![Texas Adapted Genetic Strategies]

Texas Adapted Genetic Strategies for Beef Cattle XI:

Marker Assisted Selection for Beef Improvement

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Introduction

Genes form the roadmap that directs transmission of certain traits from parents to offspring. Genes occupy specific locations on chromosomes, which are threadlike strands of DNA and proteins found in the nucleus of plant and animal cells. Each gene plays a part in determining a particular characteristic of an organism by directing the formation of a specific protein and can copy itself each time a cell divides. Base pairs are nitrogen compounds connecting strands of DNA and RNA, with different arrangements of molecules making up different pairs. The complete set of chromosomes and genes peculiar to any organism is called that organism's genome.

The cattle (bovine) genome comprises 30 pairs of chromosomes with some 3 billion base pairs, many of which determine function and appearance (including insuring that each animal has four legs with hooves, a head with two ears, two eyes and one mouth, a tail, a digestive system with four compartments, and so on). Most genes act together at a particular location on a chromosome to produce either (1) dominant characteristics (e.g., coat color, polledness, most of the hybrid vigor in crossbreeds); (2) additive effects (e.g., those characteristics which allow estimates of breeding values and expected progeny difference – EPD); or (3) epistatic effects (actions between genes located at different parts of a chromosome, also associated with hybrid vigor). Some genes, however, fail; although such "genetic trash" remains on the chromosome, it no longer plays an active part in the genome.

Because most genes are responsible for the "functioning" animal, only about 15% of the total number can be effectively selected, although no one will really know the exact number subject to selection until the entire bovine genome has been sequenced and mapped. This 15% comprises all genes with dominance effects and all those additive genes affecting performance.

Dominant genes tend to have visible, major effects not influenced by the environment (e.g., polledness, coat color), while additive genes tend to have small, individual effects that accumulate through selective breeding to produce performance increases. Additive genes can affect more than one trait, but at different levels and at different times. Importance of additive genes in selection for traits is determined by the amount of genetic variation produced by such genes. The "average additive effect" of a gene may account for only 1% of genetic variation, so genes which contribute 2%, 3%, 5% or 10% of a variation are called "major genes;" because they have a large effect relative to other genes, such genes assume major importance in beef cattle breeding.

Tests can help locate specific genes on particular chromosomes, and several *dominant* genes in cattle have been found (e.g., the polled/horned gene and the black/non-black (red) gene). However, few *additive* genes have been located. Instead, performance in cattle seems to be associated with the presence on specific chromosomes of particular DNA, called "markers." These bits of DNA are closely associated with the genes nearest them on the chromosome and are passed along from parents to offspring with these genes, giving desired increases or decreases in performance when selection is applied (hence, the term "marker assisted selection" or MAS). The actual genes involved in specific performance of a particular trait may not yet have been precisely identified, but we know these desired genes have a great chance of being inherited with their closely linked markers. In other words, we know that if a marker is present, inheritance of a trait can be predicted even if the gene which controls that trait has not yet been identified. Although hundreds of markers are now known, only a few are so closely linked that they will be passed on with the genes (or qualitative trait locus — QTL) of interest.

Several well-known benefits of DNA testing include validation of parentage, testing for diseases or genetic defects, and testing for qualitative inherited traits (Thallman, 2004). Testing for markers and other QTLs of interest can be done at or shortly after birth, reducing the time and cost involved in collecting data from older animals or from carcasses. With each animal tested, DNA testing will provide more information, especially about traits that are difficult to measure or that are sex limited (e.g., milk production). Such testing also provides greater opportunities to select for traits with genetic antagonisms (e.g., birth weight/calving ease).

Commercially Available Markers

Until 2000, no markers were commercially available, but now several companies offer at least one of five QTL markers (see Van Eenennaam, 2004, meat quality and tenderness).

Marbling Effect. In addition to markers for coat color, polledness, and some genetic diseases, the earliest commercially available markers for beef cattle determined the presence or absence of a mutation in the thyroglobulin gene (TG), located on the bovine chromosome 14 (BTA 14) (GeneSTAR marbling -- Genetic Solutions, Commonwealth Scientific and Industrial Research Organisation, Aus-

tralia). Thyroglobulin is involved in the creation of energy-yielding fat stores within muscle fibers. Presence or absence of the marker is noted by the number of stars given an animal, e.g., an animal without the marker has 0 Stars, with one copy of the marker, 1 Star, and with two copies, 2 Stars. Frequency of the high-marbling allele is greatest in the Wagyu breed, intermediate in Bos Taurus, and least in Bos indicus (Hetzel, 2003). (The test for this marker is offered in the United States through Bovigen Solutions. Results from the test can be combined with the GeneSTAR Tenderness markers, discussed below, with an animal receiving a total of from 0 to 6 Stars.)

