ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi: 10.1111/j.1365-2052.2010.02063.x

A genome scan for quantitative trait loci influencing carcass, post-natal growth and reproductive traits in commercial Angus cattle

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Summary

To gain insight into the number of loci of large effect that underlie variation in cattle, a quantitative trait locus (QTL) scan for 14 economically important traits was performed in two commercial Angus populations using 390 microsatellites, 11 single nucleotide polymorphisms (SNPs) and one duplication loci. The first population comprised 1769 registered Angus bulls born between 1955 and 2003, with Expected Progeny Differences computed by the American Angus Association. The second comprised 38 half-sib families containing 1622 steers with six post-natal growth and carcass phenotypes. Linkage analysis was performed by half-sib least squares regression with GRIDQTL or Bayesian Markov chain Monte Carlo analysis of complex pedigrees with LOKI. Of the 673 detected QTL, only 118 have previously been reported, reflecting both the conservative approach to QTL reporting in the literature, and the more liberal approach taken in this study. From 33 to 71% of the genetic variance and 35 to 56% of the phenotypic variance in each trait was explained by the detected QTL. To analyse the effects of 11 SNPs and one duplication locus within candidate genes on each trait, a single marker analysis was performed by fitting an additive allele substitution model in both mapping populations. There were 53 associations detected between the SNP/duplication loci and traits with $-\log_{10}P_{nominal} \geq 4.0$, where each association explained 0.92% to 4.4% of the genetic variance and 0.01% to 1.86% of the phenotypic variance. Of these associations, only six SNP/duplication loci were located within 8 cM of a QTL peak for the trait, with two being located at the QTL peak: *SST_DG156121:c.362A>G* for ribeye muscle area and *TG_X05380:c.422C>T* for calving ease. Strong associations between several SNP/duplication loci and trait variation were obtained in the absence of any detected linked QTL. However, we reject the causality of several commercialized DNA tests, including an association between *TG_X05380:c.422C>T* and marbling in Angus cattle.

Keywords Association analysis, beef, *Bos taurus*, genome scan, linkage analysis, QTL.

Introduction

During the twentieth century, breeders made enormous changes in the growth, stature and body composition of American Angus cattle through selection based on phenotypes and expected progeny differences (EPDs) (Northcutt & Wilson 1993). For example, the average height of registered American Angus cattle increased from 116 cm in 1964 to 135 cm in 1984 (Brown & Franks 1964; Northcutt & Wilson 1993). Since their inception and promotion in the US beef industry in the early 1970s, EPDs have been used to develop livestock that meet industry expectations, and selection on EPDs has resulted in economically desirable genetic trends in each trait for which EPDs are published by

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Accepted for publication 8 March 2010

the American Angus Association (St. Joseph, Missouri, USA) (http://www.angus.org/Nce/GeneticTrends.aspx). To aid in the early identification of beef cattle with superior genetic merits, researchers have identified QTL for numerous economically important traits. Ideally, these QTL could be incorporated into marker assisted selection (MAS) schemes in which the contributions of numerous QTL to a breeding objective are considered simultaneously. The rate of genetic improvement achieved by MAS may be substantially greater than that achieved by selection based upon EPDs for traits that are determined post-mortem, occur late in life, are lowly heritable, or are difficult and (or) expensive to accurately measure (Davis & DeNise 1998). Considering the potentially significant economic benefits expected from the discovery of QTL influencing traits of importance to producers and consumers, research groups world-wide have focused on the identification of QTL in beef cattle.

Several QTL scans have been conducted using *Bos taurus taurus* × *Bos taurus indicus* crosses or experimental *B. t. taurus* crosses, and these designs appear to assist in the detection of QTL, but hinder the implementation of MAS (Stone *et al.* 1999; Kim *et al.* 2003) and the identification of the underlying quantitative trait nucleotide (QTN) (Sellner *et al.* 2007). *B. t. indicus* and *B. t. taurus* diverged approximately 250–500 000 years ago (Miretti *et al.* 2002), and mutations with fixed allelic differences have accumulated approximately every 2 kb within these genomes (Taylor *et al.* 2006). Consequently, the confidence intervals for QTL detected in experimental crossbred populations will contain thousands of mutations with fixed differences between *B. t. indicus* and *B. t. taurus* alleles, which cannot statistically be differentiated from the causal QTN (Sellner *et al.* 2007). As the experimental crosses do not represent commercial populations, all discovered QTL must be validated for marker phase relationship and magnitude of effect within each population in which the test is anticipated to have utility, before commercialization can occur (Van Eenennaam *et al.* 2007). Diverse populations are likely to possess different marker-QTL phase relationships, and the extent of linkage disequilibrium may vary as a result of allele frequency differences caused by drift or selection (Allan & Smith 2008). Because of these issues, few of the QTL discovered thus far have been commercialized as tests that can be utilized by producers for MAS.

