Selection Enhanced Estimates of Marker Effects on Means and Variances of Beef Tenderness[1]

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Introduction

Historic surveys of retail beef have identified beef tenderness as a critical issue to consumer acceptability of beef and suggested continued investigation of pre-harvest and post-harvest interventions to improve beef tenderness (Morgan et al., 1991). Koohmaraie (1996) identified the protease μ-calpain (***CAPN1***) and its inhibitor calpastatin (***CAST***) as major factors affecting post-mortem tenderization in meat. Genetic markers in *CAPN1* (Page et al., 2002; White et al., 2005) and *CAST* (Casas et al., 2006; Morris et al., 2006) are commercially available to beef producers. However, early studies evaluating these markers had low frequency of rare homozygote animals and occasionally ignored those animals from analysis (White et al., 2005; Morris et al., 2006) – removing the opportunity to evaluate mode of inheritance (additive or dominance) for a genetic marker. Therefore, selection was used in 2 populations (Angus and MARC III – ¼ Angus, ¼ Hereford, ¼ Red Poll, and ¼ Pinzgauer composite) to equalize the allele frequency of *CAPN1* haplotypes and *CAST* genotypes to enhance estimates for slice shear force (**SSF**) of: 1) effect size, 2) mode of inheritance, and 3) interaction between *CAPN1* and *CAST* (Tait et al., 2014a; Tait et al., 2014b). Furthermore, these studies evaluated the potential for genotype specific residual variances and found these models to fit significantly better than single residual variance models for *CAST* genotypes.

Genetic Markers

The *CAPN1* haplotypes evaluated in this study were based on two previously identified SNP: CAPN1_316 (BTA 29; rs17872000) (Page et al., 2002) and CAPN1_4751 (BTA 29; rs17872050) (White et al., 2005). The CAPN1_316 marker segregates C and G alleles, whereas CAPN1_4751 segregates C and T alleles. The CAPN1_316 and CAPN1_4751 SNPs were used to define haplotypes within the *CAPN1* gene. Haplotypes of interest in these studies were: CAPN1_316 allele C with CAPN1_4751 allele C (**CAPN1-CC**), CAPN1_316 allele G with

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CAPN1_4751 allele C (**CAPN1-GC**), and CAPN1_316 allele G with CAPN1_4751 allele T (**CAPN1-GT**). Additionally, a SNP in *CAST* (BTA7; rs109221039) (Casas et al., 2006) segregating C (**CAST-C**) and T (**CAST-T**) alleles was selected to increase the frequency of CAST-C in these populations.

Populations, Selection, and Tenderness Phenotype

Angus and MARC III composite populations from a previous calving ease selection experiment (Bennett, 2008) were chosen for selection of *CAPN1* and *CAST* markers based on initial marker allele frequencies. The Angus population was selected for the 2 *CAPN1* haplotypes expected to be most divergent for tenderness (White et al., 2005) (CAPN1-CC and CAPN1-GT) and MARC III was selected to equalize the 3 most prominent *CAPN1* haplotypes (CAPN1-CC, CAPN1-GC, and CAPN1-GT). Both populations were selected to increase the CAST-C allele. Selection occurred for 3 years (Angus) or 4 years (MARC III), and then 3 years of progeny were evaluated (Figure 1). Haplotype and allele frequencies during the evaluation phase for Angus were: CAPN1-CC = 0.530, CAPN1-GT = 0.363, and CAST-C = 0.348. Haplotype and allele frequencies during the evaluation phase for MARC III were: CAPN1-CC = 0.267, CAPN1-GC = 0.326, CAPN1-GT = 0.385, and CAST-C = 0.397.

