Multiple-Trait Selection in a Single-Gene World

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Introduction

Sequencing of the bovine genome and the ongoing process of discovery of associations between observable DNA sequence variants and animal performance will be one of the great endeavors of 21st Century cattle breeding. Despite our knowledge, hopes, and dreams, the specific path this developmental effort will take remains largely terra incognita. However, we can make a few informed predictions about some things that probably will occur:

- We will find (and indeed have found) DNA sequence variants that influence, and occasionally control, traits of economic importance;
- We will continue to record performance data and calculate EPDs much as we do today. Performance recording to identify outstanding candidates for selection, and progeny testing of sires, will continue;
- In the future, EPDs will be derived from a combination of performance records and DNA sequence information to provide better genetic predictions, although the precise nature of the predictors and the relative importance of DNA markers are not yet clear.
- The capacity to screen large numbers of animals for substantial numbers of genes (e.g., 10 to 100) in a single assay will emerge, but issues of cost and potential impact remain.

The challenge we face is how to begin to utilize genetic markers with a minimum of wasted effort, without losing useful genetic resources along the way, and in a way that permits the industry and its customers to all reap appropriate benefits. Mature technologies, like BLUP, that we have come to rely on will require a make-over, and the BIF guidelines are going to start getting thicker again. There will be arguments, lies, damn lies, and statistics. It's going to be a great time to be in the cattle business!

Genetic Markers—What Are the Options?

This is hardly a crystal ball. In fact, it is more like a future seen through a glass darkly. However, some types of DNA markers do seem to be emerging as potentially more useful than others for the beef industry. At any rate, we need to become comfortable with the different sorts of markers and be able to recognize their pros and cons. We also need to acknowledge the present and likely future structure of the industry and of National Cattle Evaluation (NCE), involving, as it does, large numbers of individual producers in federation with one another and reliant on others for much of the genetic information generated in the system.

The categories of genetic markers available for use in marker-assisted selection were ably reviewed by Garrick and Johnson in the 2003 Genetic Prediction Workshop. I will use those categories for this presentation.

Gene-assisted selection (GAS).

In this situation, a known quantitative trait locus (QTL) presents two or more alternative DNA sequences and the different sequences have been shown to be associated in a causal way with variation in economically important traits. The different DNA sequences commonly (though not inevitably) differ by a single base substitution and commonly result in both a change in the gene product and a change in the functionality of that gene product. The result is a change in animal performance. Changes such as these are often referred to as functional mutations (changes in DNA sequence that produce changes in gene function), though I prefer to call them functional sequence variants, since the term "mutation" has connotations involving evolutionary history (which is often not known) and often implies an unwarranted distinction between the "normal" and the "abnormal".

The knowledge that a gene affects an economically important trait does not tell us anything about the size of the effect or the importance of the gene in a particular breed or breeding system. Effects may be large or small and must be determined. In a few cases, animal characteristics are exclusively defined by a single gene. Examples include red versus black color, horned versus polled, and various genetic disorders that are often the result of recessive gene action.

One example of a gene that takes several different forms and has a very large effect on a quantitative trait is the myostatin gene. In this case, the common ("functional") form of the gene results in regulation of muscle growth to produce a "typical" muscling pattern. However, several different sequence variants are known to exist in this gene, all resulting in a loss of regulatory function and, when homozygous, in expression of double-muscling, with an associated increase in carcass lean percentage and decrease in marbling score.

Markers in linkage disequilibrium with favorable QTL sequences (LD-MAS).

In this situation, DNA sequence variants have been identified and one or more of the variants has been shown to be reliably associated with differences in animal

performance. The presumption is that these markers are extremely close to an associated functional sequence variant in a QTL and that the favorable marker sequence can be reliably (though not perfectly) used to predict the presence of the favorable QTL sequence. The presence of a tight association between marker and QTL sequences generally suggests that these associations are reflective of evolutionary history and that the association between a favorable marker and a QTL sequences is likely to be consistent within at least some populations, perhaps only within a breed, but potentially (though not certainly) across cattle as a whole.