Historically, experimental designs for QTL mapping in cattle sampled a limited number of parental chromosomes, and thus these experiments could only detect those few QTL that are heterozygous within the parents, regardless of family size (Casas *et al.* 2003; Mizoshita *et al.* 2004; Alexander *et al.* 2007). On average, each half-sib analysed family identified 3 to 5 QTL per trait (Chamberlain *et al.* 2007; Allan & Smith 2008), which likely represents a fraction of the actual number of QTL segregating within a population (Mizoshita *et al.* 2005). In addition, the limited size of available families resulted in underpowered QTL scans that significantly underestimated the number of QTL that contribute to phenotypic variation within a population (Bogdan & Doerge 2005). Of the 2344 QTL identified to date in cattle, 219 influence fertility, 227 meat quality, 242 growth and 741 milk yield or composition traits (http://www.animalgenome.org/QTLdb/cattle.html, release 9, accessed 9/23/2009). However, because QTL location is frequently poorly estimated, the number of unique QTL is likely to be somewhat smaller. As the previous genome scans have found only a limited number of QTL influencing any one trait to be segregating in commercial populations, genetic improvement by MAS in cattle has been hindered by the inability to test for a sufficient number of QTL to economically justify the cost of testing.

By using two of the largest commercial cattle mapping populations assembled to date, we have captured the majority of the chromosomes represented within the US Angus breed. Mapping within commercial populations offers the advantage of expedited collection of DNA samples, from cryopreserved semen, and pedigrees and phenotypes, from breed associations. Additionally, any QTL identified can immediately be incorporated into the breeding programme for that population via selection on linked markers (Schnabel *et al.* 2003). This experimental design also allows the flexibility of using multiple analytical approaches to exploit both linkage information from close pedigree relationships and linkage disequilibrium information from more distantly related individuals if high density genotyping has been conducted. Finally, by independently analysing 11 SNPs and one duplication locus within candidate genes, this design allows us to explicitly analyse their individual effects on 14 economically important traits and to compare these effects relative to the position of nearby QTL detected within the whole-genome QTL scan.

Materials and methods

Animals and traits

The first mapping population consisted of a pedigree of 1769 registered Angus artificial insemination (AI) sires, spanning 29 generations and born between 1955 and 2003, which represent the major sire lines within the US breed. The second population comprised 38 half-sib (HS) families containing 1622 steers born at the Circle A Ranch (Iberia, Missouri, USA) between 1997 and 2002. The animals within the AI sire population form 10 paternal lineages, with all males having DNA samples, for which 77.9% have DNA samples represented on their maternal grandsires, and the lineages are inter-related through the sires' maternal pedigrees. In addition, 10 of the AI sires had half-sib families of ≥ 19 AI sons that were also represented in the AI sire population, and two of these sires also had sons in the HS mapping population (Table S1).

doi: 10.1111/j.1365-2052.2010.02063.x

DNA for each of the AI sires was obtained from cryopreserved semen provided by AI organizations, the National Animal Germplasm Program, the University of Maryland Wye herd, and numerous breeders of registered Angus cattle. Pedigree data, EPDs and EPD accuracies (Spring, 2005 evaluation) for birth weight (BW), calving ease direct (CED), calving ease maternal (CEM), carcass weight (CW), fat thickness (FAT), marbling score (MARB), maternal milk (MILK), mature height (MH), mature weight (MW), ribeye muscle area (REA), scrotal circumference (SC), weaning weight (WW), yearling height (YH) and yearling weight (YW) were obtained from the American Angus Association (Table 1). DNA from each steer was harvested from whole blood (10 ml) samples collected and stored in vacuum tubes with 15 mg of EDTA (Covidien). Phenotypic data for BW, WW, CW, FAT, MARB and REA were collected on each of the steers at the Circle A Ranch or abattoir (Table 1). For both populations, genomic DNA was isolated by proteinase K digestion followed by Phenol:Chloroform:Isoamyl alcohol extraction, and ethanol precipitation (Sambrook *et al.* 1989).

