would give us a lot of knowledge on how these genes operate, having markers that are tightly linked to the genes is going to be sufficient for most genetic improvement purposes. For ease of implementation, LD MAS would be the one that I would focus on.

We have talked about the ability to measure phenotypes and we can do that very well within nucleus herds. But we're not interested in improving the nucleus. We're interested in improving commercial performance. One of the things that LD MAS would offer is that we can evaluate associations between markers and traits at the commercial level even without knowing the pedigree of the breeding stock. LD markers help us to extend the phenotypes we're collecting; without markers, the phenotype of an individual contributes to the estimated breeding value of that individual and its relatives. By using LD markers, we can extend that phenotype's information to unrelated animals.

Dr. Rothschild: I personally think LE MAS is the most valuable to the swine industry. Just look at recent examples – Halothane, RN, MC4R, CAST are a few of the best examples of LE. However, LD markers are also very effective; for example, ESR is linkage LD and is very useful and quite successful.

Dr. DeNise: But if we had the actual gene, that would be the most helpful, because then you do not have to worry about the LD.

Dr. Rothschild: I agree in part, as some LD is within the gene of interest. The most successful ones, with the list of HAL, RN, MC4R, are the causative mutations. They are the most successful without a doubt, but the LD ones within the same gene are also very useful.

Dr. McKeith: I think there are a variety of other mutations out there that we do not pick up with that test.

Dr. Rothschild: I know that is what everybody is beating on the drum about, but I think there are a variety of mutations out there in other genes than HAL that *may* cause stress.

Dr. Dekkers: In breeding programs, there are multiple stages of selection, and the early stages have very limited information. I think that is what we want to focus on as far as implementing markers. When we have to make those selection decisions, having marker information will be most beneficial.

Dr. DeNise: Breeding programs would still have the same fixed cost in this scenario, so the only advantage we'd get is a reduction in the number of boars we're going to test. Therefore, we have to be confident that we are going to be able to select the best boars based on markers.

Q What about the market share vs. product differentiation?

Dr. Dekkers: Breeding companies have to determine what creates and drives market share. I think we want to include genomics when we discuss those market drivers. We could tie in genomics to select for a trait, like growth rate, for example. Having this technology can certainly increase market share if there is true value being expressed.

Dr. Rothschild: I see the role of genomics as product differentiation, and that can have a big effect on market share.

Dr. Muir: If part of differentiation is important to your customer to create a bigger market share for their product, and you can provide the means for them to differentiate their product, then that will drive market share for the genetic product. Therefore, I think the two are related.

Q Do we need to understand these genes?

Dr. Rothschild: We absolutely have to understand the genes and the biology involved. We do not use genome-wide BLUP presently because we do not have all those markers yet and we have not measured all of those phenotypes simultaneously. Care must be taken in that by selecting all of these genes and organizing directions for different traits, we will get nowhere quickly or we

will move them in the wrong direction. If we do not understand what some of the genes are that we are changing, we run the risk of affecting negatively the biology.

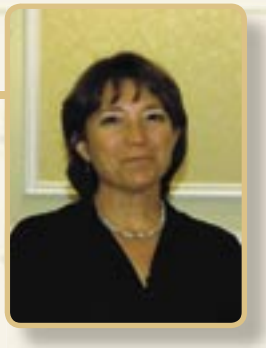
Dr. Johnson: With BLUP, we looked at the genome as a 'black box,' and we just selected on the phenotype and how it related; we did not worry so much about what was happening to gene frequencies. Actually, we did not need to understand that. We just chose on the traits and we made sure that we knew the genetic parameters involved. We knew that if we put this index in place, we were going to make progress on the index and on the traits involved. Why is it any different now?

Dr. Rothschild: Halothane is a good example. We did not need to use BLUP then to produce more muscular animals. We just selected the bigger, more muscular animal. However, when gene frequency was low for halothane mutation, it did not really matter. We got to a certain point where the HAL stress gene frequency got large enough in the industry that it became problematic and we recognized that there was a problem. Had we known that there was a gene with a specific allele that affected stress susceptibility, we would just remove the phenotype and the respective genotype. If we are actually going to go to the bottom of the sequence and get all these SNPs and recognize all these genes, I think it behooves us to understand what some of them do.

Dr. Dekker: I do not think one has to exclude the other. If you do GMAS, you will identify which genes are going to be important and be able to find them.

Genomics Overview

Sue DeNise, Ph.D.



The company I work with, MMI Genomics, is a genotyping company that was part of Celera, the company that sequenced the human genome. It became clear to my company that we had to do something radical if we were going to try to have an impact through genomics and have a future in this industry. We took the strategy that had been used in sequencing the human genome and applied it in livestock. We focused on three species: bovine, swine and chicken, because we thought that value could be created for our customers. We sequenced to the three genomes to 1X coverage, which means in bovine and swine, we sequenced about three billion bases. We did alignments of those bases and identified single nucleotide polymorphisms (SNPs) that could be used to identify genomic regions that were associated with economically important traits.

We identified over 700,000 putative SNP markers in cattle. Not all of those are going to be real because there are errors when we sequence at that depth, but the majority of them are real. We looked at 500,000 base pairs (bin) at a time and we found that we had a 99% coverage of the human genome, which means that if humans and cattle are similar in sequence, and we know that genetically there is a lot of homology, we are going to be able to create a very dense map of the genome of cattle.

In collaboration with our partner, Cargill, we have developed a bovine discovery map which includes over 6,500 markers, creating a map of greater density than a half-centimorgan. This map has been validated in seven breeds of cattle, ensuring that it can be used in a variety of research programs. In swine, we have been collaborating with Monsanto, and we identified over 600,000 punitive SNP markers and, again, we have over 99% coverage of the human genome. Moreover, in chicken, we mapped all of our chicken sequence back to the chicken sequence that is available to the public. We have identified over 96,000 markers. We were sequencing at the rate of a mammalian genome a month at the time these were completed. Thus, within three months, we had all of this sequence data available for research.

We proposed to utilize these dense SNP markers in whole-genome association studies to identify regions of the genome that were influencing economically important traits. We had a belief that if we could identify a substantial amount of the genetic variation that contributed to traits, then we should be able to greatly affect the phenotype. We made a decision very early on that if we were going to do a gene-by-gene basis or QTL-by-QTL basis, it would be very difficult to create a product that would really create value for our customers and, therefore, we had to go after it in a big way.

Livestock breeding companies have been highly successful using quantitative genetics. We have a very good way of estimating the genetic potential of individuals by knowing an individual's own phenotypic value and those of their relatives. The challenge is combining information obtained at the DNA level to understand the underlying genetic causes of the phenotypes. Therefore, there are a number of steps between DNA and the expression of the phenotypes, and that is where many challenges are going to lie for the future.

As we get down to the gene level to understand the sequence that influences quantitative traits, we are going to require a map that is much denser than currently available and those markers are single nucleotide polymorphisms, or SNPs. Single nucleotide polymorphism is a single base substitution at a location in the genome. They are highly abundant. We found them in roughly one per 200 base pairs. They are distributed throughout the genome, including genes, and, unlike microsatellites, are genetically very stable. Microsatellites mutate very rapidly as evidenced from data generated from our parentage genotyping business. SNPs can be scored as a plus/minus

marker and are amenable to high throughput analysis. Microsatellites require a lot of scientist oversight in order to get high quality data, but with SNP markers, we can do approximately 180,000 genotypes a day. At the conclusion of a research project utilizing SNP markers in a commercial population or population we want them to be used within, they can be diagnostic onto themselves.

