

**A STUDY OF CATTLE DISPOSITION:
EXPLORING QTL ASSOCIATED WITH TEMPERAMENT**

A Senior Honors Thesis

by

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ABSTRACT

A Study of Cattle Disposition: Exploring QTL Associated with Temperament (April 2008)

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In any production setting, cattle disposition (temperament) has a great impact on handling and performance. Thus, behavior can be economically important, yielding the rationale for study. Wegenhoft (2005) previously identified several quantitative trait loci (QTL) for disposition, including a partially paternally imprinted QTL at 0cM on bovine chromosome (BTA) 8 that overlaps a region on human chromosome 8 associated with Schizophrenia in humans. The objective of this study was to identify a candidate gene influencing behavior in this region. Two genes from the human Schizophrenia region, bone morphogenetic protein 1 (*BMP1*) and bridging integrator 3 (*BIN3*), were initially chosen because they were reported to be imprinted in humans and mice, and were expected to map to BTA8. Two other genes, cathepsin B (*CTSB*) and farnesyl-diphosphate-farnesyltransferase 1 (*FDFTI*), were chosen as they mapped closer to the predicted QTL location and reported functions suggested a role for these in behavior.

Amplicons from each of these 4 genes were sequenced, using genomic DNA from Texas A&M Angleton resource herd animals, to find single nucleotide polymorphisms (SNP). There were no SNP within the amplicon for *BMP1*, but 3 were found in *BIN3*, 7 in *CTSB* and 4 in *FDFT1*. Complementary DNA was synthesized from total RNA from muscle and liver samples collected at slaughter, and was sequenced to analyze SNP in transcribed regions to investigate the imprinting status of *BIN3*, *CTSB* and *FDFT1*. There was no evidence of imprinting of these genes. Microsatellites within each gene were amplified to genotype the entire population. Genotypes from the Angleton herd were used to update linkage maps for BTA8 and 11. Genotypes from the Texas A&M McGregor Genomics Project herd were used with 133 other markers to construct linkage maps for each of the 29 autosomes in this population. There were QTL for various component traits of behavior (aggressiveness, nervousness, flightiness, gregariousness, and overall disposition) measured at 4 different times (weaning, feeding, slaughter or time of first calving) on BTA3, 6, 12, 16, 26 and 29 under a Mendelian model, and on BTA3, 6, 11, 12, 13, 19, 22 and 29 under a parent-of-origin model.

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INTRODUCTION¹

Among the many traits of importance when evaluating cattle, temperament stands near the top of the list. Cattle disposition affects several aspects of the production process, including individual animal performance and ease of handling. Calmer animals are preferred in most production settings because they are easier to work with and transport, and they have been shown to perform better. Docile cattle have been found to yield a higher average daily gain (Voisinet et al., 1997b; Fell et al., 1999), higher dressing percentages (Petherick et al., 2002) and more tender meat (Voisinet et al., 1997a). Therefore, behavior differences have the potential to be economically important in livestock production, yielding the rationale to study genetic factors affecting temperament.

Behavior has been studied in cattle for decades, although its complex nature and the environmental impacts on phenotype make it quite difficult to determine genetic mechanisms affecting disposition. In recent years, quantitative trait loci (QTL) studies have developed as a way to investigate such complex traits. Most behavioral QTL studies have been performed in mice; few QTL studies have been done in cattle. There have been 3 previous studies in dairy cattle (Spelman et al., 1999; Schrooten et al., 2000; Hiendleder et al., 2003), and 2 in beef cattle (Schmutz et al., 2001; Wegenhoft 2005). Wegenhoft (2005) found QTL associated with disposition in the Texas A&M Angleton Resource herd on bovine chromosomes (BTA) 1, 4, 8, 9, 16 and 18. All QTL had obvious nearby candidate genes, except for that on BTA8.

This QTL at 0 centi-Morgans on BTA8 is the basis for this investigation. The QTL appears to have a large parent-of-origin effect, indicating the existence of imprinting of the gene affecting behavior. Two genes, BMP1 and BIN3, were chosen for study as candidate genes based on their position within the QTL region and reported imprinting status in human and mouse. Two other genes, CTSB and FDFT1, were

¹ This thesis follows the style and format of *The Journal of Animal Science*.

chosen as candidates for study as they mapped much closer to the 0 centimorgan position of the QTL on BTA8, and had reported function that could impact behavior.

Portions of each of these 4 genes were amplified and sequenced to discover single nucleotide polymorphisms (SNP) within transcribed regions. Single nucleotide polymorphisms discovered in transcribed regions were used to investigate the imprinting status of each gene.

Microsatellite markers within each of the 4 genes were also amplified in order to be genotyped in the original Angleton population. These genotypes were used to update the linkage maps of BTA8 and 11 from Wegenhoft (2005).

These microsatellite markers were also amplified and genotyped in the Texas A&M McGregor Genomics Project herd. The scores for these 4 markers were added to those of 133 other markers to construct linkage maps for each of the 29 autosomes in the McGregor population. A QTL analysis was then performed, using the linkage maps constructed and disposition scores from this population.

The results of this study and future work will lead to a greater understanding of genetic mechanisms affecting behavior, which may have a very real and practical application for breeding programs in the beef and dairy industries.

LITERATURE REVIEW

Introduction

Since the beginnings of genetic study, there has been research to find genes responsible for a given phenotype. While some traits are simple, influenced only by a single gene, many more are quite complex and are affected by many genes throughout the genome. Those complex, quantitative traits, such as height and weight, have been investigated for decades, but limited analysis methods have hindered researchers' ability to discover the full complement of associated genes. However, recent advances in genetic mapping, detection methods for quantitative trait loci (QTL), and statistical analyses have enabled researchers to find and study regions of the genome harboring those genes, and hence have allowed more thorough studies of the genetic basis of complex traits.

Behavior, or disposition, is one of these complex traits, and has been studied in cattle for several decades all over the world. Most investigations have studied heritability by looking at individual behavior among parents and offspring through several generations, but recent QTL studies have allowed a more thorough investigation of genes affecting disposition. This review covers previous behavioral genetic research in cattle as well as the 4 candidate genes to be investigated in this study.

Candidate Gene Discovery

A QTL is a region within the genome that contains a gene having some effect on the quantitative trait in question, and often times there are many QTL associated with a single trait (Doerge, 2003). The general methodology for QTL detection and mapping is based on the ability to create linkage maps for a population using several different types of genotypic markers (Doerge, 2003). These markers must be genotyped across the population under study to create genomic linkage maps; the linkage maps are then compared to phenotypic data from the population for the trait under investigation to find associations and assert relative locations of QTL in the genome. To ensure that both genotypes and phenotypes segregate in the experimental population, 2 divergent lines

are often used in the breeding plan (Knott and Haley, 1992; Doerge, 2003). Once these associations have been drawn and QTL locations identified, one can investigate nearby genes in order to determine the candidate gene affecting a specific complex trait.

Quantitative trait loci associated with behavior have been identified in several species. Neiderhiser et al. (1992) used recombinant inbred mice strains to identify QTL associated with different behaviors, including avoidance tendencies, exploratory behavior and mating patterns. A similar study in recombinant inbred strains of *Drosophila melanogaster* investigated locomotor behavior differences, finding 12 QTL and 13 corresponding candidate genes affecting locomotion and activity (Jordan et al., 2006). In honeybees, QTL associated with stinging behavior were mapped in the progeny of an F₁ queen, generated from a cross between a low-defensive European colony and a high-defensive African colony (Hunt et al., 1998). According to Hunt (2007), paternally inherited genes have a greater influence in defensive behavior than maternally inherited genes in reciprocal F₁ crosses.

Studies of Cattle Behavior

Cattle disposition affects several aspects of the production process, including individual animal performance and ease of handling. Calmer animals are preferred in most production settings because they are easier to work with and transport, and they have been shown to perform better. Docile cattle have been found to yield a higher average daily gain (Voisinet et al., 1997b; Fell et al., 1999), higher dressing percentages (Petherick et al., 2002) and more tender meat (Voisinet et al., 1997a). However, despite the great economic importance of disposition in cattle production, relatively few QTL studies have been done, perhaps due mostly to the long generation interval in this species.

Most previous investigations to find QTL associated with disposition in cattle have been performed in dairy cattle, often in conjunction with reproductive studies (Spelman et al., 1999; Schrooten et al., 2000; Hiendleder et al., 2003). Hiendleder et al.

(2003) found QTL on bovine chromosomes (BTA) 5, 18 and 29 for temperament in a German dairy cattle population.

On the other hand, only 2 studies have been done to find QTL and candidate genes associated with disposition and temperament in beef cattle. Schmutz et al. (2001) analyzed heritability as well as microsatellite markers in 130 calves out of 17 full-sib families in the Canadian Beef Cattle Reference Herd and found QTL on BTA1, 5, 9, 11, 14 and 15. There was some attempt in this study to investigate those reported QTL regions for candidate genes. Unfortunately, this study only reports QTL associations for single markers, rather than flanking markers, and confidence intervals are not reported.

Most recently, Wegenhoft (2005) studied a crossbred population of Brahman and Angus cattle that were a Texas A&M resource herd in Angleton, Texas, and took disposition scores at weaning based on a scale of 1 (calm) to 5 (wild). Wegenhoft (2005) performed a whole genome scan using microsatellite markers and found QTL on BTA 1, 4, 5, 8, 9, 13, 16, 17, 18, and 25.

Basis for Proposed Research

Wegenhoft (2005) found QTL on the 10 chromosomes previously mentioned, and all but one QTL region contained obvious candidate genes affecting behavior and temperament. This QTL, located at 0 centimorgans (cM) on BTA8, was also of interest because of a large parent-of-origin (imprinting) effect. Wegenhoft (2005) estimated that this QTL was partially paternally imprinted, which signifies that the allele from the dam is being expressed in the offspring, while the allele from the sire is being partially repressed. Although a candidate gene is not evident in cattle, the QTL overlaps with a comparative region in humans associated with Schizophrenia. Among 9 imprinted genes in the human Schizophrenia region, 3 were found to be paternally imprinted (Nikaido, 2005). These include the genes encoding bone morphogenetic protein 1 (BMP1), bridging integrator 3 (BIN3), and N-acylsphingosine amidohydrolase I (ASAHI). Comparison of sequences for these 3 genes in murine and bovine placed *BMP1* and

BIN3 on BTA8, but not *ASAHI*. Therefore, *ASAHI* was not pursued for further study. Thus, the choices for initial research were *BMP1* and *BIN3*.

Candidate Genes Under Investigation

The *BMP1* gene, localized to chromosome 8q21 (Martin-Burriel et al., 1997), at approximately 60.2Mb (Bovine Genome Database, Build 3.1; <http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/>), codes for a Ca^{2+} -dependent metalloprotease that has a wide range of functions from formation of the extracellular matrix to regulating developmental processes connected with the transforming growth factor- β (TGF- β) signaling pathway (Ge and Greenspan, 2006b). Bone morphogenetic protein 1 possesses a procollagen-C proteinase activity, enabling it to cleave the prodomains from collagen fibers to allow them to assemble as necessary to form extracellular projections (Ge and Greenspan, 2006b; Hopkins et al., 2007). The proteinase activity of *BMP1* also regulates TGF- β signaling in several ways (Figure 1).