Markers

Microsatellite markers that possess a large number of alleles and are easy to score were chosen ($N = 416$) from published genetic maps (Barendse *et al.* 1997; Kappes *et al.* 1997); in addition, 11 SNPs and one duplication locus representing candidate genes or commercialized tests were selected for genotyping (Table S2) (Barendse *et al.* 2004; Buchanan *et al.* 2002; Grisart *et al.* 2002; Blott *et al.* 2003; Grisart *et al.* 2004; Cohen-Zinder *et al.* 2005; Nkrumah *et al.* 2005; Morsci *et al.* 2006). The forward PCR primer for each microsatellite marker was synthesized with one of four fluorescent dye labels, and multiplexed PCRs were developed based on allele size distributions, fluorescent labels, and the empirically determined ability of each marker to co-amplify (Schnabel *et al.* 2003). Between two and nine markers were co-amplified in each of 69 multiplexes, with PCR $MgCl_2$ concentration, primer volume, and annealing temperature optimized for each multiplex PCR to maximize the number of amplified loci per reaction and to obtain the same relative fluorescence intensities for each marker (Tables S3 & S4). PCRs were performed in 5 μl reactions on an ABI GeneAmp 9700 thermocycler (Applied Biosystems).

SNPs and the duplication locus were amplified by allele-specific PCR along with a *16S* rRNA gene fragment used as a positive control for the PCR. Each primer ending at a SNP locus was designed with a mismatch at the third base from the 3′ end, relative to the bovine genomic sequence (Table S5). Weakening the primer annealing by providing a partial primer mismatch minimizes the likelihood of extension when there is also a primer mismatch at the position of the SNP. PCR annealing temperatures and

Table 1 Phenotype or expected progeny difference statistical summaries.

Population[1]	Trait[2]	Unit	Count[3]	Accuracy[4]	St. Dev.	Kurt	Skew	Average	Min	Max
HS	BW	Kilograms	1614	–	4.50	0.28	0.25	38.32	22.68	56.25
	FAT	Centimetres	1587	–	0.51	0.16	0.48	1.42	0.18	3.56
	HCW	Kilograms	1598	–	35.34	−0.09	−0.16	340.51	215.91	450.42
	MARB	USDA marbling score	1593	–	1.08	1.08	0.96	1.08	1.08	1.08
	REA	Square centimetres	1585	–	8.84	0.59	0.26	76.19	49.68	117.42
	WW	Kilograms	1610	–	39.09	−0.02	0.28	224.27	116.12	353.81
AI	BW	Kilograms	2853	2798	1.09	−0.05	0.01	0.96	−2.63	4.90
	CED	% unassisted births in first-calf heifers	2853	2852	1.08	0.89	−0.67	1.08	1.08	1.08
	CEM	% unassisted births in first-calf daughters	2853	2852	1.08	1.58	−0.85	1.08	1.08	1.08
	CW	Kilograms	2466	905	4.38	1.24	−0.08	1.28	−19.05	18.60
	FAT	Centimetres	2466	900	0.05	1.33	0.02	0.00	−0.28	0.25
	MARB	% difference in USDA marbling score	2466	910	1.08	1.30	0.58	1.08	1.08	1.08
	MH	Centimetres	2605	1604	1.55	0.65	0.16	1.14	−4.06	7.62
	MW	Kilograms	2605	1605	15.97	0.64	−0.08	12.54	−48.08	75.30
	MILK	Kilograms of weaning weight due to milk and mothering ability	2853	2799	4.32	0.02	−0.26	6.75	−7.71	20.87
	REA	Square centimetres	2466	901	1.10	1.13	0.49	0.45	−4.00	5.29
	SC	Centimetre	2807	2158	1.35	0.39	0.21	0.30	−4.67	5.61
	WW	Kilograms	2853	2810	6.66	0.04	−0.44	14.07	−8.62	38.56
	YH	Centimetres	2795	1995	1.04	1.05	0.25	0.76	−2.79	5.33
	YW	Kilograms	2853	2803	12.29	0.04	−0.48	25.88	−13.61	70.31

[1]HS, half-sib families; AI, artificial insemination sires.

[2]Traits measured in half-sib family members are phenotypes, but in the AI sires are expected progeny differences (EPDs).

[3]Count includes individuals with genotypes inferred by GENOPROB.

