QUANTITATIVE TRAIT LOCI MAPPING AND CANDIDATE GENE ANALYSIS FOR GROWTH AND CARCASS TRAITS ON TWO BOVINE CHROMOSOMES

A Thesis presented to the Faculty of the Graduate School

University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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AUGUST 2006

The undersigned, appointed by the Dean of the Graduate School has examined the thesis entitled

QUANTITATIVE TRAIT LOCI MAPPING AND CANDIDATE GENE ANALYSIS

FOR GROWTH AND CARCASS TRAITS ON TWO BOVINE CHROMOSOMES

Presented by Ping YAO

A candidate for the degree of Master of Science

And hereby certify that in their opinion it is worthy of acceptance.

Dr. Jeremy F. Taylor

Dr. Robert D. Schnabel

Dr. Gary S. Johnson

To my wife and son,

Xiaohong Liu and Daniel L. YAO

ACKNOWLEDGEMENTS

I wish to acknowledge deep appreciation and gratitude to my advisor, Dr. Jeremy Taylor for his guidance and support during my study. Dr. Taylor has not only expanded my knowledge on various academic subjects, but has also broadened my knowledge on a practical side as well. I appreciate the time and friendship shared by Dr. Taylor.

Special thanks are given to Dr. Robert Schnabel, my lab supervisor, for his guidance and knowledge in developing my techniques to perform this study.

Thank you, Dr. Gary Johnson, for being on my graduate committee and for your teaching, advice and support.

I would like to extend my thanks to the graduate students, faculty and staff in the Division of Animal Sciences.

Thanks to David Gust, the owner of the Circle A Ranch of Iberia, MO for donating over 5,300 Angus DNA samples and carcass records.

I would like to sincerely thank my fellow lab-mates: Natalya, Matt, Erin and Jae-Woo who shared bench work and fun with me, and who encouraged and helped me.

Last, but most importantly I would like to dedicate this thesis to my loving wife Xiaohong and our son Daniel, for their love, understanding and continuous support throughout the course of this study.

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CHAPTER I

INTRODUCTION

Background

Quantitative traits are complex and are controlled by the additive effects of multiple genes, interactions between genes and environmental effects. Most of the economically important traits in livestock such as meat quality, milk yield and growth are quantitative. The goal of mapping quantitative trait loci (QTL) in livestock is to identify the causal genes and nucleotides (Quantitative Trait Nucleotides; QTN) that underlie QTL so that the identified QTN may be used to select elite animals in breeding programs.

Growth, meat quality and milk production are among the most important traits for the beef and dairy cattle industries. With an escalating number of cattle being marketed in value-based pricing systems (Schroeder et al., 2002), meat quality grade and marbling are becoming increasingly important in obtaining carcass premiums for beef producers. Numerous factors, including the regulation of lipogenesis, genetic predisposition, age, nutrition and management affect the rate of marbling deposition. Provided nutrition is not limiting and that animals are appropriately vaccinated, genetics is the most important factor affecting meat quality and marbling score.

Growth traits of cattle are highly heritable and the three most commonly measured growth traits: birth weight, weaning weight and yearling weight are highly correlated (Kim et al., 2003). Growth directly affects gross feed efficiency and is vitally important to producer profitability. During the previous several decades the genetic

component of milk yield of dairy cattle has increased by at least 3,000 kg due to the use of quantitative genetics and BLUP for the prediction of breeding values. Milk yield and composition are polygenic traits and many QTL controlling milk yield have been detected on different chromosomes in several dairy cattle populations.

Identifying the genes affecting quantitative traits of economic importance in agricultural species has the potential to significantly increase the rate of genetic improvement through the use of marker-assisted selection.

Objectives

The primary objective of the present study was to identify the regions on chromosomes 4 and 14 containing QTL influencing growth and carcass traits in Angus cattle. The secondary objective was to evaluate the effects of diacylglycerol O-acyltransferase 1 (*DGAT1*), thyroglobulin (*TG*) and Leptin (*LEP*) on growth and carcass traits in Angus cattle.

CHAPTER II

LITERATURE REVIEW

QTL Mapping

Recent developments in molecular biology and statistical methodologies for quantitative trait loci (QTL) mapping have made it possible to localize economically important QTL and will expedite genetic improvement via marker-assisted selection, gene introgression, and positional cloning in livestock species (Andersson, 2001).

Cattle have been selected for growth, meat and milk for hundreds of years. These traits are influenced by genes and environmental factors. Advances in molecular and quantitative genetics now allow the dissection of genetic variability underlying complex traits into discrete QTL effects. A QTL is defined as a small chromosomal segment inherited according to Mendelian transmission patterns and that affects a trait of interest in that individuals that inherit alternate QTL alleles have mean phenotypes that differ. QTL mapping is a phenotype driven approach to the identification of gene function. As such it permits the discovery of new genes that control complex traits. QTL are natural genetic variations that exist in different cattle populations which may be under natural and artificial selection.

A resource population and variable markers are the basis for QTL mapping. The bovine genetic map now comprises over 3,800 microsatellite marker loci (Ihara et al., 2004). Most of the beef cattle QTL mapping experiments perfomed to date have been based on half-sib families, backcross (BC) or F_2 designs (Georges et al.,1995; Zhang et

al.,1998; Kim et al., 2003). In order to map a QTL onto the linkage map, associations between marker genotypes and phenotypes are detected through various statistical approaches that are based on ordinary least squares, maximum likelihood or Markov Chain Monte Carlo methods.

Carcass Trait QTL

USDA beef quality and yield grades are composite indices of factors that affect the palatability and cutability of meat. These factors include carcass maturity, firmness, texture and color of lean and the amount and distribution of marbling within the lean. Beef carcass quality grading is based on degree of marbling and maturity. Similarly beef yield grade is based upon the area of the ribeye muscle and the fatness and evenness of distribution of fat on the exterior of the carcass. Thus, there are several key factors that determine the yield and quality of beef. However, the majority of these traits are difficult or are expensive to measure in sufficient numbers of cattle to allow effective selection based upon predictions of genetic merit. These are the traits for which the detection of QTLs through mapping experiments and the use of these QTLs in marker-assisted selection programs is most justified (Casas et al., 2003). Genetic markers and linkage maps provide the tools necessary to detect these QTLs (Stone et al., 1999; Casas et al., 2000; Kim et al., 2003).

However, while many QTLs have been detected in beef cattle, only a few genes have been identified in which functionally significant mutations control variation in phenotype and which are currently being used commercially as tests for cattle breeding.

These include *DGAT1*, *TG*, and *LEP* which have been associated with variation in beef quality (Thaller et al., 2003; Barendse et al., 2001; Geary et al., 2003), but have yet to be shown to be the causal QTNs underlying the QTL in these chromosomal regions.

Genome-wide linkage analysis is the traditional method to identify QTL and has been successful for mapping important QTLs. However the success of QTL mapping is limited by: the low heritability of most complex traits; the low resolution of genome scans using microsatellite markers which are usually spaced about 10 centimorgans (cM) apart throughout the genome; the imprecise definition of phenotypes and inadequate experimental designs which limit the power to detect QTL (Weller et al.,2005).

Candidate gene resequencing studies based on functional relevance is an practical alternative to linkage analysis. In these hypothesis–based studies, genes are selected either according to their location in a genomic region where previous linkage studies have demonstrated the existence of a QTL, or on the basis of physiological function often in other mammals. Several genes such as *DGAT1* have successfully been identified by this method.

However, the rate of QTN discovery in beef cattle for growth and carcass traits has been low. One of the main reasons for this has been the lack of a whole genome sequence of bovine or closely related mammalian species. In 2001, the draft human sequence became available and using the bovine-human comparative map it became possible to infer sequence and gene organization in bovine from that in human. By examining the orthologous regions in human to a region harboring a QTL in cattle, it is

possible to identify putative candidate genes that may underlie the QTL. Additionally by designing PCR primers based upon sequence within these candidate genes that is highly conserved among closely related species, it is usually possible to produce the sequence of the orthologous bovine gene and thus identify SNPs and other genetic variants that segregate within cattle. These mutations can be statistically tested for their effects on phenotype and also molecularly tested for functionality to provide evidence either for or against their candidacy as the causal mutation underlying the QTL.

Several reports have identified QTLs affecting carcass trait using genome scans (Casas et al., 2001, Mizoshita et al., 2004, Mizoguchi et al., 2006). Casas et al. (2001) reported a QTL influencing hot carcass weight and Mizoshita et al. (2004) reported marbling and longissimus muscle area QTLs on BTA4. The marbling QTL in that Japanese Black population located at 55-cM and confidence interval was from 52 to 62-cM. The longissimus muscle QTL in the same population located at 60-cM and confidence interval was from 52 to 67-cM. Mizoguchi et al. (2006) detected a marbling QTL at 51-cM on BTA4 using 258 offspring of purebred Japanese Black cattle after a primary screen. In the additional paternal offspring population, Mizoguchi et al. (2006) found a marbling QTL at 63-cM on BTA4.

Casas et al. (2003) and Mizoshita et al. (2004) reported marbling QTL on BTA14 while McNeil and Grosz (2002) reported a ribeye muscle area QTL and Kim et al. (2003) and Mizoshita et al. (2004) reported a QTL influencing hot carcass weight on BTA14. Casas et al. (2000), Casas et al. (2003) and McNeil and Grosz (2002) reported QTL

affecting fat depth and yield grade on BTA14. Stone et al. (1999) reported a QTL influencing longissimus muscle area, Moore et al. (2003) reported at backfat QTL and Mizoshita et al. (2004) reported a slaughter weight QTL on BTA14.

A genome wide scan for QTL affecting carcass traits in a *Hereford* × composite double backcross population was conducted spanning 2,413 cM on 29 bovine autosomes using 229 microsatellite markers and two rib eye area QTL on BTA14 were detected at 40-cM and 46-cM. (MacNeil and Grosz, 2002)

Studies by Casas et al. (2000), Ashwell et al. (2004), and Moore et al. (2003) detected a QTL for fat traits on BTA14. Casas et al. (2000) identified a QTL for fat thickness between 10 and 20-cM on BTA14. The support interval for this fat thickness QTL placed the QTL at between 0 and 16-cM.

Mizoshita et al (2004) detected several carcass QTLs in a Japanese Black (Wagyu) cattle population, which included a carcass weight QTL located on BTA14 (22 to 39-cM), and a QTL for beef marbling score on BTA4 (59 to 67-cM).

Two candidate genes located at the centromeric region of BTA14 affecting fat production have been identified. The *DGAT1* gene has been associated with an effect on milk fat content in dairy cattle (Grisart et al., 2002) and an effect on intramusclar fat deposition in beef cattle (Thaller et al., 2003). The second gene, Thyroglobulin, has been shown to be associated with intramuscular fat deposition in long-fed cattle (Barendse et al., 2001).

Table 1. QTL detected on BTA14*

Trait Name	Starting Marker	Ending Marker
Longissimus Muscle Area	RM180	RM180
Fat Depth	RM180	RM011
Fat Percentage	BM1508	BM302
Fat Depth	BMS1678	RM180
Yield Grade	BMS1678	BM8215
Marbling Score	BL1029	BMS1304
Fat Content	BM1508	BM4630
Fat Percentage	BMS1747	BMS1747
Fat Yield	BM1747	BM1747
Protein Percentage	BMS1747	BMS740
Ribeye Muscle Area	BMS108	BM2394
Ribeye Muscle Area	BM2934	BM2934
Fat Percentage	BM1508	BM8215
Fat Yield	BM1508	BM1508
Fat Percentage	BM1508	RM180
Protein Percentage	BM1508	BMS1747
Milk Yield	BM1508	BMS1747
Fat Percentage	BM1508	BMS1678
Fat Percentage	BM1508	BM302
Fat Yield	BM1508	BM1508
Fat Percentage	BM1508	BM1508
Protein Percentage	BM1508	BM1508
Fat Yield	BM1508	BM1508
Fat Percentage	BM1508	BM1508
Fat Percentage	BM1508	BM1508
Milk Yield	BM4305	BM4305
Birth Weight	BMC1207	BM1577
Birth Weight	BMS1899	RM137
Average Daily Gain on feed	CSSM66	BMS1747
Average Daily Gain on feed	BMS1747	TG-2
Average Daily Gain on feed	BMC1207	BM1577
Initial body weight	BL1009	BM8215
Slaughter weight	BMS740	BMS1899
Hot Carcass Weight	RM192	BMS1899
Marbling Score	BMS947	BMS947

*http://bovineqtl.tamu.edu/

Table 2. QTL detected on BTA4

Trait Name	Starting Marker	Ending Marker
Birth Weight	BMS648	CA088
Hot Carcass Weight	BL1024	MAF70
Postweaning Average Daily Gain	BL1024	MAF70
Meat Tenderness	BL1024	BMS1237
Longissimus muscle area	BM6437	BM6458
Marbling Score	BM6437	BL21

*http://bovineqtl.tamu.edu/

Growth Trait QTL

Growth traits are highly heritable and expected progeny differences (EPD) can be of tremendous value in selecting breeding stock. Birth weight, weaning weight and yearling weight are the three main growth traits and all are highly correlated (Kim et al., 2003). Calf birth weight is the main factor causing dystocia (Johnson et al.,1988). So identifying genes affecting birth weight, but not sequent growth will provide tools with which to break the genetic antagonism between birth weight and subsequent growth. The objective of most beef cattle producers is to select sires that produce calves with large weaning and yearling weights but with moderate birth weights.