Confidence in the value and potential for widespread use of LD-MAS markers increases when these markers are known to exist within a gene of biological importance and when the sequence variants that define the marker are "functional" in their own right; i.e., when the different marker sequences result in changes in a gene product. This is certainly not a necessary condition for LD-MAS: the marker can be any detectable sequence variant, so long as it is very tightly linked to the QTL. However, when a marker is found within a gene of known effect, it adds confidence in the value of the marker and seems to be the situation for many of the markers of current interest in cattle.

In an excellent review of marker-assisted selection for beef palatability characteristics in last year's B.I.F. proceedings, McPeake (2003) noted that several of the markers of interest for palatability traits are themselves functional sequence variants within genes that may be anticipated to affect marbling and tenderness. These include sequence variants in both the thyroglobulin gene (which is the basis for the GeneSTAR marbling test) and the leptin gene (which appears to affect appetite and therefore potentially affects fat deposition).

These marker sequence variants may or may not be the actual cause of the observed differences in performance. Garrick and Johnson (2003) chronicled the process by which a marker in the diacylglycerol acyl transferase gene associated with milk composition and a marker in the growth hormone receptor gene associated with milk production were shown to be the actual causal changes in the QTL. However, the conduct of LD-MAS and GAS are not substantially different so long as the markers in LD-MAS are validated for each population and are very tightly linked to their associated QTL sequence variants. Both these elements are absolutely critical. Confidence will be greater in use of markers in GAS but LD-MAS can still be very effective.

Markers in linkage equilibrium with favorable QTL sequences (LE-MAS).

These markers have an association with a QTL, but the direction of the association can vary among individuals and cannot be predicted for the population as a whole. Thus in some families, the marker may have a positive association with performance while in other families the association is negative. As a result, the nature (or "phase") of the association between the marker and the QTL must be determined for each family, and the marker will be used primarily to discriminate among offspring of individual sires. To date, LE-MAS has been used (or at least discussed) mainly in dairy cattle, where elite proven sires can have the marker-QTL phase determined from estimated breeding values of their progeny-tested sons and used in screening additional progeny and grandprogeny for evaluation. In most cases, LE-MAS can be used in some, but not all, families within a population. For that reason, LE-MAS may be useful for evaluation of offspring of individual sires in individual breeding programs, but, barring discovery of a particularly important or interesting LE marker, seems less likely than the markers used for GAS or LD-MAS to be incorporated into NCE.

Assessing the Potential Importance of Genetic Markers

The potential impact of a genetic marker can be assessed in a reasonably straightforward way as the additive genetic variation in the trait of interest that can be attributed to the marker (σ^2_{A-M}). For a codominant marker with two alternative forms (i.e., when the heterozygote is exactly intermediate to the two homozygotes), this may be assessed as:

$$\sigma^2_{A-M} = 2p(1-p)a^2$$

where a is ½ the difference in mean performance between individuals that are homozygous for different marker sequences and p is the frequency of the marker in the population of interest. Analogous equations exist for dominant or recessive markers or when the marker has more than two alternative forms. The potential impact of a genetic marker thus depends on both its effect and its frequency in the population. We can also express the marker's impact as a marker heritability (h^2_M) by dividing σ^2_{A-M} by the phenotypic variance (σ^2_p):

$$h^2_M = [2p(1-p)a^2]/\sigma^2_p .$$

This h^2_M can be compared to the reported heritability of the trait (h^2) to assess how much of the genetic variation can be accounted for by the marker.

For a given marker, h^2_M will be largest if the frequency of the marker is close to 0.5, and h^2_M will decrease if the marker frequency is either very high or very low. Also, as the frequency of a favorable marker is increased to above

0.5 by selection, its future value for further improvement declines. At this time, it is nearly impossible to make general statements about "expected" values for h^2_M. However, if we exclude sequence variants with obvious visual effects (like those in the myostatin gene), we see a few situations where h^2_M might account for up to 25 to 30% of h^2, representing situations where a marker might contribute to, but not dominate, the selection process. For $h^2 = .5$ and $h^2_M = .25\ h^2$ at p = 0.5, h^2_M and h^2 would be expected to decline as the frequency of the marker increases as:

P	h^2	h^2_M
0.50	0.50	0.125
0.75	0.48	0.100
0.90	0.46	0.050

In these calculations, changes in h^2 assume that the total additive variance is also reduced as the marker frequency increases; this may or may not actually happen.

