GENE TESTING FOR PRODUCTION AND CARCASS TRAITS: WHAT DOES IT MEAN TO A RANCHER?

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INTRODUCTION

Marker assisted selection (MAS) is a process that enables the accurate selection of specific segments of DNA that are associated with a measurable difference or effect on a complex trait, like weaning weight or marbling score. MAS can be an effective way to increase or decrease the frequency of specific DNA sequences in a population. It is important to note that many genes control complex traits, like marbling or tenderness; they are polygenic in nature. Markers for specific variations in DNA sequences are available for only a few genes that contribute to marbling or tenderness. There are many other 'unmarked' and unknown genes, as well as the production environment, that affect the observed phenotypes for these traits. Therefore, MAS selection will only account for a portion of the genetic variation. Measures of net genetic merit for a trait, such as Expected Progeny Differences (EPD) should be considered when making selection decisions even when marker information is available. EPD provide an estimate of the overall (net) merit of the genes an animal has for a trait including the 'marked' and 'unmarked' genes. MAS should be seen as an additional method of selection, but not a replacement of proven selection tools like EPD.

Several of the following sections provide background information on DNA and DNA markers. If you are already familiar with these concepts you may skip ahead to the section labeled 'Benefits of Marker Assisted Selection.'

WHAT IS A DNA MARKER?

All living organisms are made up of cells. Within most cells, there is a nucleus that contains several large molecules called **deoxyribonucleic acid (DNA).** DNA is the material that carries genetic instructions, is transmitted from one generation to the next, and determines the differences in many of the physical characteristics of individuals and species. Each large DNA molecule, which is sometimes packaged in a tightly wound coil, is called a **chromosome**. Chromosomes come in pairs, one inherited from each parent. Cattle have 30 pairs of chromosomes. Collectively, all the pairs of chromosomes are called the **genome**. Every cell nucleus contains a copy of all the chromosomes. Therefore, each nucleated cell contains the complete genome of the animal.

DNA is a large double helix molecule that looks like a twisted ladder (see figure below). Each rung of this ladder is composed of a pair of **nucleotides**. The pairs are 'G' with 'C', and 'A' with 'T'. These pairs are strung together in a long sequence that codes for specific **amino acids**. Amino acids are linked together to form larger molecules called **proteins**. A sequence of DNA that codes for all the amino acids that makes up a single protein is called a **gene**. There are thousands of proteins in the body, which are coded for by thousands of genes. It is the interaction and structure of these proteins that determines the appearance or

phenotype of individual. The term **genotype** refers to the genetic sequence of an individual for a particular marker or gene.

The sequence of DNA can vary from one individual to another. In fact, the sequence of DNA for a gene on one chromosome can be different than the sequence for the gene on the other chromosome in the pair. These genetic variants of a gene are called **alleles**. The variation in their sequence may change the amino acid sequence for which they code and can result in a change in the structure of the protein they encode. Alternately, the variation may result in production of different quantities of the protein (**expression**). Differences in either the protein structure or the level of expression can have an effect on phenotype.

Each individual receives one-half of its genetic make up from their father (sire) and one-half from their mother (dam). So, one-half of the chromosomes in a cell are from the sire and one-half from the dam. If the DNA sequence for a specific gene is the same on each chromosome in the pair, then the individual is said to be **homozygous**. If the DNA sequences are different for the gene, that is, there is a variation, then the individual is said to be **heterozygous**.

Not all DNA is made up of genes. There are large pieces of DNA whose purpose is unknown. Some DNA helps regulate the expression of other genes. A genes coding sequence may be broken into several sections. The term **locus** can be used to describe a specific sequence of DNA within or outside of gene coding sequence.

MARKER BASICS

A DNA marker is simply a sequence of nucleotides that uniquely identifies a location of in the genome. This location can be in a gene or nearby a gene and used to identify a specific allele. Mutations, or changes in the coding sequence of a gene, can be used as DNA markers. These mutations may or may not cause a change in the protein product. Variation nearby a gene can also be used as a DNA marker.