Casas et al. (2001) detected a postweaning average daily gain QTL on BTA4 from two half-sib families, which were developed from a Belgian Blue× MARC Ⅲ and a Piedmontese ×Angus. Individuals inheriting the Belgian Blue allele gained weight faster and were heavier than those inheriting the MARC Ⅲ allele. Using three-generation mapping population of Angus and Brahman, Kim et al. (2003) found a birth weight QTL at 98-cM flanking by markers BPGM and RM88 on BTA4.

A half-sib family design involving purebred Japanese black cattle was used to scan 29 bovine chromosomes using 342 microsatellite markers spanning 2,664 cM (Mizoshita et al., 2004). Five growth related QTLs were located on BTA14 including slaughter weight and carcass weight in the interval from 29 to 51-cM. Two of the three detected growth QTLs were located in regions previously reported by Kim et al. (2003) to harbor growth QTL. Mizoshita et al. (2004) used 17 microsatellite markers to confirm

the QTL for five growth related traits (weight at 9 months before fatting, weight at slaughter, cold carcass weight, ADG before fatting, ADG from 9 to 30 months during fatting) on BTA14. Their results indicated that five growth related traits were positively affected by the same haplotype.

Kneeland et al. (2004) reported the identification and fine mapping of QTL for birth weight (BWT), preweaning ADG (PWADG), and postweaning ADG on feed (ADGF) in a commercial line of *Bos taurus* using an identical-by-descent haplotype sharing method. On BTA14, they found three haplotypes associated with each of the three growth traits. Haplotype BMS1678-130/BMS1941-111, BMC1207-151/BM1577-152, and BMS1899-117/RM137-149 all had significant positive effects on BWT. The three haplotypes spanned three regions, 26.0 to 26.7-cM, 36.2 to 46.2-cM and 52.0 to 67.7-cM. For PWADG, the three haplotypes that showed significant associations covered one chromosome region, 26.7 to 50.8-cM. Two regions 17.0 to 24.0-cM and 36.2 to 46.2-cM, harboring haplotypes CSSM66-198/BMS1747-89, BMS1747-89/TG-2, and BMC1207-153/BM1577-143 were found to have significant associations with ADGF.

Most of the confidence intervals for QTL locations are typically in the 10 to 30-cM range which is insufficient for positional cloning or for the effective use of the QTL information in a breeding program. Linkage disequilibrium (LD) has been suggested for the high-resolution mapping of QTL (Kruglyak, 1999).

Candidate Genes

Studies with different populations are required to properly characterize the

robustness of associations of polymorphisms in candidate genes with economically important traits across beef cattle populations before this sort of genetic information can be used efficiently in breeding and management decisions.

DGAT1

The *DGAT1* gene on bovine chromosome 14 was the first positionally cloned QTL in an outbred mammal (Grisart et al., 2002). *DGAT1* encodes acylCoA: diacylglycerol acyltransferase, spans 8.6-kb and comprises 17 exons ranging in size from 42 to 436-bp. While the first two introns are respectively 3.6 and 1.9-kb long, the remaining 14 introns are on average only 92.4-bp long. All introns conform to the GT-AG rule and are strictly conserved between human and bovine. The human and bovine *DGAT1* nucleotide (coding) and protein sequences are respectively 89.5% and 92.5% identical. The nonconservative K232A substitution in bovine *DGAT1* has a major effect on milk fat content, milk yield and composition (Grisart et al., 2002; 2004). The K allele increases fat yield, fat percentage and protein percentage, while decreasing milk and protein yields. The K232A *DGAT1* polymorphism is essentially neutral with respect to present day selection indexes in European dairy cattle where the K and A alleles are segregating at intermediate frequencies.

TG

The Thyroglobulin gene (*TG*) is located on chromosome 14 and codes for thyroglobulin which stores the thyroid hormones T3 and T4. There have been different reports about the association of an *TG* polymorphism with marbling in different breeds

(Barendse et al., 2001; Casas et al., 2005). Barendse et al. (2001) reported a significant association between the '3' allele and increased marbling scores in 1,750 Australian feedlot cattle including Angus, Shorthorn and other steers. Casas et al. (2005) demonstrated that *TG* had an effect on fat thickness but no effect on marbling score in 479 Brahman cattle. This *TG* marker was found to be associated with differences in the expression of intramuscular fat in the longissimus muscle in a small number of German Holstein cattle, but no association was found in Charolais (Thaller et al., 2003). *TG* has been found to have a significant effect on fat thickness in several different populations (Casas et al., 2000;2003; Moore et al., 2003). Commercially available DNA tests for *TG*, including GeneSTAR Marbling, evaluate a SNP polymorphism in the 5' untranslated region of *TG*. Rincker et al. (2005) tested the relationship between the GeneSTAR Marbling marker and intramuscular fat deposition (IMF) EPD in early weaned Simmental steers fed a high-energy diet and found that the marker did not efficiently predict IMF.

LEP

Leptin, a 16-kDa protein secreted from white adipocytes, has been implicated in the regulation of food intake, energy expenditure, and whole-body energy balance in rodents and humans. The gene encoding leptin was identified by positional cloning and a mutation in the gene leads to the profound obese phenotype of the *ob/ob* mouse(Zhang et al., 1994). The leptin gene is located on BTA4 (Stone et al., 1996; Pomp et al., 1997). Leptin is synthesized and expressed predominantly by adipocytes (Houseknecht et al., 1998). Studies have found associations between serum leptin concentration and carcass

adipose depots and other carcass characteristics of beef cattle (Minton et al.,1998; Geary et al., 2003). Leptin has been considered a candidate gene for performance, carcass, and meat quality traits in beef cattle (Fitzsimmons et al., 1998; Buchanan et al., 2002; Lagonigro et al., 2003). Several SNPs have been reported in the bovine leptin gene (Buchanan et al., 2002; Lagonigro et al., 2003; Nkrumah et al., 2005) and associations between polymorphisms within exon 2 (Buchanan et al., 2002; Nkrumah et al., 2004) or the promoter region (Crews et al., 2004; Nkrumah et al., 2005) of the gene with carcass and meat quality traits have been reported in beef cattle, with not all associations being consistent across studies.

CHAPTER III

MATERIALS AND METHODS

Resource Population and Phenotypes

DNA samples were obtained from the Circle A Ranch in Iberia, Missouri 5,485 Angus steers produced by matings among registered Angus bulls and commercial Angus cows. The steers had records for twelve growth, feed efficiency and carcass traits along with pedigree, birth, weaning, yearling and slaughter dates. These steers were formed into half-sib families and there were 36 families with at least 30 progeny with a total of 1,541 steers that were used in this study. Among the traits analyzed were: average daily gain from weaning to yearling (ADG), yield grade (YG), hot carcass weight (CWT; final weight of the trimmed carcass), pre-slaughter weight (WT; at 20 months of age), birth weight (BWT), weaning weight (WWT), carcass ribeye muscle area (CREA; measured between the 12^{th} and 13^{th} ribs), carcass backfat (CFAT; subcutaneous fat thickness between the 12^{th} and 13^{th} ribs), marbling score (MRB). All of these traits were statistically preadjusted for fixed effects of birth year. Weaning weight was also adjusted for weaning age, pre-slaughter weight and hot carcass weight were also adjusted for slaughter age. A Box-Cox transformation was used to establish normality of the data after pre-correction for fixed effects.

Figure 1 indicates that the transformed marbling scores follow a normal distribution. The quantile-quantile (Q-Q) plot is a graphical technique for determining if data are derived from populations following a specific distribution. Here the X-axis

represents data from normal distribution and Y-axis represents transformed marbling.

Table 3 provides means, standard deviations and the number of records for traits analyzed in the half-sib steer families by QTL Express.

Markers

Microsatellite markers (n = 42) and three SNPs within *LEP*, *DGAT1* and *TG* (Tables 4 and 5) were chosen primarily from public databases or published marker reports (http://www.marc.usda.gov). The forward PCR primer for each marker was synthesized with one of three fluorescent dye labels. Multiplexed PCR reactions were developed based on the detected allele size ranges, fluorescent label and the empirically determined ability of each marker to co-amplify. Between four and eight markers were co-amplified in each reaction. Multiplex PCR was performed using 5-μl reactions on an ABI 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) as described in Schnabel et al. (2003). The PCR products were separated on an ABI 3700 Automated Sequencer and sized relative to the GS400HD internal size standard (Applied Biosystems). Fluorescent signals from the dye-labeled microsatellites were detected using GENESCAN 3.1 (Applied Biosystems) and genotypes were assigned using GENOTYPER 3.7 (Applied Biosystems). Additionally, both sires and their sons were genotyped for the *LEP*, *DGAT1* and *TG* mutations (Barendse et al. 2001; Grisart et al. 2002; Nkrumah et al., 2004,2005). The SNPs were amplified by allele-specific PCR and genotypes were scored visually on 2% agarose gels with a co-amplified 16S rRNA gene fragment which was used as a positive control for the PCR.

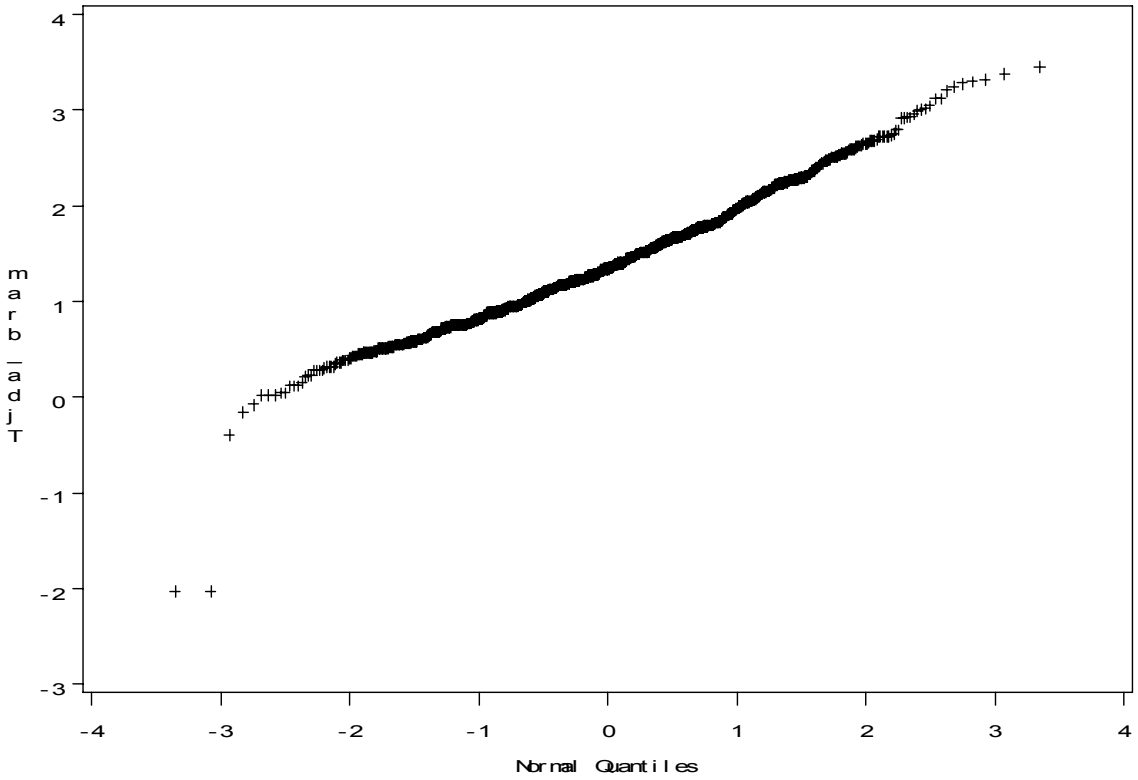

Figure 1. QQ-Plot of transformed marbling scores.