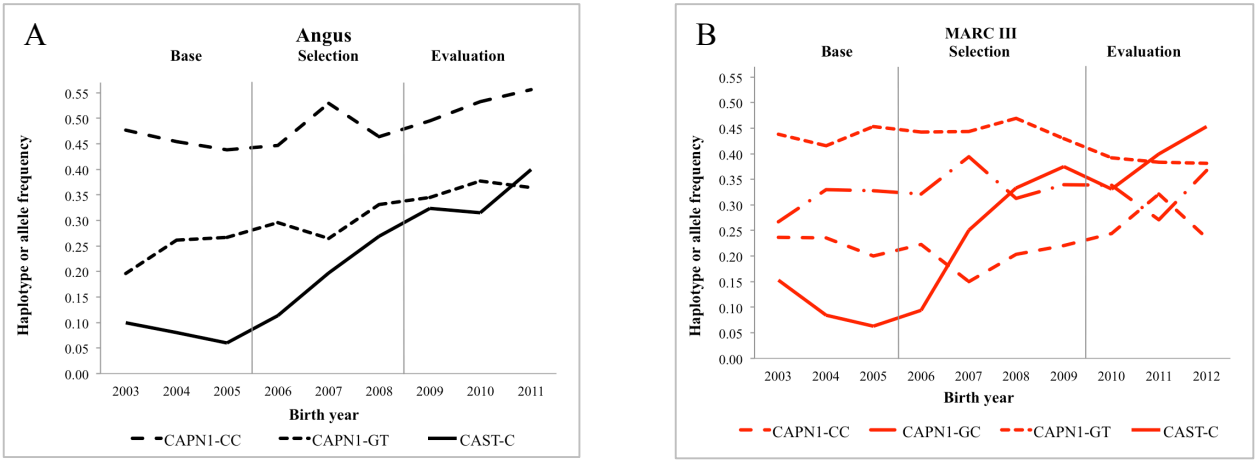

Figure 1. Haplotype or allele frequency by birth year in Angus (A) and MARC III composite (B) populations selected to equalize *CAPN1* and *CAST* genetic markers using marker assisted selection. Adapted from: A – Tait et al. (2014a) and B – Tait et al. (2014b)

Only steers were evaluated for carcass traits (Angus n = 199; MARC III n = 254). All steers within a population were harvested on a single day within each year at a commercial abattoir (Angus average age = 433 d; MARC III average age = 452 d). Carcasses were weighed hot, electrically-stimulated, and chilled using the facility's proprietary system. At 36 h postmortem, carcasses were ribbed between 12th and 13th ribs and camera-measured carcass data were collected. A LM steak from the 13th rib region was returned to the U.S. Meat Animal Research Center to evaluate SSF at 14 d postmortem (Shackelford et al., 1999).

Statistical Analysis

Haplotypes and genotyping errors were identified with GenoProb software (Thallman, 2008). GenoProb genotypes were used for the analysis. A single trait animal model utilized MTDFREML software (Boldman et al., 1995) to estimate the heritability and genetic marker effects within each population independently. Fixed effects modeled were: year of birth (3 yr for each population), age of dam (2, 3, 4, or ≥ 5 yr), covariate age (days) of steer, *CAPN1* diplotype (Angus = 3 classes; MARC III = 6 classes), and *CAST* genotype (3 classes).

Genotype specific residual variance models. Genotype specific residual variance models were analyzed using SAS version 9.3 (SAS Inst., Cary, NC) software, providing the additive genetic variance matrix from the MTDFREML heritability analysis and defining heterogeneous residual variances based on *CAPN1* or *CAST* genotypes in the MIXED procedure. A likelihood ratio test was performed to test whether the genotype specific residual variance model fit better than the single residual variance model.

Results and Discussion

Estimates of *CAST* genetic effects on SSF were significant in both Angus ($P < 0.001$) and MARC III ($P < 0.01$) steers (Table 1). Furthermore, the additive mode of inheritance for *CAST* genetic effect was significant in both Angus ($P < 0.001$) and MARC III ($P = 0.05$) steers, whereas the dominance mode of inheritance was not significant in Angus ($P = 0.43$) nor MARC III ($P < 0.22$) steers (Table 1). The *CAST* genotype additive effects were similar in direction and scale between Angus (-1.257 ± 0.261 kg / CAST-T allele) and MARC III (-0.902 ± 0.464 kg / CAST-T allele) steers (Table 2).

Estimates of *CAPN1* genetic effects on SSF were significant in Angus ($P < 0.001$) but not significant in MARC III ($P = 0.12$) steers (Table 1). The lack of significance in MARC III steers is likely a function of more *CAPN1* diplotypes being evaluated. The CAPN1-GT haplotype effect contrasted to CAPN1-CC haplotype was larger in MARC III steers (1.153 ± 0.483 kg) than in Angus steers (1.049 ± 0.246 kg), but was also less precisely estimated in MARC III steers (Table 2). Furthermore, in MARC III steers, CAPN1-GC was not significantly different ($P = 0.45$) from the average of the CAPN1-GT and CAPN1-CC effects on SSF (Tait et al., 2014b). Therefore CAPN1-GC can be assumed to have ½ the additive effect of CAPN1-GT when contrasted to CAPN1-CC. In both Angus and MARC III populations, no interaction was found between *CAPN1* and *CAST* genotypes ($P \geq 0.40$; Table 1).