The two main classes of markers used to identify different alleles in beef cattle are **microsatellite markers** and **single nucleotide polymorphisms** (SNPs, pronounced *snips*). Microsatellite markers are di-nucleotide repeats where the count of the number of pairs of nucleotides varies. Microsatellites are in the non-coding sequences of DNA and are used to identify alleles of a **quantitative trait loci (QTL)** via association between the markers and phenotype. A QTL is simply an area of the genome that is likely to harbor a gene that influences a quantitative trait and for which a set of markers can be used to track inheritance of the various alleles of the gene. SNPs are single base pair variations in the DNA coding sequence. SNPs used as DNA markers may be the causative mutation that results in an alternate form of the protein, it may be in the coding sequence but not cause a change in amino acid sequence, or may be nearby a gene in the non-coding sequence of DNA. Microsatellites or SNP marker panels may be used to identify or validate parentage of individuals.

DNA markers can be used to track the inheritance of simple traits controlled by a single gene or complex traits controlled by many genes. Examples of simple traits include coat color, horn status, and some genetic diseases; complex traits include traits like weaning weight, tenderness and marbling which are controlled by many genes. DNA markers simply identify a sequence of DNA just as ear tags identify individual calves.

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BENEFITS OF MARKER ASSISTED SELECTION

Marker assisted selection can be used to increase the frequency of desirable forms of a gene within a population by selection of parent stock that carry the gene. Selection of parents that are homozygotes for the desired allele ensures that all gametes (sperm or egg cells) produced by that parent have the desired allele. Of course, to use MAS, markers must be available for the trait and alleles of interest. The potential benefits of MAS are greatest for traits that (Van Eenennaam, 2005; Dekkers, 2003):

1. have low heritability (traits with observed or measured values that are poor indicators of breeding value),
2. are difficult and/or expensive to measure (disease resistance),
3. cannot be measured until later in life potentially after the animal has reproduced (carcass or maternal traits),
4. are not routinely measured or selected for currently (tenderness), and
5. are genetically correlated with another trait you do not want to change. (e.g. At the polygenic level the traits may be positively correlated, but selection for a marker of a gene for marbling that is not associated with increases carcass fatness might be desirable. This would entail selection for marbling genes without pleiotropic effects on fatness.)

The expected benefits of changing allele frequencies and the resulting changes in phenotype vary depending on the type of trait. The following categories are ordered from greatest to least expected benefit from MAS (Van Eenennaam, 2005):

1. simply inherited traits (coat color, horn status, genetic defects),
2. carcass quality and palatability attributes,
3. fertility and reproductive efficiency,
4. carcass quantity and yield,
5. milk production and maternal ability, and
6. growth trait performance.

The ranking above follows from a number of considerations including the relative difficulty of measuring and recording performance data, the amount of genetic and phenotypic variation in trait and their proportion (heritability), the age at which performance records are collected on the animal, and how much performance data is currently available.

A number of production traits, including carcass marbling and tenderness are likely to benefit from MAS due to a number of reasons. Although both traits have moderate levels of heritability, data collection of both measures is relatively difficult to obtain. In fact, collection of Warner-Bratzler Shear force data is especially challenging due to the costs of obtaining large numbers of steak samples from sire identified animals and then obtaining shear force values from qualified labs. Obviously, the animals from which these observations are obtained are not able to reproduce, thus, selection focuses on the sires of animals with desirable characteristics. Bulls with carcass trait and tenderness evaluations are typically 4-5 years of age and have sired a number of progeny. The ability to select young sires with desirable genetics would minimize progeny test costs and focus data collection efforts on bulls with desirable genotypes.

