

CARCASS MERIT PROJECT: DNA MARKER VALIDATION

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The Carcass Merit Project (CMP) was initiated in 1998 stemming from concern over the frequency of unsatisfactory eating experiences due to inadequate tenderness. The project was funded by America's beef producers through the \$1 per head checkoff, by the participating breed associations, and by MMI Genomics, Inc.

The primary goal of the project was to provide the tools and mechanisms to genetically identify superior animals in the U.S. beef cattle population that will produce progeny with the greatest potential for meeting the demands of consumers. The participating breed associations generated individual databases that allowed the development of EPDs for important carcass traits, including Warner-Bratzler shear force (WBSF) and sensory attributes. In addition, genetic markers for economically important carcass and consumer satisfaction traits were validated in the general U.S. beef cattle population. The markers evaluated resulted from previous checkoff-funded research at Texas A&M University (TAMU).

At completion, carcass data have been collected on over 8,200 progeny of project sires. DNA marker analysis was completed on progeny of 70 sires representing 13 breeds.

Four universities, the USDA Agricultural Research Service, and 13 breed associations cooperated with the National Cattlemen's Beef Association (NCBA) on the Carcass Merit Project. Shear force and sensory panel data was collected at Kansas State University. MMI Genomics performed the primary DNA laboratory work for the study. Dr. Dan Moser of Kansas State University acted as the facilitator and liaison to the breed associations.

Texas A&M University conducted the quality control testing for the project and performed the DNA marker statistical analysis on an individual sire basis. An independent validation of the TAMU analysis, as well as a breed-wide and project-wide analysis of marker data was performed by the U. S. Meat Animal Research Center. A secure data-base containing all the relevant data for the project has been maintained by Cornell University. Colorado State University economists have estimated economic returns to producers using carcass EPDs for cattle selection.

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Project Objectives

- Generate data from which genetic evaluations for tenderness and other sensory traits could be computed.
- Develop methodology and procedures for collection of information necessary for further development of EPDs for carcass traits.
- Validate DNA markers discovered in previous checkoff-funded research at TAMU for use in industry-wide marker-assisted selection programs for improvement of carcass traits.
- Measure costs and returns of implementing EPDs for carcass traits for the alternative genetic selection programs and combinations of management x genetic improvement of carcass traits.
- Breed comparison was strictly precluded from being an objective.

Project Design

The logistics for the project are described in Figure 1. All US beef breed associations were invited to participate. The following breeds participated in the project:

- Angus
- Brahman
- Brangus
- Charolais
- Gelbvieh
- Hereford
- Limousin
- Maine-Anjou
- Red Angus
- Salers
- Shorthorn
- Simmental
- Simbrah
- South Devon

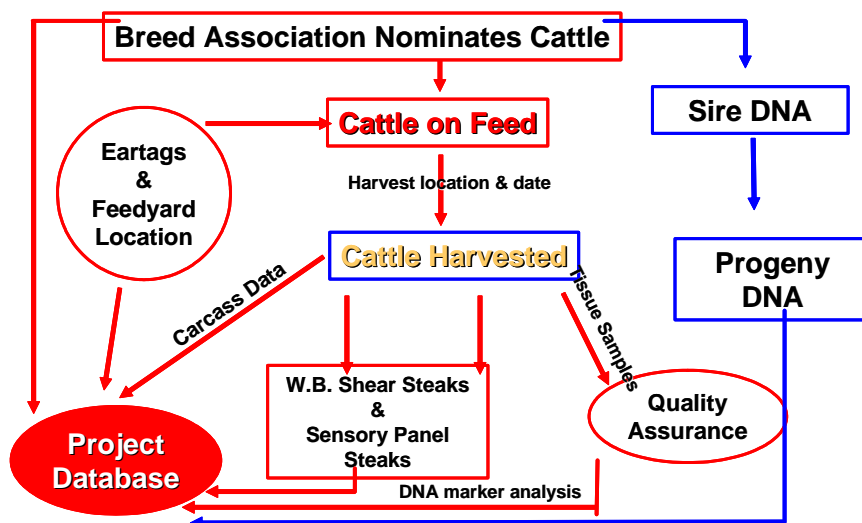


Figure 1. Carcass Merit Project Flowchart

Commercial cows were inseminated to several of the most widely used AI sires of each of the breed associations cooperating and supporting the research project. It was the responsibility of each breed association to select the sires and provide the leadership and all costs associated with nominating cattle for the study. The associations were also responsible for the semen, AI, collection of feedlot performance data, blood collection, shipping of blood samples, and the development of EPDs for their respective breeds. Breed identity was coded to prevent breed associations and/or breeders from comparing breeds.

Up to ten bulls from each breed were designated as “DNA sires.” Fifty progeny of each of these sires were used for DNA analysis and shear force measurements. Five of the

ten DNA sires for each breed were designated “sensory sires.” Sensory panel data were collected on all fifty progeny of each of the sensory sires. Additional bulls were allocated by breed based on registration numbers for EPD analysis. Twenty-five progeny of each of these “EPD sires” were allotted for shear force measures.

Progeny were fed at several locations and ultimately harvested at several cooperating processors. Age at which cattle were started on feed and other appropriate information were collected by each participating breed association. Breed associations were encouraged to minimize the number of contemporary groups and to harvest each group in a single day, whenever possible.

Carcass data, including carcass weight, ribeye area, fat thickness, marbling score and percentage of internal fat, were collected. In addition, researchers obtained one steak from each progeny of every sire and two steaks from each progeny of the DNA sires designated for the sensory panel component of the project. Steaks were shipped to Kansas State University to collect WBSF values and for trained sensory panel evaluation. Steaks measured for shear force were cooked fresh at 14 days post-mortem, whereas sensory panel steaks were frozen and later thawed for trained sensory panel evaluations. Overall tenderness (OT) is a linear function of two sensory panel traits, myofibrillar tenderness (MT) and connective tissue tenderness (CT). Higher scores for all sensory traits, which also include flavor and juiciness, are more desirable. Because WBSF is the force required to shear through cooked meat, higher values are less desirable.

The project was not designed to provide comparisons among breeds and consequently, no valid breed comparisons can be drawn. A breed’s average relative to the overall project average is due to management as much as genetics. Furthermore, some breed associations bred their sires to cows of the same breed, while other breed associations used cows of breeds known to be above average for tenderness. There is not a reasonable statistical approach to adjust for these differences in genetics of the cows.

Phenotypic Results

Analysis of the phenotypic data showed significant variation among all breeds for shear force. Ranges of average shear force values for sires within breeds were from 1.90 lb. to 6.62 lb (Dikeman *et al.*, 2003), indicating that every breed has significant variation in tenderness, and opportunity to improve this trait.

Greater than 8,200 progeny of over 300 sires representing 14 breeds were harvested for collection of carcass and meat quality data. The analysis excluded data from 883 progeny because of incorrect animal or carcass identification. There were 7,319 progeny used in carcass and WBSF analyses and 2,422 progeny with sensory panel data.

Carcass traits of the project cattle were representative of the beef industry with average hot carcass weight of 771 lb, fat thickness of 0.48 in, ribeye area of 13.2 in², yield grade of 2.8, and marbling score of Small²⁰.

Although the cattle were young, mostly from AI sires, and managed optimally, 26% of the steaks had WBSF values > 11.0 lb (considered tough) and 19.4% had sensory panel tenderness scores of < 5.0 (5 = slightly tender; 4 = slightly tough).