The situation shown above, with relatively rapid change in h^2_M, is different from what we expect from performance-based selection, where h^2 seldom changes noticeably over time. We generally believe that h^2 stays about the same because as selection progresses, new genes or gene variants come into play and the contributions of these variants are automatically picked up in the performance records to bolster and maintain heritability. However, when selection is based on a specific marker, fixation of that marker terminates its utility and continued selection response requires discovery of new markers. The discovery of new markers is anticipated to occur, but it will not necessarily happen in a way that maximizes selection response. Thus we will likely continue to keep performance records for a long time, even for traits that may be difficult to measure. We also usually don't expect h^2 to differ much among breeds, but h^2_M certainly can differ, depending on the frequency of the marker. Thus knowledge of p in the population of interest is extremely important.

Hetzel (2003) reports that individuals that are homozygous for alternative markers in the thyroglobulin gene differ by 3.5 to 11% in marbling score. The phenotypic average marbling score in the Angus database for steer carcasses harvested at less than 480 d of age is about 6.0, with a heritability of 0.36 and a phenotypic standard deviation of about 0.75 (A.A.A., 2004). If the average effect of the GeneSTAR marbling marker is taken to be 8%, that would be equivalent to a value of a (= ½ the difference between homozygotes) of about 0.24 in marbling score. At p = 0.5, that gives $h^2_M = 0.038$. That value seems small, but recognize that a market allows the genotypic value to be directly observed rather than just predicted from phenotypic information. Decisions about the utility of marker information are best made based upon the size of the marker effect and the frequency of the marker in the population of interest. The value of h^2_M, however, gives an idea of the extent to which the marker accounts for σ^2_A. In this example, GeneSTAR would account for only about 11% of the additive variance in marbling score. Put another way, if the total additive variance for marbling score is 0.27, it would require 9 independent genes with effects similar to those of the GeneSTAR marker to explain all the additive variance in marbling score. This result is not surprising given the size of the GeneSTAR marker effect and shows that while the GeneSTAR marker may be a useful tool, lots of other opportunities to improve marbling scores remain.

We can anticipate the discovery of additional genetic markers for various traits over the next few years, and the discovery process will likely expand with sequencing of the bovine genome. Issues of additivity of marker effects will soon arise. The GeneSTAR markers now include three separate sequence variants, leading to 27 possible genotypic classes. If we add a leptin marker, we get to 81 genotypic classes. We cannot assume that effects of multiple markers will be additive, and each will have its own h^2_M, depending on marker frequencies in the population. On the other hand, we should not assume that marker effects will necessarily not be additive, at least on some scale. In sheep, several different genes are known to have major effects on ovulation rate. All of these genes were discovered in different populations, but Davis (2003) reported that when a crossbred ewe was created that carried one copy of each of three of the markers (Booroola, Invermay, and Woodlands), the ewe has ovulation rate of 5 and 8 at 1.5 years of age and 12 at 2.5 years of age. So in this case, these three genes were at least additive in their effects on ovulation rate. But the ewe still only had triplets at her first lambing.

The validation of genetic marker effects in different populations and the assessments of effects of genetic markers on other traits is a critical endeavor. This issue has been addressed in part through the activities of the National Beef Cattle Evaluation Consortium (Quaas, 2003; Pollak, 2004). The validation process is extremely important. We can likely anticipate that as the effect of a genetic marker on a trait of interest increases, the potential for correlated effects on other traits will likewise increase. Thus markers with the largest effects are most easy to use but probably also have the greatest risk of other undesirable effects, whereas markers with smaller effects may be less likely to affect other traits but are harder to incorporate into NCE.

Development of a Scheme for Proactive Incorporation of Genetic Information into NCE

One of the most significant impediments to effective use of genetic markers in NCE relates to the current selective genotyping and reporting of marker information. That situation is likely to get worse before it gets better, but eventually it needs to get better, and we need to start developing a vision of how to make it better. Over the years, the breed associations have become the recognized repository for performance data. In that role, they have provided genetic evaluation services for their members and driven development of new EPDs. If genetic markers are to have a long-term impact on genetic improvement, I believe the breed associations will need to take control of the process in a proactive way that allows them to interact with commercial labs providing DNA testing from a position of strength and with a sustained focus on the needs of their members.