Table 3. Summary statistics for Box-Cox transformed traits in Angus steers.

Trait	# Records	Mean	Std. Dev.	Min.	Max.
BWT (kg)	1540	20.28	4.60	-1.27	37.68
WWT (kg)	1536	225.42	68.34	-1.01	458.62
ADG (kg/day)	1537	0.92	0.45	-0.80	2.73
CWT (kg)	1541	551.79	176.61	-0.87	1117.00
CFAT (cm)	1535	-0.59	0.26	-1.61	0.31
CREA (cm^2)	1532	3.54	0.94	-1.30	7.56
MRB (units)	1538	1.39	0.59	-2.03	3.45
YG (units)	1529	1.72	0.63	-1.21	4.02
WT (kg)	1417	605.73	153.93	137.23	1076.00

Table 4. Marker information on BTA4

Marker	Position cM (Haldane)	# HS Genotyped (%)	# Heterozygous Sires	# Alleles
BMC1410	0.00	1507 (96%)	15	9
BL1024	3.85	1487 (94%)	26	8
BMS1788	8.74	1511 (96%)	12	11
BMS827	25.85	1222 (77%)	27	10
BMS1172	28.24	1496 (95%)	25	6
DIK2956	33.11	1529 (97%)	23	13
BMS1840	45.34	1508 (96%)	22	11
BMS885	53.15	1282 (81%)	29	10
INRA072	63.03	1479 (94%)	14	9
BMS2571	70.26	1493 (95%)	23	9
BMS2809	76.94	1418 (90%)	25	8
UASMS2	89.52	1421 (90%)	23	2
UASMS3	89.53	1401 (89%)	14	2
Lep_Ex2	89.53	1448 (92%)	17	2
RM088	103.41	1508 (96%)	23	10
BR6303	108.89	1458 (92%)	23	7
AGLA227	111.18	1389 (88%)	15	3
DIK4542	125.58	1527 (97%)	25	6

Table 5. Marker information on BTA14

Marker	Position cM	# HS Genotyped (%)	# Heterozygous Sires	# Alleles
DGAT1	0.00	1484 (94%)	6	2
CSSM66	5.39	1158 (73%)	25	10
DIK4015	10.53	1317 (83%)	25	9
BMS1747	11.00	1485 (94%)	25	8
TG	12.48	1487 (94%)	15	2
CBDIKM009	14.57	436 (27%)	1	3
DIK4438	14.58	1536 (97%)	7	4
BM1508	18.57	168 (10%)	15	6
RM180	36.38	1532 (97%)	11	8
RM011	47.76	1410 (89%)	31	10
BMC1207	56.75	1371 (87%)	31	12
BL1029	64.81	1327 (84%)	26	13
BM1577	68.67	485 (30%)	15	5
BMS108	73.38	1028 (65%)	30	12
BMS1304	73.39	795 (50%)	26	4
BMS1899	74.73	1343 (85%)	27	11
BMS2513	74.74	1166 (74%)	22	5
BMS947	75.53	1232 (78%)	28	14
NRKM020	80.01	1507 (96%)	22	4
DIK2648	80.96	425 (27%)	0	5
NRKM005	81.99	394 (25%)	25	5
DIK2742	82.50	1340 (85%)	25	11
BM4513	85.84	1505 (95%)	21	11
RM66	87.32	1516 (96%)	18	3
BM4305	89.42	1442 (91%)	23	8
BM2934	90.05	1486 (94%)	27	8
BMS2055	100.77	1086 (69%)	28	7
BM6425	102.23	1156 (73%)	30	10
BL1036	107.34	1415 (90%)	25	14

Data Analysis

GENOPROB (Thallman et al., 2001a,b) was used to verify genotype scoring using published marker positions (http://www.marc.usda.gov). Individual genotypes with low probability as defined by GENOPROB (pGmx < 0.98) were excluded from further analysis.

QTL Express

Half-sib regression based on least squares using the program QTL Express (Seaton et al., 2002) was used to analyze each sire family individually under a half-sib model to estimate the segregation status of each sire. We used the simple single QTL model:

$$Y_{ij}=a_i+b_i x_{ij}+e_{ij}$$

Where Y_{ij} is the phenotype of half-sib individual j sired by bull i. Parameter a_i is the effect for the i^{th} half-sib family and x_{ij} is the difference in the conditional probability of individual j inheriting alternate QTL alleles from the i^{th} sire. Parameter b_i represents the allele substitution effect for a putative QTL segregating within the i^{th} family. Marker genotypes are used to estimate identity by descent probabilities at 1-cM intervals along the chromosomes given the genotypes of all markers in the linkage group. These probabilities are used to calculate genotype probabilities for a putative QTL at each position and the trait values are regressed on these coefficients to generate F-ratios which test for the presence of a QTL at each chromosomal location. Data permutation with

5,000 replicates was used to establish chromosome-wise significance thresholds and with 1,000 replicates to establish genome-wise significance levels for each sire and each trait (Churchill and Doerge, 1994).

Association Analysis

For the half-sib population, ANOVA was used to analyze *LEP*, *DGAT1* and *TG* associations with the transformed growth and carcass traits. The genotype frequencies of each polymorphism were examined for deviations from Hardy-Weinberg equilibrium using χ^2 tests. Tests of linkage disequilibrium between pairwise SNP genotype combinations were also performed using χ^2 procedures. Single marker association analyses were carried out to evaluate the relationship between different genotypes of each SNP and growth and carcass traits. Data were analyzed using the GLM procedure of SAS using a statistical model which included fixed effects of SNP genotype (three genotype classes per SNP). Tests of fixed genotype effects were performed using the model residual as the error term.

CHAPTER IV

RESULTS AND DISCUSSION

QTL Analysis Results

Table 6 lists all identified QTL on BTA14, the trait affected, sire ID and the significance thresholds for the half-sib family. BTA14 appears to harbor three QTL affecting marbling score at 44, 98 and 107-cM; three QTL affecting hot carcass weight at 65, 75 and 87-cM;, three ribeye area QTL at 65, 75 and 90-cM; two QTL influencing yield grade at 66 and 79-cM; two weaning weight QTL at 79 and 107-cM and one birth weight QTL at 82-cM.

Table 7 lists all identified QTL on BTA4, the trait affected, sire ID and the significance thresholds for the half-sib family. Four birth weight QTL at 8, 20, 84 and 125-cM; four marbling QTL at 5, 21, 45 and 109-cM and four weaning weight QTL at 9, 53, 77 and 103-cM were detected.

Carcass QTL on BTA14

There is clear evidence for three marbling QTL detected across the half-sib sire families by QTL Express located at 44, 98 and 107-cM on chromosome 14. One sire is segregating for the QTL at 44-cM, a second sire is segregating for the QTL at 98-cM, and a third sire is segregating for the QTL at 107-cM (**Figure 2**). Mizoshita et al. (2006) detected several carcass QTLs in a Japanese Black (Wagyu) cattle population which included a carcass weight QTL located on BTA14 at 22 to 39-cM (Stone et al.,1999).

Table 6. QTL on BTA14 detected by QTL EXPRESS.

Sire	Trait	Position cM	F	*P*-value
33749	BWT	83	7.75	0.05
31433	CFAT	77	10.38	0.01
28578	CFAT	87	8.49	0.05
31365	CFAT	107	6.13	0.10
28578	CREA	65	13.88	0.01
28628	CREA	75	12.98	0.01
28709	CREA	67	7.82	0.05
31365	CREA	87	7.81	0.05
31651	CREA	89	11.82	0.05
28645	CWT	60	11.05	0.01
28349	CWT	75	7.79	0.05
28662	CWT	87	10.76	0.05
28709	CWT	90	8.47	0.05
31433	CWT	85	8.94	0.05
31617	CWT	67	7.68	0.05
33774	CWT	87	7.69	0.05
28645	MRB	107	12.45	0.01
28543	MRB	98	7.47	0.10
28635	MRB	44	6.79	0.10
28662	ADG	90	13.99	0.01
28645	ADG	61	9.57	0.05
28349	ADG	92	6.92	0.10
33774	ADG	87	7.55	0.10
28349	WT	79	9.65	0.05
28543	WT	82	10.37	0.05
28349	WWT	75	7.67	0.05
31651	WWT	106	7.68	0.10
28578	YG	67	13.10	0.01
31433	YG	82	20.30	0.01
31617	YG	63	9.43	0.05
28564	YG	78	8.29	0.10
28628	YG	53	6.30	0.10
28645	YG	70	6.45	0.10

Table 7. QTL on BTA4 detected by QTL EXPRESS

Sire	Trait	Position cM	F	*P*-value
28709	BWT	7	15.76	0.01
28635	BWT	112	6.31	0.05
28728	BWT	125	8.85	0.05
31446	BWT	19	10.53	0.05
31530	BWT	125	10.92	0.05
31651	BWT	84	7.47	0.10
28628	CFAT	12	8.91	0.05
33749	CFAT	92	7.59	0.05
31433	CREA	104	10.7	0.01
28564	CREA	5	7.09	0.05
31560	CREA	107	7.85	0.05
28645	CWT	80	9.20	0.01
31575	CWT	2	7.54	0.05
33749	CWT	81	9.60	0.05
28645	MRB	20	11.12	0.01
28564	MRB	42	6.26	0.05
31617	MRB	109	9.54	0.05
31651	MRB	7	10.32	0.05
31720	MRB	11	7.23	0.05
33749	MRB	103	8.40	0.05
28645	ADG	77	13.83	0.01
33749	ADG	83	11.61	0.01
31560	ADG	103	7.16	0.10
31446	WT	77	11.98	0.01
31731	WT	29	10.8	0.05
33749	WT	78	7.34	0.05
31651	WT	90	7.41	0.10
31433	WWT	57	11.65	0.01
28645	WWT	9	7.74	0.05
28662	WWT	97	7.66	0.05
31446	WWT	77	10.51	0.05
31731	WWT	70	8.68	0.05
28628	YG	125	11.54	0.05
28645	YG	48	6.18	0.10

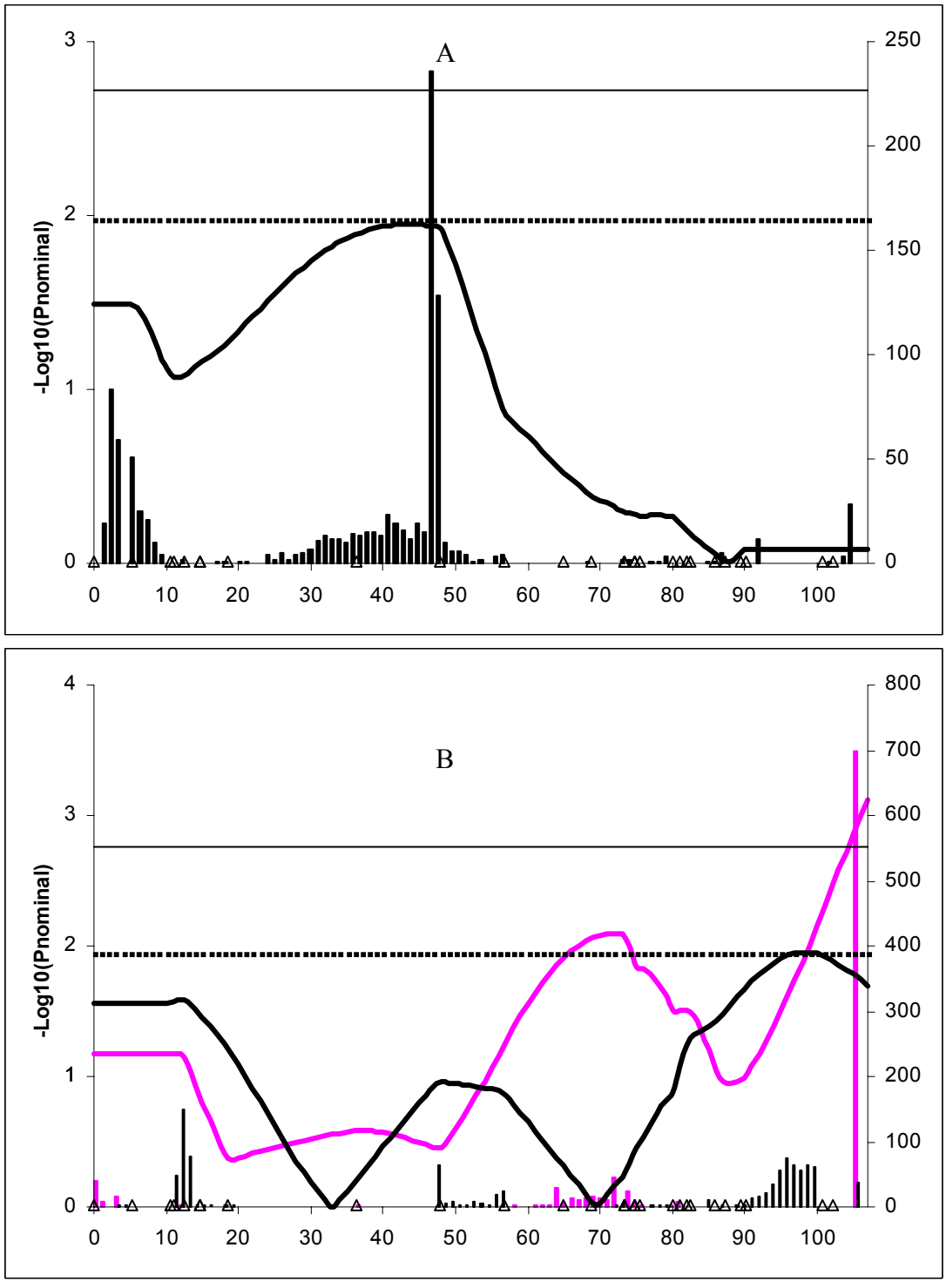

Figure 2. Marbling QTL on BTA14 analyzed by QTL-Express. Bars represent bootstrap replicates, X-axis denotes map distance, triangles indicate the marker positions and A and B indicate different QTLs.