Heritabilities and Genetic Correlations

Data from 2,615 progeny of 70 sires were used to estimate heritabilities and genetic and phenotypic correlations (Table 1) using an animal model with relationships among sires (dams were assumed unrelated) in a series of 4-trait analyses. The identity and paternity of these carcasses were verified by DNA marker data. The genetic correlations between WBSF and the sensory panel tenderness scores are highly negative (favorable) and therefore WBSF is a useful measure of tenderness. The genetic correlations between marbling and sensory tenderness are much closer to zero. Furthermore, WBSF is a heritable trait, and hence, it will respond to selection. Therefore, EPDs for WBSF can be computed for all sires in the CMP and can be generated on an ongoing basis if new phenotypic information is generated. Four breeds (Simmental, Simbrah, Shorthorn, and Hereford) have published shear force EPDs mostly based on data collected in this project. The CMP data has augmented the carcass EPDs of many breeds and has allowed one breed, (Maine-Anjou) to publish its first carcass EPDs of any kind.

Table 1. Heritabilities and genetic and phenotypic correlations.

Trait Name	Trt	WBSF	MT	CT	CL	FL	JC	MB	FT	KPH	HCW	REA
Shear Force	WBSF	0.43	-0.99	-0.79	0.41	-0.65	-0.65	-0.56	-0.28	0.47	0.02	0.23
Myofib Tnd	MT	-0.68	0.29	0.92	-0.26	0.79	0.74	0.38	0.14	-0.86	0.20	-0.51
Cn Tiss Tnd	CT	-0.63	0.82	0.25	-0.24	0.74	0.62	0.19	0.22	-0.82	0.38	-0.53
Cooking Loss	CL	0.27	-0.06	-0.04	0.14	-0.16	-0.21	-0.72	-0.16	-0.02	0.16	0.28
Flavor	FL	-0.14	0.24	0.23	-0.03	0.18	0.98	0.35	-0.23	-0.61	-0.17	-0.63
Juiciness	JC	-0.05	0.26	0.14	0.02	0.43	0.29	0.56	-0.11	-0.39	-0.14	-0.66
Marbling	MB	-0.23	0.21	0.13	-0.14	0.14	0.20	0.76	0.20	-0.19	-0.27	-0.36
Fat Thick	FT	-0.10	0.07	0.09	-0.08	0.05	0.02	0.22	0.24	0.37	0.27	-0.19
Internal Fat	KPH	0.01	-0.03	-0.02	-0.04	-0.06	-0.08	0.03	0.13	0.42	0.76	0.37
Hot Carc Wt	HCW	-0.07	0.12	0.15	0.02	0.03	-0.01	0.10	0.31	0.19	0.24	0.24
Ribeye Area	REA	0.07	-0.06	-0.04	0.08	-0.05	-0.08	-0.04	-0.11	0.06	0.41	0.30

Heritabilities are on the diagonals in bold black, genetic correlations are above the diagonals in black, and phenotypic correlations are below the diagonals in blue.

DNA Component of the CMP

The objectives of the DNA component of the CMP were to validate and characterize 11 quantitative trait loci (QTL) for carcass and meat quality traits that were discovered in previous checkoff-funded research at TAMU (the Angleton Project). The Angleton Project used a resource population (Figure 2) comprised of greater than 600 progeny in large, full-sib families (produced by embryo transfer) of a double, reciprocal backcross design between Angus and Brahman.

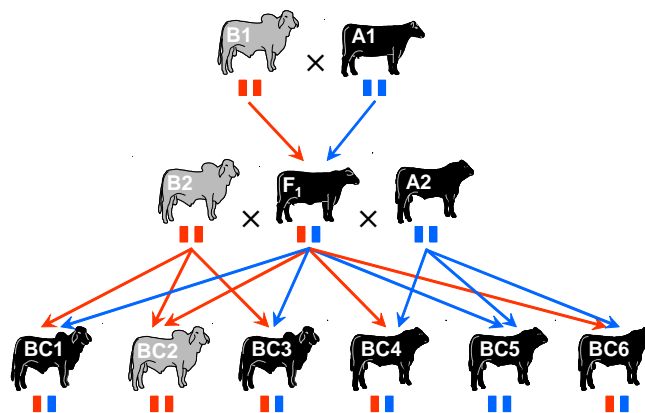


Figure 2. Design of Angleton Project

Validation of QTL discovery projects is necessary because of the substantial risk of false positive results, even in large, well-designed projects. However, failure to validate a QTL does not necessarily imply that the QTL was a false positive; it may simply mean that the QTL was segregating in the resource population used for discovery, but not in the population used for validation.

Characterization of QTL involves determining which QTL are segregating in each breed, how many sires per breed appear to be segregating for each QTL, and which traits are affected by each QTL. In other words, characterization seeks to determine the potential utility of the QTL in genetic improvement programs.

Segregation of QTL in the CMP occurs within paternal half-sib families (Figure 3). Some sires segregate QTL, but many are homozygous at the QTL. The QTL analysis involved 70 sires with 2,615 progeny with DNA marker data and phenotypes in 210 contemporary groups. There were 1,458 progeny with sensory data and DNA marker data.

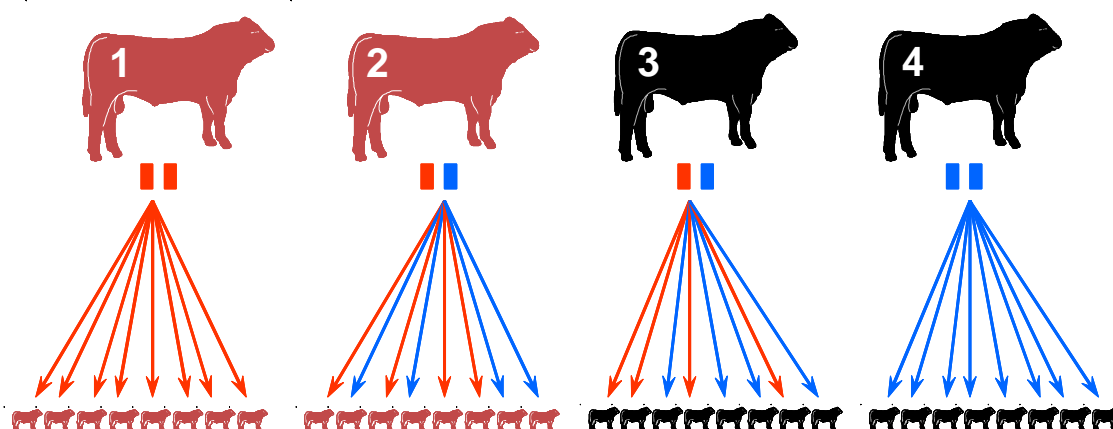


Figure 3. Segregation of QTL in Paternal Half-Sib Families

Individual Sire Analysis

As marker data were collected on the progeny of each sire, an analysis of the sire's progeny was performed and reported to the respective breed association. Figure 4 shows an example of such a report. Each QTL was evaluated for the trait for which it showed the greatest association in the Angleton project. The model used was:

$$y_{ij} = X_j\beta_j + Q_{ij}\alpha_{ij} + e_{ij}$$

where y_{ij} is a vector of observations on the progeny of sire i for trait j , X_j relates observations to contemporary groups, β_j is a vector of fixed contemporary group effects, Q_{ij} is a vector of probabilities that each progeny of i inherited QTL allele A of sire i minus the probability it inherited QTL allele B from its sire, α_{ij} is the fixed within-sire effect of QTL allele A minus the effect of QTL allele B on trait j , and e_{ij} is a vector of residuals.