So just to give you an idea of the resolution between microsatellite markers and SNP markers, if we have a map of California from Sacramento to Reno, and if we think about our DNA being a linear piece of real estate, then the interstate highway is similar to a strand of DNA. If we are interested in getting to the Sugar Bowl Ski Resort, it could be rather difficult if we only have the major exits to choose to exit the interstate. That might be like microsatellite markers. On the other hand, if we saturate that region with a number of different exits, which are like SNP markers, then we have a pretty good chance of getting to the point at which we want to be. In designing our research strategy, we knew that we had to be able to saturate the genome in order to have a chance of finding some of the important QTLs in a commercial population.

It is relatively easy to identify QTLs that are segregating within families and explore the region where that QTL is identified. On chromosome two, for example, we can attack the region with a number of different markers and it will allow you to develop a test that works within that family for that specific gene. However, we are learning that there are interacting genes on other chromosomes that will influence the effects of that QTL. Also, if the results of an experiment as described above are transferred to other populations, they often do not work very well for a variety of reasons. The QTL you identify in one family may not replicate in other families. If we have all of the genome saturated with SNP markers so that all of the individuals are genotyped at once, then we may have a chance of uncovering those interacting genes.

This technology is allowing us to put the whole puzzle together and figure out exactly what combination of markers provides the optimum phenotype. If we equate within family studies as panning for gold, we find small nuggets one at a time. If we use a dense, saturated SNP map, we can find all the nuggets at one time.

Our vision of the future is to have a set of "SNPs on a chip," or a predetermined series of SNPs, that tell us about the underlying genetic mechanisms of specific traits, and those traits may contribute to a production system in a number of different ways. They could influence breeding and management; they could contribute to source verification and parentage identity or branding of commercial products.

In regards to value creation opportunities, we think that there are going to be a number of unique opportunities at different levels. We think that breeders and producers will utilize this technology as a unique breeding tool where they can increase the accuracy of selection and be able to target traits that are difficult to measure with traditional selection. For producers and feeders, perhaps they will be able to sell individual animals into a marketing program that's really designed for their genetics, i.e., the Japanese market. They might also be able to sort and manage individual animals to optimize their genetic potential. Finally, for the packers and processors, they might be able to make purchasing decisions or guarantee palatability characteristics or meat quality characteristics. They might be able to forward market products and they might be able to create new branded products. So we think that there are a number of different opportunities, and it remains to be seen exactly how the industry and the research will combine to create these new products.

Consider a traditional selection program in a pyramid-type scheme. At the very top of the pyramid there is an elite breeding population where we are doing most of the traditional selection, and then those unique and elite animals are used to multiply our populations, and then, finally, at the bottom of the pyramid, we create animals that produce the commercial product. We can see marker-assisted selection working in the elite breeding population to create lines with the optimum alleles for the future. We could use it at the multiplier level to identify matings to produce the best parents for the commercial product and, finally, we could produce animals with customer-designed traits for the commercial product. I think the future of pig breeding will contain all kinds of new opportunities, both in the public and private areas, for creating new products and enhancing selection strategies.

Q Dr. DeNise mentioned 'SNPs on a chip' and some other genomic tools will be available soon. How soon?

Dr. DeNise: We will have a SNP panel available for cattle and canine parentage by the fall of 2004. They will be readily available for anyone who wants to do parentage analysis. For predicting phenotypes, I think it's a couple of years away. Nevertheless, it is coming quickly.

Q Dr. DeNise, you mentioned three genomic advantages regarding capturing the value of genomics – breeding tools for genetic companies, management tools for producers and feeders and purchasing/branding for packers-processors. Which avenue would you vote as the most value for swine?

Dr. DeNise: I think it will be the packer-processor that will benefit the most because they will directly provide consumers with preferred products. However, genomics are going to have to be implemented, I think, at the breeding company level.

Dr. Johnson: Do you think it is going to have more value enhancement power vs. reducing the cost of production?

Dr. DeNise: Both are value opportunities. Product differentiation is a real advantage with genomics. We as an industry have already done an excellent job of making animals efficient and productive. Now we are looking at incremental changes. Whereas, the heritabilities are so much higher with the traits of interest for the packer-processor. They have ready value if you can convince a processor that they can get more for a specific product. I think that is where genomics has a real value position.

Q **How much advantage will a whole-genome approach have over the more traditional QTL-by-QTL or gene-by-gene approach?**

Dr. DeNise: We believe that it has very distinct advantages over traditional QTL discovery approaches. However, there are increased costs of the research and the design of the experiments are critical. We have to have good phenotypes in our research population of thousands of animals and we have to measure almost all the phenotypes that create value so that we get all of the interactions among those genes. I think we end up with a lot of information that is valuable. But if we don't have the resources to do it well, and we don't have all the SNPs to create a dense map, then the gene-by-gene approach has been successful and identified a number of really important genes. The whole-genome approach certainly allows a breeding company to apply science and create product advantages. We believe that it is going to be an important strategy, especially for a commercial breeding company that wants to create long-term value and to create a competitive edge.

Dr. Rothschild: To me, it is a stock portfolio approach. It seems, in this industry, that everyone wants to be critical of everyone else's discoveries. This undermines our own efforts in the long run. Instead, we need to figure out how to sell genomics to the public, whoever the company is that is developing the gene markers. Furthermore, we need to take a portfolio approach to genomics and utilize all the science and tools available. There are varieties of approaches that will work in genomics. The one thing that nobody has proven yet, except maybe MMI is doing a good job of trying to make this work, but nobody has proven that, in the long term, the companies that are using the technologies can make money off the technologies. We need to look at this hard and develop some value strategies for the industry.

Discovery Projects in Long-Term Selection

Rodger Johnson, Ph.D.

I probably have the longest ongoing selection for a trait in a research herd of livestock. Long-term selection lines have value because they create relatively large differences from controls when we know the selection background. And I would have drawn a little of this experience from what we've done at Nebraska, where we've had selection now for 23 generations for components of litter size. Figure 6 describes the four lines that we have.

There are actually two control lines, so there are five lines. We have an index line and then two lines I call IOL and COL (Figure 7). In 1979, we produced a composite population of Large White Landrace and, in 1981, implemented selection for ovulation rate and embryo survival in what has now become known as the Nebraska Index Line. This is the line that Monsanto Swine Genetics has sampled and now has possession of.

We continued that selection for 11 generations to 1992, and since then, it's been selected for increased litter size, total born and/or born alive in more recent years. This line has undergone 23 generations of selection for components of large litter size. Alongside it, there's been a control line, that's the far right-hand side of the diagram, that's been selected randomly.

In 1989, we created a couple of sublines from the index line and the control line that were selected for ovulation rate and uterine capacity. I call one the IOL line because it was derived from the index line and then selected for ovulation rate and uterine capacity, and the other one is called COL because it was derived from the control line and then underwent the same selection. After eight generations of that kind of selection, we simply switched both lines to selection for increased numbers born alive.