Stone et al. (1999) reported a QTL located at 19-cM on BTA14 for which the Brahman allele conferred a larger longissimus muscle area compared to the Hereford allele. Casas et al. (2000) identified a QTL for fat thickness between 10 and 20-cM on BTA14 in progeny from a Brahman x Angus sire mated to mostly of MARC III dams. The support interval for the fat thickness QTL indicated that the most likely QTL location was between 0 and 16-cM. The QTL at 98 and 107-cM identified within an Angus population was not supported by previous reports.

QTL on BTA4

The results of the half-sib family analyses by QTL-Express indicate that there are four birth weight QTL segregating in five sires; one sire is segregating for the QTL at 8-cM, a second sire is segregating for the QTL at 20-cM, a third sire is segregating for the QTL at 84-cM and three sires are segregating for the QTL at 125-cM **(Figure 5**). There are two CFAT QTL segregating at 12 and 92-cM both in two sires. Two sires were also segregating for the CREA QTL at 105-cM. There is clear evidence for two CWT QTL located at 4 and 80-cM. For MRB, six sires were segregating for QTL at four positions; two sires for the QTL at 5-cM, one sire for the QTL at 21-cM , one sire for the QTL at 45-cM and two sires for the QTL at 109-cM (**Figure 3**). There are four WWT QTL segregating in five sires located at 9, 53, 77 and 103-cM . For ADG and YG, there was one QTL segregating at 76-cM and 125-cM, respectively. Two WT QTL were detected to be segregating in three sires, one sire segregating at 29-cM and two sires segregating for a QTL at 77-cM.

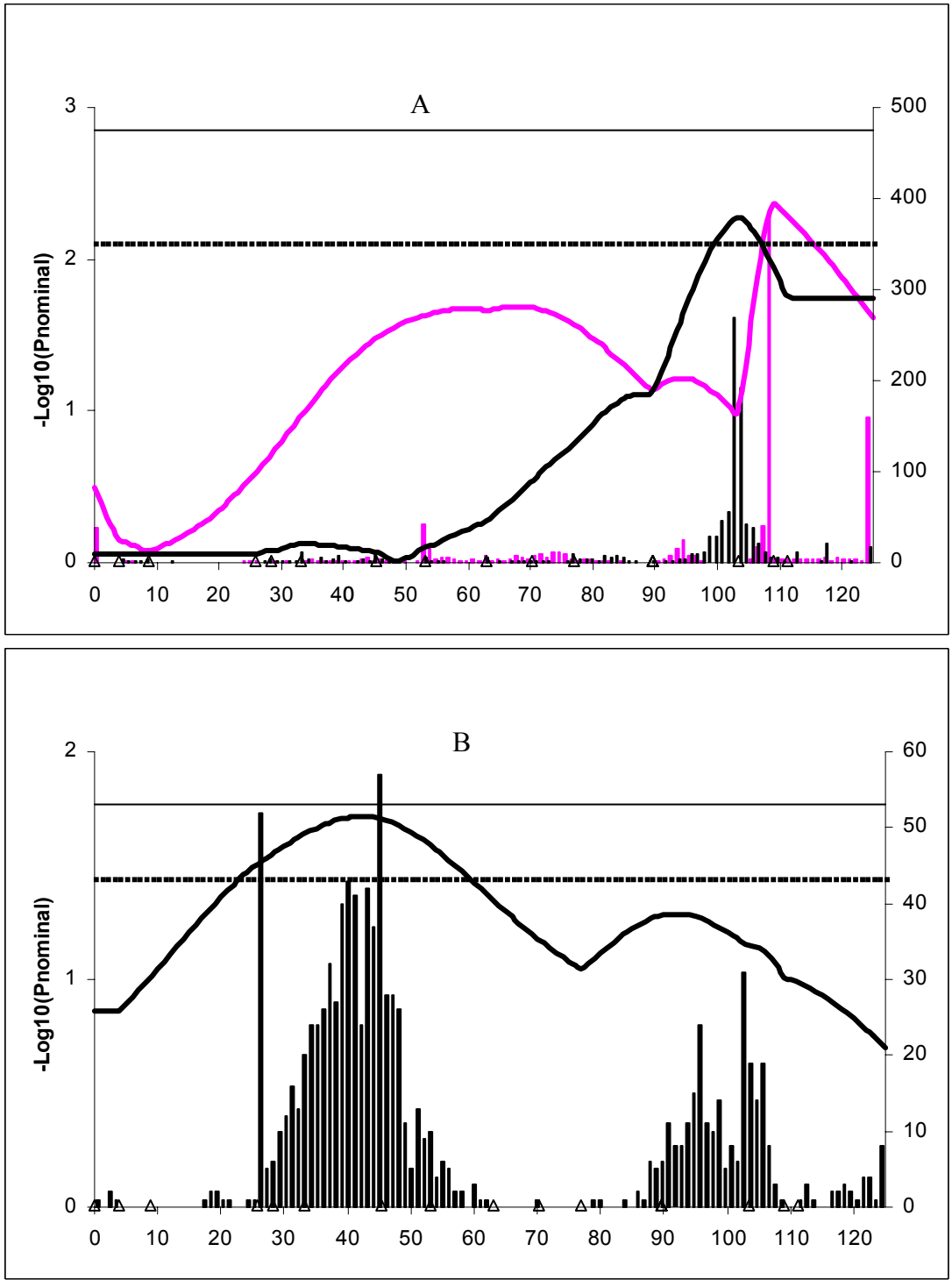

Figure 3. Marbling QTL on BTA4 analyzed by QTL-Express. Bars represent bootstrap replicates, X-axis denotes map distance, triangles represent marker position and A and B indicate different QTLs.

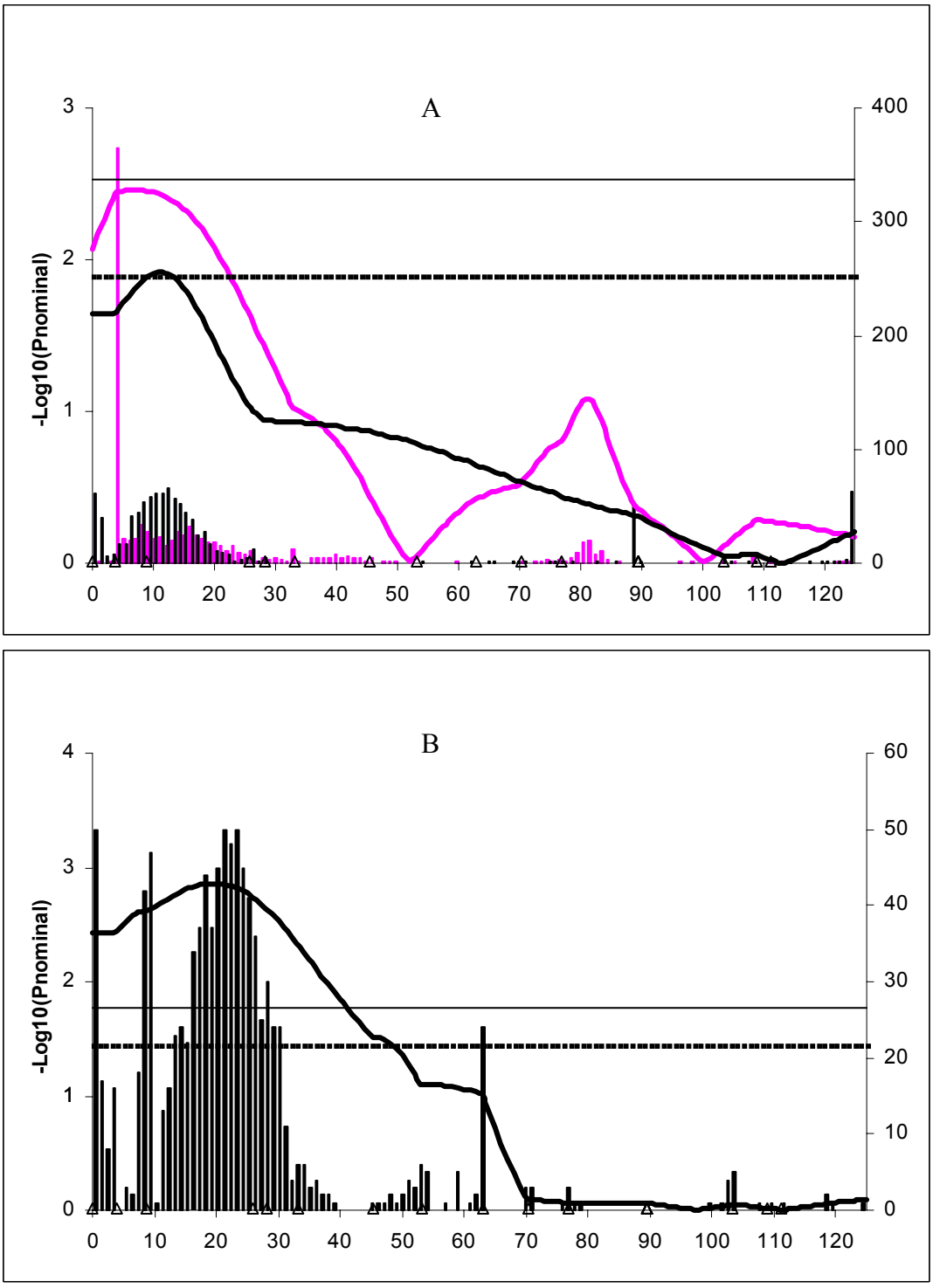

Figure 4. Marbling QTLs on BTA4 analyzed by QTL-Express. Bars represent bootstrap replicates, X-axis denotes map distance, triangles represent marker position and A and B indicate different QTLs.