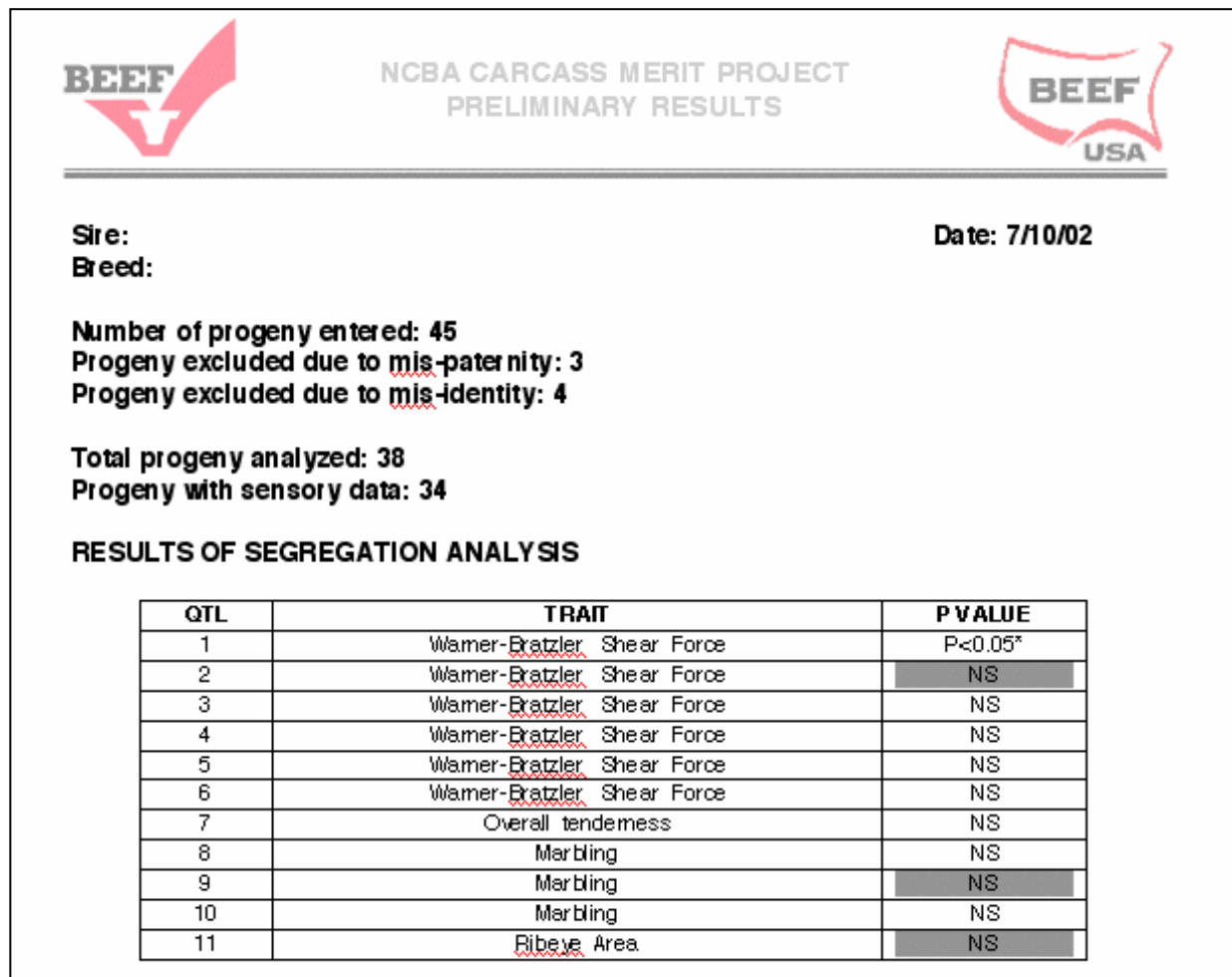


Figure 4. Example of an Individual Sire Report

Overall Analysis – Single Trait, Fixed QTL Model

Upon completion of data collection, the entire dataset was analyzed together to determine which QTL were segregating and which traits they influenced. The first model used was:

$$\mathbf{y}_j = \mathbf{X}_j\boldsymbol{\beta}_j + \mathbf{Z}_{sj}\mathbf{s}_j + \mathbf{Q}_j\boldsymbol{\alpha}_j + \mathbf{e}_j$$

where \mathbf{y}_j is a vector of observations on trait j , \mathbf{Z}_{sj} relates observations to sires, \mathbf{s}_j is a random vector of residual polygenic breeding values of sires, \mathbf{Q}_j is a block diagonal matrix of the \mathbf{Q}_{ij} 's, $\boldsymbol{\alpha}_j$ is a vector of the α_{ij} 's (one element per sire), \mathbf{e}_j is a vector of residuals and the remaining terms are as previously defined. Sires were considered unrelated and \mathbf{Q} was computed at the QTL position estimated in the TAMU Angleton project. The \mathbf{Q} matrices were computed by an extended version of the GenoProb software (Thallman *et al.*, 2001a,b; Thallman *et al.*, 2002).

Table 2 contains significance levels from the fixed QTL analysis. It addresses the question “Does the QTL have an effect on the trait and is it segregating in the set of sires that were sampled in this breed?” A value of + indicates weak evidence ($P < 0.10$) that the QTL affects the trait. A value of ++ indicates moderate evidence ($P < 0.05$) and +++ indicates strong evidence ($P < 0.01$) that the QTL influences the trait. An empty cell indicates that we do not have sufficient evidence to conclude that the QTL influences the trait, but it does not imply that we have evidence that the QTL does not influence the trait (it could be that we simply do not have enough evidence to decide). No QTL should be expected to influence every trait, but we should expect that some QTL will influence several traits. Therefore all QTL were tested for effects on all of the traits.

Table 2. Significance levels from Fixed QTL analysis

Level of Significance	Quantitative Trait Locus										
	1	2	3	4	5	6	7	8	9	10	11
Shear Force						+++		++			
Overall Tend.						++		++			
Myofibrillar Tend.						+		++			
Conn. Tiss. Tend.						+++		+			
Juiciness				+		+++	++			+	
Flavor									+	+	
Marbling											+++
Ribeye Area			+			++	++		+		
Fat Thickness				+++		++					
Hot Carc. Wt.							+++	+++			
KPH Fat		+				++					
Yield Grade	++					+					

+ ($P < 0.10$)

++ ($P < 0.05$)

+++ ($P < 0.01$)

Table 3 presents the number of sires with highly significant QTL effects for each trait by QTL combination. It addresses the question “Is a specific sire segregating at the QTL?” and then counts the affirmative answers for each trait. It is the number of sires in the analysis that have strong evidence ($P < 0.01$) of the QTL segregating for the indicated trait. A significant overall result can occur from either a few sires with strong evidence or a larger number of sires with weaker evidence. Therefore, it is useful to look at the results from both perspectives.