Table 1. Significance of *CAPN1* and *CAST* genetic effects, modes of inheritance, and fit of genotype specific residual variance models for 14-day slice shear force in Angus and MARC III cattle populations; Adapted from Tait et al. (2014a) and Tait et al. (2014b)

Type of effect	Angus	MARC III
CAPN1, *P*-Value	< 0.001	0.12
CAST, *P*-Value	< 0.001	< 0.01
CAPN1 × *CAST* interaction, *P*-Value	0.55	0.40
CAPN1 Additive effect, *P*-Value	< 0.001	NA[1]
CAPN1 Dominance effect, *P*-Value	0.19	NA[1]
CAST Additive effect, *P*-Value	< 0.001	0.05
CAST Dominance effect, *P*-Value	0.43	0.22
CAPN1 Genotype specific residual variance model, *P*-Value	0.05	0.03
CAST Genotype specific residual variance model, *P*-Value	2.5×10^{-4}	5.0×10^{-4}

[1]NA = Not available because 3 *CAPN1* haplotypes were selected and evaluated in MARC III population.

Table 2. Estimated genotypic effects (± SE) and variance components for 14-day slice shear force under single residual variance or *CAST* genotype specific residual variance models in Angus and MARC III cattle populations; Adapted from Tait et al. (2014a) and Tait et al. (2014b)

Type of residual variance model	Angus	MARC III
Single		
CAPN1-GT – CAPN1-CC effect, kg	1.049 ± 0.246	1.153 ± 0.483
CAST-T additive effect, kg	-1.257 ± 0.261	-0.902 ± 0.464
σ_g, kg	1.23	1.88
σ_e, kg	1.79	3.58
h^2	0.32	0.22
***CAST* genotype specific**		
CAPN1-GT – CAPN1-CC effect, kg	1.080 ± 0.224	1.081 ± 0.465
CAPN1-GC – ((CAPN1-CC + CAPN1-GT)/2) effect, kg	NA[1]	0.312 ± 0.417
CAST-T additive effect, kg	-1.240 ± 0.341	-0.940 ± 0.553
σ_g, kg	1.23	1.88
$\sigma_{e\text{-}CC}$, kg	2.82	4.86
$\sigma_{e\text{-}CT}$, kg	1.99	3.98
$\sigma_{e\text{-}TT}$, kg	1.22	2.54
h^2_{CC}	0.16	0.13
h^2_{CT}	0.27	0.18
h^2_{TT}	0.50	0.35

NA[1] = Not available because CAPN1-GC haplotype was not evaluated within Angus population

Genotype specific residual variance models were more strongly supported for the *CAST* genotype specific residual variance models than the *CAPN1* genotype specific residual variance models in both Angus ($P = 2.5 \times 10^{-4}$ vs. $P = 0.05$, respectively) and MARC III ($P = 5.0 \times 10^{-4}$ vs. $P = 0.03$, respectively) populations (Table 1). In both populations, the most tender *CAST* genotype (CAST-T homozygote) also had the smallest genotype specific residual variance (Table 2). Furthermore, there was a progressive trend amongst *CAST* genotype specific residual variances where the more tough the expected mean, the larger the genotype specific residual variance (and hence phenotypic variance) (Figure 2). In comparison, *CAPN1* genotype specific residual variance models were not as strongly supported in Angus ($P = 0.05$) and MARC III ($P = 0.03$) populations (Table 1) and the genotype with the smallest genotype specific residual variance was a different heterozygous genotype in each population (Tait et al., 2014a; Tait et al., 2014b).

The economic value in the multi-trait selection objective for *CAPN1* and *CAST* genetic markers should be driven by the risk of an animal with a particular genotype producing beef that is "tough" (above some SSF threshold). Single residual variance models will have a different proportion of animals above some tough designation threshold than *CAST* genotype specific residual variance models and this could have important ramifications for selection emphasis on *CAST* markers depending on which distribution is assumed for the *CAST* genotypes.

The observation of *CAST* genotype specific residual variance models fitting significantly better than single residual variance models in replicated populations provides novel, powerful information about the *CAST* genetic effects on beef tenderness. Additionally, the progressive nature of these residual variances where the most tender genotype has the smallest residual variance and the toughest genotype has the largest residual variance provides a unique opportunity for application or utilization of this marker. This knowledge may someday be extended to national cattle evaluation programs by modeling tenderness to have a different heritability based on genotype at a single genetic marker.