[4]Number of animals in the AI sire population for which the EPD accuracy >0.05.

doi: 10.1111/j.1365-2052.2010.02063.x

setup for SNPs and the duplication locus are in Tables S3 & S4.

Genotypes

Genotyping of the 1622 steers from the HS population and 1769 sires from the AI population was attempted for the 416 microsatellite markers, 11 SNPs, and one duplication locus (Table S2). Microsatellite alleles were separated on an ABI 3730 Automated Sequencer or an ABI 3100 Automated Sequencer, with fragment sizes determined relative to the GeneScan 500 LIZ internal size standard (Applied Biosystems). Fluorescence signals were detected using GENESCAN v3.1 (Applied Biosystems) and fragment sizes were determined by GENEMAPPER v3.7 (Applied Biosystems). SNPs in the *ATP-binding cassette subfamily G member 2* (*ABCG2*) (Cohen-Zinder *et al.* 2005), *adiponectin* (*ADIPOQ*) (Morsci *et al.* 2006), *growth hormone receptor* (*GHR*) (Blott *et al.* 2003), *leptin* (*LEP*) (Buchanan *et al.* 2002; Nkrumah *et al.* 2005), and *somatostatin* (*SST*) (Morsci *et al.* 2006) genes and a duplication locus in the *ADIPOQ* promoter (Morsci *et al.* 2006) were genotyped by the visualization of amplification products on a 2% standard agarose gel. However, the SNPs in the *thyroglobulin* (*TG*) (Barendse *et al.* 2004) and *acyl-CoA:diacylglycerol acyltransferase* (*DGAT1*) genes (Grisart *et al.* 2002, 2004) were genotyped as PCR restriction fragment length polymorphisms and scored on 1.5% (*DGAT1*) and 3% (*TG*) agarose gels [50% standard agarose and 50% high resolution NuSieve 3:1 agarose (Cambrex Bioscience, Rockland, ME, USA)]. SNP genotype counts for each population are in Table S6.

Seven of the microsatellite loci amplified poorly in the multiplex reactions, three were essentially monomorphic, and the 16 BTAX microsatellites were excluded from further analysis (Table S2). The remaining 402 loci spanned 2820 cM of the bovine autosomes, resulting in an average marker interval of 7.56 cM (Table S7).

Data analysis

GENOPROB (Thallman *et al.* 2001a,b) was used to identify misinheritances, genotype errors, infer missing genotypes and estimate the probability that a genotype was correctly scored (pGmx) using the full pedigree and marker positions from the USDA MARC cattle mapping database (http://www.marc.usda.gov/genome/cattle/cattle.html). Pedigree relationships for all genotyped animals were assembled into a single pedigree to capture relationships among females that were not genotyped. Genotype and grand-parental origin probabilities were estimated for each of the genotyped animals, and genotypes with low probability (pGmx < 0.98) were excluded from further analysis. Subsequently, 1 717 936 genotypes on 13 165 animals with pGmx ≥ 0.98 were generated, of which, 224 708 genotypes were on females (Fig. 1).

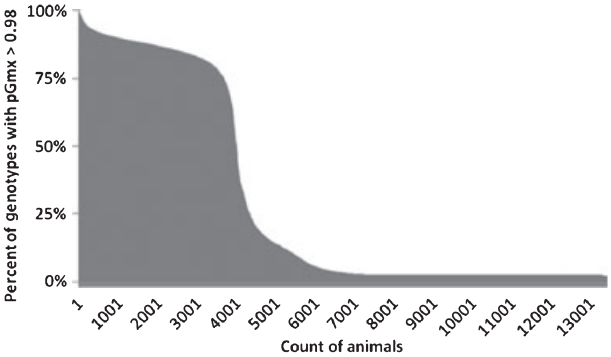

Figure 1 Cumulative distribution of numbers of animals by percentage of highly supported (pGmx ≥0.98) genotypes. 100% indicates that an animal had pGmx ≥0.98 for all 402 genotypes.

Genome scan for QTL

Two complementary approaches were used for QTL analysis. Thirty-eight sires from the HS sire population and 10 sires from the AI sire population with 19 or more progeny (Table S1) were individually analysed by half-sib least squares regression using GRIDQTL (Seaton *et al.* 2006) to identify QTL and determine the segregation status for each sire and trait combination. Each progeny included in the analysis had pGmx ≥ 0.98 for at least 75% of the 402 possible genotypes from the GENOPROB analysis. Chromosome- and genome-wide significance levels were determined by permutation analysis with 1000 data permutations for each sire and each trait (Churchill & Doerge 1994). As the number of offspring varied per sire, F statistics were transformed to $-\log_{10}P_{nominal}$ values to allow comparisons between families.