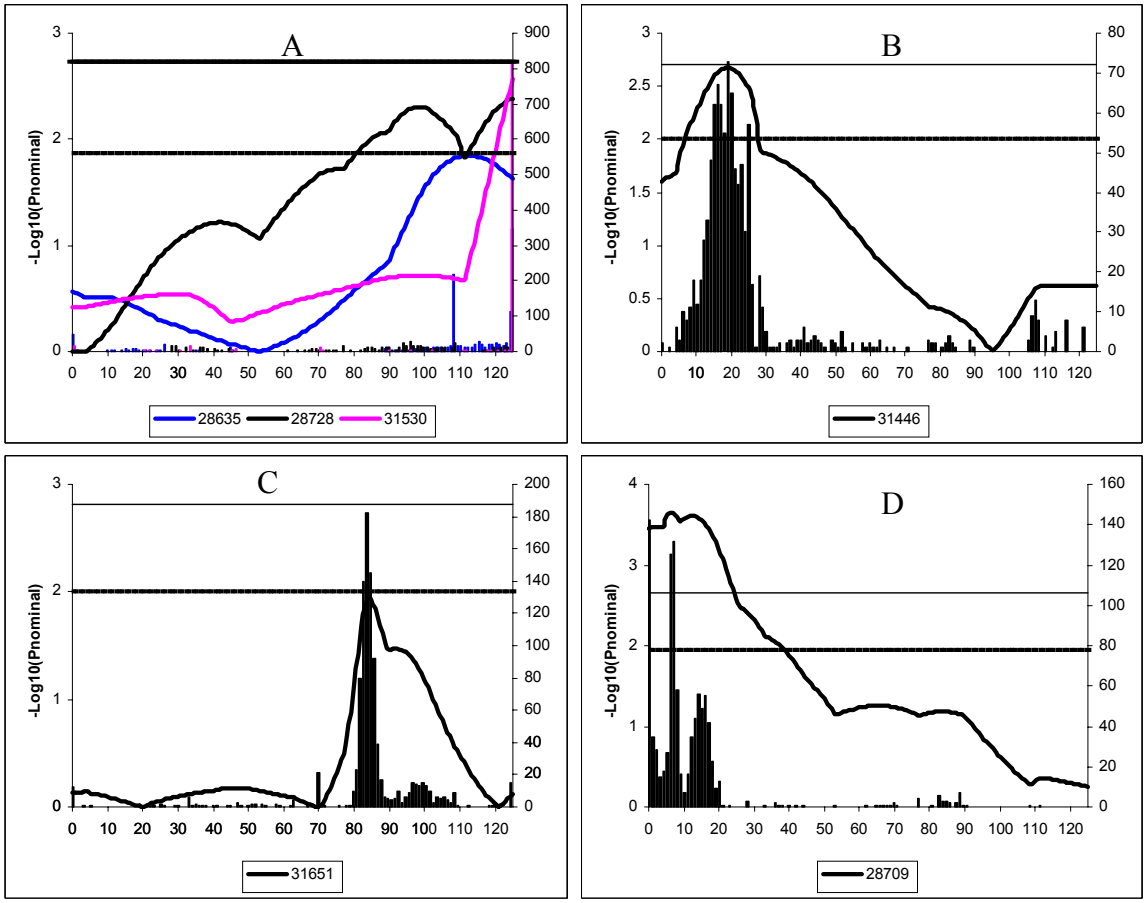

Figure 5. Birth weight QTLs on BTA4 analyzed by QTL-Express. Right axis is for the number of bootstraps. Bars represent bootstrap replicates, X-axis denotes map distance and A, B, C and D indicate different QTLs.

Candidate Gene Tests

For the half-sib families, ANOVA was used to analyze *LEP*, *DGAT1*, and *TG* associations with the transformed growth and carcass traits. Table 8 indicates that the K232A mutation within *DGAT1* is not associated with growth and carcass traits since all of the P-values were larger than 0.05. The *UASMS2* SNP is a cytosine/thymine (C/T) substitution at position 528 in the bovine leptin promoter. *UASMS3* is a cytosine/guanine (C/G) substitution at position 1,759 in the bovine leptin promoter. *Lep_exon2* is a cytosine/thymine (C/T) substitution within the second exon of the Leptin gene. The frequency of the *UASMS2* T allele was 0.2851, and of the *UASMS3* G allele was 0.5778, while the frequency of the *Lep_exon2* C allele was 0.5406 in the half-sib steers. The frequency of the TG T allele was 0.29 in the half-sib steers. The frequency of the *UASMS2* T allele and of the *UASMS3* G allele in Angus were close to the allele frequencies reported in the TX line (Nkrumah et al., 2005). The genotype frequencies of the three Leptin SNPs deviated from Hardy-Weinberg equilibrium proportions in the population ($P<0.001$) possibly due to the fact that Angus cattle were not random mating. The overall averages and standard deviations for the traits tested are presented in Table 3. The effects of the *UASMS2*, *UASMS3*, *Lep_exon2* and *TG* genotypes on the analyzed traits are presented in Tables 9, 10, 11, and 12, respectively. The genotypes for *Lep_exon 2* and *TG* had no effect on marbling, but the *UASMS2* T allele and the *UASMS3* G allele were significantly associated with marbling score ($P<0.001$).

The *Lep_exon 2* C allele was associated with weaning weight, hot carcass weight,

WT, and intramuscular fat. Weaning weight, hot carcass weight, WT, and intramuscular fat were higher for animals with the *Lep_exon2* CC and CT genotypes.

Birth weight and CREA were higher for animals with the *UASMS2* CC genotype. Weaning weight and intramuscular fat were higher for animals with the *UASMS2* TT genotype. The *UASMS3* GG genotype had higher CREA and WT than the CC and CG genotypes. *TG* was not significantly associated with any trait except for CWT where animals with the 33 genotype had higher weights than those with 22 or 23 genotypes.

Barendse et al. (2001) reported an association between the '3' allele and higher marbling scores in Australian feedlot cattle including Angus, Shorthorn, and some steers of other breeds. Casas et al. (2005) demonstrated that *TG* had an affect on fat thickness but no effect on marbling score in Brahman cattle. The *TG* marker was found to be associated with differences in the expression of intramuscular fat in the longissimus muscle in a small number of German Holstein cattle, but no association was found in Charolais (Thaller et al., 2003). *TG* has been shown to affect fat thickness in several different populations (Casas et al., 2000,2003; Moore et al., 2003). Commercially available DNA markers, including GeneSTAR Marbling (Rincker et al., 2006), evaluate a polymorphism in the 5' untranslated region of *TG*. Rincker et al. (2005) tested the relationship between this *TG* SNP and intramuscular fat deposition expected progeny difference in early weaned Simmental steers fed a high-energy diet and found no effect. In our Angus population the linkage analyses indicates that there is no marbling QTL in the region of *TG* on BTA14, since the three detected marbling QTL are located at 47, 70

and 107-cM. *TG* has no effect on marbling in our Angus population, but animals with '3' allele had higher carcass backfat and carcass ribeye area. The linkage analysis results indicated that there were no carcass backfat and carcass ribeye QTL near the *TG* gene.

Leptin is the hormone product of the obese (Leptin) gene located on BTA4 (Stone et al.,1996; Pomp et al., 1997). Studies have found associations between serum leptin concentration and carcass adipose depots and other carcass characteristics of beef cattle (Minton et al.,1998; Geary et al., 2003). Leptin has been considered a candidate gene for performance, carcass, and meat quality traits in beef cattle (Fitzsimmons et al., 1998; Buchanan et al., 2002; Lagonigro et al., 2003). Several SNPs have been reported in the leptin gene (Buchanan et al., 2002; Lagonigro et al., 2003; Nkrumah et al., 2005). Associations between polymorphisms within exon 2 (Buchanan et al., 2002; Nkrumah et al., 2004) or the promoter region (Crews et al., 2004; Nkrumah et al., 2005) of the leptin gene with carcass and meat quality traits have recently been reported in beef cattle. In the Circle A Angus population, *UASMS2* and *UASMS3* were associated with marbling as well as several other carcass traits. Animals with the *UASMS2* T allele and with the *UASMS3* G allele had higher marbling scores. The Leptin gene is located at about 90-cM on BTA4 and a marbling QTL was detected near 100-cM.

Table 8. Association of *DGAT1* genotypes with growth and carcass traits.

Trait[1]	DGAT1 genotype			*P*-value
	AA[2] (0.7816)[3]	LA (0.2092)	LL (0.0092)	
BWT (kg)	21.44±0.13	20.86±0.25	20.54±1.22	0.1145
WWT (kg)	246.01±1.94	247.23±3.69	254.70±17.1	0.8504
ADG (kg/day)	1.13±0.01	1.11±0.02	1.35±0.10	0.0912
CWT (kg)	607.97±4.89	608.37±9.34	635.39±43.71	0.8233
CFAT (cm)	0.18±0.02	0.13±0.07	0.04±0.14	0.5857
CREA (cm^2)	3.76±0.02	3.84±0.0	3.70±0.24	0.3301
MRB (units)	1.41±0.01	1.33±0.03	1.43±0.15	0.1085
YG (units)	1.96±0.01	1.91±0.03	2.03±0.15	0.3597
WT (kg)	641.95±4.60	651.72±8.64	675.3±40.01	0.4503

[1]Transformed trait
[2]Least squares means ± SE
[3]Genotype frequency

Table 9. Association of *Lep_Ex2* genotypes with growth and carcass traits.

Trait[1]	Lep_Ex2 genotype			*P*-value
	CC[2]	CT	TT	
	(0.4326)[3]	(0.2953)	(0.2161)	
BWT (kg)	21.35±0.20	21.51±0.26	20.58±0.32	0.0636
WWT (kg)	250.37±2.99	240.28±3.95	241.14±4.55	0.0712
ADG (kg/day)	1.14±0.01	1.14±0.02	1.12±0.02	0.8678
CWT (kg)	616.04±7.74	606.56±10.08	590.30±11.67	0.1839
CFAT (cm)	0.23±0.02	0.04±0.06	0.10±0.04	0.0176
CREA (cm^2)	3.83±0.04	3.72±0.05	3.72±0.06	0.1774
MRB (units)	1.42±0.03	1.36±0.03	1.33±0.04	0.1531
YG (units)	1.92±0.02	1.96±0.03	1.97±0.04	0.5768
WT (kg)	655.04±7.31	643.81±9.53	625.46±11.38	0.0900

[1]Transformed trait
[2]Least squares means ± SE
[3]Genotype frequency

Table 10. Association of *UASMS2* genotypes with growth and carcass traits.

Trait[1]	*UASMS2* genotypes			*P*-value
	CC[2] (0.4326)[3]	CT (0.5645)	TT (0.0028)	
BWT (kg)	21.42±0.21	21.60±0.19	14.22±5.47	0.3387
WWT (kg)	245.95±3.15	248.52±2.75	269.56±60.41	0.7739
ADG (kg/day)	1.15±0.01	1.09±0.017	0.79±0.43	0.0339
CWT (kg)	615.35±7.99	607.23±7.02	597.26±161.31	0.7451
CFAT (cm)	0.088±0.049	0.224±0.029	NA	0.0367
CREA (cm^2)	3.77±0.044	3.81±0.03	2.73±1.05	0.4734
MRB (units)	1.36±0.02	1.44±0.02	2.19±0.57	0.0424
YG (units)	1.96±0.029	1.93±0.025	2.07±0.54	0.7806
WT (kg)	655.62±7.52	646.06±6.51	741.94±136.08	0.5030

[1]Transformed trait
[2]Least squares means ± SE
[3]Genotype frequency

Table 11. Association of *UASMS3* genotypes with growth and carcass traits.

Trait[1]	*UASMS3* genotypes			*P*-value
	CC[2]	CG	GG	
	(0.1269)[3]	(0.3587)	(0.5144)	
BWT (kg)	21.37±0.37	21.38±0.21	21.42±0.18	0.9837
WWT (kg)	240.19±5.53	247.60±3.20	248.44±2.69	0.4009
ADG (kg/day)	1.18±0.03	1.10±0.02	1.13±0.017	0.1432
CWT (kg)	608.17±14.04	607.72±8.21	613.38±6.92	0.8551
CFAT (cm)	0.11±0.05	0.046±0.05	0.23±0.02	0.0151
CREA (cm^2)	3.78±0.07	3.75±0.045	3.86±0.03	0.1694
MRB (units)	1.28±0.04	1.46±0.02	1.41±0.02	0.0100
YG (units)	1.99±0.05	1.96±0.02	1.90±0.02	0.1329
WT (kg)	640.82±13.38	643.14±7.62	654.81±6.50	0.4154

[1]Transformed trait
[2]Least squares means ± SE
[3]Genotype frequency

Table 12. Association of *TG* genotypes with growth and carcass traits.

Trait[1]	*TG* genotypes			*P*-value
	22[2] (0.4773)[3]	23 (0.4478)	33 (0.0749)	
BWT (kg)	21.44±0.16	21.30±0.17	20.72±0.43	0.2918
WWT (kg)	243.63±2.46	249.34±2.53	245.48±6.25	0.2697
ADG (kg/day)	1.13±0.015	1.12±0.01	1.16±0.038	0.5612
CWT (kg)	597.55±6.25	615.53±6.38	626.59±15.58	0.0608
CFAT (cm)	0.14±0.04	0.191±0.03	0.12±0.08	0.5935
CREA (cm^2)	3.71±0.03	3.82±0.03	3.97±0.08	0.0073
MRB (units)	1.39±0.02	1.39±0.02	1.30±0.05	0.2728
YG (units)	1.96±0.02	1.95±0.02	1.87±0.05	0.3585
WT (kg)	641.20±5.97	646.68±5.97	657.80±14.39	0.5290

[1]Transformed trait
[2]Least squares means ± SE
[3]Genotype frequency

CHAPTER V

SUMMARY AND CONCLUSIONS

The first objective of this study was to identify genomic regions on BTA4 and BTA14 which harbor QTL affecting weight and carcass traits in the commercial Angus population. Many QTL were identified for most of the analyzed phenotypes of the 36 half-sib families using QTL Express. BTA14 appears to harbor three QTL affecting marbling score at 46, 90 and 106-cM, three QTL affecting hot carcass weight at 61, 75 and 87-cM, three ribeye area QTL at 65, 75 and 90-cM, two QTL influencing yield grade at 66 and 79-cM , two weaning weight QTL at 79 and 107-cM and one birth weight QTL at 82-cM. Since *DGAT1* was located at 0-cM and *TG* at 12-cM on BTA14, none of the detected QTL would lead us to consider either of these genes as candidates for the detected QTL. There were also several QTL detected on BTA4; four birth weight QTL, four marbling QTL, four weaning weight QTL, two hot carcass weight QTL, two ribeye QTL and one yield grade QTL. The QTL mapping interval is not fine enough so that more high density markers are needed to find the positional candidate genes. Leptin is a candidate gene for the carcass fat QTL since there was a CFAT QTL at 90-cM. *UASMS2* and *UASMS3* were located 13-cM from the marbling QTL at 103-cM which explains their association with marbling.