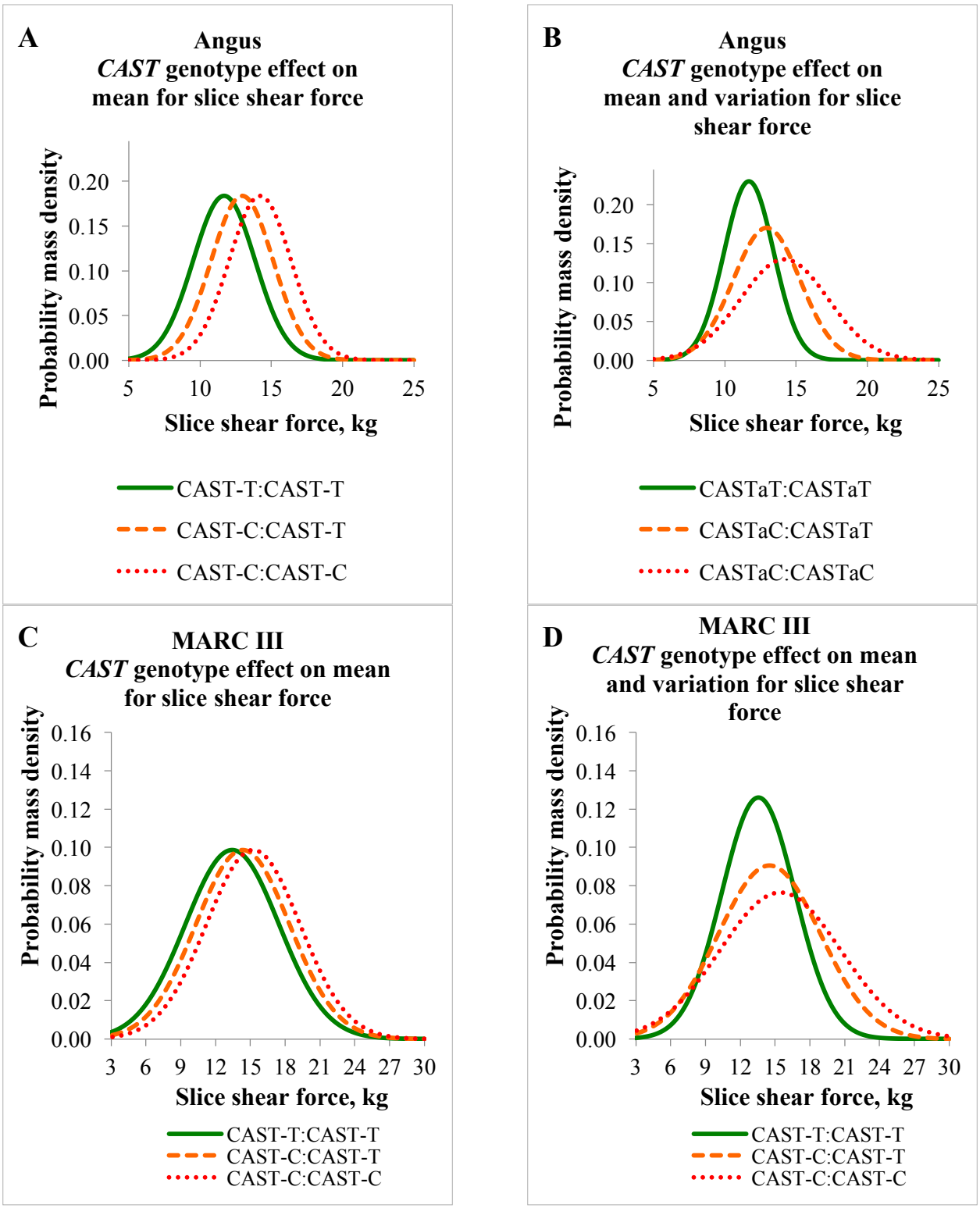

Figure 2. Additive effects of *CAST* genotype on LM slice shear force in Angus (A & B) and MARC III (C & D) populations under single residual variance model (A & C) or *CAST* genotype specific residual variance model (B & D). Adapted from: A & B – Tait et al. (2014a) and C & D – Tait et al. (2014b)

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