LOKI v.2.4.5 (Heath 1997) was used to perform multipoint QTL interval analysis in the AI sire population using a Bayesian Markov chain Monte Carlo approach to analyse the entire pedigree while simultaneously estimating the total number and position of QTL. This analysis was performed using 2854 registered animals that had ≥ 22% of their genotypes (average 80.81%) satisfying pGmx ≥ 0.98. LOKI does not allow the use of weights required to model heteroscedastic residual variances and we restricted analysis to EPDs with accuracies >0.05, but did not account for the variation in accuracies among observations. An initial burn-in of 1000 iterations was followed by 500 000 iterations, with parameter estimates collected at each iterate. A Bayes Factor (BF) was generated as the posterior/prior probability ratio supporting a QTL at each 1 cM position on each autosome. Substantial support against the null hypothesis of no QTL was accepted with a BF ≥ 5, and strong support with a BF ≥ 10 (Jefferys 1961; Goodman 1999).

A QTL was considered significant if it was detected in two or more families with chromosome-wide $P \leq 0.05$ support and/or by both analytical methods with a LOKI BF ≥5.0. We also considered QTL detected in only a single family with a

doi: 10.1111/j.1365-2052.2010.02063.x

chromosome-wide $P \leq 0.01$ support or detected only by LOKI with a BF ≥ 10 to be significant. Statistically significant QTL for the same trait that were found within the average marker resolution of 8 cM (Table S7) of each other were considered to be a single QTL. A chromosome was considered to harbour multiple segregating QTL for a trait if each detected QTL was separated by at least one marker and the QTL were ≥ 8 cM apart. The reported map location corresponds to the QTL position with the highest statistical support. For consistency, all analyses used a sex-averaged genetic map calibrated in Haldane cM units.

As confidence intervals (CI) for QTL location are usually computed under single QTL models, they produce very large regions, even for large-effect QTL (Ron & Weller 2007; Kim *et al.* 2009), which can lead to a significant underestimation of the number of QTL that are present within the genome. Additionally, when multiple QTL for a trait are located on a chromosome, it is improper to use bootstrapping methods to estimate the CI of a QTL (Schnabel *et al.* 2005). Instead of using CI estimated under a single QTL model to provide an indication of the genomic location of a QTL and thus of how many QTL are located within specific regions of the genome, the location of a QTL was supported by multiple sires from separate mapping populations, and independent analytical methods (GRIDQTL and LOKI).

Variation explained by detected QTL

An additive effects model was fitted using the microsatellite nearest each QTL to estimate the amount of genetic or phenotypic variation explained by each QTL in a single marker analysis using the model:

$$Y_k = \mu + \delta_{\mathrm{ki}} M_i + e_k$$

where when the AI population was analysed: Y_k is the EPD for the kth animal, μ is the overall mean, M_i is a column vector of additive allelic effects for the ith microsatellite marker and δ_{ki} is a row vector containing elements that define the numbers of each allele in M_i that are present in individual k, and e_{k} is the random residual for each animal's EPD. This analysis weighted residuals according to the accuracies of the EPDs according to Morsci *et al.* (2006). A total of 1951 registered animals from the AI population with all animals having EPDs for BW, CED, CEM, MILK, WW and YW; 98% for SC and YH, 94% for CW, FAT, MRB and REA; and 91% for MW and MH were used in this analysis. For the HS population, Y_k is the phenotype for the kth animal and e_{k} is the random residual for each animal's phenotype. In total, 1622 steers from the HS population were used in the analysis, with ≥ 98% animals having BW, CW, FAT, MARB, REA and WW phenotypes.

To estimate the genetic or phenotypic variation explained by all identified QTL for each trait the following additive model was used:

$$Y_k = \mu + \delta_{k1} M_1 + \ldots + \delta_{\mathrm{kn}} M_n + e_k$$

where model terms are as previously described, n QTL were assumed identified for the trait, and the AI and HS populations were analysed separately. Animals with ≥ 80% of genotypes with pGmx ≥ 0.98 were included in this analysis and animals with missing genotypes for any marker were considered as being homozygous for an 'unknown' allele.