Figure 8 shows that the index line is currently producing 13 to 14 pigs per litter. While not all of them

Figure 6

NE: Selection for Components of Litter Size

- 23 generations
- Index line (NIL)
  - –OR, ES, TB, BA
- IOL: derived from NIL
  - –OR, UC, BA
- COL: derived from, Control
  - –OR, UC, BA
- Controls

Figure 7

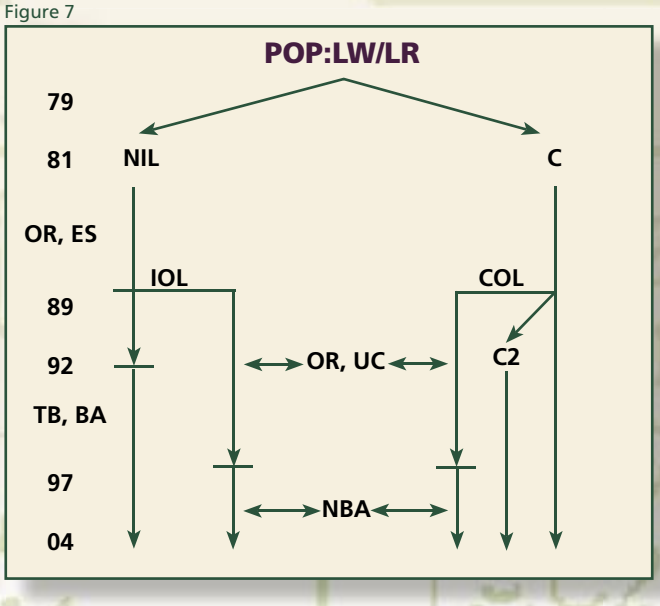


Figure 8

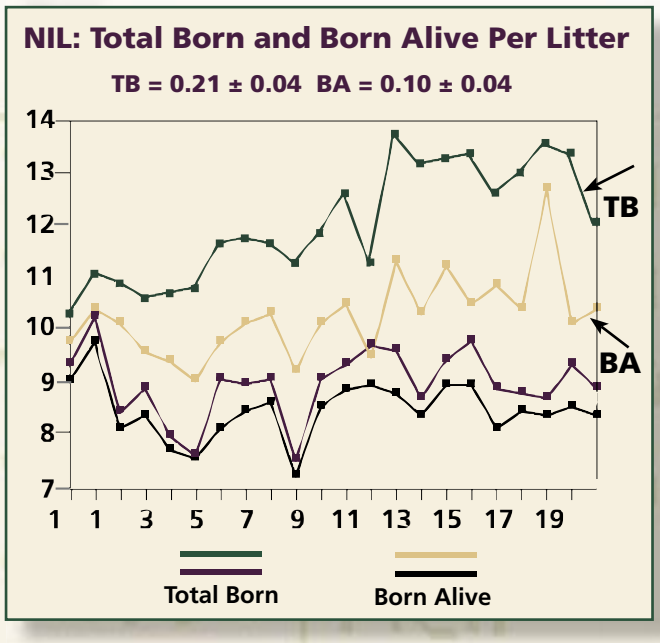
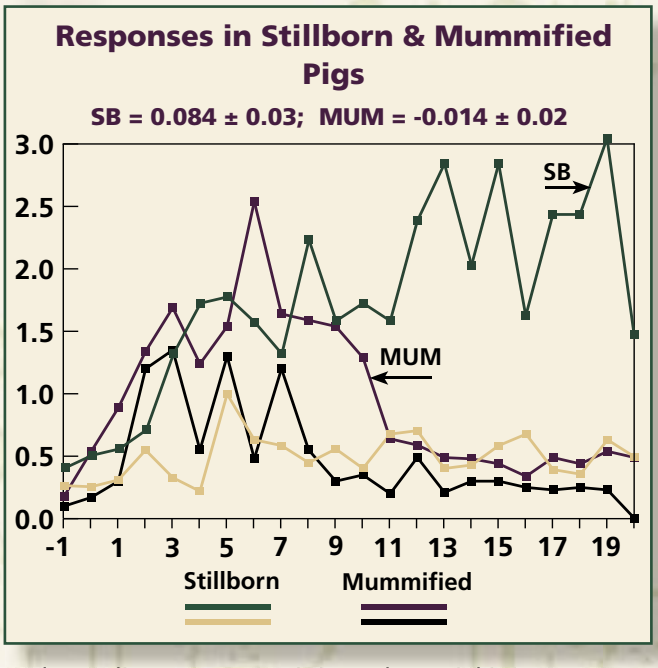


Figure 9



are born alive, we can see it is a substantial improvement over the control line. We've created a pretty large difference between the lines in litter size. But we've also created a very interesting physiological model, because some of those pigs are born as stillborn pigs and understanding the nature of those losses is important (Figure 9).

Initially, we were also getting a substantial increase in number of mummified piglets, but that has gone in the opposite direction during our later generations when we selected for uterine capacity. That population has now become a very valuable resource for physiological studies to look at follicle recruitment and ovulation rate.

We have used genome scans in this herd. We created an F2 population in which we did an initial QTL search for genes affecting reproduction traits. More recently, composite interval mapping was used and the population has been used in expression profiling studies with tissue from the interior pituitary, as well as the follicles.

We're now doing some fine mapping with SNPs in projects in cooperation with Monsanto. We're focusing on specific chromosomes, and more recently, we've used the population as a resource to study possible genetic mechanisms involved in PRRS resistance.

Figure 10 shows a summary of the QTL search. The traits involved range from ovulation rate to numbers born alive, fully formed, stillborns, mummified piglets, birth weight, weaning weight, age of puberty, nipple number; anywhere from one to five or six QTL have been identified for each of those traits. The focus now is on certain positions where those genes are thought to be located. We have done gene expression work with anterior pituitary and three genes in particular are expressed differently in the anterior pituitary, between

Figure 10

QTL Search Summary

Trait	Cassady *et al* (2001)	Mendelian	Imprinted
OR	1*	0	1
BA	1	1	0
FF	1	1	0
STB	2	3	1
MUM	0	5	3
BWT	—	1	1
WWT	—	0	0
AP	6	7	1
NN	5*	3	5

- 21 Mendelian QTL
  - 7 additional
- 12 Imprinted QTL
  - 6 paternal
  - 3 maternal
  - 3 partial

the selection and control line. None of those genes are in positions where the QTL scan indicates there is a gene, so these may not be the QTL. They are probably secondary genes that are being controlled by genes at other positions. Once again, none of those genes reside at areas on the genome where there is a peak for a QTL.

In summary, I would say we have learned that litter size is a very complex trait. We have identified several positions and genes that influence it, but none of them by themselves have large effects. Dr. Dekker noted that litter size was lowly heritable and it was going to be a trait for which marker-assisted selection would have high value, but it would be more difficult to find the genes. I think this confirms what he said. Most of the genes expressed that we found are not in positions where there are QTL, so I think we're going to need several markers or genes to enhance selection response, and that's what we're working on.

So in the future, we are going to try and fine-map these genes and our specific goals are to identify genes responsible for ovulation rate and uterine capacity, number of stillborns and number of live pigs per litter.

Future Direction of Meat Industry & Pork Quality Improvements via Genomic Advancements

Floyd McKeith, Ph.D.

As far as the future of the meat industry, I think one of the biggest things that have happened in the last decade involves the consolidation of the meat industry. Basically, five or six companies drive the entire swine industry as far as processing and marketing fresh pork products. One of the other big things that's happening and impacting the entire meat industry is case-ready packaging. That is where products are processed or cut centrally, packaged and distributed out into the retail chain. In addition, I think that this whole concept of genomics will lead the way for branded products. If we go to a number of stores today, we see Tyson's brand, Cargill's brand, Smithfield's brand, etc., directly on the products. Moreover, I think that will drive the packer-processor to be much more concerned about quality and consistency than they have ever been before.