The eventual mix of performance and marker data that will contribute to NCE is still unknown, but it appears likely that the EPD of progeny-tested sires will remain the gold-standard for genetic evaluation for quite a while. Within that context, here are a few suggestions that breeders and their associations might consider.

Identify an array of genes and markers of importance to the breed.

These would include known genes (for GAS) and marker genes with documented associations with performance (for LD-MAS) that are important to a breed. The marker gene arrays might well differ for different breeds. Such an array might also include a set of informative microsatellite markers that could be used for parentage testing and as a way to link newly discovered markers back to older animals. "Several" markers should be identified on each chromosome, with the exact number defined by future technological developments and cost. These microsatellite arrays would probably be breed-specific but might well include a mixture of some markers common to all (or most) breeds and some unique to a specific breed.

Such an array of genes and markers would necessarily have to have the capacity to evolve over time, as new genes or more informative genetic markers are discovered. Inclusion of a set of microsatellite markers would facilitate this evolution by allowing some genotypes for newly discovered genes to be "inferred" from their position and phase relative to the microsatellite markers rather than determined in the laboratory. Techniques for using microsatellite markers to predict probabilities for the various marker genotypes in animals that have not been genotyped have been presented by Thallman et al. (2001) and will continue to develop. Note that identifying the markers of interest does not imply that all animals will necessarily be genotyped for all (or any) of them. It simply means that a target array of potentially useful markers has been identified, providing guidelines for breeders. The decision about how many animals to genotype will likely depend on the development of cost-efficient, chip-based "multiplex" assays that allow genotypes to be determined for many genes in a single assay. The industry has been waiting for this technology to emerge for quite a while, and is still waiting, but its eventual development seems likely.

Develop a DNA collection strategy.

Access to DNA from the influential animals in the breed will be required for widespread use of marker information in genetic improvement, and access to marker information will likewise be required on substantial numbers of their progeny and mates to facilitate marker discovery and validation. Therefore, easy access to DNA from "many" animals in the breed will be required. At the moment, the most promising and economical way to do this seems to be to adsorb several drops of blood onto cards made of fluoroacetate paper. A card might have "several" sections, each containing a few drops of blood that could be cut out and submitted for DNA analysis, thereby allowing repeated analyses of DNA from the same animal when necessary as new markers are discovered. Storage requirements for the cards are very modest, although they do require physical (as opposed to electronic) storage.

Guidelines for which animals to include in this DNA repository will need to be developed. But we should not rule out the possibility that a registration application or a weaning weight record might someday automatically be accompanied by a blood sample. In any case, some sort of breed policy on DNA collection and storage seems warranted.

Develop a genotyping strategy.

If marker information is going to have a widespread impact on NCE, it is important that the breed associations become the repositories for marker information. Effective use of markers will require that certain animals in the breed be regularly genotyped, although we don't yet fully know just who these animals should be. We likewise can anticipate that genotyping will remain selective, although we cannot yet project the numbers of animals that will be genotyped.

We should anticipate that widespread use of a sire would trigger genotyping of that sire for the current marker array and of a sample of his progeny as needed for validation or future gene discovery. Even if a full multiplex DNA analysis costs a few hundred dollars, genotyping of 10 or 20 potential legacy sires each year would be a reasonable endeavor, and when extended to capture the sires and maternal grandsires of many offspring would be a rich source of genetic information. Such a database would also allow rapid predictions of frequencies of newly discovered

sequence variants in different breeds, allowing calculations of h^2_M and assessment of potential contributions of markers to selection response.

Incorporate marker information into NCE.

Incorporation of marker information into NCE will involve a major developmental effort: conceptually straightforward but technically challenging. We have started to scratch the surface, but just barely, and the operational considerations outlined above will greatly influence the probability of success. The objective is to be able to combine performance records with marker data in a way that will both increase accuracy and avoid bias in resulting EPDs. Equally challenging will be the capacity to work with an evolving set of markers; many animals will not have marker information, and those that do will likely often be genotyped for only a few of the available markers. A breed policy of relatively comprehensive genotyping of influential sires (and of their progeny as necessary for marker validation) could improve the situation, but access to comprehensive, consistent marker data across the breed is unlikely to be realized in the short term or necessarily warranted in the long term.