Table 3. Number of Highly Significant Sires

No. of Highly Sig. Sires	Quantitative Trait Locus										
	1	2	3	4	5	6	7	8	9	10	11
Shear Force	2	1	1	1	1	6	1	3	1		
Overall Tend.	1	1			1	1	1	2		3	
Myofibrillar Tend.		1			1	1	2	2		1	
Conn. Tiss. Tend.	1	1			1	3	2	1		2	
Juiciness	1	1	2	2		3	1	1	1	2	
Flavor			1		1		1				1
Marbling		1	1	1	2		1	1	1	2	1
Ribeye Area	1		3		1	3	3		1	1	
Fat Thickness	1			3	1	1	1	2	1	2	2
Hot Carcass Wt.	1		2		1		4	4	1	1	3
KPH Fat	1	2		2	2	3				2	1
Yield Grade	2			1	1	2				1	1

Analysis of the marker data with a single trait model fitting QTL as fixed effects validates that at least some of the previous QTL have significant effects, and are good candidates both for marker-assisted selection, and for further study. Most QTL seem to have pleiotropic effects, where the QTL influence two or more traits.

Breed Analysis

Marker data were also analyzed by breed to validate which markers were segregating in each breed, as well as in the overall population. Tables similar to the previous two were constructed for each breed and distributed to the respective breed associations. As expected, significance of marker effects varies widely across breeds, indicating the degree of heterozygosity for each QTL also varies by breed. However, because there were only between one and ten sires per breed, the within-breed analyses are not generally very powerful. More emphasis should be placed on the combined analyses for evaluating the effects of the QTL.

Multiple Trait, Random Regression QTL Model

The single trait analyses presented above involve a very large number of statistical tests. With so many tests, it is expected that a number of false positive results would occur. Because the number of significant results exceeds the expected number of false positives substantially, it appears likely that at least several of the QTL evaluated are segregating in the CMP families. However, it is not clear which ones are real and which are spurious.

There are several sources of information that are not considered by the single trait analysis.

- Most QTL probably influence a number of traits, to different degrees. In fact, due to chance, a real QTL would probably show significance in the single trait analysis for some, but not all, of the traits that it influences. Obviously, those traits that it has the greatest influence on are more likely to show significance.
- If a sire is segregating at a real QTL, then his progeny that inherit QTL allele A instead of allele B should differ for all traits that the QTL influences. The magnitudes of those differences should be in proportion to the degree of influence that the QTL has on each of those traits. Furthermore, if allele A is defined as the allele with favorable effect on the trait most influenced by the QTL, then the directions of differences between alleles A and B for secondary traits should tend to be consistent across sires.
- If a QTL is not real, then in a single trait analysis, we should not be surprised if one set of sires contributes evidence of segregation for one trait and a different set of sires contributes evidence of segregation for another trait. Furthermore, the directions and magnitudes of differences between alleles A and B for secondary traits would likely be inconsistent across sires, although residual correlations will prevent complete independence of these effects.

The single trait analysis cannot take the above factors into account, but these factors are considered by a multiple trait analysis in which the QTL effects are fit as random. Such a model was implemented to help discern the real QTL from the false positives.

Unfortunately, the multiple trait, random QTL model increases the computational requirements by several orders of magnitude and software to implement it is more specialized than that required for the single trait analysis.

Given the simple structure of the CMP pedigree, a random regression model similar to the fixed effects model described previously is a logical choice. The random regression model has essentially the same terms as the fixed effects model:

$$\mathbf{y}_j = \mathbf{X}_j\boldsymbol{\beta}_j + \mathbf{Z}_{sj}\mathbf{s}_j + \mathbf{Q}_j\mathbf{a}_j + \mathbf{e}_j$$

where \mathbf{a}_j is a vector of the random effects of sire QTL allele A minus allele B, nested within sire, and the remaining terms are the same as previously defined. However, in this model, records for t different traits are analyzed simultaneously. The (co)variance of sire effects can be represented as $\boldsymbol{\Sigma}_s \otimes \mathbf{I}$, where $\boldsymbol{\Sigma}_s$ is a matrix of sire (co)variance parameters among traits and the (co)variance of QTL effects, \mathbf{a} , can be represented as $\boldsymbol{\Sigma}_q \otimes \mathbf{I}$, where $\boldsymbol{\Sigma}_q$ is a matrix of QTL (co)variance parameters among traits and \otimes represents the operator for the Kronecker product of two matrices. The (co)variances of the residuals of a progeny with all traits measured are represented as $\boldsymbol{\Sigma}_e$. Sires were considered unrelated.

The size of the mixed model equations (MME) is the same per trait as for the model in which the QTL were considered fixed (210 equations for contemporary groups, 70 for sire breeding values, and 70 for QTL segregation effects, for a total of 350 equations per trait). Because the computations required to invert a matrix are roughly proportional to the cube of its size, two, three, and four trait analyses require roughly 8, 27, and 64, respectively, times as much computation as a single trait analysis. Furthermore, the single trait, fixed QTL analysis required estimation of only two variances, whereas two, three, and four trait analyses require estimation of 9, 18, and 30 (co)variance parameters, respectively. Consequently, the number of iterations required for variance component estimation is considerably greater with more traits.

This model was initially attempted with the Mixed Procedure of SAS. However, it would run only if the residual correlations were constrained to zero ($\boldsymbol{\Sigma}_e$ was forced to be diagonal). This compromise was accepted because those correlations were not of primary interest and were thought to only help to reduce the noise in the analysis. However, this led to results that looked too good to be true being presented at the workshop. It was discovered, after the December 2003 BIF Genetic Prediction Workshop, that covariances among QTL were able to account for some of the covariance among residuals and this biased upward the estimates of QTL variances and the significance of QTL effects. Subsequently, all analyses have included residual correlations.

After the workshop, a group consisting of Steve Kachman, Janice Rumph, Dick Quaas, Rohan Fernando, Dale Van Vleck, Kathy Hanford, Gerhard Moser (Genetic Solutions, Australia), John Pollak, Dan Moser, Elizabeth Dressler, and Mark Thallman met to discuss statistical and computational options for proceeding with the analysis. Steve

Kachman tested MATVEC (Kachman and Fernando, 2002; Wang *et al.*, 2003) and Janice Rumph tested ASREML for analysis of the CMP data with the random regression model. The ASREML software worked well for some trait combinations, but did not provide estimates when marbling was included in the analysis. The MATVEC software (developed jointly by Tianlin Wang, Rohan Fernando, and Steve Kachman) rapidly provided estimates that agreed with estimates Mark Thallman had obtained by doing a Cholesky transformation of the data to account for residual correlations with SAS (an approach that was slow and that complicated the interpretation of the results).

For each QTL, multiple trait analyses were run at one or two cM intervals throughout the region spanned by the marker data to estimate the position of each QTL from the CMP data. All analyses described subsequently are conditional on those estimated positions.

One of the primary questions to be answered in the CMP project was “which of the 11 QTL could be shown to segregate in the U. S. beef cattle population?” When QTL are fit as random effects, the typical approach to hypothesis testing is to use the likelihood ratio test (LRT) to test whether the variance due to the QTL is zero. However, with multiple traits, there were a variety of hypotheses that could have been tested and it was not clear which of them would be most powerful (have the greatest likelihood of detecting a QTL that is real). Furthermore, testing all possible hypotheses would probably decrease the power. The group agreed on sets of two or three biologically related traits that spanned the economically important traits that were measured.

Another challenge in testing hypotheses with multiple trait models is that the test statistics may depart substantially from the textbook distributions, which are based on asymptotic theory. Permutation testing (Churchill and Doerge, 1994) is one way to determine the sampling distribution of the test statistic, but it requires repeating the analysis thousands of times. Therefore, it is only feasible for analyses that can be conducted very rapidly.