Today, when those stores are purchasing their case-ready product with their name on it, if it doesn't look good or doesn't meet consumer expectations, they're no longer going to go back and purchase that brand, which will, in turn, impact the profitability of that packer or processor.

Speaking of consistency, we just finished an audit and product consistency was one of the biggest economical issues brought up by the meat industry. They see traits such as size, color, shape and taste as part of consistency. The industry is putting a lot more emphasis on quality and consistency.

The other thing that appears to be evolving is that several companies are developing programs or brands that are designed for very specific uses. For example, white-tablecloth restaurants want high-marbled; something different that they want to market into that group as a specialized product. We have a large export demand. We are trying to specialize in those types of products as well as a variety of other niche markets, and all of those will involve genomics, in some form.

There is no doubt, genomics will play a huge role in the future of branded products. Economics will dictate the speed in which they are adopted and utilized. Will the food service market be big enough for a large genetics company to go after, to design pigs to fit into that? In addition, probably one of the most complex parts of this is being able to market the entire animal profitably. Can we utilize genomics to help the pork chain capture additional value for the entire animal? On the other hand, do we have to depend on one or two pieces to carry the load financially?

In addition, we talk a lot about pH, color, water-holding capacity, palatability and even marbling has resurfaced as a significant area of discussion. The big challenge that I see is if the industry has the ability to measure value and pay for it. As we look at contemporary processing facilities that are carrying 1,000-plus animals per hour, we do not have the technology to measure those traits that rapidly. We are taking subsamples and trying to characterize value. It is more difficult to get that one-on-one relationship to pay producers back for those specific characteristics.

Q What will consumers pay for?

Dr. McKeith: I used to think there were many people that were price driven, but I am not sure if that is true today. Look at the fact that there is as much beef today as we have had in a long time, and it's three times the price of pork. We cannot create enough middle meats. We cannot create enough prime at a $50 premium over choice. Tonight we will go out, spend $25 or $30 on a steak, and not think anything about it. I think there is clearly an opportunity to capture more value than we currently are. The amazing thing is, if we want to talk about value, the most expensive pork item we can buy is back ribs right now at $4.50 to $5.00 a pound and only half of it is eatable. If people like that, they will pay five bucks and never think twice. The value of the belly is for $5 more than the head, so I can't find or understand any direct signals on what they are willing to pay for. I mean, it is not lean meat and things like that. If they like the taste or they like the product, then they will buy it, so I think it is probably a marketing phenomenon.

Cargill's marketing people, for example, probably understand much more about marketing and consumers than most of us in academics, but it is a challenging question on how we capture the more value from the consumer. Right now, pork is probably at the bottom as far as value. We can buy boneless pork loin for a little over $2 a pound and boneless chicken breast might be $4 a pound. Little meats from beef would be $6 a pound and up, and we have not seen a tremendous upsurge in pork consumption just because it is the cheapest.

Dr. Paszek: We refer to it very often as customer solutions. Raw pork put in the right customer solution demands a very different value. Integrating those inputs together into products and customer solutions, that's where we have to be very inventive in order to create value and capture it.

Q Can genomics improve consistency?

Dr. Rothschild: We have some data that shows that if we select a MC4R genotype, we will reduce the variation in growth rate by 2% or 3%, so we can definitely improve the consistency.

Dr. McKeith: I think the environment definitely exacerbates it.

Dr. Johnson: If we really wanted to work on improving consistency, we would spend a lot of time on the environment for it creates most of the variation for most traits. If we could find out what the environmental

variables are that lead to inconsistency or variation, there would be more opportunity to reduce variation. There is more opportunity to reduce variation by working on the environment, than there is by working on genetics.

Dr. Rothschild: Reducing environmental problems will not help solve genomics, though. We should look for improvements in both genomics and management.

Dr. Johnson: We can invest a lot of money in genomics to try to address the inconsistency and variation and we will fail, because it's the other variable that creates most of the variation. With genomics, we can improve the problems with lack of consistency on the bottom end of production. We can do this through selection and move more of the animals out of that bottom end of production.

Dr. Muir: Another variable are genes that are linked to environmental sensitivity and not homozygosity. That is why we are trying to produce a hardy animal, to be less environmentally sensitive and stay consistent.

Dr. Johnson: Even a set of clones is going to have a lot of variability.

Q Traceability has gotten to be a fairly important issue lately. Does genomics have a role in traceability issues?

Dr. Rothschild: Genomics could very easily be used as an insurance policy. Most of us buy life insurance, but we do not ever plan to use it ourselves. It is not much value to us; its value is to somebody else. I believe traceability products from a genomics standpoint are incredible insurance policies. If we are forced to use it, it can be an incredible value. They can also be used to assure consistent products from a specifications standpoint. For example, if the purchaser of that product says I want to make sure I have the right specifications, he can check the genomics of the product and he knows they have it or they don't. Genomics will be a very valuable tool in the future.

Predicting Impact of Genetic Response

Bill Muir, Ph.D.

How much additional response can we make to a selection using markers? The question is not can MAS produce a response to selection – the value of MAS is in the advantage it offers over conventional breeding technology. The big message is that when the phenotype is difficult or expensive to measure, then marker-assisted selection can greatly increase our ability to make response to selection.

In addition to the type of trait being selected, the heritability of the trait also has a major consideration. When we examine how heritability impacts the utility of MAS, theory shows that, providing the QTL's are known (or at least very closely linked makers), its effectiveness is greatest for traits of low heritability because, in that case, the phenotype is a poor indicator of a genotype.

For traits of low heritability, initial theoretical examination showed that MAS could increase response to selection by as much as 500%. However, a decade of experimentation and simulations has since demonstrated a much more moderate response. These shortcomings were found to be due a critical assumption: that the quantitative trait loci (QTL, or closely linked markers) affecting such traits was known. In actuality, these QTL associations are found by statistical estimation and hypothesis testing based upon similar data breeders would use to make selection decisions, i.e., have the same limitations of a high environmental variance. Thus, QTLs for traits of low heritability are difficult or impossible to locate.

How do we overcome the problem with capturing the value of genomics with low heritable traits? An alternative method to implement MAS has been developed to overcome these limitations and has been termed genome-wide marker-assisted selection (GMAS). The power of genomics has been the simultaneous use of all genes (or markers) in the genome for discovery. Using that same concept, GMAS uses all markers, significant or not, to maximize the accuracy of prediction. This technology utilizes the population-wide LD that Jack Dekkers discussed that is generated from very closely linked markers due to random genetic drift.

The method is based on a Bayesian framework and requires a prior estimate of the amount of the variation due to genetic causes, and a dense marker map of at least 1cM spacing across the genome, and all individuals are genotyped for all markers. Simulations conducted in my laboratory were used to examine the efficiency of the method. Results showed remarkable predictive ability, with the relative efficiency actually increasing as the heritability decreases. This result shows that it is possible to overcome current limitation of MAS and realize the power of the technology.

Some people would say this is a ridiculous idea because it is cost prohibitive right now. I would predict that within a few years, we'll be down to the point where they only cost like $30 to get 3,000 genotypes per individual; particularly if we get chip technology for genotyping or some of those technologies Dr. DeNise's group is working on.