In animals with marker data, a portion of the genetic variation that would normally be incorporated into the EPD can be partitioned off and attributed to the marker genotype. If several markers are available, then several portions of the underlying additive variance can be carved out, but those marker effects will be additive (i.e., the total marker effect will equal the sum of the individual marker effects) only if the markers are independent, both in their location on the chromosomes and in their effects on the trait of interest. For the foreseeable future, the additive variance that exists independently of the markers (i.e., the residual polygenic variance) will remain very important and cannot be overlooked or devalued in NCE. In addition, we must recognize that average marker effect, even when large and clearly significant, will not necessarily be the same for all animals or all sires. Interactions between the marker(s) and the polygenic genetic background should be anticipated and methods to account for variation in expression of marker effects among sires should be considered.

When animals do not have marker data, predictions of EPDs will continue to rely heavily on performance records, but marker data from relatives can provide useful supplemental information. The challenge will be to deal with different subsets of markers among the different ancestors and relatives.

A proactive, breed-centered program to manage and utilize marker data can provide important dividends. Chief among them will be a capacity to focus the gene discovery process in areas that are most important to the breed. As shown above for the GeneSTAR marbling marker, our current markers provide potentially useful but hardly complete indications of genetic merit. The process of gene discovery needs to continue. Even if we were to be successful in finding a marker of very large effect for some trait, fixation of the marker would quickly lead to a need for additional, new markers, or to a return to performance-based selection.

The capacity to monitor and validate marker effects becomes particularly important if a marker should "stop working" or if an outstanding sire emerges that has the "wrong" marker. Even with LD-MAS (and especially with LE-MAS), associations between the marker and the QTL can break down or reverse due to recombination between the two sites. Attention to progeny-test results can allow prompt recognition of such events and trigger a reassessment of marker relationships.

Even with GAS, changes in marker effects can occur. Other, unknown sequence variants, inherited from ungenotyped ancestors, can cause unexpected results. For example, an animal tested for one of the myostatin mutants that causes double-muscling might be shown to not carry that mutation but could still express and transmit double-muscling if it inherited one of the other mutations that are known to exist for that gene.

Conclusions

The purpose of this presentation is not to list things that should be done. Instead, the focus is on things that could be done and should be considered. Selective, comprehensive testing of high-impact sires for available markers seems clearly warranted to provide ready access to frequencies of the different markers within different breeds and to provide the baseline information necessary to properly validate markers in their progeny. Incorporation of marker data into NCE will occur, though the methodology to be used in that incorporation is still emerging.

References

A.A.A. 2004. Angus Sire Evaluation Report - Spring 2004. American Angus Association, St. Joseph, MO. Available: http://www.angus.org/sireeval. Accessed Apr. 5, 2004.

Davis, G. 2003. Major genes affecting ovulation rate in sheep. Proc. Intern. Workshop on Major Genes and QTL in Sheep and Goats, CD_ROM communication 2-06, INRA, Toulouse, France.

Garrick, D. J. and P. L. Johnson. 2003. Examples of marker-assisted selection in sheep and cattle improvement in New Zealand. Proc. Genetic Prediction Workshop, pp. 5-23. Available: http://www.beefimprovement.org. Accessed Mar. 31, 2004.

Hetzel, J. 2003. GeneSTAR markers—delivering productivity improvement to the beef industry. Proc. Genetic Prediction Workshop, pp. 30-34. Available: http://www.beefimprovement.org. Accessed Mar. 31, 2004.

McPeake, C. A. 2003. Marker assisted selection for beef palatability characteristics. Proc. Beef Improvement Fed. 35th Annu. Res. Symp. Annu. Meeting, Lexington, KY, May 28-31, pp. 67-73.

Pollak, E. J. 2004. Validation of DNA testing for carcass traits. Brown Bagger Series 2—Beef Cattle Genetics: Fine-Tuning Selection Decisions, U. S. National Beef Cattle Evaluation Consortium. Available: http://www.nbcec.org. Accessed Apr. 3, 2004.

Quaas, R. L. 2003. Validating genetic tests for quantitative traits. Proc., pp. 24-29. Available: http://www.beefimprovement.org. Accessed Mar. 31, 2004.

Thallman, R. M., G. L. Bennett, J. W. Keele, and S. M. Kappes. 2001. Efficient computation of genotype probabilities for loci with many alleles: II. Iterative method for large, complex pedigrees. J. Anim. Sci. 79:33-44.