The TGF- β family of proteins includes many other bone morphogenetic proteins (*BMP2-BMP11*). Inactivation of inhibitors allows these TGF- β family proteins to interact with cell surface receptors to, in turn, initiate transcription of genes involved in cell development, as shown in an investigation to study patterning in *Xenopus*. Chordin, which holds bone morphogenetic protein 4 (a protein in the TGF- β family) inactive, is cleaved by *BMP1*. The cleavage of chordin releases *BMP4*, and allows it to signal the cell to specify ventral fates (Wardle et al., 1999; Ge and Greenspan, 2006b; Hopkins et al., 2007). Bone morphogenetic protein 1 also cleaves the prodomains from bone morphogenetic protein 2 and 4 to activate them, much as is done with collagen fibers (Ge and Greenspan, 2006b; Hopkins et al., 2007; Jasuja et al., 2007).

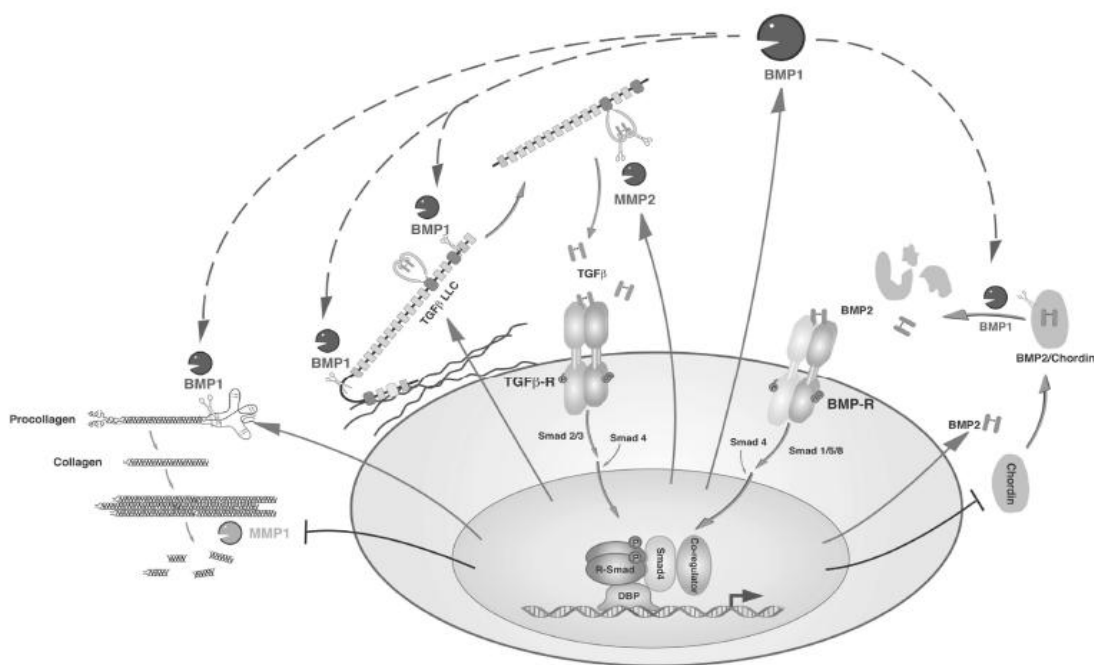


Figure 1. Proposed involvement of BMP1 metalloprotease in TGF- β signaling pathway. (Ge and Greenspan, 2006a)

Recently, researchers have shown that BMP1 is involved not only in cleaving prodomains to yield mature, functional proteins, but also cleaves and activates latent protein complexes. One such complex, GDF11, has its prodomain severed, but remains noncovalently bonded to it as a latent complex. This complex, when cleaved and activated by BMP1, may play some role in neural cell differentiation, indicating a potential role for *BMP1* in behavior (Ge et al., 2005; Ge and Greenspan, 2006a, b; Hopkins et al., 2007). The GDF11 complex shows a great deal of structural and functional similarity to GDF8, or myostatin, in acting as a negative feedback inhibitor (Ge et al., 2005). Myostatin performs its function in muscle cells, whereas GDF11 is an

inhibitor in neuronal cells. The apparent mechanism discovered by Ge et al. (2005) indicates that activated GDF11 inhibits the ability of nerve growth factor to induce neuron differentiation in a target cell, and arrests the cell in that state. Without the GDF11 inhibitor, nerve growth factor induced the neuronal development of PC12 cells from the rat adrenal medulla (Ge et al., 2005).

Bridging integrator 3, located at ~67.3 Mb on BTA8 (Bovine Genome Database, Build 3.1; <http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/>), has vital functions in cell segregation and cytokinesis. In a study in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* cells, a *BIN3* homolog was found to be crucial for cell separation and F-actin localization, with mutant cells often being multinucleate and having widely dispersed actin (Routhier et al., 2001). Routhier et al. (2001) showed that F-actin was localized to cell ends in wild-type *BIN3* cells, with mutants having patches throughout and rarely having formed the F-actin ring that serves to pinch cells apart during cytokinesis.

Other studies seem to indicate that BIN3 works as part of a chaperone protein in the cell. Molecular chaperones are proteins that use the energy gained from adenosine-triphosphate hydrolysis to perform the proper folding of proteins bound to them. Chen et al. (1994) suggested that tubulin and actin are both bound to a heteromeric chaperone, containing a *BIN3* homolog that uses adenosine-triphosphate hydrolysis to fold them into the proper structure to be assembled into longer filaments. Thus, BIN3 could have a great deal of influence on the proper formation of actin and tubulin filaments, both very important in segregation of cellular materials during division, and in the final separation stages of cytokinesis. If BIN3 is not active, this chaperone cannot help the cell to form cytoskeletal filaments as is necessary for cell division (Chen et al., 1994; Routhier et al., 2001).

Proposed Additional Candidate Genes

In August 2006, Build 3.1 of the bovine genome was released, and many rearrangements in the assembled sequence relative to Build 2.1 were observed. While

originally, *BMP1* and *BIN3* were shown to map quite close to the position of the QTL on BTA8 (Build 2.1), their locations on BTA8 changed substantially in the new assembly. Consequently, 2 additional genes were chosen based on their position relative to the QTL location and function in the cell. Farnesyl-diphosphate farnesyltransferase 1 (*FDFT1*) and cathepsin B (*CTSB*) are located on BTA8 at roughly 6 Mb (Bovine Genome Database, Build 3.1; <http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/>), very close to the position of the QTL in question.

Farnesyl-diphosphate farnesyltransferase 1, also known as squalene synthase, catalyzes the first committed step in the synthesis of sterols, or cholesterol, in the cell (Funfschilling et al., 2007). Thus, it has a very important role in many cellular processes, as Funfschilling et al. (2007) verified by showing that knockout mice died in early embryonic stages. That study also looked specifically at neuronal cells, which must synthesize their own purified cholesterol, and found that *FDFT1* mutants do not lose function in that cell type. Therefore, while *FDFT1* is critical for cholesterol synthesis, it is apparently not in neuronal tissues.

However, Schweitzer et al. (2005) found that levels of expression of *FDFT1* in mouse cardiac tissue varied depending on animal activity. Additionally, it was shown that access to varying amounts of exercise alters the levels of expression of *FDFT1*, and differing levels of expression correlate with different degrees of success in spatial maze performance (Schweitzer et al., 2006). These findings are relevant to the current study and suggest a role for *FDFT1* in behavior.

Cathepsin B has been shown to potentially have a great impact in the neuronal secretion pathway, particularly involved with Alzheimer's disease. A major cause of this degenerative disease is buildup of the neurotoxic β -amyloid protein, contributed mainly by the regulated secretory pathway of neurons (Hook et al., 2005). Hook et al. (2005) showed that this extracellular β -amyloid is generated through β -secretase processing of the amyloid precursor. This β -secretase activity was being performed by

cathepsin B, inhibition of which blocked the conversion of the immature amyloid protein into the extracellular β -amyloid that can become harmful.

Objective

This study aims to discover and characterize candidate genes associated with the QTL on BTA8 found to have an association with cattle disposition. Using information about the QTL region obtained by Wegenhoft (2005), possible candidates have been chosen and will be further investigated to determine if they are a source of genetic variation in behavior traits. Those genes will be characterized to determine if they fit the criteria reported for the QTL on BTA8. Through this analysis, we will gain a greater insight into the genetic effects and genes responsible for cattle temperament and disposition.

MATERIALS AND METHODS

Single Nucleotide Polymorphism Discovery

Primer design. Target sequences for amplification were identified using the Bovine Genome Database (Builds 2.1 and 3.1; <http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/>). Oligonucleotide primers, to amplify bovine gene fragments, were designed using Primer v0.5 (Lincoln et al., 1991). Design criteria included: optimal primer length (20bp), minimum primer length (18bp), maximum primer length (22bp), optimal primer melting temperature (58.0 °C), minimum acceptable primer melting temperature (53.0 °C), maximum acceptable primer melting temperature (63.0 °C), minimum acceptable primer GC% (20), maximum acceptable primer GC% (80), salt concentration (mM) (50.0), DNA concentration (nM) (50.0), maximum number of unknown bases (Ns) allowed in a primer (0), maximum acceptable primer self-complementarity (number of bases) (8), maximum acceptable 3' end primer self-complementarity (number of bases) (6), GC clamp (how many 3' bases) (0), restriction sites which flank region of interest (0), and product length ranges (100-150bp, 150-250bp, 250-400bp). Forward and reverse primer pairs were selected with similar melting temperatures, GC content from 40% to 60%, and minimal self-complementarity and pairwise matches between primers. Primers (Table 1 and Appendix A) were designed to anneal within exons or the 3' untranslated region (UTR), so that they were able to amplify both genomic and complementary DNA (cDNA) templates. The Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990, <http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) was used to align primers to the bovine genome to verify that the sequences were unique.

Primer Optimization. A gradient of annealing temperatures from 50 °C to 65 °C was tested to optimize the polymerase chain reaction (PCR) for each pair of primers. Each 25 µl PCR reaction included 40 ng bovine genomic DNA template, 1 unit of Taq polymerase, 0.2 µM forward primer, 0.2 µM reverse primer, 1X Buffer (50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.1% Triton X-100), 1.5 mM MgCl₂, and 0.1 mM

Table 1. Gene Specific Primer Sets for Discovery of Single Nucleotide Polymorphisms

Locus	Primer	Sequence (5' to 3')	GC % ¹	T _A (°C) ²	[Mg ²⁺] (mM)	gDNA (bp) ³	cDNA (bp) ⁴
<i>BMP1</i>	BMP1F BMP1R	GTGGTCGTAGGCACACTCAG ACCTGGGCCATCTCTAGCAC	59	56.0	1.5	436	94
<i>BIN3</i>	BIN3F BIN3R	CAAGCCAAGGTGGAGAAGTA AGTCCAGTCGGCTGTTGTAG	60	56.0	1.5	366	168
<i>CTSB</i>	CTSBEF CTSBER	CTCTGGAGCCTGGA ACTTCT GCAGGAAGTCCGAGTACACA	57	64.0	1.5	1003	285
<i>FDFT1</i>	FDFT1_E8F FDFT1_E8R	AACTCTGACCCCTGTTCCAC GACTGGCAACTCACCTGCTA	49	64.0	1.5	826	826

¹Percentage GC content of amplicon

²Annealing temperature

³Expected amplicon size from genomic DNA

⁴Expected amplicon size from cDNA

2'-deoxynucleoside 5'-triphosphates (dNTP). With the use of a PTC-0200 DNA Engine (MJ Research, Inc., Waltham MA), reactions were denatured at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 sec, the annealing temperature (50 °C to 65 °C) for 30 sec, 72 °C for 30 sec, and a final extension of 7 min at 72 °C. The annealing temperature resulting in the most robust product was considered optimal (Table 1).