I call GMAS a holistic approach, because this method will work for any trait regardless of heritability or type of trait and the same genome scan for all traits. Thus, the same selection indexes used in breeding programs can still be used; the only difference is the BLUP equations

are augmented with genomic information. In addition, it will be possible to make meaningful advances for traits which are difficult or impossible to measure on live animals, such as disease resistance or meat quality.

We developed a mixed-model approach to incorporate a genome-wide scan into a quantitative breeding program. We used individual markers at each locus and did not worry about haplotypes as recommended by the original authors of the idea. I found that works quite well. We did not worry about false positives or false negatives. We just used them all. We got away from the entire problem of saying, "these are markers associated with this trait." Once we estimated the effect of each marker across the entire genome, we just used a prediction equation that incorporated all the markers.

For the genetic parameters, we took the total genetic variation and divided it by the entire number of markers. This assumes that every marker contributes the same amount of genetic variation. I have done some simulations since then to look at how bad the assumption is, and how does it influence the results. Research shows that it works quite well. Moreover, that is where we are trying to go with this technology. We are trying to predict which are the best animals to breed. And we can't necessarily say which of those markers was associated with the gene of interest, but it can predict very accurately which animals to breed.

In this simulation, we genotyped all animals across the entire genome or 3,000 genotypes that were spaced one centimorgan apart. We did this for three generations, because we wanted to follow the inheritance patterns. We also measured the phenotypes for each of those animals in the first three generations. Then, in the first generation of selection, all you do is genotype. All the animals have 3,000 SNPs done, and then we predict the breeding value of that animal just based upon the genotype, so we have that predicted breeding value.

In the original paper by T.H.E. Meuwissen, he looked at marker spacings of 1, 2 and 4 centimorgans and, as the spacing got further apart, the accuracy decreased, which you would expect. If we look at the accuracy of prediction in his study (Figure 11), with 1cM spacing for a trait with a heritability of 0.5, it is greater than 0.71, which means that GMAS is more accurate than selecting on a phenotype itself.

There are many simplistic assumptions in his simulations and this is where I extended Meuwissen's results. He looked at haplotypes, assumed a heritability at 0.5, and also assumed that a QTL was always bracketed by markers that was exactly in the middle. These were very limiting assumptions. What I did was relax all those assumptions and let both the location and magnitude of the QTL be random. Thus, in some cases, a QTL was not bracketed by any markers, and in others, multiple QTL would be bracketed by the same set.

The only thing we could fix was the placement of the markers. So I let the markers be 1cM apart. I also wanted to know, how does this compare for a trait of low heritability? We know that for traits of high heritability, such as 0.5, there is limited need for MAS, the phenotype is an excellent indicator of genotype. Further, none of the important traits in animal breeding have a heritability that high. Heritabilities are usually in the range of 0.3, with many much lower, particularly reproductive traits which have a heritability around 0.1.

Also, I wanted to see how this technology compared to state-of-the-art methods which do not use marker information, i.e., phenotypic animal model BLUP. BLUP uses the relationship matrix, whereas GMAS uses the genome matrix, thus, one of the advantages of GMAS is that we do not actually have to pedigree our populations. Naturally one can combine both technologies if pedigrees are available to maximize the accuracy of selection.

Another issue that has came up is, how many generations of training are really needed before one can use GMAS, and how long can one do genotype-only selection? In other words, after one trains the model, can one then do selection just based on the genotyping for a long period? To address this question, I looked at different numbers of generations for training and, in each, looked at how the accuracy of selection was maintained in subsequent generations.

Results of the simulations show that as expected for GMAS, the more generations of training we do, the

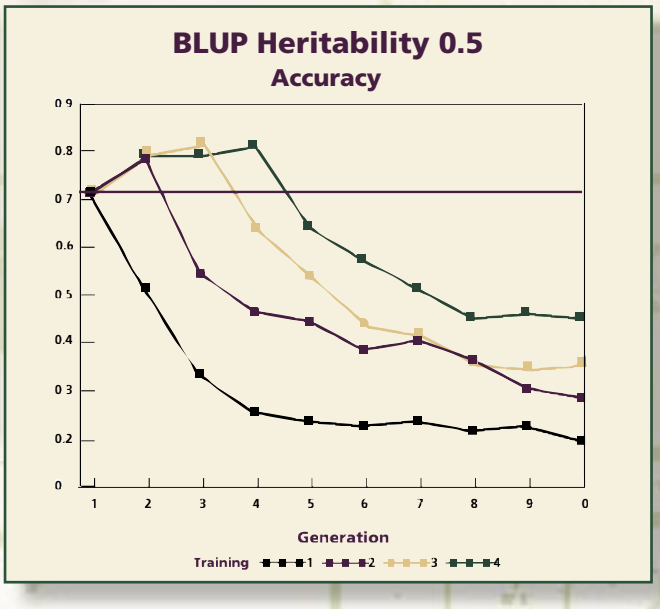
Figure 11

Results GMAS
Correlation Between Predicted and True Breeding Values (Meuwissen *et al* 2002)

Statistic	Marker Spacing (cM)		
	1	2	4
Accuracy (r)	0.732	0.708	0.666
Heritability of the Index (r^2)	0.535	0.501	0.443

Genotypic Information More Accurate than Phenotype

Figure 12

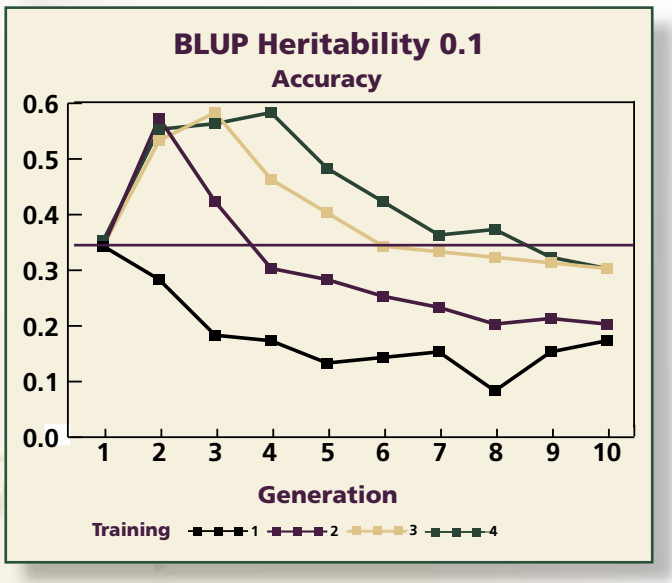


better it is at prediction. For a trait of heritability of 0.5, the accuracy of selection with GMAS reached about 88%, whereas the BLUP line only reaches 82% accuracy (Figure 12). Note that the accuracy of BLUP rapidly dropped off in generations where the genotype is predicted based only on ancestors' information, whereas GMAS continued at a higher accuracy.

Now, what I really want to talk about is traits of low heritability, because that is where the power of genomics comes in. For a trait with a heritability of 0.1, the accuracy of selection is up to almost 70% with GMAS and three generations of training, while BLUP is only hitting about 60% (Figure 13). So now, we can see where GMAS really hits its stride, if you will, is when heritability is low. This is in contrast to traditional MAS where it starts to fail as the heritability decreases. Now we have a technology that actually excels in that case. So I see this as the future of genomics in animal breeding, the only real issue is if we can get the price down.