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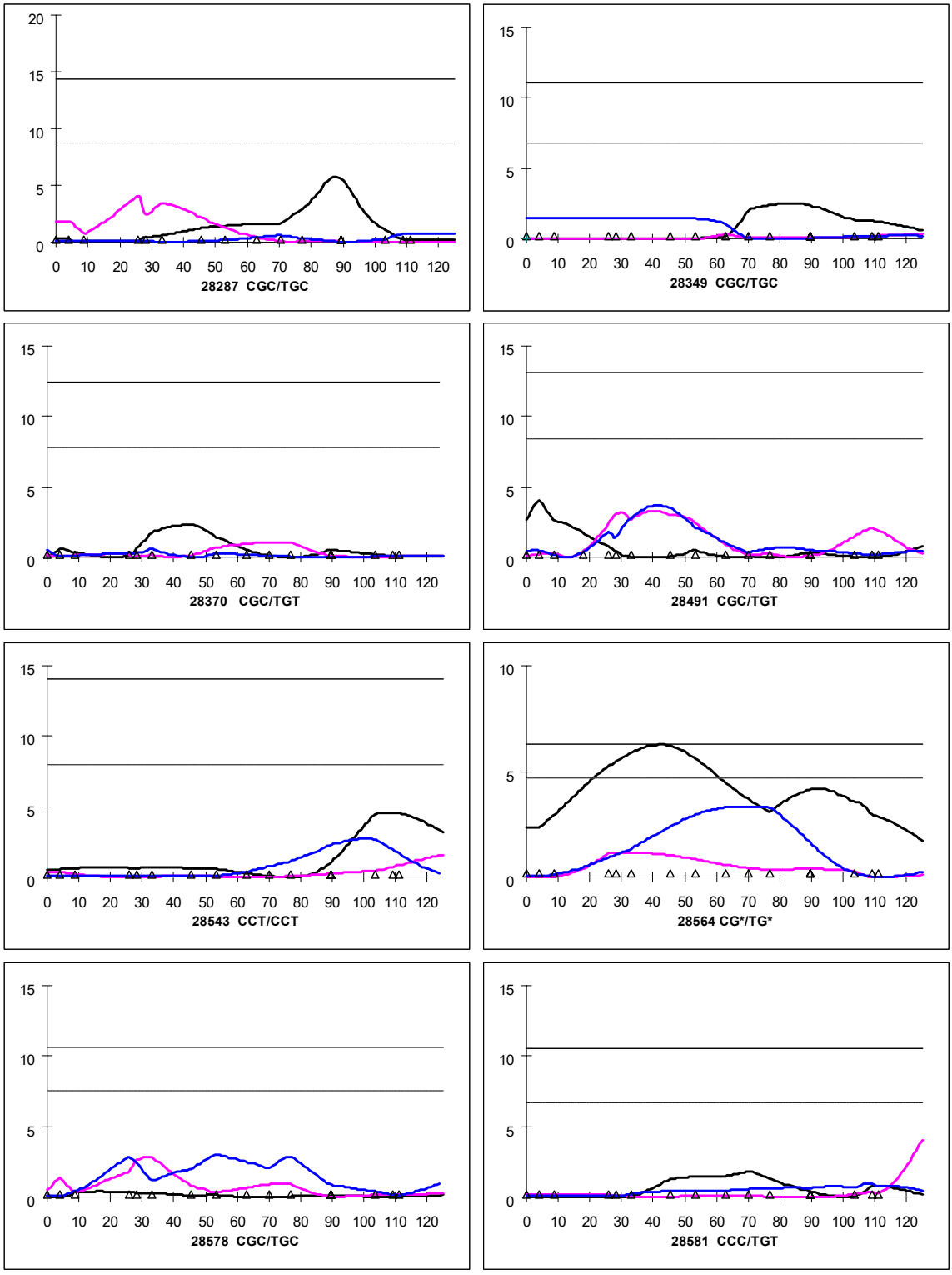
Appendix I

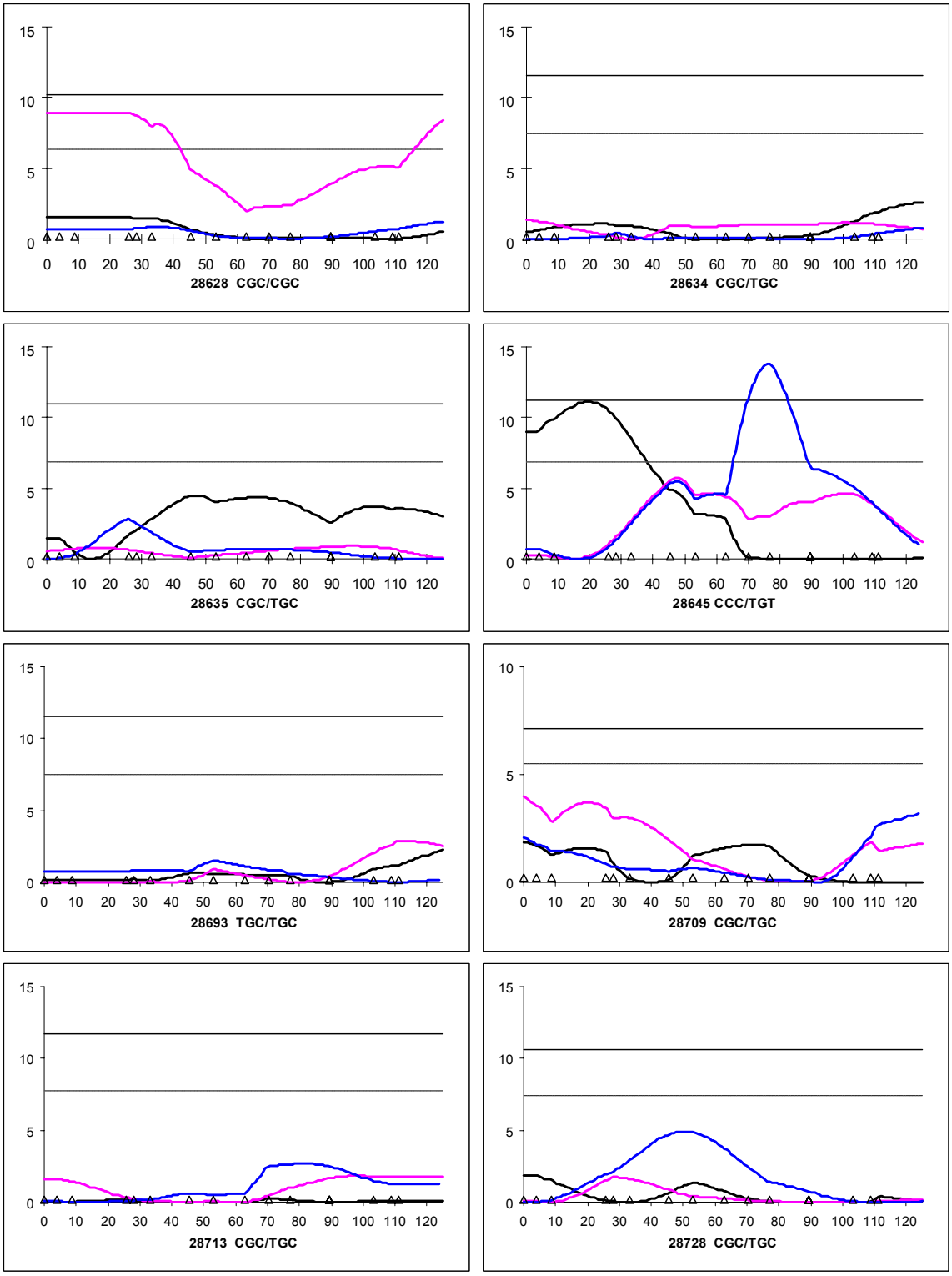
Interval Analysis of Circle A Sire Half-sib Families for BTA4

* F-statistic profiles CFAT (pink), MRB (blue), REA (black). Graph legends contain sire ID and haplotype at the Lep_ex2, UASMS2 and UASMS3 loci respectively.
* P=0.05 (solid) and P=0.01 (dashed) significance thresholds are indicated for each sire.

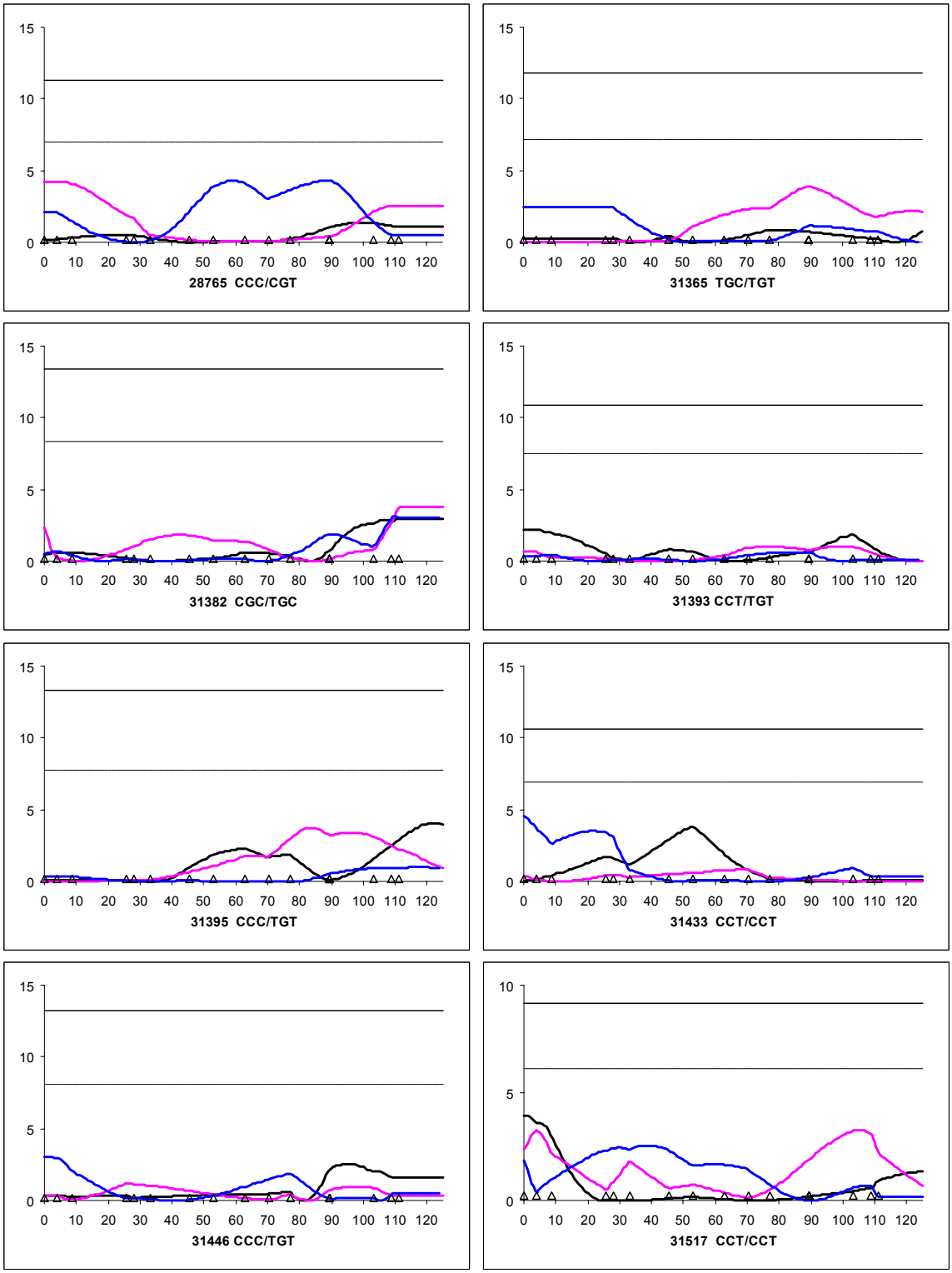
* F-statistic profiles CFAT (pink), MRB (blue), REA (black). Graph legends contain sire ID and haplotype at the Lep_ex2, UASMS2 and UASMS3 loci respectively.
* *P*=0.05 (solid) and *P*=0.01 (dashed) significance thresholds are indicated for each sire.