Radu Totir (a postdoctoral researcher working with Rohan Fernando at Iowa State) performed multiple trait hypothesis tests and permutation tests using the random regression model in MATVEC, which is feasible for such analyses. The significance levels resulting from those tests are reported in Tables 4 and 5. Significance levels are the probabilities that the variation accounted for by the QTL is due to chance (probability of obtaining a spurious result). Therefore, smaller numbers indicate stronger evidence supporting the effect of the QTL on a trait.

Several QTL showed significant effects for two or more traits. QTL 6 had significant effects on shear force, overall tenderness, and ribeye area. QTL 7 was significant for ribeye area, hot carcass weight, and juiciness. QTL 8 had significant effects on shear force, overall tenderness, ribeye area, hot carcass weight, and flavor. QTL 10 had significant effects on overall tenderness and juiciness. In addition, QTL 4 and 5 had significant effects on fat thickness, and QTL 11 had noteworthy effects on marbling.

Table 4. χ^2 significance levels from multiple trait hypothesis tests^{a,b}.

Trait	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11
Shear Force	0.50	0.50	0.50	0.32	0.25	0.0003	0.50	0.02	0.16	0.49	0.50
Overall Tnd	0.50	0.50	0.50	0.37	0.44	0.0007	0.50	0.02	0.49	0.003	0.50
Fat Thick	0.10	0.50	0.48	0.08	0.05	0.12	0.20	0.50	0.10	0.34	0.33
Marbling	0.15	0.40	0.44	0.31	0.32	0.11	0.12	0.50	0.06	0.37	0.007
Fat Thick	0.19	0.34	0.50	0.15	0.12	0.23	0.32	0.50	0.17	0.38	0.40
Marbling	0.17	0.41	0.48	0.31	0.21	0.16	0.11	0.50	0.11	0.45	0.01
Internal Fat	0.50	0.08	0.50	0.38	0.16	0.47	0.49	0.50	0.44	0.43	0.44
Ribeye Area	0.50	0.50	0.18	0.44	0.32	0.05	0.009	0.05	0.50	0.50	0.50
Hot Carc Wt	0.50	0.50	0.19	0.47	0.50	0.47	0.01	0.003	0.50	0.50	0.49
Juiciness	0.50	0.23	0.40	0.18	0.24	0.10	0.10	0.50	0.50	0.12	0.38
Flavor	0.50	0.34	0.47	0.26	0.22	0.31	0.28	0.02	0.50	0.10	0.34
Overall Tnd	0.50	0.38	0.42	0.41	0.49	0.02	0.48	0.03	0.50	0.24	0.49
Flavor	0.50	0.45	0.50	0.50	0.28	0.30	0.34	0.03	0.32	0.11	0.33
Overall Tnd	0.50	0.29	0.50	0.50	0.45	0.01	0.35	0.02	0.50	0.24	0.50
Juiciness	0.50	0.33	0.46	0.11	0.50	0.11	0.05	0.49	0.50	0.06	0.41
Overall Tnd	0.50	0.37	0.49	0.31	0.50	0.01	0.45	0.09	0.50	0.14	0.46

^aTwo or three traits were analyzed together as indicated by the groupings in the table. For each cell, the hypothesis tested was that the variance in the indicated trait (and associated covariances) due to the corresponding QTL was zero (with the remaining QTL variances and covariances included in the model).

^bOne additional analysis was run for QTL 8: Shear Force (P<0.03), Overall Tnd (P<0.01), and Hot Carc Wt (P<0.0005).

Table 5. Significance levels from multiple trait permutation tests^a.

Trait	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11
Shear Force						0.008		0.040		n.t.	
Overall Tnd						0.001		0.030		0.004	
Fat Thick				0.043	0.030	0.180			0.200		n.t.
Marbling				n.t.	n.t.	0.110			0.079		0.002
Ribeye Area						0.011	0.008	0.037			
Hot Carc Wt						n.t.	0.006	0.002			
Flavor								0.022			
Overall Tnd								0.015			
Juiciness						0.107	0.024			0.050	
Overall Tnd						0.021	n.t.			n.t.	

^aTwo traits were analyzed together as indicated by the groupings in the table. Only selected hypotheses were tested by permutation. Cells containing "n.t." indicate that a QTL effect for that trait was not included in the analysis; the hypothesis for the other trait is that the QTL influences that trait (1 df).

The analyses were performed in groups of two or three traits, as indicated in Table 4. The hypotheses tested were that the QTL variance (and the associated covariances) for a specific trait were zero. Thus, the full model included QTL (co)variance parameters for all traits in the model, and the null hypothesis model included (co)variance parameters for all traits, except the one being tested. The significance levels in Table 4 are “textbook” values, computed from a χ^2 distribution with degrees of freedom equal to the number of parameters constrained (number of traits) and the resulting probability divided by two to account for the null hypothesis being on a boundary of the parameter space (Self and Liang, 1987; Littell et al., 1996).

As is evident from Table 4, significance levels can differ considerably, depending on the set of traits included in the model. For example, overall tenderness is included in three different two-trait analyses in Table 4. In the cases of QTL 6 and QTL 8, overall tenderness was most significant when paired with another trait with a significant effect on that trait. However, in the case of QTL 10, overall tenderness was most significant when paired with the trait (shear force) that showed the least evidence of being affected by QTL 10.

Table 4 also demonstrates that significance levels can differ substantially when a third trait is added to a two-trait analysis, depending on whether the QTL affects the third trait, or not. In the case of fat thickness and marbling, adding internal fat to the model decreased levels of significance for the first two traits rather uniformly. However, when hot carcass weight was added to the analysis of shear force and overall tenderness for QTL 8 (results not shown), the p-value for shear force increased slightly to 0.03, for overall tenderness decreased slightly to 0.01, and for hot carcass weight decreased substantially to 0.0005.

Tests were also performed for single trait analyses using the random regression model (results not shown). They followed the same general pattern as the single trait fixed QTL tests presented in Table 2.

Permutation tests were performed to obtain more reliable significance levels where the values in Table 4 appeared to at least approach significance. Table 5 contains significance levels from 2,000 permutations for selected hypotheses. A small proportion of the permutations failed to converge and were discarded. In most cases, the values in Table 4 were more significant for a set of two-trait analyses than for the three-trait analyses. Consequently, all of the permutation tests were for two-trait analyses. Where values are presented for both traits in a pair, the hypothesis being tested is the same as was described for Table 4.

However, when one trait in a pair is unaffected by the QTL, the power of detecting the QTL for the second trait is improved by dropping the QTL effect for the first trait from both the alternative and null hypotheses, resulting in a one degree of freedom test for the second trait. In this case, the effect of the QTL on the first trait is not tested. Where this approach was used in Table 5, a significance level is given for the second trait and the first trait is indicated by n.t. (not tested).

More impressive significance levels could undoubtedly have been obtained by testing additional trait combinations, especially trait combinations suggested by preliminary analyses. However, such significance levels would need to be adjusted for the multiple (and perhaps selective) tests of hypotheses that would not be independent. It was not feasible to use permutation testing to obtain appropriate significance thresholds for such an approach.

Nonetheless, an approach that considers the correlated effects of a QTL on a subset (determined by the data) of the traits measured seems to offer the potential for increased power, provided that appropriate significance thresholds can be established. Much work remains to be done on QTL analyses with true multiple trait models.