Another advantage of GMAS is in getting the right combinations. One of the big problems with MAS, or genomics in general, is not just finding the best genes; it is getting them all into one individual. This process can only occur through recombination; thus, we need to have many meiotic events to get them all together in one individual. If we can get meiosis to occur in a test tube and then use cloning like they did in cattle, where they were able to get five generations in two years, we can really speed up the process of getting the right genes into the elite individuals you want them in. I see this as a far distant goal of the technology, but many technical issues of getting meiosis to occur in cell culture and cloning selected cells still needs to be worked out.

Figure 13



Note that the object of this method is not gene discovery. The goal is predicting the best breeders. However, gene discovery may be an important part of the IP process, because they may result in patents or development of other products based on knowledge of gene function. If gene discovery is one of the primary goals of genomics, rather than increasing response to selection, then GMAS may not be the best choice. Of course, information from the training generations can potentially be used to identify potential marker-QTL associations, but the power of the method for that purpose has not been investigated.

Commercial Application, Commercial Development of Genomics in Swine Breeding

Albert Paszek, Ph.D.

My experience from academia, industry, swine breeding organizations and pork production has taught me importance of value creation in pork production. People/organizations wish to use genomics for value creation. From a food production standpoint, whatever changes we make in swine populations, it is with food in mind, because food is the primary value capture point.

It is important to create value, as well as recognize, measure and capture it. We want to use genomics as input to swine generations, affecting value creation and capture for important economic traits. Let's use genomics to make sure that at points of the production pyramid (or breeding pyramid) where we create value, we can measure and capture it.

That value could be different for maternal traits vs. carcass traits. Value could be measured by quantity, quality and efficiency. The industry understands well that, in order to create and capture value in a swine breeding production pyramid, we have to take advantage of the breeding/production pyramid. Changes probably have to happen in one generation in order to create and capture value in another generation, because of animal multiplication. It's important to put it in the right spot in the pyramid in order to maximize value creation and capture. With genomics we have a chance to integrate G&E/nutrition inputs. We need to develop better metrics in order to create values with genomics and measure and capture it.

I've always thought that genomics probably has one of its greatest value for enhancing pork in the area of meat quality because you can't measure these types of traits very well on live animals. We do measure these traits, but it is also rather difficult to assign precise economic values to these traits. For example, I do not know how strongly we should select on meat color.

I also wonder whether we're describing traits correctly. Should we be looking instead at something/some traits related to animal processing or another measure of animal processing result like eating quality

or healthfulness of the meat product? Maybe we need to look beyond traits of intramuscular fat, color, water-holding capacity, etc. We can measure those very traits relatively well by now. However, if we were to put those into a selection index, I still am not sure what economic weights should we apply on a continuous basis in selected herds.

Dr. McKeith: I think part of it is almost like the niche market concept. If you are with a major integrator and your main goal is to export products, the selection pressure on color should be higher, because for you to get premium value, your pork needs to be darker color and potentially more marbling to fit that market. Consistency of color is probably more important for our domestic market.

Dr. Johnson: But then, is the net effect of this to the breeder that we need more sublines and smaller populations to fit unique markets? There is going to be a market for pork without very much intramuscular fat and you cannot have that on the same line.

Dr. McKeith: That is right. I think there will be niche lines, and it appears to me that many of the companies and the integrators are working with breeding companies to use genomics to develop a dedicated line for their company.

Q Will the pork chain pay for the changes made possible through genomics?

Dr. Muir: If we cannot show them a real value or reasonable return that influences their bottom line, they are not going to pay for it. Breeding companies have to invest in it. They have to be paid for it. However, they have to sell it to their customers who see a real return. In addition, they have to sell it to the packers. And they have to sell it to the consumer who races to the supermarket because he didn't pull anything out of the freezer that day, and they look down and they have to buy the product to make sure it's worth the extra buck they're going to spend on it.

Dr. Dekkers: A breeding company can profit in two ways from investment in genomics. One is to increase the price per unit of semen, for example. The other is to increase market share by selling more breeding stock. It probably has to be a combination of the two. I think increasing market share is an important factor to consider when evaluating the benefit from investments in genomics.

Dr. McKeith: Another part that fits into that is, "what are we looking for?" Do we market improved palatability, tenderness or juiciness through genomics? What is the value if half of the pork is enhanced? Does that overwhelm the need to select for those traits? Should we be spending time on selection when we could use some other tool to fix that?

Dr. Rothschild: That is an excellent point. One of the genes that we have developed increases pH about a tenth of a pH unit and makes the meat better naturally. However, if you pump that product, the one with the better genotype improves significantly more also.

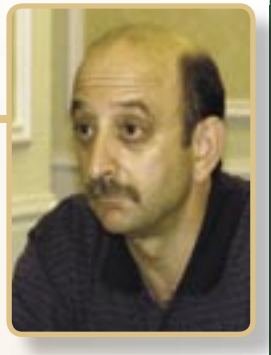
Dr. McKeith: The trends are for more branded products. And branded products mean somebody thinks that product has some value so they put a name on it so it can be easily recognized. The packer knows they can make more money on branded products. The question is whether the producer who buys a specific genetic company's breeding stock and sales to the packer, can they recoup what they paid for those advanced genetics produced from a genomics investment?

Dr. Rothschild: The trend towards branding certainly supports genomics, because it puts a value downstream. The real question, I think, in terms of is there value in genomics, is can we easily demonstrate that there is value? We can make all kinds of arguments about whether a quarter of a pig is worth something in improving litter size, but the problem is that if we have a line of pigs that are sold by a company, and it produced a quarter pig more, that is only valuable if the integrated producer raises the pigs well enough to actually see the quarter pig extra. In many cases, the average producer is doing such a poor job of raising pigs, all these advantages of genomics are being lost at that level. It goes back to environment and management.

Dr. Paszek: I agree. There are quite a few factors to consider. Some are related to production and some of them are related to marketing.

Dr. Dekkers: The industry is not uniform. For one sector, we would focus on one approach, and for another sector, we would focus on another approach. If we work with an integrated company, then it may be easier to sell the value of increased growth rate or the value of increased meat quality, because they can see the value of that going through their system.

Value of the Swine Sequencing Project: New Discoveries and Uses



Max Rothschild, Ph.D.

People got very excited when they saw the covers of Science and Nature about the human genome being sequenced. They were told you could unlock the secrets of the human genetic code. Some of the secrets include determining genetic reasons for disease or normal conditions such as growth and development. So, it's clear to us that deciphering the human genetic code has real value. This begs the question, "are similar opportunities possible from learning about the pig genome, and is there value from the knowledge?"

How are genes currently being discovered? Before SNPs were the buzzword, we were finding SNPs but were calling them polymorphisms, and we examined candidate genes and we found a number of valuable SNPs. They also have to be validated in commercial herds. Validate; that word is very interesting to me for this discussion. It does not mean just repeated; it means for this real world problem also 'find the value of.' Moreover, you can do QTL scans. We have done those, too, in our lab. You find positional candidates eventually, and again, you validate them in commercial herds. Finally, expression studies produce candidate genes or pathways and again, you have to validate them in commercial herds. So the point I would make here is, the discovery is only part of what is important. The validation and understanding of how it fits into commercial aspects is also very important.