Screening of Bovine Bacterial Artificial Chromosome Library. The 3.5X TAMBT bovine bacterial artificial chromosome (BAC) library (Cai et al., 1995) that has been pooled for PCR-based screening was utilized to isolate BAC containing the gene-associated microsatellite markers. The 71 bovine BAC super pools, each representing BAC DNA from 12 96-well plates, were screened by PCR using 40 ng BAC DNA template in 25 µl reactions as described above. A genomic DNA positive control and no template control were included with each set of reactions. The 12 single pools corresponding to each positive super pool were subsequently screened. Finally, DNA representing the 8 rows and 12 columns from positive plates was screened, with the intersection of a positive row and positive column identifying a single BAC.

Upon identification of a positive clone, the BAC was streaked onto an LB/Agar plate containing 12.5 µg/ml chloramphenicol (CM) and grown at 37 °C overnight. A single colony from the streaked plate was used to inoculate 50 ml of LB containing 12.5 µg/ml CM, which was incubated overnight at 37 °C and then BAC DNA was extracted using standard alkaline/lysis mini-prep procedures (Sambrook et al., 1989).

Amplicons as templates for confirmation sequencing were generated from 40 ng BAC DNA in 50 µl reactions using 2 units of Taq polymerase and the same conditions as previously described. These PCR products were cleaned using Princeton Separations' (Adelphia, NJ) PSIClone HTS 96-well PCR clean-up plate kit as per the manufacturer's instructions. Sequencing reactions were then performed for each BAC clone as a 10 µl reaction using 100 ng of the cleaned PCR product, 0.5 µM forward primer, 1X Sequencing Buffer (60 mM Tris-HCl, pH. 9.0, 1.5 mM MgCl₂), and 1 µl of Big Dye v1.1 Terminator mix (PE Corp. Applied Biosystems, Foster City, CA). The template, primer, buffer and water were mixed and denatured at 98°C for 2 min, followed by snap cooling

on ice for 2 min. Big Dye v1.1 terminators were then added and the reaction was cycled 25 times at 96 °C for 10 sec, 50 °C for 5 sec, and 60 °C for 4 min. Dye terminators and salts were removed from the sequencing reactions using Qiagen's (Valencia, CA) DyeEx 96-well sequencing clean up plate as per the manufacturer's instructions. Cleaned sequences were dried down, resuspended in 10 µl Hi-Di Formamide and loaded onto an ABI 3130xl (Applied Biosystems, Foster City, CA). Samples were denatured at 98 °C for 2 minutes, snap cooled on ice for 2 minutes, injected at 1.6 kV for 15 sec, and run at 8.5 kV for 6000 seconds. Sequences were aligned by BLAST to the bovine genome to verify amplification of the intended product.

Sequencing of Angleton Grandparents. Genomic DNA from Angus and Brahman grandparents in the Angleton herd was amplified using 50 µl reactions and sequenced, as described previously. A BAC clone and no template control were included as positive and negative controls, respectively. Sequence files from the ABI 3130xl (Applied Biosystems, Foster City, CA) were imported into Sequencher 4.8 (Gene Codes, Ann Arbor, MI) and aligned into contigs. Reported differences in base pairs between sequences were investigated, and single nucleotide polymorphisms (SNP) were recorded as confirmed after viewing the sequence chromatograms. The Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990, <http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) was used to align short sequences containing each SNP to determine if it was in an intron, exon, or UTR and thus if it was useful for the imprinting study.

Investigation of Imprinting Status of Candidate Genes

Synthesis of cDNA. Genomic DNA from Angleton population calves was amplified, sequenced and aligned in Sequencher 4.8 in the manner previously described to determine which of the calves were heterozygous for SNP in coding or 3' UTR regions. RNA from these heterozygous individuals was extracted from liver and muscle tissues using TRIzol Reagent (Gibco BRL, Gaithersburg, MD), per the manufacturer's instructions.

Once extracted, the RNA was DNased with Promega RQ1 DNase. In a 100 μ l reaction mixture, 10 μ g RNA was added to 1X RQ1 DNase buffer (40 mM Tris-HCl (pH 8.0), 10 mM MgSO₄ and 1 mM CaCl₂), 10 μ g RQ1 DNase and DEPC-water. Samples were incubated at 37 °C for 30 min and 13 mM EDTA was then added. Samples were heat shocked at 65 °C for 10 min, and loaded into the NanoDrop ND-1000 UV-Vis Spectrophotometer (Wilmington, DE) to determine RNA concentration (~100 ng/ μ l).

Synthesis of cDNA was performed using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). In a 10 μ l reaction mixture, 0.5 μ g DNase treated RNA was added to 1 mM dNTP, 1 μ g Oligo(dT) and DEPC-water. Samples were incubated at 65 °C for 5 min, then cooled on ice for 1 min. To each reaction mixture, 1X RT buffer (20 mM Tris-HCl (pH 8.4), 50 mM KCl), 5 mM MgCl₂, 10 mM DTT, and 40 units RNaseOUT Recombinant RNase Inhibitor. This mixture was incubated at 42 °C for 2 min, and 50 units of SuperScript II RT were added to each. This was incubated at 42 °C for 50 min, followed by termination at 70 °C for 15 min, and cooling on ice. Then, 2 units of *E. coli* RNase H were added, followed by incubation at 37 °C for 20 min.

Sequencing. Amplicons for expressed SNP were generated in 50 μ l reactions from both genomic DNA and cDNA for each calf. These PCR products were cleaned, sequenced and imported into Sequencher 4.8 (Gene Codes, Ann Arbor, MI) in the same manner as described previously. Sequences for each individual were compared to examine differences between genomic and expressed products.

Microsatellite Genotyping

Microsatellite Primer Design and Optimization. Target microsatellites were identified by uploading the FASTA sequence of entire genes from the Bovine Genome Database (Builds 2.1 and 3.1; <http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/>) into RepeatMasker (<http://www.repeatmasker.org>). Simple dinucleotide repeats were chosen, and if such a repeat could not be found in the gene itself, DNA sequences near the gene were used. Oligonucleotide primers (Table 2) to amplify microsatellites within

or near genes of interest were designed using Primer v0.5 (Lincoln et al., 1991) as described previously. Predicted sequences of amplicons obtained from these primer sets are found in Appendix B. A gradient of annealing temperatures was used to optimize the conditions for PCR of 25 μ l reactions with the same cycle conditions as above.

Genotyping. A forward primer carrying a 5' fluorescent dye was used with an unlabeled reverse primer for genotyping on an ABI 3130xl (Applied Biosystems, Foster City, CA). Prior to genotyping the Angleton and McGregor populations, 40 ng DNA from 16 grandparents from each population was amplified in 25 μ l reactions by PCR to verify that the microsatellite was polymorphic. To visualize the genotypes, 0.5 μ l of the PCR product, 0.2 μ l Rhodamine-X MapMarker for *BMP1* and *BIN3* microsatellites (BioVentures, Inc., Murfreesboro, TN) or 0.2 μ l Rhodamine-X MapMarker 1000 for *FDFT1* and *CTSB* microsatellites, and 9.2 μ l Hi-Di Formamide (Applied Biosystems, Foster City, CA) were combined in a 10 μ l volume. Samples were loaded onto an ABI 3130xl (Applied Biosystems, Foster City, CA), injected at 15 kV for 15 sec and run at 15 kV for 1800 sec. Genotypes were analyzed in GeneMapper 4.0 (Applied Biosystems, Foster City, CA). If polymorphic, the entire Angleton and McGregor populations were genotyped with each primer set shown in Table 2.

Construction of Linkage Maps

Linkage maps were constructed for both the Angleton and McGregor populations. Wegenhof (2005) constructed maps of every chromosome for the Angleton population, using 313 markers (mostly microsatellites). The genotyping data from the 4 genes in this study were used to update the maps of BTA8 and 11. In the McGregor population, genotypes from the 4 genes in this study were combined with that of 133 other markers to construct maps for every autosome. Genotype data were formatted for CRI-MAP V2.4 software according to instructions by Green et al. (1990). The twopoint, build, flips and chrompic options were used with default settings; likelihood of difference score threshold 3.000, phase unknown likelihood tolerance 3.000, and phase known likelihood tolerance 3.000. Markers were excluded if they

Table 2. Specific Primer Sets for Microsatellite Amplification and Genotyping

Locus	Primer	Sequence (5' to 3')	GC % ¹	T _A (°C) ²	[Mg ²⁺] (mM)	gDNA (bp) ³	Fluorescent Label Used
<i>BMP1</i>	BMP1_MS1F	CCAAGAACAGTAGCCACCAG	47	57.6	1.5	173	HEX ⁵
	BMP1_MS1R	CAGGACGTTTCATCTGACCT					
	BMP1_MS2F	ATGGGGTTGATACAAGGGTT	41	52.0	1.5	153	NED ⁴
	BMP1_MS2R	ATGGGGTGACAAAAGTCAGA					
<i>BIN3</i>	BIN3_MS1F	AGCAACTGAGTGAGACTGGG	36	57.6	1.5	335	6-FAM ⁵
	BIN3_MS2R	ATCAGGATTCTTTCCTGCC					
<i>CTSB</i>	CTSB_MS2F	AGCCTCACACATGGATTCTT	52	51.0	1.5	399	6-FAM ⁵
	CTSB_MS2R	TCATCCCATTACCATTACGG					
<i>FDFT1</i>	FDFT1_1F	AGCAATTCTTTCTGATATGG	33	50.0	1.5	257	HEX ⁵
	FDFT1_R2	GTACTATTTCAAGGGGTCGC					

¹Percentage GC content of amplicon

²Annealing temperature

³Expected amplicon from genomic DNA

⁴5' modification ordered from Applied Biosystems (Foster City, CA); NED = 2-chloro-5-fluoro-7,8-fused phenyl-1,4-dichloro-6-carboxyfluorescein

⁵5' modification ordered from Integrated DNA Technologies (Coralville, IA); HEX = hexachloro-fluorescein, 6-FAM = 6-carboxyfluorescein

could not be placed into a framework map at LOD = 3.000. Because only a small subset of the population was genotyped for markers on BTA9, the PUK_LIKE_TOL and PK_LIKE_TOL were reduced to 2.000 for this chromosome. Sex-averaged framework maps with distances in Kosambi map function units (cM) were built for each autosome.

Quantitative Trait Locus Analysis

Disposition Scoring. In the Angleton project, disposition scores were taken twice using a 1 to 5 scale, with 1 being calm and 5 being crazy. The first score was assigned in pens after blood samples were collected at weaning. The second score was assigned in pens immediately prior to slaughter.

In the McGregor Genomics project, disposition of calves was scored one month after weaning by a panel of 4 evaluators. Calves were grouped in pens of about 15 animals and then released into a 20m alleyway in pairs. Two evaluators were at each end of the alley. The animals were left in the alley for 2 to 3 min, and then one animal was cut back into the pen with the others and the animal remaining in the alley was scored. Animals were scored on a 1 to 9 scale for aggressiveness, nervousness, flightiness, gregariousness, in addition to overall disposition. Aggressiveness refers to the animal's desire to hit evaluators, where 1 is non-aggressive, and 9 is extremely aggressive. Nervousness refers to the animal's behavior in regard to walking and running, vocalization, and physically shaking, where 1 is totally calm and 9 is extremely nervous. Flightiness refers to an animal's desire to keep away or get away from evaluators, where 1 is totally quiet and 9 is extremely flighty. Gregariousness refers to an animal's desire to get back to the group of individuals from which it came and how it acted in a pair as compared to being separated where 1 is totally willing to be separated from the group and 9 is unwilling to be separated. Overall disposition is scored as a separate trait (as opposed to being an average of the others), where 1 is completely docile and 9 is crazy. Each of the component traits of disposition were scored again in the steer progeny about one week prior to slaughter by a single evaluator. An overall disposition score was also assigned in the pens immediately prior to slaughter (as for the

Angleton population). Heifers and cows were also scored for their overall disposition each year at time of calving.