Multiple Trait, Random, Gametic QTL Model

Another of the primary results of a random QTL analysis is the amount of variance in phenotypes that can be explained by the QTL. This can be most easily interpreted as the proportion of phenotypic variance explained by the QTL, just as heritability is the proportion of phenotypic variance explained by breeding value. In a sire model, the variance due to breeding value is estimated by multiplying the estimate of sire variance by four because every calf inherits exactly half of its breeding value from its sire.

Unfortunately, with the random regression model, the transformation from the QTL variance parameter to the phenotypic variance explained by the QTL is not so simple, because the values in \mathbf{Q} for each calf differ, depending on the amount of information contributed by the DNA markers. Calves with informative markers on both sides of and close to the QTL position have \mathbf{Q}_{ijk} very close to either +1 or -1 (are essentially fully informative, but calves with no marker information have $\mathbf{Q}_{ijk} = 0$ (are completely uninformative) and yet other calves have an informative marker on only one side of, and at varying distances from, the QTL position. The latter calves have intermediate values of \mathbf{Q}_{ijk} and are partially informative. While every calf in the DNA analysis had marker data at most of the QTL, very few of them were completely informative at every QTL.

A fully informative calf has a residual that is “truly residual” to the QTL model, but a completely uninformative calf has a residual variance that consists of the “true residual variance” plus variance due to segregation at the QTL (expected to be half of the phenotypic variance accounted for by the QTL). Partially informative calves have residual variances somewhere in between. Therefore, calves differ in the proportion of phenotypic variance accounted for by the QTL when the random regression model is used.

Furthermore, the relationship between the QTL variance parameter and phenotypic variance explained by the QTL changes with the position of the QTL. This is most evident when there is marker data on only one side of the QTL. The estimated QTL variance increases as the putative distance between the QTL and the markers increases, but the phenotypic variance explained by the QTL must remain the same. A similar phenomenon is well known in the analysis of QTL as fixed effects: as the putative distance between a QTL and markers to one side increases, the magnitude of

the QTL effect estimate increases. It is not possible to distinguish between a modest QTL close to the markers and a large one far away.

Consequently, the random regression model is not well-suited to estimation of the phenotypic variance accounted for by the QTL. However, the gametic model proposed by Fernando and Grossman (1989) is well-suited to this problem because the QTL effect for each progeny is considered in the model, instead of only the sire's expected contribution to each of his progeny at the QTL, as in the random regression model. The multiple trait gametic model is:

$$\mathbf{y}_j = \mathbf{X}_j\boldsymbol{\beta}_j + \mathbf{Z}_{uj}\mathbf{u}_j + \mathbf{Z}_{uj}\mathbf{v}_{mj} + \mathbf{Z}_{uj}\mathbf{v}_{pj} + \mathbf{e}_j$$

where \mathbf{Z}_{uj} relates observations to individuals, \mathbf{u}_j is a random vector of residual polygenic breeding values of the individual, \mathbf{v}_{mj} and \mathbf{v}_{pj} are random vectors of maternal and paternal, respectively, gametic values of the individual at the QTL, and the remaining terms are the same as previously defined. The (co)variance of QTL effects, can be represented as $\boldsymbol{\Sigma}_q \otimes \mathbf{G}$, where $\boldsymbol{\Sigma}_q$ is a matrix of QTL (co)variances among traits. Sires were considered unrelated.

Because the segregation of QTL alleles from sire to progeny is accounted for in \mathbf{v}_{pj} instead of in \mathbf{e}_j , a clean estimate of the phenotypic variance accounted for by the QTL is available. It is equal to twice the appropriate diagonal element of $\boldsymbol{\Sigma}_q$.

The size of the mixed model equations (MME) is considerably greater for the gametic model. There are 2,615 progeny, 2,615 dams, and 70 sires (5,300 individuals) in each of \mathbf{u}_j , \mathbf{v}_{mj} , and \mathbf{v}_{pj} , plus 210 contemporary group equations, for a total of 16,110 equations per trait.

These data were analyzed in a set of four-trait analyses using an extended version of the MTDFREML software package (Boldman *et al.*, 1995). Because MTDFREML uses sparse matrix techniques, the computational requirements do not increase as rapidly with size of the MME as was indicated previously, but the large number of (co)variance parameters to be estimated (30) requires that a large number of iterations be performed. Additional analyses were performed with modified starting values for subsets of parameters to ensure that convergence was achieved. The computational demands of this approach currently preclude its use in permutation testing.

Table 6 contains the estimated percentages of phenotypic variance accounted for by each of the 11 QTL for each trait. The amount of variance explained indicates the magnitude and practical significance of the QTL effect. As is the case for all analyses reported herein, each QTL was analyzed separately; no multiple QTL analyses have been performed. Values of at least 5% are indicated in blue. We should expect much of the variation in truly quantitative traits to be accounted for by a number of QTL that each account for a relatively small proportion of the variation, especially when evaluating data that are pooled over 13 breeds.

As expected, most of the QTL with significant evidence of segregation account for some of the variance in a number of traits. Typically, one or a few closely related traits will be most influenced by a QTL and a number of other traits will be influenced to a lesser extent.

Table 6. Percentage of phenotypic variance accounted for by each QTL.

Trait	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11
Shear Force	1	0	1	3	3	12	0	6	3	1	0
Overall Tnd	0	1	1	1	0	10	2	8	0	4	0
Myofib Tnd	1	2	0	1	0	9	2	8	0	4	0
Cn Tiss Tnd	3	1	0	0	0	12	4	8	0	2	0
Cooking Loss	4	2	5	0	3	1	0	0	2	2	1
Flavor	1	1	0	3	2	1	3	3	2	5	3
Juiciness	0	4	1	6	0	6	7	0	0	5	3
Marbling	1	2	2	2	1	4	4	1	4	1	8
Fat Thick	3	1	3	5	6	4	3	2	2	2	2
Internal Fat	0	7	0	3	5	2	0	1	2	1	1
Hot Carc Wt	1	0	2	0	0	2	6	10	0	1	3
Ribeye Area	0	0	4	2	3	7	7	3	0	1	1

Correlations Among QTL Effects on Multiple Traits

Whenever QTL appear to have effects on multiple traits, it is useful to know whether the allele that is favorable for one trait is favorable or unfavorable for others, or whether there are antagonistic relationships that decrease the difference in net merit between the genotypes of the QTL. To answer this question, correlations among the effects of QTL 6, 7, and 8 are reported in Tables 7, 8, and 9, respectively. These correlations were obtained from a series of 19 four-trait analyses for each of the QTL and each correlation represents the average of from 1 to 6 analyses in which that correlation was estimated. The proportions of phenotypic variance accounted for by the QTL in these tables are each averages of five to seven four-trait analyses.

If a QTL has pleiotropic effects on multiple traits, then it should be expected to influence all of those traits in the progeny of sires that are heterozygous for the QTL and no traits in progeny of sires that are homozygous for the QTL. Furthermore, assuming pleiotropy, the directions of QTL effects should be consistent across heterozygous sires and the magnitudes of effects should at least be proportional, with respect to traits, across sires. If these conditions are met, then the expected values of the correlations of QTL effects among the traits will be either 1 or -1.

Not all genetic correlations are due to pleiotropy; some certainly are due to multiple, linked genes that affect different traits. Therefore, we should not expect all correlations among QTL effects to be 1 or -1, but it does seem reasonable to expect such correlations to tend to be of large absolute value. The effect of the QTL correlations is taken into account in multiple trait hypothesis tests and, thus, the correlations themselves do not directly provide additional information from which to decide which

QTL are most likely to be real. However, the QTL correlations may help to explain why multiple trait tests differ from each other or from the single trait tests.