So what value do genes or mutations or SNPs or sequence really have? It depends on the frequency of the alleles. That is extremely important when you talk about value. We've discovered genes that affect a trait, where the frequency is nearly fixed in certain populations. A good example of that, frankly, is the IGF2 marker that a major genetics company is selling now. Actually, it is fixed in nearly all the important sire lines. It is a great story, but it does not have much commercial value. So it all boils down to your allele frequency – the size and the effect of it, and the trait value (Figure 14).

Figure 14

Candidate Genes and Gene Tests Identified and Used in the Industry

Candidate genes	Traits	Industry use
HAL	meat quality/stress	yes
KIT	white color	yes
MC1R	red/black color	yes
MC4R	growth and fatness	yes
RN, PRKAG3	meat quality	yes
AFABP, HFABP	intramuscular fat	??
CAST	tenderness	yes
IGF2	carcass composition	yes
ESR, PRLR, RBP4	litter size	yes
FSHB	reproduction	unknown
NRAMP, SLA	disease susceptibility	unknown
FUT1	disease susceptibility	yes
Trade secret tests	several traits	yes

Growth rate and backfat, their values depend on the markets they're sold in. Meat quality might have more value. As crazy as it may sound, producing an extra ear on a pig as a dog toy might have value in certain markets. Therefore, you have to understand the trait. I like to think of two values that companies need to employ. Real value; that's dollars produced for the product that is sold. Then there is the marketing value. A number of people made the point that ESR, our gene test, had much more marketing value initially than it had actual trait improvement value. In the long run, of course, the ESR gene marker had real value in litter size. That's certainly also the case with MC4R, which has enormous product value in some situations.

When you look at all the markers currently available, there are approximately 20 to 50 being used by breeding companies. But the exciting thing is that somewhere between 100 and 700,000 markers are being developed. Experience has taught us that it takes 18 months or less to get them into actual herds.

What is the real value of the swine sequencing project for new discoveries and use? The real value of sequencing comes from not just the old style – one gene, one transcript, one protein produces one phenotype – but the technology now is that we can look at new technologies – sequencing, transcript profiling, protein quantification and function – so there are all these other opportunities that Dr. NeNise outlined. The problem is, all these technologies involve considerably more investment than the old technologies did.

I have four areas where I think we can quantify the value of genomics.

1). Speed of discoveries will be increased. There's no doubt in my mind it would be a lot easier if I had the whole sequence to figure out where I, as a scientist, am headed. You can use positional candidates or biological candidates more rapidly. You can compare gene sequences from different phenotypes more quickly. You can use comparative genomics and examine genetic structure and look for causative mutations more easily. Therefore, those all fit into speed of discoveries. The downside is they require more investment.

2). Ability to verify discoveries in different genetic backgrounds. One of the big lies that I think geneticists told themselves was that if a gene didn't work in all backgrounds, then it wasn't the right gene. In fact, we now know that is not correct. We needed to go back and look at how genes interacted in pathways so we could verify the value of each gene in different backgrounds and look for different mutations in the same gene. Of course, knowing the sequence would really speed this along. So as Dr. DeNise and Dr. Paszek have said, gene discoveries can be applied to all commercial lines in a much more rapid fashion. Moreover, additional mutations can be discovered. This requires considerably more investment in phenotyping.

3). Variety of traits affected can be increased. There is no doubt that sequencing costs will go down. But I would argue that phenotyping costs have gone up, because now, all of a sudden, we're not just weighing them and measuring them but measuring expensive genotypes. The likely targets now will most certainly be traits like disease resistance, healthiness and longevity. Moreover, there are some large marketing advantages with these traits. This will require more accurate description of phenotypes and, again, more cost.

4). Greater speed at which genetic improvement can be implemented. That offers an opportunity to leapfrog over past, existing selection programs. Accuracy is increased. Of course you may have an excellent pig breeding program for the gene improvements to be valuable. The only other thing I would argue, and this is obviously the central part of this discussion, is, will customers pay for fixed or improved products? The expected outcomes are faster delivery through marker- or gene-assisted selection, definitely better genetic lines and better branded products or better-directed products.

What are the expected outcomes of this technology? I would say increased discovery will certainly yield better products throughout the entire pork chain. We'll see better genetic lines that will yield better products for producers, packers and consumers. But, genomics are only going to modify the base product, which must be good to start with. I don't think they can change the route to market immediately. Therefore, that is a negative.

There is also going to be increased pressure to sell genomics to pay for the expenditures. In the long term, genomics are going to pay for themselves, but maybe not in the short term. When talking about genomics, we need to realize that we're at the beginning of this technology. There are more new discoveries this technology has yet to deliver. It's exciting, and we're only at the beginning.

Dr. Johnson: Dr. Rothschild has made a point that has always been important to me, and that is that the value of genomics will not be realized until we get phenotypes to go with all that genetic information to accurately interpret and understand it. We need good phenotyping experiments as ongoing efforts with collection of genomic data to fully utilize all of this genetic information.

Q Dr. Rothschild mentioned that we need to be able to demonstrate value and show that it is there with genomics. Are there tools that we need to create or ways that we can prove that we have created value?

Dr. Rothschild: I used to be a big proponent for using genomics, regardless of management to improve litter size. All you need to do is look at the data on the effects of the ESR gene across populations to increase litter size. Genotypes go from negative effects in poorly managed herds to well over a pig per litter for the right ESR genotype, and those aren't just random estimates; they are well correlated to the quality of management. If one is selling their genomics to a guy that can't figure out how to get his sows to reproduce or hit the right number in the crates, then they are never going to see a value. That's why, at least in my mind, if we are really going to capture value from genomics, it's got to be a combination of traits that management can't affect too much and on these 'knock them dead' traits like disease resistance.

Dr. Johnson: It is critical to keep track of those metrics to preserve the value that we are creating through genomics. We need to make sure that the end-user preserves that value and recognizes it when it gets to the consumer. That's certainly part of the branding concept, too. I think when we talk about alignment between a genetics company and a producer, a swine production company, we certainly have to be able to talk and communicate about what is important.

Dr. Muir: There is the need to validate that certain line or certain gene effects have value over the average of relevant environments, and then, in that case, we need to know what those relevant environments are so that gene or that line can be validated across them. There is also likely to be situations where their rank changes or interactions with distinct environments, maybe their nutritional systems and, in that case, then perhaps their value in customizing a line or identifying a line that has the greatest value in each of those distinct environments.

Q Does genomics have a place in helping set up or determine a set of specifications?

Dr. Rothschild: I believe they do. The MC4R – one allele is a growth allele which is selling really well in the United States, but the lean allele is selling really well in Europe, so there is a case where we can differentiate within a line by allele, but the reverse may not be true. If only one allele is good and the other allele is quite bad, then we are going to remove the bad allele.

Right now, breeding companies are essentially selling breeding stock to large multipliers to produce their own sublines. We can at least foresee that a breeding company is going to need more lines to produce that, because Cargill is not going to want something that sold to Hormel, for example. If it looks the same, they are going to want to make sure that it is different. And that is one case where genomics might be really advantageous, when we can say "these specifications for the 25 most important alleles are in a different combination than one company or some other company got."

This supports the genomics argument. The customer who wants to develop their own product can use genomics to make sublines for each of the products. That's what genomics can do.

Dr. Johnson: Genomics could help speed up development of those sublines.

Q Is there any relative value when looking at epistatic vs. additive/dominant?

Dr. DeNise: We have some anecdotal evidence from mouse genetics and some human research that says that epistasis is going to be important.