LIMITATIONS/CHALLENGES OF MARKER ASSISTED SELECTION FOR PRODUCTION AND CARCASS TRAITS

Selection based on marker information for a single gene with the exclusion of other sources of genetic information such as EPD will yield poor results for overall improvement in a trait. Ignoring information about the net merit of an animal's genotype via phenotypically derived genetic predictors such as EPD is discouraged. For instance, both marbling and tenderness are complex traits controlled by many genes, with only a few genes that have useful markers associated with them. More response to selection will be obtained if both MAS and EPD are used. EPD should, when available, be the primary driver in selection decisions, with marker data playing a secondary role for selection of specific known alleles.
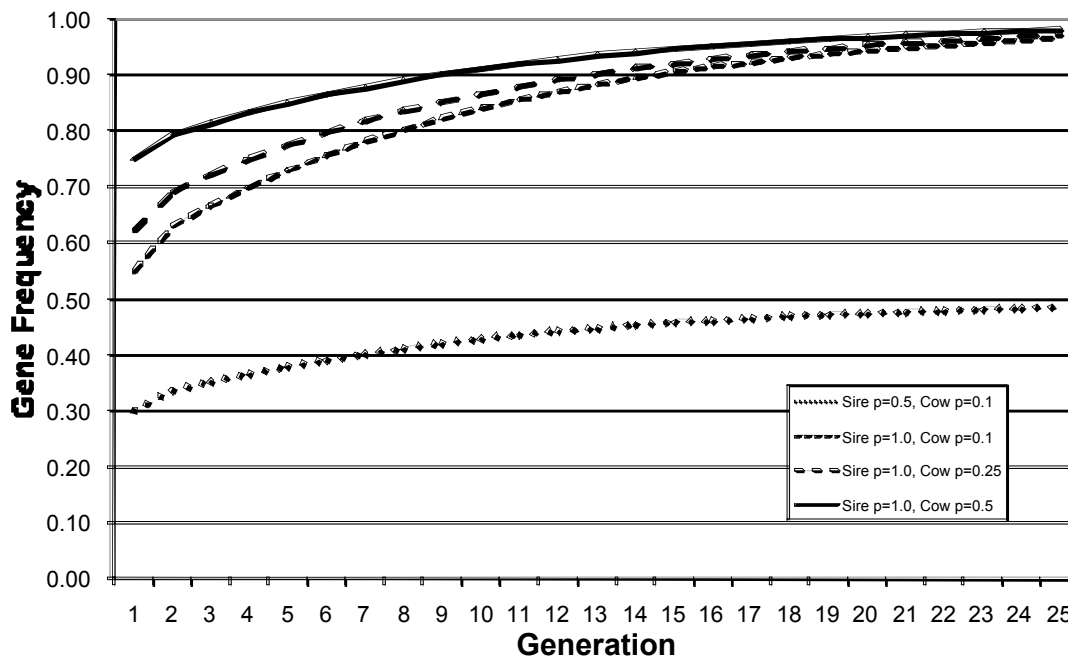
Several potential limitations or challenges to implementation of DNA marker technology by the seedstock producer or commercial cow-calf producer? One of those is the frequency of the favorable allele. Several scenarios will illustrate the practical challenges. One scenario is a population of animals with a favorable allele frequency of 90%; the favorable allele is almost fixed in the population. Is it worthwhile to test a large number of animals animals to find the 5-10% that don't carry the favorable allele? Likely not, unless the trait affected by the gene is very valuable and the marker accounts for a large portion of genetic variation in that trait. An alternative is that if the gene is at a very low frequency in the population, it may take a long time, many generations, to actually increase that gene to a level where much benefit is observed. If the population has an allele that is not very frequent but accounts for 80% of the genetic variation in a particular trait, it might be worth going after. It's important to know these two things interact with each other – the frequency of the alleles and the magnitudes of the effect impact the cost-benefit ratio.

Mode of inheritance is also important. Is the trait co-dominant or recessive? If it's recessive the gene will need introgressed into the population such that both the sire and dam populations are carriers to ensure that calves have a high probability of inheriting the recessive allele from both parents. In cases where the recessive trait is the desirable phenotype calves have to be homozygous recessives before the favorable effect is observed. Selection for recessive traits is difficult and time consuming. For example, consider selecting for red coat color from a herd of only black (which is dominant to red) animals.