SNP/duplication locus association analysis

An additive allele substitution model was fitted to estimate the amount of genetic or phenotypic variation in each trait explained by each SNP and duplication locus in a single marker analysis using:

$$Y_k = \mu + \alpha G_k + e_k$$

where when the AI population was analszed: Y_k is the EPD for the kth animal, μ is the overall mean, α is the effect of an allele substitution at the tested locus, G_k takes the values 0, 1 and 2 for genotypes *AA*, *AB*, *BB* respectively, and e_k is the random residual for each animal's EPD. This analysis weighted residuals according to the accuracies of the EPDs according to Morsci *et al.* (2006). For the HS population, Y_k is the phenotype for the kth animal and e_k is the random residual for each animal's phenotype. As the number of offspring varied per sire, F statistic results were transformed to $-\log_{10}\mathrm{P}_{\mathrm{nominal}}$ values to allow comparisons between families. Animal counts for each population and model are listed in Table S8.

Results

Every autosome was found to harbour multiple carcass, growth and reproduction- related QTL with high levels of statistical support (Table 2). In total 56 BW, 25 CED, 31 CEM, 69 CW, 56 FAT, 69 MARB, 38 MH, 48 MILK, 52 MW, 59 REA, 41 SC, 54 WW, 24 YH and 51 YW QTL were detected. Of the 673 detected QTL regions, only 118 have previously been reported (Tables 3 & S9). While numerous mapping studies have been performed for milk production traits in dairy cattle, we considered a MILK QTL to have previously been reported if a milk fat or protein yield or concentration QTL was located within 15 cM. We postulate that variation in these traits may affect the overall energy content of milk, which influences the maternal component of a calf's weaning weight.

On average, each chromosome was found to harbour 23.2 QTL and 1.7 QTL per trait (Table 2). On average, only 2.3 chromosomes did not contain a significant QTL for each trait. Table 3 provides the average allele substitution effect estimated by GRIDQTL, the average difference between alternate homozygotes, and the average frequency of the economically desirable allele for each trait estimated by LOKI. Analysis of the markers most closely linked to each detected QTL revealed that these QTL explain a substantial amount

doi: 10.1111/j.1365-2052.2010.02063.x

Table 2 Number of QTL found per autosome by trait.

Bos taurus autosome	Trait[1]															
	BW*	CED	CEM	CW*	FAT*	MARB*	MH	MILK	MW	REA*	SC	WW*	YH	YW	Total	QTL/Mb
1	2	2	1	1	2	3	1	3	1	3	1	3	0	2	25	0.155
2	4	0	2	5	1	5	0	1	2	0	0	1	0	0	21	0.149
3	4	1	0	6	1	1	3	1	2	3	0	2	1	3	28	0.219
4	2	0	3	0	1	1	2	1	1	0	2	1	2	3	19	0.153
5	2	1	1	2	4	5	3	1	2	3	2	2	2	2	32	0.254
6	2	2	1	2	3	4	1	2	1	3	1	2	1	1	26	0.212
7	2	0	2	4	2	4	2	4	2	3	3	3	0	5	36	0.321
8	3	3	1	3	1	4	3	0	1	3	1	2	1	1	27	0.231
9	0	0	3	1	1	3	0	0	4	2	3	2	0	2	21	0.194
10	3	1	1	3	0	2	1	1	2	1	2	1	1	1	20	0.188
11	3	0	0	3	2	3	0	3	2	4	3	4	0	3	30	0.272
12	2	1	0	1	1	1	3	3	4	1	1	0	1	2	21	0.246
13	0	0	0	1	3	3	0	3	0	4	1	2	0	0	17	0.201
14	1	1	2	4	6	1	3	2	1	2	1	1	0	2	27	0.332
15	3	0	2	3	1	2	3	4	2	3	2	3	0	1	29	0.343
16	3	1	1	2	4	2	1	3	1	4	1	3	2	1	29	0.372
17	2	1	1	2	3	1	0	3	2	2	1	1	0	1	20	0.261
18	2	0	0	0	1	1	0	1	3	2	1	1	1	0	13	0.197
19	1	1	1	0	1	4	0	0	3	2	4	3	1	3	24	0.367
20	2	2	0	3	1	1	1	0	3	1	1	2	1	2	20	0.264
21	1	2	0	3	3	1	2	1	1	2	1	1	2	2	22	0.318
22	1	0	3	2	1	2	1	0	1	2	2	1	2	3	21	0.340
23	2	1	1	3	3	2	1	3	2	1	1	2	1	1	24	0.450
24	0	0	3	3	2	3	0	1	2	2	0	1	1	2	20	0.308
25	2	1	1	1	1	1	1	2	0	0	1	3	0	4	18	0.409
26	2	2	0	2	0	3	3	1	2	1	1	4	1	2	24	0.464
27	1	0	1	3	2	3	1	1	2	2	1	2	1	0	20	0.410
28	3	2	0	2	2	1	1	2	1	1	2	0	1	1	19	0.412
29	1	0	0	4	3	2	1	1	2	2	1	1	1	1	20	0.385
Total	56	25	31	69	56	69	38	48	52	59	41	54	24	51	673	0.264