Effects of this marker on marbling scores for animals that have received the specific gene from both parents (homozygous animals) range from 3.5% to 11%. In Waygu cattle, this effect ranges from 14% to 20% and affects quality grade differences by 16% to 19%. This marker also has been confirmed by the National Beef Cattle Evaluation Consortium (NBCEC) in Simmental x Angus cattle, with a statistically insignificant increase in marbling score but a statistically significant (18%, comparing 0 Stars to 2 Stars) increase in the percentage of animals grading Choice. The gene apparently increases average marbling scores just enough to allow carcasses that would have graded High Select to grade Low Choice.

Merial has released Igenity-L, a marker for specific forms of the hormone leptin that code either for cytosin (C) or for thymine (T). Leptin is produced by the obese gene and is synthesized and secreted by white adipocytes. Leptin regulates body weight, food intake, energy expenditure, reproduction and certain immune functions (Nkrumah, 2005). Cattle homozygous for thymine (both genes code for thymine, L-tt) tend to be higher quality-grading with reduced cutability, while cattle homozygous for cytosin (L-cc) tend to be higher yield- grading with reduced quality grades. Dairy cattle homozygous for thymine produce 3.3 pounds more milk per day than do cows homozygous for cytosine (Buchanan, 2003).

Tenderness Rating. Genetic Solutions has developed the two-gene GeneSTAR tenderness rating, based on two DNA marker tests (T1 and T2). (However, the National Beef Cattle Evaluation Consortium has not validated these tests in the U.S.) Both T1 and T2 test markers for the bovine calpastatin gene (CAPN1) located on BTA 29. Calpastatin is a naturally occurring enzyme inhibiting normal tenderizing of meat during post-mortem aging by regulating the enzyme calpain (which increases tenderness). Results from the two tests are added to yield from 0 to 4 Stars; neither marker has been found to have significant correlated effects on traits other than tenderness.

In trials with 8,000 cattle from seven different breeds, the T1 marker was associated with a 0.8 lb difference in Warner Bratzler Shear Force (WBSF), predicted to reduce the proportion of unacceptably tough carcasses from 21% to 8%. T1 results indicated an 8% to 10% increase in tenderness in cattle with 2 Stars as compared to cattle with 0 Stars. Bos Taurus breeds had greater frequency of the T1 marker, while Bos indicus breeds had the lowest (Hetzel, 2003); for example, Brahman cattle have fewer 2-Star and more 1-Star animals than do British breeds.

The T2 test found that for Angus and Santa Gertrudis cattle, the marker for the SNP 316 (single nucleotide polymorphism) marker is consistently linked to tenderness and explains most of the T1 effect (Hetzel, 2003). A higher percentage of Angus cattle were given 3 Stars or 4 Stars than were Santa Gertrudis cattle.

Another tenderness marker, TenderGENE, uses the specific amino acid substitutions at SNP 316 (as in T2) and SNP 530 to rank animals for WBSF tenderness (also as in T2, Genetic Solutions) (Gibb, 2003). The most desirable tenderness combinations involve SNP 316 C and SNP 530 G (see Table 1), although the SNP 530 maker is not recommended for Bos indicus cattle. TenderGENE was developed by the U.S. Meat Animal Research Center and validated by Frontier Beef Systems, GeneSeek, and the NBCEC; this marker test is now owned by Merial.

Table 1 lists T2 and TenderGENE marker results for tenderness yields from SNP 316 and SNP 530 genotypes; genotype score 5 represents the greatest effect on tenderness and genotype score 1, the least.

TABLE 1.

Tenderness Rank	SNP 316	SNP 530	Genotype Score
1	CC	GG	5
1	CC	GA	4
3	CC	AA	3
3	GC	GG	3
5	GC	GA	3
5	GG	GG	3
7	GG	GA	2
7	GC	AA	2
9	GG	AA	1

The reported difference between 316CC/530GG (1st tenderness rank) and 316GG/530AA (9th tenderness rank) was 1.8 lbs WBSF. For Santa Gertrudis cattle, the difference between 316CC and 316GG was 0.84 lbs. WBSF (Quaas, 2003).

Genaissance Pharmaceuticals recently announced a third tenderness marker (White et al., 2005), located near the CAPN1 gene. This new marker can more reliably be documented in both Bos taurus and Bos indicus populations than can the SNP 530 marker.

Collection of DNA

Cattle can be tested for markers from DNA extracted from blood, tissue, semen, or hair follicles. Current testing procedures are relatively expensive ($10 to $45 per test), but the technology increasingly is attracting attention. Each of these DNA tests is offered only by one company, but several companies provide specimen collection services.

Interpreting and Using Results

Producers should exercise caution in the interpretation and use of DNA marker test results, especially with regard to trait selection. The following caveats should be kept in mind:

- None of the markers accounts for all the additive genetic variation (e.g., breeding value or EPD) in the trait influenced by the gene's associated QTL. An animal's phenotypic records and subsequent EPD already may account for some or all of the variation attributed to the marker. Also, because genes with additive effects act in concert, applying selection pressure to any one gene may trigger undesirable interaction effects among genes.