To illustrate the importance of gene frequency, data was simulated data for a single gene locus. Consider a homozygous sire with two copies of the favorable allele and he's mated to a population of cows that have a gene frequency of about 50%. Before the calves have a gene of 90% frequency, 8 generations of selection are required. See solid black trend line in Figure 1 below. If a heterozygous bull is used (he has a 50% gene frequency), it's really difficult to make much headway. When this bull is mated to cows with a favorable allele frequency of 10%, progress will be slow. The round dotted line in Figure 1 illustrates this scenario. One may select for 25 generations and still the gene frequency in the calves is less than 50% because the cows are low and the bulls are low. The point is selection with DNA markers is not an overnight fix. Genetic testing and selection using markers, particularly single-gene markers, and not panels that account for large portions of variation, this can be a real challenge.
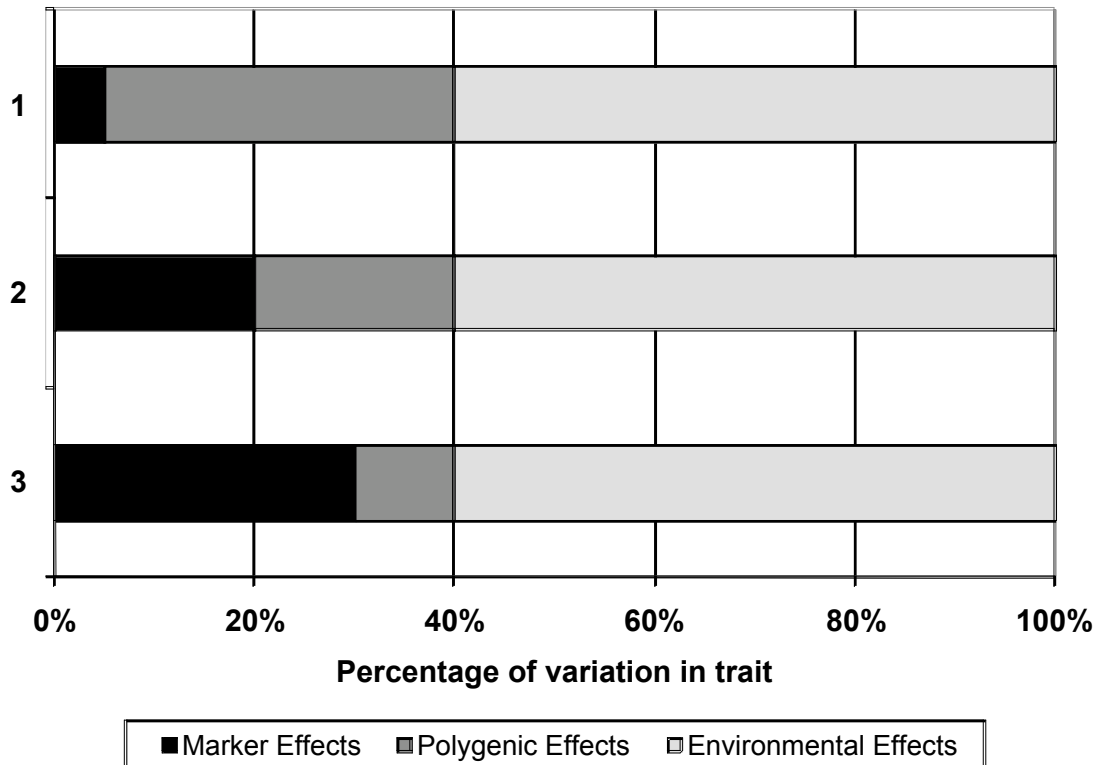
Figure 1. Frequency of favorable allele in cow herd when using heterozygous and homozygous sires and cows with allele frequencies of 10%, 25% and 50%.

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Frequency of Favorable Allele in Calves

To date, markers for only a few genes affecting production traits are available. These markers account for a relative small amount of the genetic variation and a smaller portion of phenotypic variation for the traits. Heritability is the proportion of phenotypic variability that is controlled by genetics. For example a trait with a 40% heritability means that 40% of the variation observed in the trait from animal to animal is controlled by genetics; 60% of the variation is then due to the environment. Figure 2 depicts the examples of three different scenarios for a trait with a heritability of 40%. Scenario 1 is a trait where the DNA marker accounts for 5% of the phenotypic variation or 12.5% of genetic variance. In scenario 2 the DNA marker accounts for half of the genetic variation and 20% of the phenotypic variation. In scenario 3, the marker accounts for 75% of the genetic variation and 30% of phenotypic variance. Current marker systems don't account for as much variation as scenario 2 or 3 with several makers in the panel. If a single marker that accounted for half of the additive genetic effect with a trait that is 40% heritable would be considered very successful. Even though the marker accounts for half of the 40% of the variation in the trait controlled by genetics there are a lot of opportunities for other genetic and environmental variation to affect phenotype.

Figure 2. Three scenarios of increasing DNA marker panel explanatory power for a trait with heritability of 40%.

Percentage of variation in trait

Legend: ■ Marker Effects □ Polygenic Effects □ Environmental Effects

Researchers continue to investigate the bovine genome and find DNA markers associated with a wide variety of traits. Interpretation of genotyping results is already fairly complicated with just a few genes on the market. Introduction of a large number of DNA marker tests will quickly generate an overwhelming amount of data. The challenge will be to make these genotypes into useful information. One way this may happen is with the inclusion of the genotype data into the computation of EPD. The augmentation of EPD with DNA marker data will reduce data overload and continue to focus selection decisions on measures of net merit. The markers will allow for improved accuracy of prediction of the EPD at an earlier age.