[1]All traits were analysed in the AI population.

*Indicates traits that were also analysed in the HS population by GRIDQTL.

of the genetic and phenotypic variation in each trait. On average, 58.4% of each trait's genetic variation and 47.1% of phenotypic variation was explained when the markers most closely linked to all detected QTL were included in the model (Table 4). Individually, the majority of the QTL-associated markers explained < 3% of the genetic variation and <1% of the phenotypic variation within each trait (Fig. 2). Comparisons between the amount of genetic and phenotypic variation explained by each marker should theoretically only differ according to each trait's heritability, as an additive model was used to analyse both EPDs in the AI sire population and phenotypes in the HS population.

Although heterozygosity for the analysed SNPs and duplication loci was, on average, 31.7% in the sires (Table S6), of the 240 analysed trait × SNP/duplication loci, 53 had $-\log_{10}P_{nominal} \geq 4$, but only six of these had a QTL identified within 8 cM of the tested SNP/duplication locus for that trait (Table S8). Of these six associations, only two loci coincided with the location of the QTL peak: *SST_DG156121:c.362A>G* for REA and *TG_X05380:c.422C>T for CED*, with $-\log_{10}P_{nominal}$ values of 9.52 and 4.42 respectively.

Discussion

The experimental designs historically used for QTL detection in livestock have analysed only a limited number of parental chromosomes, and most genome scans performed in *B. t. taurus* have identified only a small number of the QTL which influence any one trait. In this study, by capturing the majority of chromosomes represented within American Angus, the experimental design ensures that the vast majority of large-effect QTL segregating within American Angus can be identified.

The analysis detected 673 putative economically important QTL distributed over 29 autosomes with an average of 48.1 QTL per trait (Table 2). LOKI did not detect any QTL with BF ≥ 5 support for FAT, which may be attributable to

doi: 10.1111/j.1365-2052.2010.02063.x

Table 3 Total QTL count for each trait, average QTL frequency from LOKI and the average QTL effect from LOKI and GRIDQTL.

	Count		LOKI		GRIDQTL	
Trait	QTL	Referenced[1]	Freq[2]	Effect[3]	AI-Effect[4]	HS-Effect[5]
BW	56	12	0.29	–0.11	1.22	4.84
CED	25	8	0.76	–0.74	11.90	–
CEM	31	6	0.82	–0.54	4.94	–
CW	69	5	0.37	1.32	9.47	36.74
FAT	56	5	–	–	0.15	0.61
MARB	69	14	0.85	0.05	0.35	1.09
MH	38	3	0.58	0.33	1.93	–
MILK	48	45	0.45	1.04	4.50	–
MW	52	3	0.54	9.77	19.50	–
REA	59	8	0.61	0.19	2.00	8.39
SC	41	0	0.52	0.18	2.16	–
WW	54	3	0.55	0.82	6.79	39.45
YH	24	3	0.65	0.08	0.84	–
YW	51	3	0.49	1.66	9.77	–

[1]Number of QTL detected in this study that have previously been reported in the literature.
[2]The average frequency of the economically desirable allele as estimated by LOKI.
[3]The average effect of the economically desirable homozygote on EPD values in the AI population estimated by LOKI.
[4]The allele substitution effect for the economically desirable allele on EPD values in the AI population estimated by GRIDQTL.
[5]The allele substitution effect for the economically desirable allele on phenotype in the HS population estimated by GRIDQTL.