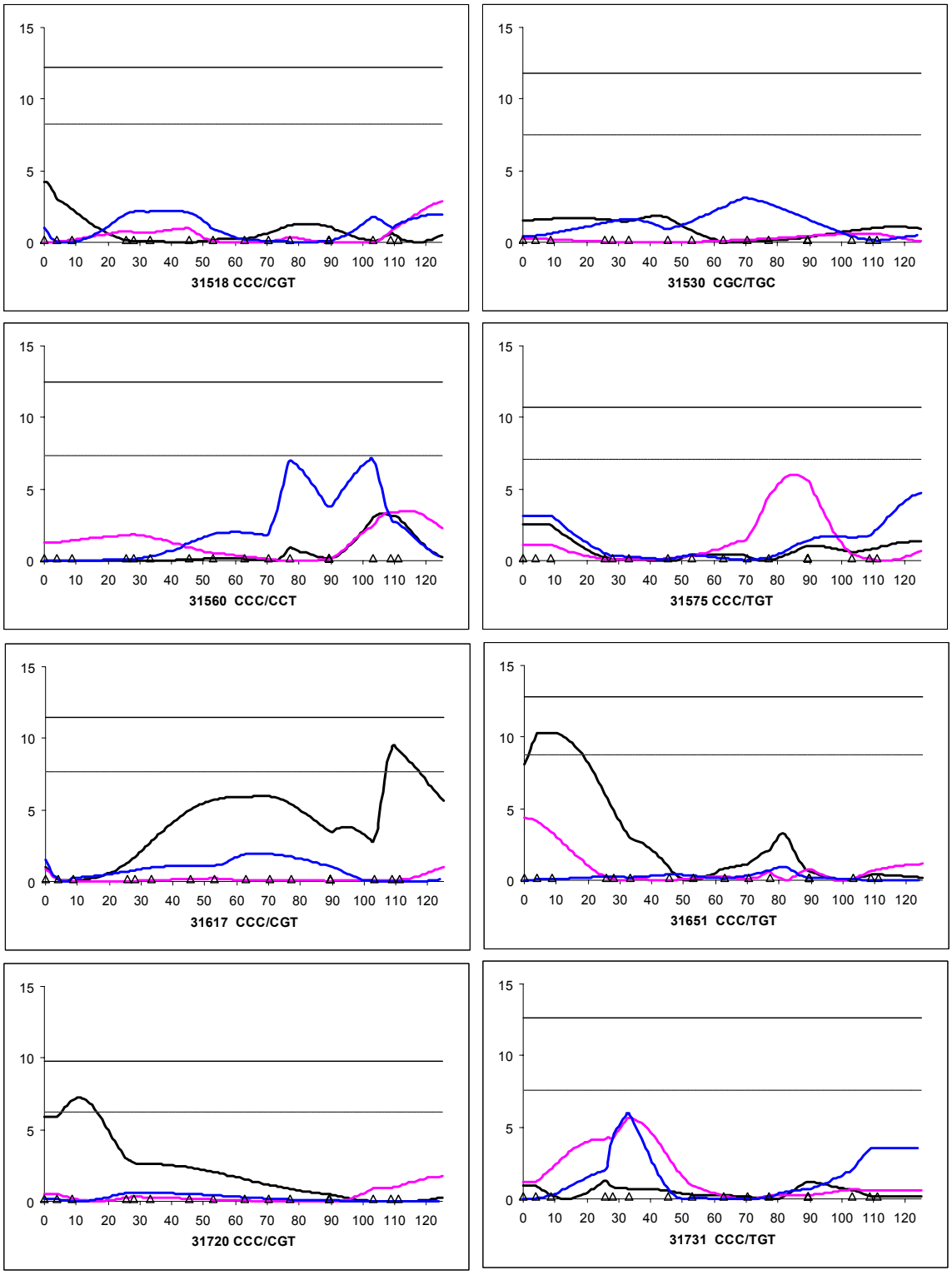
* F-statistic profiles CFAT (pink), MRB (blue), REA (black). Graph legends contain sire ID and haplotype at the Lep_ex2, UASMS2 and UASMS3 loci respectively.
* *P*=0.05 (solid) and *P*=0.01 (dashed) significance thresholds are indicated for each sire.



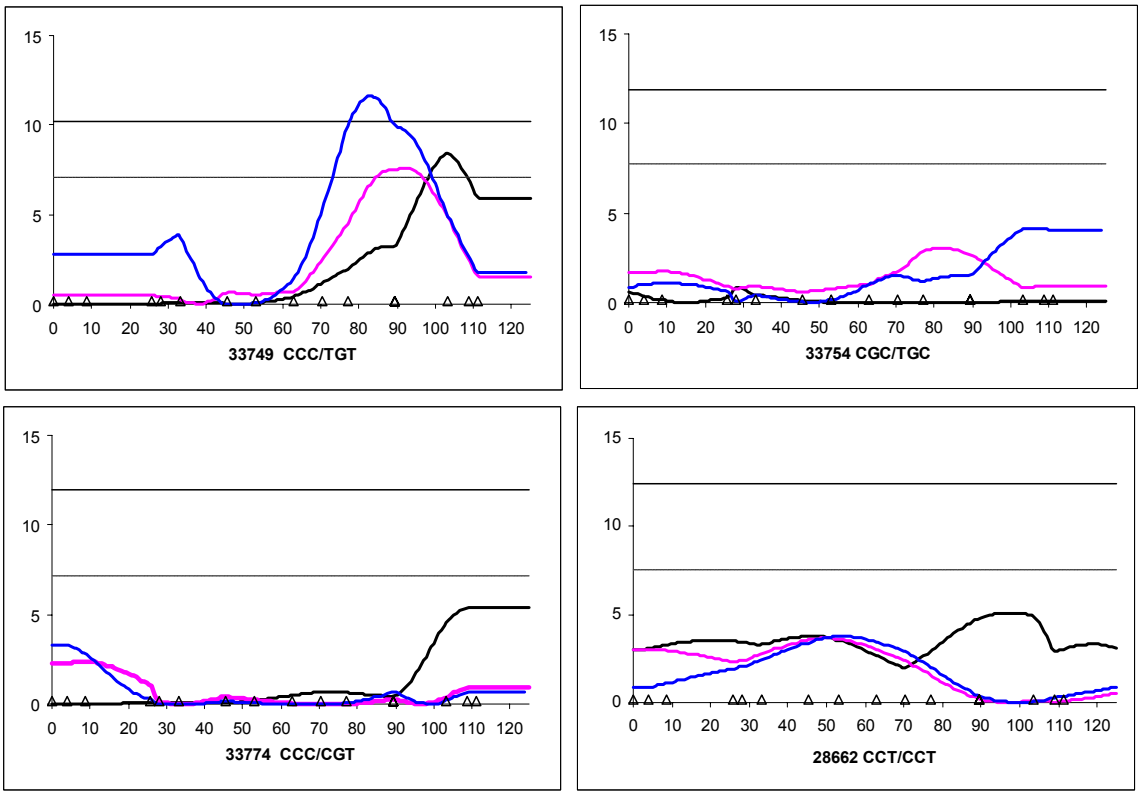
* F-statistic profiles CFAT (pink), MRB (blue), REA (black). Graph legends contain sire ID and haplotype at the Lep_ex2, UASMS2 and UASMS3 loci respectively.
* *P*=0.05 (solid) and *P*=0.01 (dashed) significance thresholds are indicated for each sire.



* F-statistic profiles CFAT (pink), MRB (blue), REA (black). Graph legends contain sire ID and haplotype at the Lep_ex2, UASMS2 and UASMS3 loci respectively.
* *P*=0.05 (solid) and *P*=0.01 (dashed) significance thresholds are indicated for each sire.



* F-statistic profiles CFAT (pink), MRB (blue), REA (black). Graph legends contain sire ID and haplotype at the Lep_ex2, UASMS2 and UASMS3 loci respectively.
* *P*=0.05 (solid) and *P*=0.01 (dashed) significance thresholds are indicated for each sire.