AVAILABLE TESTS:

The National Beef Cattle Evaluation Consortium (NBCEC) is an organization that provides the "Good Housekeeping" seal of approval for DNA tests for beef cattle production traits. The NBCEC has taken on the task of validation of DNA marker claims made by commercial genotyping labs. The NBCEC is a group of researchers at some of the land-grant universities who work in quantitative and molecular genetics. Validation is initiated when a commercial lab request the NBCEC validates their marker claim. The NBCEC organizes a resource population to test that claim against and then reports its findings in the scientific literature.

The markers I will discuss today are primarily those validated by the NBCEC as well as some that are pending validation. Genotyping companies are aggressively trying to add genetic markers to their panels. The validation data was reported in late 2006 so some of the panels have changed slightly.

Bovigen Solutions has a product line called GeneStar and in that line they market GeneStar Marbling and GeneStar Tenderness. The GeneStar Marbling makers were validated in the Charolais and Angus populations. The NBCEC found that the marker panel didn't account

for a significant amount of variation in marbling. There are two markers, TG5 and an anonymous SNP called M2.

Another company that is actively marketing DNA marker panels is Igenity, a division of Merial. They have a product that looks at tenderness that is called TenderGene. It's a Warner Bratzler shear-force tenderness measurement and was validated in Brangus, Charolais, Angus, Red Angus and Brahman populations. Total population size was 1,209 head. The NBCEC did validate a significant result for the panel of markers and individual markers within those panels. There is a statistically significant amount of genetic variation explained by this particular panel.

Tenderness is a trait that is difficult to measure. The difficulty and expense in collecting Warner Bratzler phenotypes makes tenderness a good candidate for DNA marker assisted selection. A couple of breed associations publish Warner Bratzler shear force EPDs. The American Simmental Association used genotype data to augment prediction of Warner Bratzler EPD. Their system uses both the phenotypes collected on progeny plus the DNA samples to compute breeding values.

The Igenity tenderness panel accounts for about 2.5 lbs. of difference in Warner-Bratzler shear force which. Although the panel explains a considerable portion of genetic variation in WBSF, one of the obvious challenges with improvement of beef tenderness is that the current beef carcass pricing system does not communicate a pricing signal for improved tenderness. There's some intrinsic economic benefit to the beef business to improve shear force. The NCBA Carcass Merit Project found that if the beef industry could improve tenderness by 10% the industry could capture an additional $200 million through increased demand for beef products (NCBA, 2004). The industry has the tools to improve beef demand and our macro-economical position by improving tenderness. However, it's hard to motivate commercial producers to spend a lot of time and money to select on Warner-Bratzler shear force when they sell calves at weaning. Not having a clear price signal to improve tenderness is a limiting factor for the adoption of marker assisted selection for tenderness. GeneStar tenderness is the Bovigen product and is very useful as well. It's statistically significant and basically uses the same set of markers.

The Igenity profile covers more traits than any other panel which is one of the reasons I've used them for an example. The traits included in the Igenity profile are end-product focused and includes: yield grade, percent product choice based on quality grade, ribeye area, hot carcass weight, 12th rib back fat, USDA marbling score and Warner Bratzler shear force. Each trait's marker panel includes several markers. For instance, the USDA marbling score panel includes five different markers. The Igenity profile is reported in figure 3 below.

Figure 3. Igenity Profile marker panels, effects and statistical significance.

	Yield Grade	% Choice Based on Quality Grade	Ribeye Area in Inches	Hot Carcass Weight lbs.	Back Fat Thickness in Inches	Marbling Score	Tenderness in lbs. of WBSF
10	0.44	44.5	0.95	45.5	0.100	85.3	-2.27
9	0.39	38.9	0.85	39.8	0.085	76.6	-1.95
8	0.33	34.3	0.74	34.1	0.070	67.4	-1.85
7	0.28	29.6	0.68	28.7	0.060	57.9	-1.54
6	0.23	24.6	0.51	23.3	0.050	48.4	-1.22
5	0.19	19.9	0.41	21.8	0.040	39.0	-1.13
4	0.15	14.9	0.27	16.6	0.033	29.6	-0.79
3	0.11	10.3	0.21	11.4	0.025	20.1	-0.42
2	0.05	5.6	0.11	5.7	0.013	10.1	-0.21
1	0	0	0	0	0	0	0
P-value	2.96×10^{-12}	1.52×10^{-05}	7.25×10^{-05}	5.03×10^{-06}	7.05×10^{-13}	1.14×10^{-05}	1.9×10^{-08}