Statistical Analysis of Disposition Scores. Wegenhoft (2005) studied the Angleton disposition scores through the analysis of covariance using the mixed model procedure of SAS (SAS Institute, Cary, NC) that included independent variables of sire-type x dam-type (ST x DT) interaction, the three-way interaction of sex x ST x DT, the regression of birth date within season-year combination and family nested within ST x DT as a random effect. Residuals from this model were used for QTL mapping in the current study.

Disposition data from the McGregor project were also studied through the analysis of covariance using the mixed model procedure of SAS (R. Funkhouser, Texas A&M University, personal communication). The models for each of the components traits of behavior measured at weaning included fixed factors of sire, family nested within sire, birth-year-season, pen within birth-year-season, sex, evaluator within birth-year-season, the two-way interaction of sex x sire, and the regressions of recipient dam's disposition within birth-year-season, and sequence within pen within birth-year-season.

For the disposition scores taken on steers in the feeding pens, the models included fixed factors of sire, family nested within sire, birth-year-season, feeding pen within birth-year-season, and the regressions of weaning overall disposition, and recipient disposition within birth-year-season. The model for disposition scores taken at slaughter included fixed factors of sire, family nested within sire, birth-year-season, feeding pen, number of knocks and the regressions of weaning overall disposition score and slaughter order nested within slaughter date within birth-year-season. The model for disposition scores for first calf heifers at time of calving included fixed factors of sire, family nested within sire, heifer birth-year-season, heifer's calf-year-season nested within heifer birth-year-season and the regression of heifer calving date nested within calf-year-season within heifer birth-year-season. Residuals from these models (R. Funkhouser, personal communication) were used in the current study.

Interval mapping. Interval mapping was performed using the QTL Express package (Seaton et al., 2002). Three files were created according to the QTL Express directions: a genotype file, a map file and a phenotype file. The genotype file contained the sire, dam, generation type, sex and genotypes for each marker for each individual. The map file contained the markers and distances between them in whole cM for each chromosome. The phenotype file contained the residuals from the statistical analyses of the progeny.

For the Angleton population, the Combined F2/Backcross Analysis model was used to detect QTL. The F2 Analysis model was used for the McGregor population. Both a Mendelian inheritance model and a parent-of-origin model were considered. Chromosome-wise and genome-wise significance thresholds were determined by using n=1000 permutations with replacement.

RESULTS AND DISCUSSION

In our investigation of *BIN3*, *BMP1*, *CTSB* and *FDFT1*, we have gained valuable information about several markers within these genes, as well their imprinting status. We have constructed an updated linkage maps for BTA8 in the Angleton herd, as well as linkage maps for each autosome in the McGregor population. This section will describe those findings and discuss their meaning for our study.

Single Nucleotide Polymorphism Discovery

Sequencing verified that bovine BAC 224R4C8 contained *BMP1*, both 318R1C8 and 14.66R4C2 contained *BIN3*, *CTSB* was contained in BAC 145R5C11, and 255R6C1 contained *FDFT1*. These clones were used as positive controls for the remainder of the experiment.

Single nucleotide polymorphisms were identified in the amplicons for *BIN3*, *FDFT1* and *CTSB*, but not for *BMP1* (Appendix B). In intron 7 of *BIN3*, a G/A transition was detected at position 46,961 from the start codon, and a C/T transition was found at position 47,053. In exon 8, a silent G/T transversion was found at position 47,165 from the start codon. In sequential order of SNP, 8% of Angus had an AA, CC, TT genotype, 26% were GG, CC, TT, and 68% were GA, CC, TT. Of the Brahmans, 53% were GG, TT, GG, 13% were GG, CC, TT, and 33% were GG, CT, GT (Table 3). The second and third SNP were in complete linkage disequilibrium.

Individuals (2 Angus and 6 Brahman) that were informative based on the SNP in *BIN3* were sequenced for *CTSB* and *FDFT1*. For *CTSB*, only one Angus sequence worked, but 7 SNP were discovered nonetheless. In Exon 5, a silent G/T transversion was found at position 4,644 from the start codon. In Intron 6, G/A and C/T transitions were discovered at positions 4,780 and 4,937, respectively. In Exon 6, there was a silent C/T transition at position 5,062 from the start codon. Finally, in Intron 7, G/A transitions were discovered at positions 5,151, 5,160 and 5,164 from the start codon. The Angus individual, in order of SNP, had a GG, GG, CT, CT, AA, AA, GG genotype.

Table 3. Single Nucleotide Polymorphisms in *BIN3*

Animal ID	Sex	Breed	SNP 1	SNP2	SNP3 ¹
			Position 46,961	Position 47,053	Position 47,165
P59	F	Angus	AA	CC	TT
2520	F	Angus	GG	CC	TT
2546	F	Angus	GG	CC	TT
P44	F	Angus	GG	CC	TT
PINETAR	M	Angus	GG	CC	TT
SHOSHONE	M	Angus	GG	CC	TT
2627	F	Angus	G/A	CC	TT
2749	F	Angus	G/A	CC	TT
P12	F	Angus	G/A	CC	TT
P48	F	Angus	G/A	CC	TT
W11	F	Angus	G/A	CC	TT
W6	F	Angus	G/A	CC	TT
G211	M	Angus	G/A	CC	TT
MR ANGUS	M	Angus	G/A	CC	TT
PINEDRIVE	M	Angus	G/A	CC	TT
POWERDRIVE	M	Angus	G/A	CC	TT
SCOTCH CAP	M	Angus	G/A	CC	TT
SKY HIGH	M	Angus	G/A	CC	TT
WRANGLER	M	Angus	G/A	CC	TT
1/4	F	Brahman	GG	CC	TT
249/3	F	Brahman	GG	CC	TT
539/1	F	Brahman	GG	C/T	G/T
G102	F	Brahman	GG	C/T	G/T
P3385	F	Brahman	GG	C/T	G/T
LA500	M	Brahman	GG	C/T	G/T
ROCKY	M	Brahman	GG	C/T	G/T
164/3	F	Brahman	GG	TT	GG

¹SNP at a coding position in Exon 8

Table 3. Continued

Animal ID	Sex	Breed	SNP 1	SNP2	SNP3 ¹
			Position 46,961	Position 47,053	Position 47,165
296/1	F	Brahman	GG	TT	GG
P363	F	Brahman	GG	TT	GG
P3735	F	Brahman	GG	TT	GG
34/3	M	Brahman	GG	TT	GG
9/118	M	Brahman	GG	TT	GG
EJL309	M	Brahman	GG	TT	GG
VA777/2	M	Brahman	GG	TT	GG

¹SNP at a coding position in Exon 8

In the Brahman animals, 37.5% of animals were GG, GG, CC, TT, AA, AA, GG; 25% were GT, GA, CT, CT, GA, GA, GA; and 12.5% of individuals had the GG, GG, CT, CT, AA, AA, GA genotype. Table 4 shows the genotype of these individuals at each SNP position.

The same 8 individuals were sequenced for the *FDFTI* amplicon and although 2 Brahman sample sequences were not successful 4 SNP were discovered. In Exon 8, a silent C/T transition was at position 25,665 from the start codon. In the 3' UTR, a C/A transversion, a G/A transition and C/T transition were found at positions 25,793, 25,948 and 25,982 from the start codon, respectively. Both of the Angus individuals were TT, AA, GG, CC at these SNP, in sequential order. Of the Brahman individuals, 50% had the CC, CC, AA, TT genotype, 25% had a genotype of CC, CC, GG, CC and 25% were TT, AA, GG, CC (Table 5).

Table 4. Single Nucleotide Polymorphisms in *CTSB*

Animal ID	Sex	Breed	SNP 1 ¹	SNP 2	SNP 3	SNP 4 ²	SNP 5	SNP 6	SNP 7
			Position 4,644	Position 4,780	Position 4,937	Position 5,062	Position 5,151	Position 5,160	Position 5,164
P44	F	Angus	GG	GG	C/T	C/T	AA	AA	GG
1/4	F	Brahman	G/T	A/G	C/T	C/T	G/A	G/A	G/A
249/3	F	Brahman	G/T	A/G	C/T	C/T	G/A	G/A	G/A
539/1	F	Brahman	GG	GG	CC	TT	AA	AA	GG
G102	F	Brahman	GG	GG	CC	TT	AA	AA	GG
P3385	F	Brahman	GG	GG	C/T	C/T	AA	AA	G/A
296/1	F	Brahman	GG	GG	CC	TT	AA	AA	GG

¹SNP at a coding position in Exon 5²SNP at a coding position in Exon 6**Table 5.** Single Nucleotide Polymorphisms in *FDFT1*

Animal ID	Sex	Breed	SNP 1 ¹ Position 25,665	SNP 2 ² Position 25,793	SNP 3 ² Position 25,948	SNP 4 ² Position 25,982
P44	F	Angus	TT	AA	GG	CC
2749	F	Angus	TT	AA	GG	CC
1/4	F	Brahman	CC	CC	AA	TT
249/3	F	Brahman	CC	CC	AA	TT
G102	F	Brahman	CC	CC	GG	CC
P3385	F	Brahman	TT	AA	GG	CC

¹SNP at a coding position in Exon 8²SNP at a coding position in 3' UTR

The *BMP1* amplicon only represented parts of 2 exons of the gene and no SNP were discovered. In order to investigate the imprinting status of this gene in future studies, primers that amplify different parts of the gene must be designed. The goal of SNP discovery in this project was to facilitate the comparison of SNP in genomic DNA and cDNA to determine if expression of transcripts was dependent on the parent-of-origin. Therefore, only a few exons of each of the 4 genes were amplified and sequenced (Appendix A).

Investigation of Imprinting Status of Candidate Genes

To investigate differences in expression based on parent-of-origin, the individual must have inherited a different allele from each parent. Imprinting can then be detected by comparing SNP in genomic DNA and cDNA. If a transcript is completely imprinted then only one of the 2 alleles would be observed in the cDNA.

Genomic DNA from calves from the Angleton population, for which muscle and liver RNA had previously been extracted, was sequenced to identify heterozygotes for the SNP in the coding and 3' UTR regions of *BIN3*, *FDFT1* and *CTSB*. Individual 215 was heterozygous G/T for SNP 3 of *BIN3* from both genomic DNA and cDNA. Likewise, individual 1001 was heterozygous G/T at *CTSB* SNP1, and 3 individuals were C/T at SNP 4 from both genomic DNA and cDNA. For *FDFT1*, one individual was heterozygous C/T at SNP 1 and C/A at SNP 2, and one individual was heterozygous G/A at SNP 3 and C/T at SNP 4 from both genomic DNA and cDNA (Table 6). Thus, there was no evidence of imprinting of these 3 genes in muscle or liver collected at slaughter.

Table 6. Genotypes at Coding SNP in Genomic and Complementary DNA

Locus	SNP Position¹	Animal ID	Sex	Breed¹	Genomic DNA	Muscle Tissue cDNA	Liver Tissue cDNA
BIN3	47,165	215	F	BAxAA	G/T	G/T	G/T
CTSB	4,644	1001	F	BBxAB	G/T	G/T	G/T
CTSB	5,062	1001	F	BBxAB	C/T	C/T	C/T
CTSB	5,062	2504	F	ABxBB	C/T	C/T	C/T
CTSB	5,062	2006	F	BBxBA	C/T	C/T	C/T
FDFT1	25,665	2803	F	BAxBB	C/T	C/T	C/T
FDFT1	25,793	2803	F	BAxBB	C/A	C/A	C/A
FDFT1	25,948	1001	F	BBxAB	G/A	G/A	G/A
FDFT1	25,982	1001	F	BBxAB	C/T	C/T	C/T

¹Base pairs from start codon

²Paternal Grandsire/Paternal Granddam X Maternal Grandsire/Maternal Granddam

(B: Brahman, A: Angus)

Wegenhoft (2005) showed that this QTL on BTA8 was at least partially paternally imprinted, indicating that the gene affecting behavior in this QTL region should follow suit. Examination of the chromatographs of the sequence data did not reveal any consistent differences in intensities of SNP alleles between genomic and cDNA samples. Based on these data and the lack of any evidence of imprinting, *BIN3*, *CTSB* and *FDFT1* are ruled out as candidates for this QTL.