Table 7. Correlations among effects of QTL 6 (proportion of phenotypic variance accounted for by QTL 6 on the diagonals).

Trait	Trt	WBSF	OT	MT	CT	CL	FL	JC	MB	FT	KPH	HCW	REA
Shear Force	WBSF	0.12											
Overall Tnd	OT	-0.96	0.10										
Myofib Tnd	MT	-0.99	1.00	0.09									
Cn Tiss Tnd	CT	-0.89	0.95	0.96	0.12								
Cooking Loss	CL	0.67	-0.86	-0.84	-0.76	0.01							
Flavor	FL	0.31	0.62	0.32	0.57	-0.57	0.01						
Juiciness	JC	0.11	0.04	0.04	0.27	-0.74	0.79	0.06					
Marbling	MB	0.23	-0.57	-0.41	-0.62	0.90	-0.85	-0.24	0.04				
Fat Thick	FT	-0.40	0.43	0.28	0.03	0.18	0.85	0.70	0.84	0.04			
Internal Fat	KPH	-0.79	0.93	0.99	0.70	-0.90	-0.43	-0.66	-0.19	0.41	0.02		
Hot Carc Wt	HCW	-0.23	0.18	0.02	-0.16	-0.51	-0.35	-0.45	0.87	0.54	1.00	0.02	
Ribeye Area	REA	0.17	0.09	0.14	0.06	0.47	-0.57	-0.77	0.35	0.91	0.21	-0.02	0.07

In the case of QTL 6, all of the correlations among WBSF and sensory tenderness are strong and in the favorable directions. This means the allele that increases shear force decreases tenderness score, where higher tenderness scores indicate greater tenderness. Therefore, selecting for the favorable allele at QTL 6 for shear force will also improve overall tenderness. This, together with the proportion of phenotypic variance (9-12%) accounted for by QTL 6, supports the conclusion that the effects of QTL 6 on tenderness are real, as indicated by the significance levels reported in Tables 4 and 5. This also suggests that all of these effects are likely due to pleiotropic effects of the same polymorphism(s) in a single gene.

Correlations of the effect of QTL 6 on flavor (FL) and juiciness (JC) with tenderness traits were weak, but in the favorable direction. Correlations of internal fat (KPH), fat thickness (FT), and marbling (MB) with tenderness were generally antagonistic.

The estimate that QTL 6 accounts for 7% of the phenotypic variance ($P = 0.011$) in ribeye area (REA) is large enough to be quite interesting. However, the correlations of the effect of QTL 6 on REA with its effects on the tenderness traits are very weak. This

Table 8. Correlations among effects of QTL 7 (proportion of phenotypic variance accounted for by QTL 7 on the diagonals).

Trait	Trt	WBSF	OT	MT	CT	CL	FL	JC	MB	FT	KPH	HCW	REA
Shear Force	WBSF	0.00											
Overall Tnd	OT	-0.99	0.02										
Myofib Tnd	MT	-0.99	1.00	0.02									
Cn Tiss Tnd	CT	-1.00	1.00	0.99	0.04								
Cooking Loss	CL	0.41	-0.33	-0.27	-0.98	0.00							
Flavor	FL	0.98	0.83	0.76	0.84	0.99	0.03						
Juiciness	JC	0.99	0.57	0.60	0.58	0.99	0.97	0.07					
Marbling	MB	-0.84	0.75	0.50	0.70	0.96	0.73	0.55	0.04				
Fat Thick	FT	0.95	1.00	0.92	1.00	0.99	1.00	1.00	0.81	0.03			
Internal Fat	KPH	0.95	1.00	1.00	1.00	0.99	0.99	1.00	0.54	0.98	0.00		
Hot Carc Wt	HCW	-0.98	0.48	0.24	0.60	-0.99	-0.23	-0.23	0.70	0.43	0.20	0.06	
Ribeye Area	REA	-0.99	0.20	-0.09	0.34	-0.99	-0.52	-0.52	0.56	0.12	-0.13	0.96	0.07

would suggest that there may be another gene, in the same general region of the genome as, but in linkage equilibrium with, the gene influencing tenderness. If this is the case, it should be possible to select for favorable effects of both genes. The lack of correlation between the QTL effect on REA and hot carcass weight (HCW) is puzzling, especially in light of the positive remaining correlations among these traits, FT and KPH.

For QTL 7, the significant effects of increased REA and HCW were the result of the same allele, but the allele that increased those traits tended to decrease JC and FL. This allele tended to improve MB and tenderness, although the proportion of variance in tenderness traits accounted for by QTL 7 was low.

Table 9. Correlations among effects of QTL 8 (proportion of phenotypic variance accounted for by QTL 8 on the diagonals).

Trait	Trt	WBSF	OT	MT	CT	CL	FL	JC	MB	FT	KPH	HCW	REA
Shear Force	WBSF	0.06											
Overall Tnd	OT	-0.97	0.08										
Myofib Tnd	MT	-1.00	1.00	0.08									
Cn Tiss Tnd	CT	-0.81	0.97	0.99	0.08								
Cooking Loss	CL	0.90	-0.14	0.00	0.46	0.00							
Flavor	FL	-1.00	1.00	1.00	0.93	-0.99	0.03						
Juiciness	JC	-0.99	0.93	0.99	0.67	-0.99	0.83	0.00					
Marbling	MB	-0.99	0.95	0.87	0.67	-0.92	0.95	0.99	0.01				
Fat Thick	FT	-0.35	0.67	0.15	0.67	-0.58	1.00	0.99	0.78	0.02			
Internal Fat	KPH	0.69	-0.44	0.24	-0.63	0.85	-1.00	-0.99	-0.47	-0.87	0.01		
Hot Carc Wt	HCW	-0.49	0.66	0.61	0.70	-0.14	0.61	-0.60	0.97	0.18	0.01	0.10	
Ribeye Area	REA	-0.81	0.86	0.88	0.91	-0.52	0.55	-0.65	0.98	-0.30	0.48	0.93	0.03

It was especially encouraging that the correlations among effects of QTL 8 were all favorable. The allele that decreased shear force improved overall tenderness, as expected, but was also associated with increased flavor, increased carcass weight, and increased ribeye area.

The effect of QTL 10 on JC is favorably correlated (results not shown) with effects on FL (0.73), MB (0.73), CT (0.86), OT (0.71), and MT (0.61). QTL 10 accounts for 2% or less of phenotypic variances in the remaining traits. The effect of QTL 11 on MB is favorably correlated (0.63) with its effect on JC. The effects of QTL 11 on remaining traits are either weakly correlated with its effect on MB or account for 1% or less of phenotypic variance. The effect of QTL 5 on FT is uncorrelated (-0.05) with its effect on KPH.

There are inconsistencies in the correlations in Tables 7-9 due to the combinations of four-trait analyses that were used, along with the variable number of records per trait. A single 12-trait analysis for each QTL would have provided more consistent correlations, but assuring convergence of four-trait analyses (each with 30 (co)variance parameters) is sufficiently challenging for today's software.

The amount of residual variance associated with phenotypes for economically important traits is one of the most limiting factors in determining which locations in the genome influence those traits. Considering observations for a large number of traits simultaneously, especially when some of the traits are inexpensive to measure, is likely to be an effective way to reduce the impact of this limitation. Therefore, it seems that

statistical developments, both computational and theoretical, in the area of multiple trait analysis of QTL should be an area of emphasis for the next several years.