Source: http://www.igenity.com/beef/profile/carcasscomposition.aspx. Accessed 11/4/2007

Igenity has added markers to their ribeye area test and account for about 1.5 square inches of ribeye area. Hot carcass weight about 46 lbs. difference, 0.10 in. 12th rib back fat, about 85 units of marbling score, so almost an entire marbling grade is accounted for by their five marbling markers, then about 2.25 lbs. of Warner Bratzler shear force. Reporting the genotype effect data so that meaningful to producers is a big challenge. One method is to determine the amount of the phenotypic standard deviation accounted for by the range of genetic effect of the panel. In terms of yield grade, the panel range represents about two-thirds of a phenotypic standard deviation. About two-thirds of a phenotypic standard deviation in rib eye area, about one-half of a standard deviation in hot carcass weight, two-thirds in back-fat, about 0.90 standard deviations in USDA marbling score and about 0.70 in Warner Bratzler shear force. Recall one of the challenges discussed earlier is the gene-frequency issue. It would be difficult to find a group of cows that are all homozygous for the unfavorable alleles.

SELECTION SYSTEMS USING DNA PARENTAGE ASSIGNMENT

Research conducted by personnel at Cornell University investigated a strategy utilizing a two-stage sire selection program in a large integrated commercial beef operation (Weaber, 2005). The prototype model was the Bell Ranch in New Mexico, a ranch with an integrated seedstock operation and about 4,000 head of commercial cows. We wanted to see if we could progeny test young bulls in large multi-sire breeding pastures. So, paternity was established on calves by genotyping them. A two stage selection system for a trait like weaning weight can generate enough genetic gain to by genotyping calves and doing a genetic evaluation based on calf phenotypes to be profitable. There's a positive return on investment. This system has been commercialized by Igenity and uses their parentage testing platform to do the paternity assignments. Colorado State University provides genetic evaluation on the pedigree derived from the genotyping. Large commercial herds can use the system to progeny test young bulls.

Another application of the paternity information is identifying calves that were sired by bulls producing dystocia events. Sampling outlier animals enables a producer to figure out if it's

an environmental problem or if it's truly a genetic problem from a hard-calving bull. Paternity assignment via genotyping can be done for less than $20 per head.

FUTURE OF MARKER ASSISTED SELECTION

The future for marker assisted selection in beef cattle is dependent on the convergence of molecular and quantitative genetics. In other words, incorporating DNA genotype results into traditional quantitative genetic evaluations is essential. The convergence will enable easier use of DNA tools by producers and enhance the reliability of EPD on young or un-phenotyped animals.

New high throughput genotyping platforms allow for dense marker profiles at a relatively low cost. The new Illumina Infinium SNP chip for beef cattle will yield about 50,000 SNP genotypes in a single assay per animal. Twelve samples can be assayed per chip and it will cost about $200 per sample. While this may seem expensive, it represents a dramatic reduction in cost and tremendous opportunity to discovery new genes and QTL that affect beef cattle production traits. An alternative method to traditional QTL discovery is an idea called whole genome selection. We know that most production traits are polygenic. Using a tool like the Infinium chip with a high-density marker panel one can divide up the genome in to small blocks. Cattle have 30 pairs of chromosomes. Dividing each chromosome into 100 blocks produces about 16 SNP markers per block. Theoretically, each of those blocks can be analyzed for association with a broad range of phenotypes. For a trait like marbling, researchers have identified nearly 60 different QTL and there might be 60 or more of these blocks, out of the 3,000 total blocks in the genome, that account for variation in the trait. Once blocks have been identified specific genetic effects can be determined for the various variants in genotype within each block. A breeding value can be estimated, then, by genotyping an animal then summing all of effects from each block. Prototype work has shown a high correlation between genome breeding value with the breeding values we would estimate via traditional methods (Jerry F. Taylor, personal communication).

Ultimately, for seedstock and commercial producers, it will be a convergence of quantitative and molecular data to produce estimates of genetic merit that will be used in selection programs. Large genotyping panels will provide much more information to base our genetic predictions on and that will be very valuable. Convergence of the traditional quantitative and the new molecular tools will dramatically enhance the usability of DNA marker technologies.

ADDITIONAL READING

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