However, imprinting status is known to vary spatially among tissues and temporally throughout development. Verona et al. (2003) reviewed many of the significant imprinting studies in mouse and human. These studies indicate not only that imprinting is regulated and will vary between some tissues and developmental stages, but that imprinting status also varies between the human and mouse. A gene that follows one imprinting pattern in the human may not necessarily follow suit in the mouse. Therefore, before these 3 genes can be completely ruled out as candidates for this QTL, other tissues (e.g. brain) and earlier developmental time points should be sampled in future studies.

Genotyping and Construction of Linkage Maps

The Angleton population was genotyped for the microsatellites associated with *BIN3*, *CTSB*, and *FDFT1* and the linkage map constructed by Wegenhoft (2005) was updated to include these new markers (Appendix C). Plis-Finarov et al. (2004) previously used several microsatellite markers to map *CTSB* and *FDFT1* in cattle. While the markers flanking the gene-associated microsatellites in the study by Plis-

Finarov et al. (2004) were UWCA47 (0 cM) and IDVGA11 (26 cM) compared with BMS1864 (0 cM) and BM3419 (29.5 cM) in the current study, the position and order of these genes is equivalent in the 2 studies. The genes *CTSB* and *FDFTI* were placed at about 12 cM and 16.5 cM, respectively, in the current study.

Originally, the BMP1_MS1 primer set was designed to amplify a microsatellite within *BMP1* on BTA8 based on Build 2.1 of the Bovine Genome Database (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/>). However, upon constructing a linkage map of BTA8 for this population, the marker could not be placed on this chromosome. The marker was instead found to belong on BTA11 (Appendix C), and it was subsequently discovered that this sequence on BTA8 in Build 2.1 had shifted to BTA11 in Build 3.1. We developed the BMP1_MS2 primer based on Build 3.1 to amplify a new microsatellite in *BMP1* on BTA8. This marker, however, was not polymorphic in this population.

The McGregor population was genotyped at the *BIN3*, *CTSB*, and *FDFTI* markers. The BMP_MS1 primer set was not used in this population, as we were interested only in the microsatellites on BTA8. As for the Angleton population, the BMP_MS2 microsatellite was not polymorphic in this population, and was not pursued any further. The genotypes for the 3 genes mentioned were added to those of 133 other markers in this population to create linkage maps for the each of the 29 autosomes (Appendix C).

Angleton Population QTL Analysis

The analysis performed by Wegenhoft (2005) that discovered the QTL associated with final disposition on BTA8 was repeated, using the updated linkage map obtained in this study (Table 7).

The analysis performed in this study verifies the position of a QTL on BTA8 near the centromere. The position has shifted slightly, but remains within 1cM of that found previously. As previously, the QTL was significant at the chromosome-wise level ($P < 0.05$) and is estimated to be partially paternally imprinted. These results add to the information known about this QTL region and will aid subsequent investigations of candidate genes near this locus.

McGregor Population QTL Analysis

As described previously in the materials and methods section, eight traits related to disposition were scored in the McGregor population. Each trait was used with the marker information from the linkage maps constructed for the 29 autosomes to find QTL on these chromosomes. These traits are highly correlated ($r = 0.827$ to 0.978), but they cannot be treated as if they were the same trait (Wegenhoft, 2005).

The analyses tested both a chromosome-wise and experiment-wise significance level for each trait for each chromosome. Those QTL found to be significant at $P < 0.05$ at the chromosome-wise level only were designated as suggestive QTL. Those found to be significant at $P < 0.05$ at the experiment-wise level were significant QTL in the population.

Table 7. Angleton Positions of Final Disposition QTL on BTA8 under Parent-of-Origin model, test statistics and size effects

BTA	Position (cM)	Flanking Markers	Test Statistics				Effects		
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE	Parent-of-Origin \pm SE
8 ^c	3	BMS1864-CTSB	6.68	19.6	4.26	0.331 \pm 0.108	-0.135 \pm 0.109	-0.299 \pm 0.109	0.315 \pm 0.110
8 ^d	2	BMS1864-CTSB	5.46	16.1	3.496	0.3223 \pm 0.1071	-0.2253 \pm 0.1043	-0.1806 \pm 0.1058	0.2728 \pm 0.1059

^aQTL genotypic value of Angus homozygotes such that $2a=AA-BB$.

^bAB heterozygote deviation from QTL homozygote midpoint such that $d=AB-0.5(AA+BB)$.

^cInformation from Wegenhoft (2005).

^dUpdated QTL information based on addition of new markers.

Under a Mendelian model of inheritance, significant QTL for aggressiveness were found on BTA 3 and 12, and suggestive QTL on 12 and 29. Suggestive QTL for flightiness and overall weaning disposition were also found on BTA12 at approximately the same location (interval from *BMS2252* to *RM094*) as the aggressiveness QTL. On BTA 26, we found a suggestive QTL for overall yearling disposition. Finally, a suggestive QTL for disposition of the heifer at calving was discovered on BTA16. These QTL positions, flanking markers, test statistics and size of effects in disposition score units are given in Table 8-12.

The effect of a QTL is the change in phenotypic value when the genotype of the QTL changes. The additive effect (a) is the half of the difference in genotypic value between the 2 alternative homozygotes. In this study, the additive effect is defined as the QTL genotypic value of Nellore homozygotes such that $2a = NN - AA$. If an effect was positive, as for the aggressiveness QTL on BTA3 (Table 8), the Nellore homozygote had a higher disposition score (worse disposition) than individuals homozygous for the Angus allele at this QTL. If the effect had a negative value, the individuals that were homozygous for an Angus allele at the QTL had a higher disposition score than homozygotes for the Nellore allele. Additive values are considered breeding values and are useful in selection for traits. Except for the QTL for disposition of the heifer at calving on BTA16 (Table 12), individuals homozygous for Nellore alleles at the other QTL had worse disposition.

Dominance effects are those due to the effects of combinations of alleles at a given locus, or QTL, which form the genotype of an individual. Values are the NA

Table 8. Positions of Aggressiveness QTL under Mendelian model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics			Effects		
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE
3 ^{***}	45	BM7225-ILSTS64	9.67	18.77	4.075	-0.4755 \pm 0.2486	0.8876 \pm 0.2321	0.9224 \pm 0.4683
6 [^]	1	CSSM22-CSSM34	7.53	14.71	3.194	0.7484 \pm 0.2458	0.4616 \pm 0.2300	-1.4989 \pm 0.4682
12 ^{***}	20	BMS2252-RM094	8.64	16.83	3.654	0.8665 \pm 0.2508	0.3612 \pm 0.2231	-1.7592 \pm 0.4809
29 [*]	21	BMC3224-BMS764	5.35	10.52	2.284	0.4161 \pm 0.2263	0.5548 \pm 0.1873	-0.7938 \pm 0.4367

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

**Significant at $P < 0.01$ at chromosome-wise level.

***Significant at $P < 0.05$ at experiment-wise level.

****Significant at $P < 0.01$ at chromosome-wise level.

Table 9. Positions of Flightiness QTL under Mendelian model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics			Effects		
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE
12 [*]	22	BMS2252-RM094	6.41	12.57	2.729	0.9403 \pm 0.2865	0.1467 \pm 0.2616	-1.8971 \pm 0.5473

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

Table 10. Positions of Overall Weaning Disposition under Mendelian model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics			Effects		
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE
12*	22	BMS2252-RM904	5.43	10.69	2.32	0.8462 \pm 0.2808	0.1379 \pm 0.2564	-1.7086 \pm 0.5364

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

Table 11. Positions of Overall Yearling Disposition QTL under Mendelian model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics			Effects		
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE
26*	33	IDVGA59-HEL11	4.08	7.98	1.733	0.3817 \pm 0.1543	0.0957 \pm 0.1312	-0.7725 \pm 0.2892

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

Table 12. Positions of Heifer Calving QTL under Mendelian model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics			Effects		
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE
16*	70	INRA48-BM3509	5.43	10.17	2.208	0.3284 \pm 0.2403	-0.8021 \pm 0.2490	-0.5322 \pm 0.4196

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

heterozygote deviation from QTL homozygote midpoint such that $d = NA - 0.5(NN+AA)$ and range from additivity to complete dominance and even to overdominance. These values are based on the interaction between the allele from the sire and the allele from the dam. The QTL for disposition of the heifer at calving was estimated to have a dominance effect favoring the Angus allele, while the remainder of the QTL under the Mendelian model were estimated to have large overdominance effects such that the Nellore-Angus heterozygotes had worse temperaments than the Nellore homozygotes.

When a parent-of-origin model was investigated, QTL for aggressiveness were found on BTA3, 6, 12, and 29 as under the Mendelian model and a suggestive QTL was also detected on BTA 22. In approximately the same locations on BTA 22 (BMS672 to BM3628) and BTA 29 (BMC3224 to BMS764), suggestive QTL were also detected for nervousness, flightiness, and overall weaning. There was a suggestive QTL for gregariousness in the same location on BTA22 and a significant QTL for flightiness on BTA 12 as for the Mendelian model. There was a suggestive QTL on BTA19 associated with overall yearling disposition scores, and a suggestive QTL for disposition of the heifer at calving on BTA13. Finally, a QTL for slaughter disposition score was found on BTA11. These QTL positions, flanking markers, test statistics and size of effects in disposition score units are given in Table 13-20.