Interpretation of the results presented here should be made in the context of both the amount of statistical evidence obtained for each QTL on the various traits and the amount of evidence in the literature from other studies.

How Can Cattle Breeders Use the Results?

The most direct and immediate way is for breed associations to compute and publish EPDs for shear force and sensory traits from the data generated by the CMP.

Use of the DNA results is contingent on a partner commercializing tests based on the QTL. This could be done either in the form of direct tests or linked markers.

The existing linked markers could be used to select among progeny and grandprogeny of the 70 legacy bulls that were evaluated in the DNA component of the CMP. While this may seem to be a small number of bulls, these 70 bulls were very influential in their respective breeds and have produced hundreds of thousands of progeny and even larger numbers of grandprogeny.

Linked markers could be commercialized quickly with relatively little development cost and could be used to improve accuracy of selection among progeny of the CMP sires. The technology would probably be used effectively by only a small proportion of the breeders in any breed, but the improved selection response in those herds would likely benefit the entire breed. Some additional development of statistical/computational methods would be required to include marker information in national cattle evaluation.

This approach would also require continued collection of phenotypes and marker data on progeny groups for the approach to be sustainable long-term. However, fewer phenotypes would be required than without the markers and accurate genetic evaluations could be obtained earlier in life (prior to breeding decisions).

The linked markers from the CMP could be used effectively in intensive breeding programs for tenderness, as shown in Figure 5. Young bulls would be progeny test-

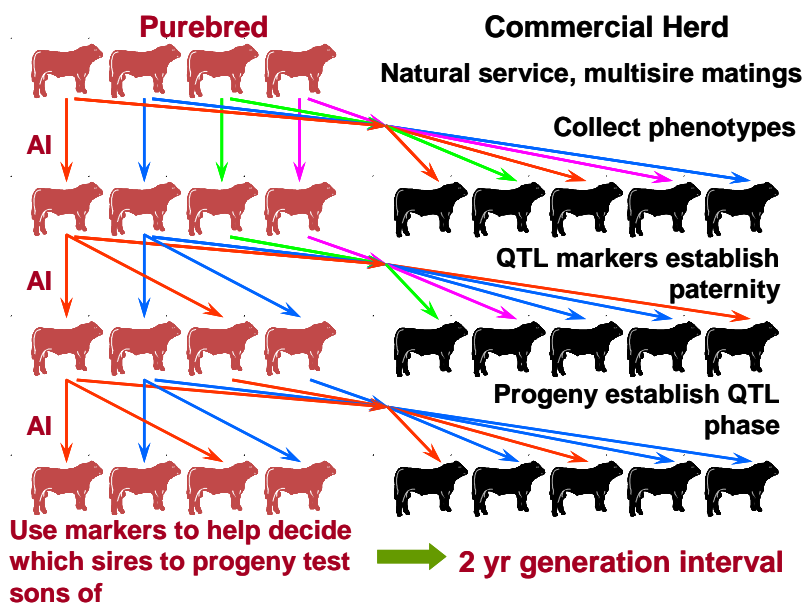


Figure 5. Progeny Testing Scheme

ed in multiple sire matings to commercial cows and, at the same time, would be mated to seedstock cows to produce the next generation of herd sire candidates. Some of the CMP markers would be used to determine paternity of the multiple sired calves. This would not necessarily be any more expensive than a paternity test based on anonymous DNA markers and should be cost-competitive with progeny testing by artificial insemination (AI) matings. The QTL effects of the sires for the markers used in paternity testing could be estimated at no additional cost and those estimates could be used with marker data on seedstock progeny of the tested sires to select the next generation of bulls to be progeny tested. Provided that phenotypes could be collected by about 14 months of age, this approach would allow marker assisted progeny testing at a two-year generation interval with the existing markers.

The Next Steps for Application of the CMP Results

Although there are several scenarios under which the CMP QTL could be used as linked markers, most commercial interest is in association (linkage disequilibrium) or functional tests. Therefore, the most promising QTL should be converted into association tests based on SNP. As illustrated in Figure 6, the CMP population and DNA samples could be an important resource to aid in converting the QTL (using Q6 as an example) into association tests as follows:

1. Identify positional candidate genes under QTL6.
2. Rank sires by evidence of segregation at QTL6.
3. Sequence portions of positional candidate gene(s) in top 8 sires.
4. The best SNPs are heterozygous in the greatest number of top sires.
5. Score those in remaining sires and test concordance with QTL6.

The SNP with greatest concordance could then be used in commercial DNA tests following validation in other populations.

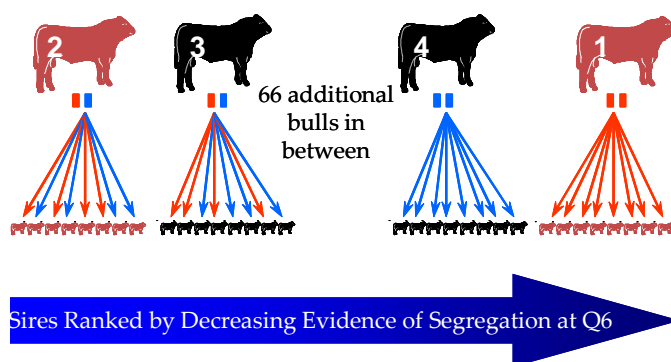


Figure 6. CMP population is a valuable resource for conversion of QTL into Association Tests

Conclusions

The primary objectives of the NCBA Carcass Merit Project were to collect data for carcass merit EPDs, including tenderness, and to attempt to validate previously discovered QTL for carcass merit in the U.S. cattle population. Both of those objectives were accomplished, but much work remains to be done in this area.

Five of the QTL (6, 7, 8, 10, and 11) show solid evidence of segregation in relevant beef industry populations. Two additional QTL (4 and 5) appear worthy of further investigation. The remaining QTL (1, 2, 3, and 9) did not show significant effects on any of the traits evaluated in the study. However, it is possible that the latter QTL have

unique alleles in Brahman and non-Brahman populations. Unfortunately, only six Brangus or Simbrah sires were analyzed in CMP, with a total of 161 progeny.

Besides the stated objectives, several other benefits have resulted from the Carcass Merit Project, both tangible and intangible. The project perhaps represents the greatest cooperative effort ever among U.S. beef breed associations. Experiences gained and goodwill generated in this project will allow further cooperative research by breeds, which will benefit the entire beef industry. The project has also raised the visibility of marker-assisted selection and genomics in the beef industry. The considerable publicity received and educational efforts undertaken by the Carcass Merit Project have moved the industry closer to embracing selection aided by DNA tests, and have improved the understanding of issues with these technologies. In addition, the project has revealed the considerable cost and coordination required for industry-wide tenderness data collection. Furthermore, the CMP has resulted in greater understanding of, and development of methods to address, statistical issues in the validation of quantitative trait loci in populations representative of the U.S. beef industry.

The most significant result of the Carcass Merit Project is the sizeable database of phenotypic information and DNA samples collected from a wide cross section of U.S. beef germplasm. Already, data and samples stored by breed associations are being used to validate DNA tests marketed to U.S. cattle producers. These resources could be extremely valuable tools for converting QTL (both those developed in the Angleton project and in public research) into more easily used association or functional tests. Having a large unbiased resource population, representative of the U.S. beef cattle population, justifies the industry's investment in this project, and stands to be the project's greatest legacy.

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