Dr. Dekkers: The question is whether you could utilize it effectively. If you have two genes with large effects that interact, this may be possible. If you have 100 QTL with interactions amongst all of them, it comes back to getting the right combination in that single package and that is not easy to put together.

Dr. DeNise: Anecdotally, we have some experience in identifying markers that act epistatically. For example, we have identified a set of 20 markers that interact. While the epistatic effect can be estimated within that set of markers, there are only a couple of those markers that have the majority of influence on the trait. Additive and dominance effects are going to be the easiest to manage initially, but as we fix combinations of genes, we will be searching for new strategies to continue improvement of the elite populations. One of those strategies will be to capitalize on epistatic effects.

Dr. Rothschild: I think something very important to remember is that most of the breeding companies have X number of lines and, while they're interested generally in epistasis, they're really interested in which genes work in which lines. So initially, I think epistasis means nothing more than background genetics in the first round. The only way that is going to be sorted out is to have an easy way to test a gene or pairs of genes across all the lines. Future generations of improving this analysis would definitely mean we are looking 10 by 10 genes or 6,000 by 6,000 genes, etc., probably at first as two-way interactions. However, initially, it is just, 'does this gene work in the three sow lines that I am trying to sell to my customers and how do I use that properly?'

Q Do we need to identify the epistatic interactions, or should we just identify association of markers with positive performance?

Dr. DeNise: In a large, well-designed experiment, the epistatic interactions will be contained in the data. The first goal of analysis would be to fit additive and dominance effects because the model for these effects is relatively simple. If the data set is large enough, you could start to fit epistatic effects. It may be possible to fit additive-by-additive and additive-by-dominance and dominance-by-dominance effects, but the model becomes increasingly more complicated. As you continue to add more markers to the analysis (additive x additive x additive, etc) it becomes exponentially more difficult to fit the effects and interpret the results. Our goal is to estimate as many of the epistatic effects as possible to learn the role of these factors in livestock improvement.

Dr. Rothschild: Some alleles exist in high frequency in some lines and not in others. By combining those in the right cross, you add value. You will need to test them both in the pure line and in the crossbreed progeny. You have to know the allele frequency and you have to know its value. The value you get is in a crossbred, but the allele frequency in the pure lines to make those crosses is important to know.

Q Are there profits in genomics?

Dr. Rothschild: Right now, there are not huge profits. All the companies have lines and these lines act as if they are existing products, so adding on new gene effects because of genomics will improve the product, but initially, I don't think they are going to make a huge profit from that. They can make, in some cases, incremental, and in others, long term or, in some cases, fluctuating profit levels based on that. I can't see how they can easily make $5 a pig to $10 a pig just on genomics. I think they can make sustained profit, but it is not without lots of sweat and development and a little luck and a variety of other issues. There is an advantage in genetic improvement but there is definitely an advantage in marketing strategies that help genetic improvement and those will lead to the profits.

Dr. Dekkers and I did some discussion a while ago on what method to select on genes. Scientifically, we take this long-term approach and make slow incremental improvements, but the business model says make quick discoveries and move on to the next one because we've got to be selling the market advantage and we've got to be selling the quick genetic turnaround.

Dr. Johnson: That's what bothers me a little bit. The long-term strategy says you don't just put all the emphasis on that one QTL and fix it immediately, because there is some long-term cost associated with that process. But, it may be the best business model.

Dr. Dekkers: The big problem with economic analysis is that we don't know what the increase of the market share is going to be or what the producer is going to be willing to pay more for this product. That's where the big dilemma is.

Dr. Rothschild: I do not think we can sell genomics if we tell the producer buying it from us that our breeding company has incrementally improved all the traits at once. However, we can say we improved the lean percentage by 3% if you buy my boar. We have to concentrate on fewer traits in the initial sales.

Dr. Johnson: Why not just choose a trait, let's say pH is important and there may be 100 genes that are responsible for pH. We can make faster progress with the gene mass approach than by selecting five of those genes of the hundred that are responsible. It seems this might be a better approach.

Dr. Rothschild: It seems like the infinitesimal model is correct, but I am not convinced. I think we can make a case for either one, but in the short term, it seems to me that before we know all of the interactions that the gene can cause, it is more important to know and select the individual genes so we can track things a little better.

Glossary of Genetic Terms

Allele – Any of the alternative forms of a gene that may occur at a given locus.

Base Pair – Two bases which form a "rung of the DNA ladder." A DNA nucleotide is made of a molecule of sugar, a molecule of phosphoric acid, and a molecule called a base. The bases are the "letters" that spell out the genetic code. In DNA, the code letters are A, T, G and C, which stand for the chemicals adenine, thymine, guanine and cytosine, respectively. In base pairing, adenine always pairs with thymine, and guanine always pairs with cytosine.

Candidate Gene – A gene, located in a chromosome region suspected of being involved in a disease, whose protein product suggests that it could be the disease gene in question.

Centimorgan – A genetic unit equivalent to 1/100 of a morgan.

Gene – A specific sequence of nucleotides in DNA or RNA that is located in the germ plasm, usually on a chromosome, and that is the functional unit of inheritance controlling the transmission and expression of one or more traits by specifying the structure of a particular polypeptide and especially a protein or controlling the function of other genetic material.

Genetic Marker – A usually dominant gene or trait that serves specifically to identify genes or traits linked with it.

Genome – The entire DNA contained in an organism or a cell, which includes both the chromosomes within the nucleus and the DNA in mitochondria.

Genotype – All or part of the genetic constitution of an individual or group.

Heterozygous – Possessing two different forms of a particular gene, one inherited from each parent.

Homozygous – Possessing two identical forms of a particular gene, one inherited from each parent.

LD MAS – Linkage Disequilibrium Marker-Assisted Selection

LE MAS – Linkage Equilibrium Marker-Assisted Selection

Linkage – The association of genes and/or markers that lie near each other on a chromosome. Linked genes and markers tend to be inherited together.

Locus or Loci – The position in a chromosome of a particular gene or allele.

Mapping – The process of deducing schematic representations of DNA. Three types of DNA maps can be constructed: physical maps, genetic maps and cytogenetic maps, with the key distinguishing feature among these three types being the landmarks on which they are based.

MAS – Marker-Assisted Selection

Meiosis – The cellular process that results in the number of chromosomes in gamete-producing cells being reduced to one-half, and that involves a reduction division in which one of each pair of homologous chromosomes passes to each daughter cell and a mitotic division.

Microsatellite – Repetitive stretches of short sequences of DNA used as genetic markers to track inheritance in families.

Peptide – Two or more amino acids joined by a peptide bond.

Phenotype – The visible properties of an organism that are produced by the interaction of the genotype and the environment.

Polymorphism – The quality or state of being able to assume different forms.

Single Nucleotide Polymorphisms (SNPs) – Common, but minute, variations that occur in human DNA at a frequency of one every 1,000 bases. These variations can be used to track inheritance in families. SNP is pronounced "snip."

Trait – A distinguishing quality (as of personal character) or inherited characteristic.

Monsanto Choice Genetics wishes to thank the members of this prestigious panel for sharing their expertise and experience in the roundtable discussion of *Understanding the Value of Genomics in the Pork Industry*. From left to right: Dr. Floyd McKeith, Dr. Bill Muir, Dr. Rodger Johnson, Dr. Sue DeNise, Dr. Jack Dekkers, Dr. Max Rothschild and Dr. Albert Paszek.

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