The parent-of-origin model is indicative of imprinting in the QTL. Complete imprinting suggests that the heterozygotes will have the same score as the comparable homozygotes, as only one allele is being expressed. In the case of paternal imprinting,

Table 13. Positions of Aggressiveness QTL under Parent-of-Origin model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics				Effects			
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE	Parent-of-Origin \pm SE	
3 ^{***}	45	BM7225-ILSTS64	7.58	21.98	4.773	-0.443 \pm 0.2484	0.7893 \pm 0.2376	0.8398 \pm 0.4689	0.3976 \pm 0.2203	
6 ^{**}	1	CSSM22-CSSM34	5.64	16.52	3.587	0.7553 \pm 0.2455	0.5588 \pm 0.2406	-1.5211 \pm 0.4678	-0.3171 \pm 0.2340	
12 ^{***}	20	BMS2252-RM094	6.6	19.24	4.177	0.9533 \pm 0.2564	0.5173 \pm 0.2440	-1.9615 \pm 0.4969	-0.3994 \pm 0.2554	
22 [*]	38	BMS672-BM3628	3.22	9.54	2.071	0.304 \pm 0.3787	0.4232 \pm 0.2916	-0.609 \pm 0.7528	-0.8569 \pm 0.3137	
29 ^{****}	23	BMC3224-BMS764	6.49	18.92	4.108	0.3753 \pm 0.2066	0.6802 \pm 0.1891	-0.6814 \pm 0.3952	-0.6382 \pm 0.2026	

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

**Significant at $P < 0.01$ at chromosome-wise level.

***Significant at $P < 0.05$ at experiment-wise level.

****Significant at $P < 0.01$ at chromosome-wise level.

Table 14. Positions of Nervousness QTL under Parent-of-Origin model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics				Effects			
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE	Parent-of-Origin \pm SE	
22*	33	BMS672-BM3628	3.28	9.71	2.109	0.6485 \pm 0.4106	0.3992 \pm 0.3048	-1.3063 \pm 0.8173	-0.823 \pm 0.3370	
29*	25	BMC3224-BMS764	3.46	10.24	2.224	0.1567 \pm 0.2304	0.484 \pm 0.2170	-0.2554 \pm 0.4384	-0.6773 \pm 0.2320	

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

Table 15. Positions of Flightiness QTL under Parent-of-Origin model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics				Effects			
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE	Parent-of-Origin \pm SE	
12*	22	BMS2252-RM094	4.33	12.76	2.77	0.9692 \pm 0.2938	0.1978 \pm 0.2849	-1.9649 \pm 0.5677	-0.1366 \pm 0.2990	
22*	32	BMS672-BM3628	3.37	9.95	2.161	0.7938 \pm 0.4211	0.4046 \pm 0.3115	-1.6047 \pm 0.8384	-0.782 \pm 0.3465	
29*	23	BMC3224-BMS764	4.28	12.59	2.734	0.1983 \pm 0.2479	0.6218 \pm 0.2269	-0.3232 \pm 0.4740	-0.7372 \pm 0.2431	

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

Table 16. Positions of Gregariousness QTL under Parent-of-Origin model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics			Effects			
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE	Parent-of-Origin \pm SE
22*	33	BMS672-BM3628	3.39	10.02	2.177	0.71 \pm 0.3883	0.289 \pm 0.2882	-1.4271 \pm 0.7730	-0.7219 \pm 0.3188

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

Table 17. Positions of Overall Weaning Disposition QTL under Parent-of-Origin model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics			Effects			
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE	Parent-of-Origin \pm SE
22**	35	BMS672-BM3628	4.2	12.38	2.689	0.6952 \pm 0.4249	0.5253 \pm 0.3189	-1.3993 \pm 0.8454	-0.9977 \pm 0.3486
29*	26	BMC3224-BMS764	4.01	11.82	2.567	0.2166 \pm 0.2287	0.5757 \pm 0.2187	-0.3678 \pm 0.4338	-0.6916 \pm 0.2336

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

**Significant at $P < 0.01$ at chromosome-wise level.

Table 18. Positions of Overall Yearling Disposition QTL under Parent-of-Origin model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics			Effects			
			F	LRT	LOD	Mean \pm SE	Additive \pm SE	Dominance \pm SE	Parent-of-Origin \pm SE
19*	0	BMS745-RM388	3.2	9.37	2.035	-0.3323 \pm 0.1567	0.1227 \pm 0.1410	0.6656 \pm 0.2867	0.1719 \pm 0.1327

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

Table 19. Positions of Heifer Calving QTL under Parent-of-Origin model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics			Effects			
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE	Parent-of-Origin \pm SE
13**	0	HUJ616-RM327	5.05	14.21	3.086	0.0787 \pm 0.2121	-0.0056 \pm 0.2235	-0.2532 \pm 0.3610	0.7679 \pm 0.2087

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

**Significant at $P < 0.01$ at chromosome-wise level.

Table 20. Positions of Slaughter Disposition QTL under Parent-of-Origin model, test statistics and size of effects

BTA	Position (cM)	Flanking Markers	Test Statistics			Effects			
			F	LRT	LOD	Mean \pm SE	Additive ^a \pm SE	Dominance ^b \pm SE	Parent-of-Origin \pm SE
11*	0	BM9067-BM7169	5.59	16.05	3.486	0.6831 \pm 0.16205	-0.0556 \pm 0.1653	-0.6311 \pm 0.3128	0.6005 \pm 0.1723

^aQTL genotypic value of Nellore homozygotes such that $2a=NN-AA$.

^bNA heterozygote deviation from QTL homozygote midpoint such that $d=NA-0.5(NN+AA)$.

*Significant at $P < 0.05$ at chromosome-wise level.

Nellore-Angus (NA) individuals (where the breed of sire is listed first) will have the same score as Nellore homozygotes (NN), and Angus-Nellore (AN) individuals will have the same scores as Angus homozygotes. If this parent-of-origin effect is the same sign as the additive effect, there is maternal imprinting, and there is paternal imprinting if the signs are different. Under these guidelines, the QTL on BTA3 and 19 were estimated to be partially maternally imprinted. The QTL on BTA6, 12, 13, 22 and 29, for every trait, were estimated to be partially paternally imprinted. Except for the flightiness QTL on BTA 12, the likelihood of odds (LOD) scores were higher for QTL estimated under the parent-of-origin model than the Mendelian inheritance model, indicating that the parent-of-origin model fits these data better.

Wegenhoft (2005) found QTL associated with final disposition in the Angleton herd on BTA 1, 4, 8, 9, 16, and 18 under the Mendelian model, and on BTA 4, 8, 16, and 18 under a parent-of-origin model. Ideally, QTL analysis in the McGregor population would serve to validate those findings, but only one QTL detected in the McGregor population overlapped a QTL detected in the Angleton population. The QTL on BTA 16 associated with disposition of the heifer at calving at 70 cM and flanked by INRA48 and BM3509 overlaps the QTL for final disposition in the Angleton population at 79cM and flanked by INRA013 and BMS462. The lack of concordance between these 2 studies was not completely unexpected, however, as disposition was scored differently in the 2 studies, and thus different phenotypes were measured.

Some of the QTL discovered in the McGregor analysis do verify regions identified in previous studies studies. Schmutz et al. (2001) discovered a QTL on

BTA11, associated with temperament, or response to isolation in a scale, and habituation, the difference between initial score upon arrival at the feedlot and a later day. That study reports a QTL at 57 cM on BTA11, whereas in this study it was found at 0 cM. However, as mentioned previously, Schmutz et al. (2001) reported associations for single markers as opposed to the flanking markers and confidence intervals were not reported.

Similarly, Hiendleder et al. (2003) performed a QTL mapping analysis in a population of German Holstein cattle, scoring milking speed and behavior as behavioral traits. Significant QTL were reported on BTA5, 18 and 29. The reported QTL on BTA29 is at 20 cM, and is significant at the experiment-wise level at $P < 0.05$ (Hiendleder et al., 2003). The QTL for various of the component traits of behavior that were detected on BTA29 in this study appear to map to the same position.

Interestingly, Voisinet et al. (1997a) discovered that individuals with a calmer score for temperament had a lower Warner-Bratzler Shear Force score, indicating a more tender meat product, and hypothesized that selection for calmer animals had indirectly selected against those susceptible to stress. However, it appears there may be another possibility. Calpain (*CAPNI*), located at ~37.5 Mb on BTA29 (Bovine Genome Database, Build 3.1; <http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/>), has been investigated as a candidate gene affecting meat tenderness in association with a QTL for that trait on BTA 29 (Page et al., 2004). Page et al. (2004) found an association with 2 SNP markers in *CAPNI* and shear force score in cattle.

The flanking markers of the QTL on BTA29, BMC3224 and BMS764, are located at approximately 32 Mb and 8 Mb, respectively (Bovine Genome Database, Build 3.1; <http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/>). The proximity of the QTL discovered in this study and that of the QTL for meat tenderness on BTA29, and the link between docility and meat tenderness (Voisinet et al., 1997a) lead to speculation about a possible overlap between these QTL. Perhaps *CAPNI* has some role in behavior, or some overarching regulation factor ties the 2 traits together. It would be worthwhile to continue to investigate the disposition QTL on BTA29 in relation to meat tenderness in order to determine what, if any, relation there is between meat tenderness and cattle temperament.

IMPLICATIONS

Cattle disposition is economically important in any production setting. More docile animals do not lend themselves to as much stress, are easier to handle, and allow for less chance of injury to handlers and themselves. Docile cattle have also been found to yield a higher average daily gain (Voisinet et al., 1997b; Fell et al., 1999), higher dressing percentages (Petherick et al., 2002) and more tender meat (Voisinet et al., 1997a).

Wegenhoft (2005) discovered a QTL associated with disposition on BTA8 with no obvious candidate gene. This study of candidate genes lends more information to the search for the gene affecting behavior within this region. Although a candidate gene has not been identified definitively, much was learned about the imprinting status of these genes, as well as microsatellite and SNP markers within them. This information has updated the linkage maps constructed by Wegenhoft (2005) and increased the knowledge of the QTL on BTA8. Additional work on the candidate genes presented in this study will yield more detailed information about their imprinting status in other tissues and at other developmental stages, to provide a more accurate understanding of their complete imprinting pattern. Continued studies of these and other candidate genes will narrow down the QTL region and allow for discovery of a gene within this region affecting behavior.

The McGregor QTL analysis has validated several of the QTL found in other studies (Hiendleder et al., 2003; Wegenhoft, 2005). Several new QTL positions were also discovered in association with various disposition and temperament traits. Future

studies may investigate these QTL regions to find candidate genes within them, as was done in this study.

As more is understood about which genes are affecting disposition traits in cattle, more can be learned about correlations between behavior and average daily gain, dressing percentages and meat tenderness. Marker association tests may eventually be developed to aid in selection for behavioral traits for breeding program use. The ability to breed for a more desirable disposition would have a great economic impact on the beef and dairy industries.

CONCLUSION

Four candidate genes affecting disposition on BTA8, *BMP1*, *BIN3*, *CTSB* and *FDFT1* were characterized in this study. Three SNP were discovered in the portion of *BIN3* amplified, 7 SNP in *CTSB*, 4 SNP in *FDFT1* and no SNP in *BIN3*. SNP in transcribed regions were used to investigate the imprinting status of each gene. There was no evidence of imprinting of *BIN3*, *CTSB* and *FDFT1* in muscle and liver collected at slaughter. Additional tissues and developmental time-points need to be investigated before these genes can be eliminated as candidates for the QTL on BTA 8. Additionally, a different portion of *BMP1* should be amplified and sequenced to find SNP elsewhere in the gene, and the imprinting investigation should be completed upon the discovery of a transcribed SNP.

The Angleton population linkage maps for BTA8 and BTA11 were updated with the addition of microsatellite markers at each of the 4 genes. In the McGregor population, these markers were used with 133 other markers to construct linkage maps for each of the 29 autosomes, and QTL analysis was performed in that population. QTL associated with several disposition traits were discovered on BTA3, 6, 12, 16, 26 and 29 under a Mendelian model, and on BTA3, 6, 11, 12, 13, 19, 22 and 29 under a parent-of-origin model. These findings did not validate all the QTL findings by Wegenhoft (2005), but that is not unexpected as different traits were scored. The new QTL should be investigated to determine candidate genes affecting behavior within these regions.

The results of this study and future work will lead to a greater understanding of genetic mechanisms affecting behavior, which may have a very real and practical application for breeding programs in the beef and dairy industries.