- If other information is available, DNA tests should never be used as the sole selection criteria. However, marker information can be combined with EPD data to aid in selection, adding precision to calculated EPD expected results. For high-accuracy EPD, simply "add" the marker data to the EPD. For example, EPD yearling weight of +25 lbs could be added to the marker for increased yearling weight of +5 lbs to yield an expected yearling weight of +30 lbs. However, DNA tests affect low-accuracy EPD data much more than they affect high-accuracy EPD; thus, adjustment factors associated with DNA tests must be "shrunk" to reflect this disparity, i.e., a high accuracy EPD should be adjusted less as a result of the DNA test. Do not use DNA test results to penalize bulls with relatively high-accuracy EPDs for given traits.

- Adjustments based on DNA markers are not the same for all tests. Such tests affect results from progeny of heterozygous parents more seriously than progeny from homozygous parents. Thallman (2004) suggests conducting simultaneous analysis of DNA test results and phenotypes, resulting in DNA-adjusted EPDs.

- Frequency of any particular marker is unknown for most breeds; such frequency could be quite low, meaning that most animals in the population have only one copy of the gene in question (heterozygous animals). Thus, selecting for homozygous animals could reduce significantly the size of an effective breeding population, increasing in-breeding. Such selection considerations assume even more importance with increasing numbers of tests and the desired QTL combinations associated with them.

- Take care that MAS does not become single trait selection. Greatest increases in performance are usually seen in the first generation produced after marker (or gene) introduction into a population. For highly heritable traits, MAS is much less effective than is traditional selection using EPD. Additionally, because genes usually act in concert, selection for a specific marker can have negative effects on overall genotype.

- Most markers are valid and useful for limited populations, primarily those in which they were discovered, e.g., at this point, principally in Bos taurus breeds. Since it is doubtful that genetic effects will be the same, unbiased parties should conduct genetic validation within and across all breeds and should evaluate economic considerations of any effect discovered (Pollack, 2004).

Use of Heterozygous and Homozygous Bulls

Bulls or cows that are homozygous for a particular gene or marker are rare, minimizing their genetic input (except through AI or ET) to the general purebred population. For example, the frequency of a desired marker in one herd was .02% (.0002). This means that the fraction of homozygous individuals in the herd would be $(0.02)^2$ or .0004; only 1 out of 2500 head – one out of 5000 bulls -- would be homozygous for the desired marker. How can producers counter such impractical situations by using either homozygous or heterozygous bulls?

Homozygous bulls. Consider the following scenario which emphasizes the value of homozygous bulls:

- If no animals in the herd are homozygous, the initial frequency of the desired gene or marker

equals (essentially) zero. Then, using a bull homozygous for the gene or marker increases the gene frequency in the calf crop to 50%, with all calves having one copy of the gene or marker inherited from their sire.

- Mating another unrelated, homozygous bull to the heifers of the first calf crop, all of which are heterozygotes, results in a second generation calf crop that is 50% homozygous for the desired gene or marker and 50% heterozygous. The gene frequency of this second generation is now 75% for the desired gene or marker.

- If another unrelated, homozygous bull is mated to the heifers of the second generation, the third generation calf crop will be 75% homozygous and 25% heterozygous for the desired gene or marker, with a gene frequency of 88%.

- In three generations, the gene frequency has been increased from 0% to 88% and the number of homozygous individuals, from 0% to 75%. However, not all of the animals in the herd are from last-generation production, so, averaged over four generations, each about 4 or 5 years long, the average gene frequency will be 53%, assuming equal population sizes in each generation

Heterozygous Bulls. Consider the same scenario, emphasizing that heterozygous bulls and their contributions to herd genetics should not be ignored:

- The first generation sired by a heterozygous bull produces 50% heterozygous calves and 50% with no copies of the desired gene or marker, yielding a gene frequency of 25%, exactly half that of the homozygous bull's first generation.

- Mating another unrelated, heterozygous bull to the heifers of the first calf crop results in a second generation calf crop that is 12.5% homozygous for the desired gene or marker (50% of each sex, approximately 6.25% homozygous males), 50% heterozygous, and 37.5% without the desired gene or marker. The gene frequency of this second generation is now 37% for the desired gene or marker.

- In the third generation, the proportions between homozygous, heterozygous and "neither" will not change, but the gene frequency for the desired gene or marker will increase to over 41%.

- The average gene frequency over the four generations will be 26%. If the heterozygous form of the gene or marker has any effect, many animals in the herd (approximately 1 in 4) will exhibit that effect (compared to 1 in 2 from using homozygous sires).

Summary

With EPD, producers can select for improvement simultaneously in several economically important traits. In the near future, the beef industry probably will begin using national animal ID to collect data at all marketing points; some of the data collected will improve the number of breeds and animals using EPD and will increase EPD accuracy.

Soon, MAS most likely will be available for a number of economically important markers, allowing seedstock producers to increase selection response over that available solely with EPD. However, producers should exercise caution against single trait selection with either MAS or EPD. And they should remember that MAS, like other new technology, is not a "silver bullet"; it should be used only to enhance other performance information already being collected.

Acknowledgment

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