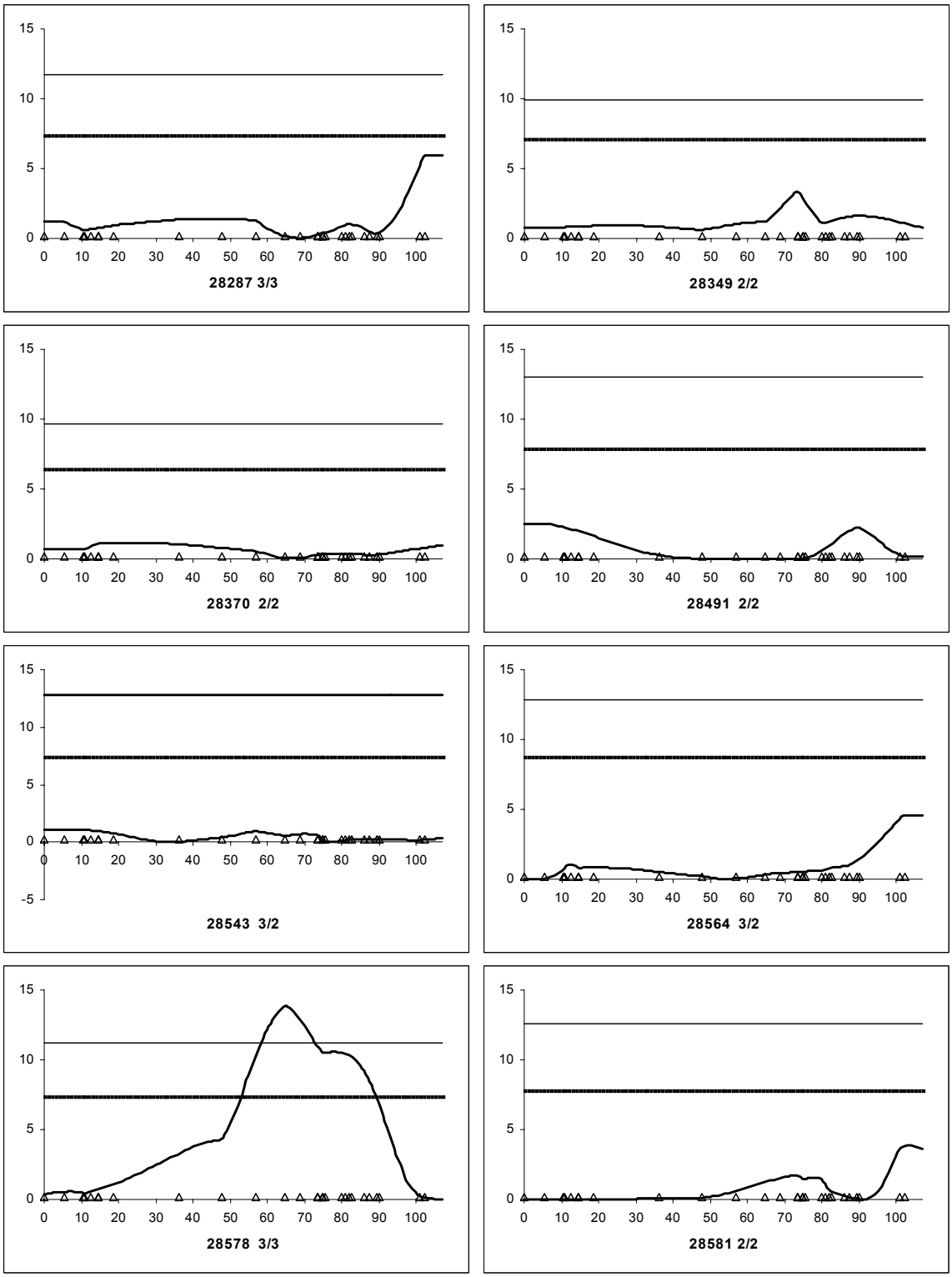


Appendix II

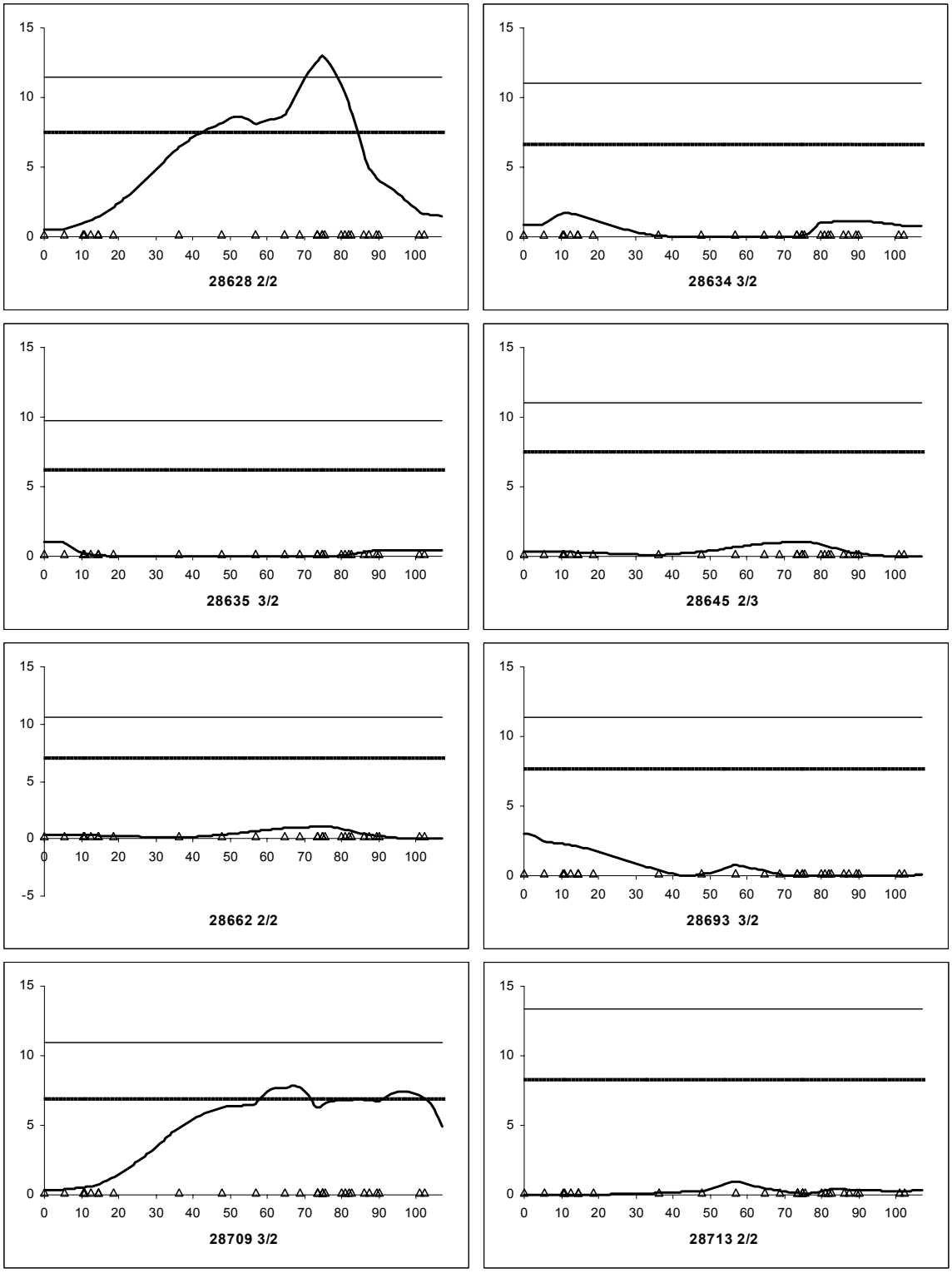
Interval Analysis of Circle A Sire Half-sib Families for BTA14

* F-statistic profiles for REA. Graph legends contain sire ID and genotype at the TG5 locus.
* *P*=0.05 (dashed) and *P*=0.01 (solid) significance thresholds are indicated for each sire.

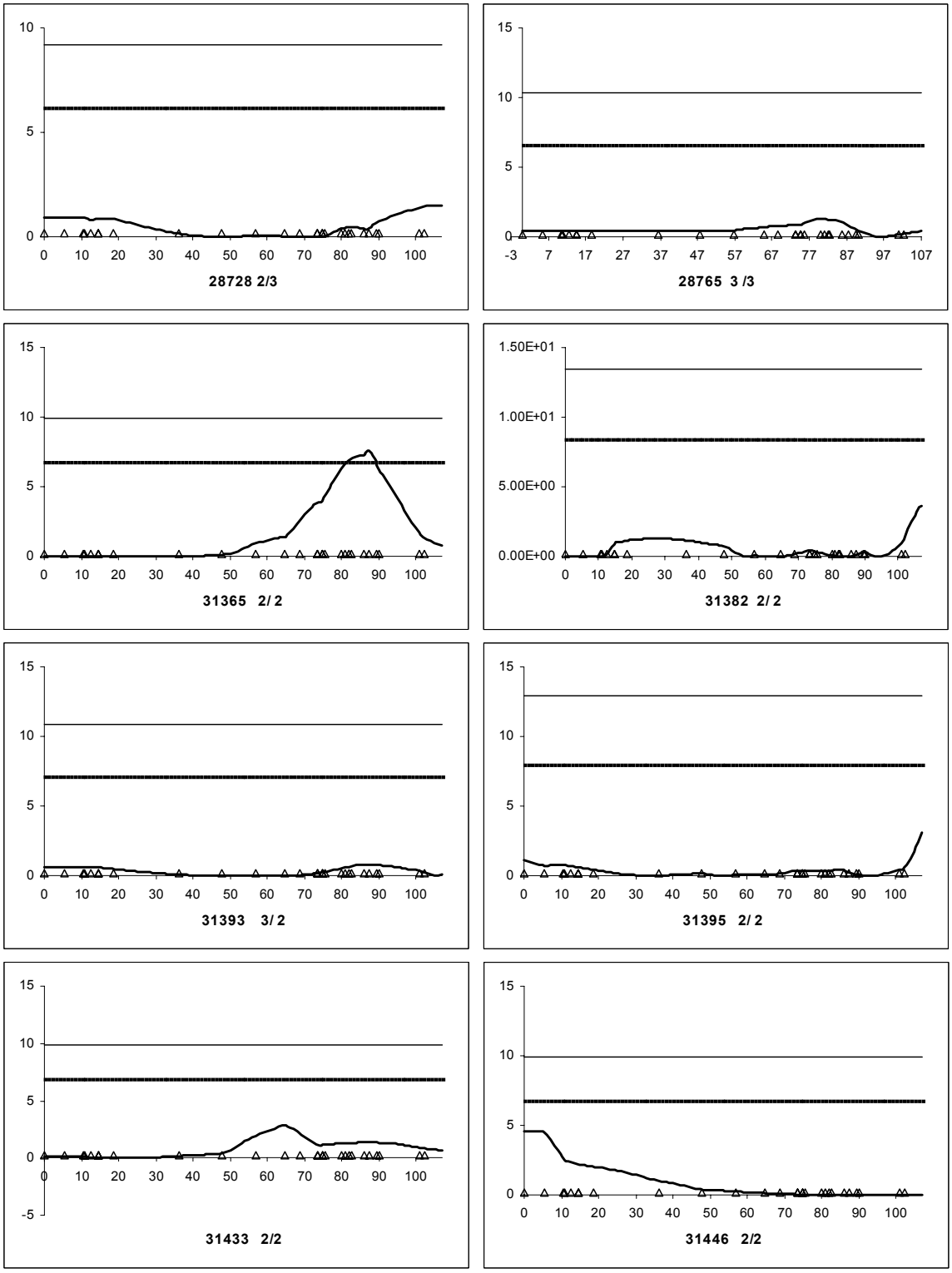
* F-statistic profiles for REA. Graph legends contain sire ID and genotype at the TG5 locus.
* *P*=0.05 (dashed) and *P*=0.01 (solid) significance thresholds are indicated for each sire.



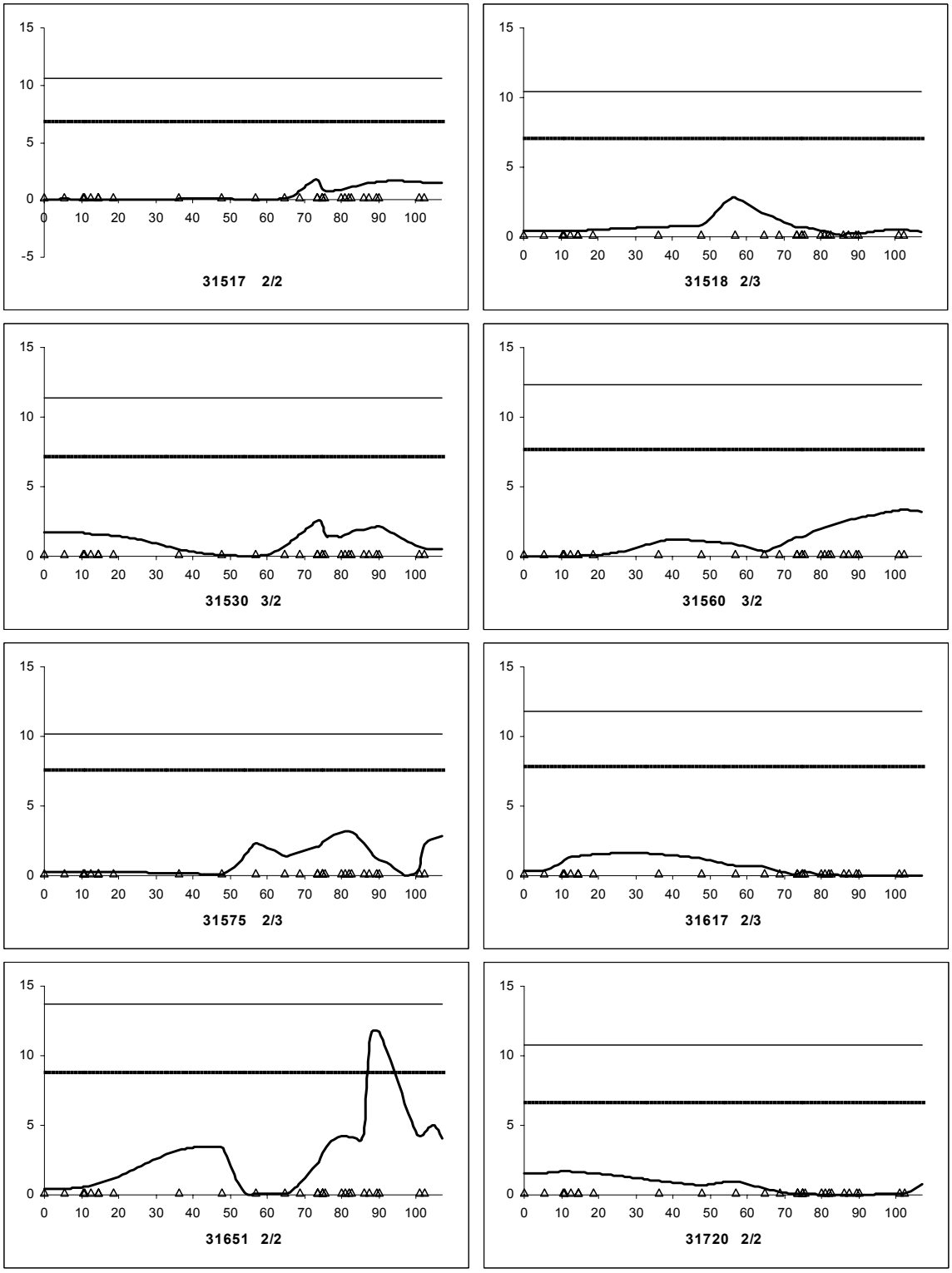
* F-statistic profiles for REA. Graph legends contain sire ID and genotype at the TG5 locus.
* *P*=0.05 (dashed) and *P*=0.01 (solid) significance thresholds are indicated for each sire.



* F-statistic profiles for REA. Graph legends contain sire ID and genotype at the TG5 locus.
* *P*=0.05 (dashed) and *P*=0.01 (solid) significance thresholds are indicated for each sire.



* F-statistic profiles for REA. Graph legends contain sire ID and genotype at the TG5 locus.
* *P*=0.05 (dashed) and *P*=0.01 (solid) significance thresholds are indicated for each sire.



* F-statistic profiles for REA. Graph legends contain sire ID and genotype at the TG5 locus.
* *P*=0.05 (dashed) and *P*=0.01 (solid) significance thresholds are indicated for each sire.