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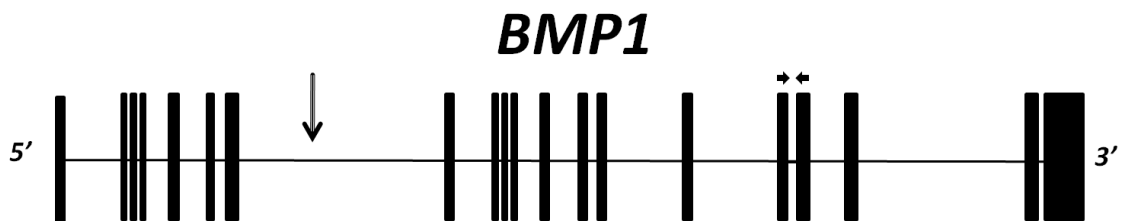
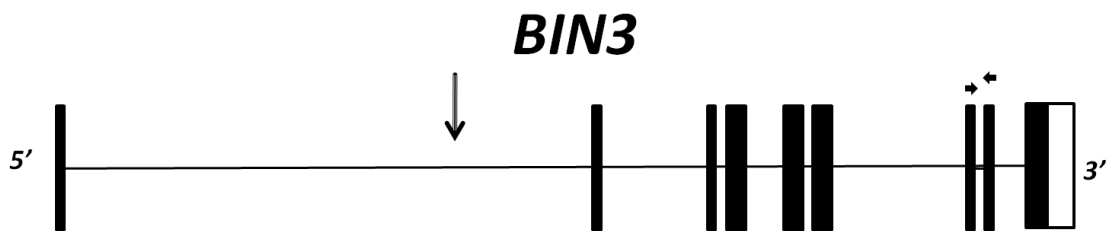
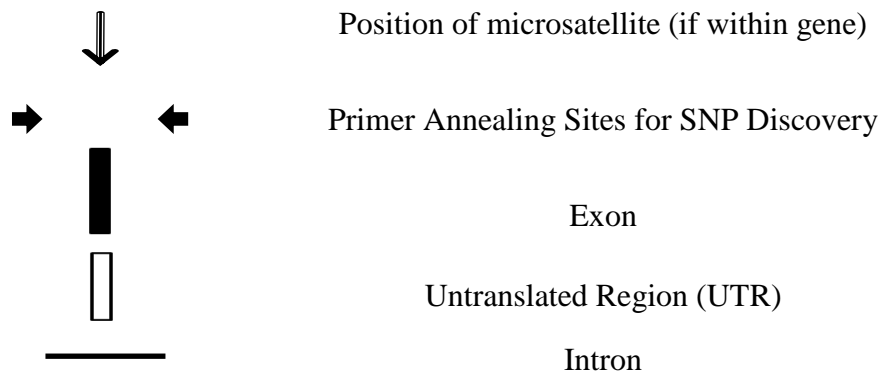
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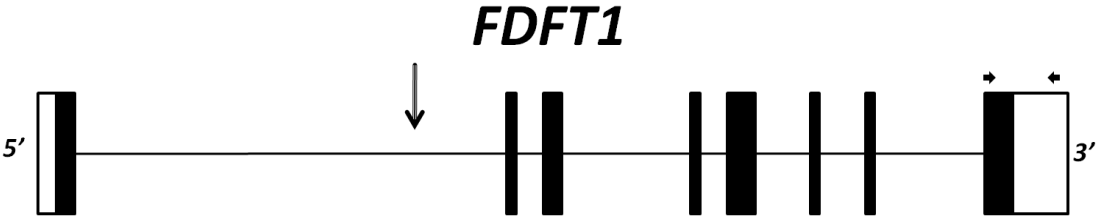
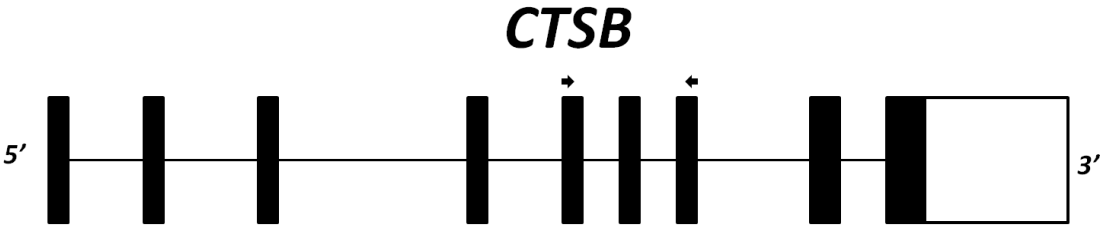
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APPENDIX A

Schematics of Candidate Genes**Legend**



Legend

-
-  Position of microsatellite (if within gene)
 -  Primer Annealing Sites for SNP Discovery
 -  Exon
 -  Untranslated Region (UTR)
 -  Intron

APPENDIX B

Sequences Obtained from Primer Sets Used for SNP Discovery²

BMP1 Genomic	GTCTGGAGGGTGTGAGGGAGAACAGAGAATCAGGAATAT GGCCAAGGGCAGGAGACGTGGGCAATAAACCAAGACCT CTGGACCCAGTTTAGGACAGTGGCCACTGCTGGTGGAT GGGGAGAGGGAGGACCTAGGAATGGGGCAATGGCAGGT ATACCCCAACATGATGCTGGGGAAAGGGCCTCTGTCCAG GCCAGACTTGCAGTTCCCATAGGGATGCCGGCCCTGTGG GCTCAGCCAGTCACCAGCCTAAGGTGCTCTGAGTGACGT GAGGCTTTGGAGCTGATGGGAAGTAGGTGATGGGTAGAT GGCATGGAGGAGCAGGCCTGGGAGTGGCACCTTACCAGC TTGACCCGGTGCCAGGGGTGCTAGAG
BIN3 Genomic	CTCCACCAGGTACCAGGAAGAAATGCTGGGRGTGGACCG GAAGCCGTGTGGTCCGACACGGCAAGTTCCTCCTTCACCA AGGGGCTAAGACACCAGATGTCCTGACACCTCCGCCTTC CCTCYACCACCCACCCGACCCCGACACACACACACAT CTTGCCCCAGTCAGGGTTCTGGGTGAGCCGTGACACCCA GCGTGTGCTCCCCAGGCCCGAGAAGAGCTTAGGCCAGT KCGGGATGACTTTGAGGCCAAGAACAAGCAGCTCCTGGA TGAGATGCCGCGCTTCTACAACAGCCGA
BIN3 cDNA	CCAGGCCCGAGAAGAGCTTAGGCCAGTKCGGGATGACTT TGAGGCCAAGAACAAGCAGCTCCTGGATGAGATGCCGCG CTTCTACAACAGCCGACTGGACTAGAGCTCTTCTCGGGCC TGG

² The sequences are reported using IUB single-letter codes for nucleotides (IUB Nomenclature Committee, 1985).

CTSB Genomic GGGCCTCTATAACTCKCATGTAGGTGAGTGTCTGCCTTTA
GCTCCCGTCCAGCCAGATGGATTTTATAAGCAGGAGTAA
TAGTGGTCTCATTTCCTATAAAGTGAAGATCAGGGTT
GAGGGTACATTGAACTCTGGGCTGAAAACTCARGTAGAA
GACAGGGCAGCCCTGTGTACACAGAGTCAGCAACTGGAA
GAGGGCAGTGTTGGCGCCACAGACTCTGGGCGACTTCCG
TTCTCATGAGCTCTCGCCCTGATTCTTCTGTCTGGCCTCTG
AGGGGCTTCCTGCTGACACCACCTTTCTCCTYGGCCCCCA
GGTTGCAGACCGTACTCCATCCCTCCCTGTGAGCACCATG
TGAACGGCTCCCGGCCCCCGTGCACCGGTGAGGGGGACA
CCCCAAGTGCAGCAAGACCTGTGAGCCCGGCTACAGYC
CGTCTACAAAGAAGACAAGCATTTCGGTAAGTGGGGTG
GGCGACCCCGCCCGGGGCCAGGGAAGCTGAAGGAGG
AGGCCAGAGCRTTGGAGTCRTAGRTCAGGAGAAGCGATG
CGGTTAGTGCCTCTTTGATGGGAGGGAGCCGCACGGTGTT
CTCTGCGTGTCTCCACCCTGAACGGAGTCTTAGACCCTCA
GTCTCGGAAGAAAGAGACCAAACCCCTCAGTCACGGGGC
AGTAATCTGGCCTAGGAGCC

CTSB cDNA GGGCCTCTATAACTCKCATGTAGGTTGCAGACCGTACTC
CATCCCTCCCTGTGAGCACCATGTGAACGGCTCCCGGCC
CCGTGCACCGGTGAGGGGGACACCCCAAGTGCAGCAAG
ACCTGTGAGCCCGGCTACAGCCCGTCCTACAAAGAAGAC
AAGCATTTCGGATGCAGYTCCTACAGCGTCGCCAACAAC
GAGAAGGAGATC

FDFT1 Genomic ACAGAATCTCCCCAACTGTCAGCTGGTCTCGCGGAGCCA
CTACTCGCCCATCTACCTGTCGTTTCGTCATGCTCCTGGCG
GCCCTGAGCTGGCAGTACCTGAGCACCCCTGTCCCAGGTC
ACAGAGGACTAYGTTTCAGACCGGGGAGCACTGACTGGCT
CGGTCTGGAGACTGAACGCCCTCCTCCCAAGCCCCTATC
TGGGAAACAGACTGACCTTCTCTTCAGGGATGGATGTGG
GCTCCTTCTCTTTTTTCCCCTMCTGTTTTAATCCCTCAAAG
AGTACTGTGGGCCTGGACCTTTAGAAACTGTGACCTGTGG
TGGAGAAAAGATAGGATTAAGGGAAAGGACAGCTCA
GCCACCTGTACTCACCTGTGCGGGGTGACTGACGCCGAA
CGTTCACGGCTGCCATCARGGAAGGGGCTGCATCCGGGG
CTGCAGAGGAGATYATAGTGTGAATACAGGCTAGAGTTA
CAATTAATGTATTTAATGCAAACAACCTTTTGAATACCT
ATCACAGTAGAAAGTGAAGTGAATTTTCTTTCCATTTCGCT
TCTTGTTTTTTTTCCATCATTGTCTCTTCCAGTGGACTT
GAATGTAGCAGGTGTGAATATTTGTAGAGTTCTAGGAAA
TATTCCTAAGAATGCAGACTGCCTGCTGCACATGAAGCCT

FDFT1 cDNA GAATCTCCCCAACTGTCAGCTGGTCTCGCGGAGCCACTAY
TCGCCCATCTACCTGTCGTTTCGTCATGCTCCTGGCGGCC
TGAGCTGGCAGTACCTGAGCACCCCTGTCCCAGGTCACAG
AGGACTATGTTTCAGACCGGGGAGCACTGACTGGCTCGGT
CTGGAGACTGAACGCCCTMCTCCCAAGCCCCTATCTGG
GAAACAGACTGACCTTCTCTTCAGGGATGGATGTGGGCT
CCTTCTCTTTTTTCCCCTACTGTTTTAATCCCTCAAAGAGT
ACTGTGGGCCTGGACCTTTAGAAACTGTGACCTGTGGTGG
AGAAAAGATAGGATTAAGGGAAAGGACAGCTCAGCC
ACCTGTACTCACCTGTGCGGGGTGACTGACGCCGAACGTT
CACGGCTGCCATCARGGAAGGGGCTGCATCCGGGGCTGC
AGAGGAGATYATAGTGTGAATACAGGCTAGAGTTACAAT
TAAATGTATTTAATGCAAACAACCTTTTGAATACCTATCA
CAGTAGAAAGTGAAGTGAATTTTCTTTCCATTTCGCTTCTT
GTTTTTTTTCCATCATTGTCTCTTCCAGTGGACTTGAAT
GTAGCAGGTGTGAATATTTGTAGAGTTCTAGGAAA

Predicted Sequences of Region Amplified by Microsatellite Primers

BMP1 Microsatellite 1 CCAAGAACAGTAGCCACCAGCTCTCTGCCTTCTTTTGCACCAG
 TTCG[TGTGTGTGTGTGTGTGTGTGTG]CATAGAGGAAGGAAAGA
 AGTGTCAGAAATGGGCCTTCAGCTAGAGGGAGAGATAGAAG
 AAAAATTCAGGTGTGGCTGTATATACTCAGGTCAGATGAAAC
 GTCCTG

BMP1 Microsatellite 2 ATGGGGTTGATACAAGGGTTAAACAAGTTAATGTACATAAAG
 TGCTTATAGGGCACTGCCTAAACATAGCAGGAATTCAGTGTT
 AGCTGCTAGTATCATTCT[TGTGTGTGTGTGTGTGTATGTG]CTC
 AGTCATGTCTGACTTTTGTACCCCAT

BIN3 Microsatellite ACAAAAAAAGAACAGCCAAGTTAGAAGTGCTCATCTCAAGCA
 ACTGAGTGAGACTGGGGAGCCTAGAACCTAATGTTTTGCTTA
 GCAAATATTGTTCCCTTATTAAACAATGCACATGTATAGCTTT
 GATAAGAGCAAAGCTTAAAGAGGAAGTCATAAAAGTAATAA
 AGTAAATCTAGATGAATTTTTCTTGTTGGGGTGGAGGAGGCA
 GGTCTTTTTAGGACATCAAAGAAGAAATCATAAAGGAAAAA
 GATAGACTTGGTCATTTTGACTTCATACTAATTAACCCCTCT
 GTATGTTAAA[CACACACACACACACACACACA]CGTAAAATG
 GAGAGGAAACAAGTAAGGCAGGAAAAGAATCCTGAT

CTSB Microsatellite

AGCCTCACACATGGATTCTTCCCAGTGGCTTGTTGCTTAAGCA
ACAGATAAATTTAGATTTGTTTTGCTTAAATAGAATTCCTG
TGTGGGAGGCAGCGTGTTCAAGTGCTGTCACGCACGTCATCTTC
CTGTACCGCTCAGCAACCTGTGACCTGAGGAGGTGACACAGT
GACCAGCTCCTTTGTGCGCGTG[CACACACACACACACACT
CACACACACACACTCACA]CTCACTGAGAGGGCATCTTCCCAG
GCCCCGGGGTGGGGTGCTGTGGCACCTGGGTCATATTTCTTGC
TCTCATCAGAGCCTCCTGCCTAAGAGTGTGACTCTAGAACTCG
CCTGTCTCTCGACCTGCAGGCCAGTCCAGGATCTAAGGCCGTA
ATGGTAATGGGATGA

FDFT1 Microsatellite

AGCAATTCCTTCTGATATGGTTGTGTATTAAAATACGGATTTT
TTTTAAGAAACAGTTTTTGTTCCTTAGGGAAGAGTAAATA
TAAAGTTCATAAAGGATTGTTATGGTTCATTGATTTGAAAGGGA
AAGATGATTAGAAATCTTTATAAAAGGAATAATTAGATTTCC
AGAGGATAATAGAGG[TGTGTGTGTGTGTGTGTGTGTGTGTGT
GTG]CTCAGTCATGTCCAACCTTTGCGACCCCTTGAAATAGT
AC

APPENDIX C

Linkage Maps Developed in Angleton Population

BTA8			BTA11		
Marker	Recombination Fraction	Distance (Kosambi cM)	Marker	Recombination Fraction	Distance (Kosambi cM)
BMS1864		0.0	TX11_BULL2F		0.0
	12.1			10.3	
CTSB		12.1	BM827		10.3
	4.6			9.8	
FDFT1		16.7	BM716		20.1
	12.8			16.4	
BM3419		29.5	INRA177		36.5
	9.3			5.5	
BM310		38.8	BMP1_MS1		42.0
	2.4			2.6	
TGLA10		41.2	TGLA327		44.6
	10.3			2.8	
TX6_BESSIE19		51.6	CSSM16		47.4
	5.7			6.7	
CSSM37		57.3	BM6445		54.1
	4.2			34.4	
TGLA13		61.5	BM746		88.5
	5.1			7.6	
RM32		66.6	TGLA436		96.1
	8.2			4.5	
LPL		74.8	BM6491		100.6

Linkage Maps Developed in McGregor Population

BTA1			BTA3		
Marker	Recombination Fraction	Distance (Kosambi cM)	Marker	Recombination Fraction	Distance (Kosambi cM)
162M1		0.0	BMC4214		0.0
	1.9			14.3	
MS60773		1.9	BMS896		14.3
	1.2			16.7	
BM6438_34		3.1	BM7225		30.9
	1.5			27.2	
SOD1M2		4.6	ILSTS64		58.1
	0.8			14.2	
TGLA49		5.4	HUJ246		72.3
	22.4			9.1	
MS34554		27.8	INRA003		81.4
	22.4			13.5	
BMS4037		50.2	BM723		95.0
	13.4			7.3	
RM326		63.6	BMS963		102.3
	12.3			7.6	
BMS4008		76.0	BMS2904		109.8
	21.3			4.8	
BM864		97.2	INRA006		114.6
	59.3			10.4	
MS70223		156.6	BMS871		125.0

BTA2			BTA4		
Marker	Recombination Fraction	Distance (Kosambi cM)	Marker	Recombination Fraction	Distance (Kosambi cM)
RM041		0.0	BMS2809		0.0
	100			19.4	
FCB11		100.0	BMS1840		19.4

BTA5			BTA7		
Marker	Recombination Fraction	Distance (Kosambi cM)	Marker	Recombination Fraction	Distance (Kosambi cM)
CSSM22		0.0	BL1043		0.0
	11.8			36.0	
CSSM34		11.8	BM9065		36.0
	9.8			21.3	
RM103		21.6	BMS2258		57.3
	16.0			8.5	
BMS610		37.6	BMS2840		65.8
	16.0			42.0	
BMS1095		53.6	IL4		107.8
				25.5	
			BM7160		133.3
BTA6			BTA8		
Marker	Recombination Fraction	Distance (Kosambi cM)	Marker	Recombination Fraction	Distance (Kosambi cM)
BMC4203		0.0	BMS1864		0.0
	16.2			7.4	
BM8124		16.2	CTSB		7.4
	5.3			0.6	
BMS2460		21.4	FDFT1		8.0
	18.4			10.8	
BM4621		39.8	RM372		18.8
	3.2			27.8	
BMS360		43.0	BMS678		46.6
	10.3			5.8	
BMS518		53.3	BM4006		52.4
	6.8			11.2	
BM143		60.1	BMS2072		63.6
	8.6			15.0	
BMS2508		68.7	BIN3		78.6
	7.2			3.8	
BM1239		75.9	BM3412		82.5
	25.7			6.6	
INRA133		101.6	BM711		89.0
	7.0			26.5	
ILSTS093		108.5	BMS2847		115.5
				100.0	
			BP2		215.5

BTA9			BTA11		
Marker	Recombination Fraction	Distance (Kosambi cM)	Marker	Recombination Fraction	Distance (Kosambi cM)
BM757		0.0	BM9067		0.0
	1.6			33.7	
BMS47		1.6	BM7169		33.7
	23.9			15.8	
BM2504		25.5	RM150		49.5
	25.2			33.4	
BMS434		50.7	BL1103		82.9
	19.7			8.4	
BMS1724		70.4	ILSTS45		91.3
	10.3			10.6	
BM4208		80.6	HEL13		101.9
	10.5				
BMS2295		91.2			
	14.0				
BMS1967		105.2			

BTA10			BTA12		
Marker	Recombination Fraction	Distance (Kosambi cM)	Marker	Recombination Fraction	Distance (Kosambi cM)
CSSM46		0.0	TGLA36		0.0
	2.9			10.4	
TGLA272		2.9	BMS2252		10.4
	15.0			15.3	
BMS1318		18.0	RM094		25.7
	17.2			13.3	
INRA71		35.2	BMS975		39.0
	15.2			19.5	
BM875		50.4	BMS2598		58.5
	12.0			4.5	
BMS2742		62.4	INRA005		63.0
	9.2			12.8	
BMS528		71.6	BMS1316		75.8

BTA13

Marker	Recombination Fraction	Distance (Kosambi cM)
HUJ616		0.0
	11.9	
RM327		11.9

BTA15

Marker	Recombination Fraction	Distance (Kosambi cM)
BMS812		0.0
	11.8	
JAB8		11.8
	9.8	
BMS1004		21.6

BTA14

Marker	Recombination Fraction	Distance (Kosambi cM)
BMS1747		0.0
	21.8	
RM11		21.8

BTA16

Marker	Recombination Fraction	Distance (Kosambi cM)
BMS357		0.0
	7.4	
BMS1348		7.4
	11.4	
BM121		18.8
	8.2	
TGLA53		27.0
	8.3	
BMS1907		35.3
	12.2	
ETH11		47.4
	22.2	
INRA48		69.6
	13.6	
BM3509		83.2
	6.9	
BMS462		90.1

BTA17

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BM305		0.0
	0.00	
BM305		0.0

BTA18

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BM2078		0.0
	91.0	
BMS1355		91.0

BTA19

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BMS745		0.0
	98.4	
RM388		98.4

BTA20

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BMS1282		0.0
	29.8	
BMS2361		29.8

BTA21

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BMS2382		0.0
	19.2	
TGLA122		19.2
	8.4	
TGLA337		27.6
	18.2	
BMS2815		45.7
	4.7	
BMS2557		50.5
	4.0	
BM103		54.5
	9.1	
ILSTS095		63.7
	11.3	
RM151		75.0
	9.3	
BM8115		84.3

BTA22

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BMS672		0.0
	38.1	
BM3628		38.1

BTA23

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BM1818		0.0
	28.5	
UWCA1		28.5

BTA27

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
INRA134		0.0
	6.9	
CSSM43		6.9

BTA24

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BMS2270		0.0
	5.7	
AGLA269		5.7

BTA28

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BMS1714		0.0
	24.8	
IDVGA29		24.8

BTA25

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
AF5		0.0
	30.1	
BM737		30.1

BTA29

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BMC3224		0.0
	38.1	
BMS764		38.1

BTA26

	Recombination	Distance
Marker	Fraction	(Kosambi cM)
BM804		0.0
	7.9	
IDVGA59		7.9
	26.3	
HEL11		34.3

CURRICULUM VITA

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Research & Work Experience:

Texas A&M University Animal Breeding & Genetics Lab, College Station, TX

Student Worker: September 2005-Present

- Routine lab maintenance, DNA extraction, PCR reactions, research in cattle genetics, independent research project in cattle disposition.

Texas A&M Senior Honors Research Fellows, College Station, TX

Research Fellow: August 2007-May 2008

- Thesis: A Study of Cattle Disposition: Exploring QTL Associated with Temperament
- Presented research to peers as well as at American Society of Animal Science Southern Section, Texas Genetics Society & TAMU Student Research Week

Texas A&M University Cereal Grain Quality Lab, College Station, TX

Student Worker: August 2004 – September 2005

- Analyzed various cereal grain crops for quality and yield productivity.

Leadership Experience:

Agriculture & Natural Resources Policy Program, Washington, D.C.

Summer 2007

- Honorable Michael McCaul, Texas Congressional District 10
- Assist in office management and attend science and ag policy hearings

Agriculture & Life Sciences Student Council, Texas A&M University

August 2006-Present

- Represented students of the college on the governing student body.
- Committee responsible for developing and implement freshman leadership organization in the college

Honors and Awards:

- 2008 American Society of Animal Science Southern Section 1st Place Undergraduate Oral Research Presentation
- 2008 Texas Genetics Society 1st Place Undergraduate Oral Presentation
- 2008 Texas A&M University Student Research Week Genetics Session & Genetics Taxonomy Winner for Oral Research Presentation
- TAMU University Honors, University Scholar & Lechner Endowed Scholar