the low variance among EPDs (0.0049 cm^2) in the AI population, possibly reflecting that progeny of these bulls were slaughtered at a fatness dependent end-point, or because only 36.5% of the analysed animals had an EPD accuracy for FAT > 0.05 (Table 1). While 42 BW, 33 calving ease, 28 CW, 23 FAT, 7 height, 41 MARB, 187 milk fat, 322 milk protein, 27 REA, 13 mature weight, 4 WW and 7 YW QTL have previously been identified, most of the QTL reported here appear to be novel, with only 118 being previously reported (http://www.animalgenome.org/QTLdb/cattle.html, release 9, accessed 23/9/2009). Our objective was to attempt to estimate an upper limit to the number of large-effect QTL within the Angus genome in comparison with those reported within the public QTL databases. Therefore, a liberal approach was used to detect QTL, which will no doubt overestimate the number of detected QTL. Repeated detection in replicated studies and fine mapping studies in our laboratory with this and other commercial populations will validate or refute these detected bovine QTL.

The discrepancies in magnitudes of estimated allelic effects and the number of QTL detected by each analytical method (Tables 3 & S9) are resulting from the methodological differences between the two analytical approaches employed. Variance component (VC) methods such as LOKI (Heath 1997) assume that QTL allele effects and the residual polygenic components are normally distributed, and estimate the genetic variance explained by a QTL by inferring the QTL genotype for all animals within the pedigree. QTL with small minor allele frequencies may not be detected in a VC analysis, as the power for QTL detection depends on the variance explained by the QTL (de Koning *et al.* 2003). On the other hand, half sib (HS) models, as implemented by GRIDQTL, estimate allele substitution effects within each family. Maternally inherited QTL alleles are assumed to be randomly distributed between siblings and independent of the allele inherited from the sire. A QTL detected in a population-based analysis performed by LOKI will not be detected by an HS analysis if the analysed sires are homozygous or if family size is small for the heterozygous sires (de Koning *et al.* 2003). On the other hand, a rare QTL may be detected in HS analysis if the segregating sire has a sufficiently large family size. As with other studies, discrepancies between the magnitude of significance levels for QTL detected by both LOKI and GRIDQTL are likely due to differences in each model's ability to represent the true architecture of QTL in a population (de Koning *et al.* 2003; Schnabel *et al.* 2005). Additionally, the larger allele substitution effects estimated by GRIDQTL could either be attributable to the smaller half-sib family size of each sire relative to the size of the entire mapping population (Luo *et al.* 2003), or could reflect a bias caused by the presence of multiple QTL in coupling phase on the same chromosomes. As the number of progeny in a half-sib family decreases, the variances associated with identified QTL are increasingly overestimated (Beavis 1998).

Differences in the amount of genetic variance explained by the markers most closely linked to each QTL in the AI and HS populations are primarily because EPDs were analysed for AI sires while phenotypes were analysed for the HS steers. However, in general, we detected more alleles at each microsatellite in the HS steer population than we did for the AI sire population (Table 4). While all of the alleles present in the HS steers that were not found in the AI sires were at very low frequency (<0.01), this suggests that the commercial Angus cows at the Circle A Ranch may have low levels of introgression from other breeds. This could lead to differences in allele frequency and phase relationships between the linked microsatellite markers and QTL, which will impact the variation explained by linked markers.

Our use of a large, multigenerational pedigree increased the power to detect QTL segregating within the full pedigree, because essentially all of the QTL of large effect that segregate within Angus are represented in this pedigree and the sample size was larger than usually analysed for livestock pedigrees. As LOKI and GRIDQTL differ in their ability to detect QTL with low allele frequencies, their combined use allows the identification of QTL that may have been missed by one approach (de Koning *et al.* 2003).

The estimates of the amount of genetic variation explained by markers closely linked to the QTL are biased

doi: 10.1111/j.1365-2052.2010.02063.x

Table 4 Genetic and phenotypic variance explained in each trait by all detected QTL.

	Count						R^2		
Trait[1]	Population[2]	Markers	Alleles[3]	Records[4]	Rank[5]	Full model[6]	Ave[7]	Min[8]	Max[9]
BW	AI	56	440	1520	380	0.5173	0.0239	0.0009	0.0804
	HS	56	592	1615	530	0.4481	0.0120	0.0004	0.0419
CED	AI	25	195	1557	170	0.3367	0.0209	0.0001	0.0564
CEM	AI	31	256	1518	222	0.4680	0.0275	0.0008	0.0845
CW	AI	69	560	1338	485	0.7086	0.0225	0.0010	0.1126
	HS	69	748	1467	657	0.5584	0.0112	<0.0001	0.0327
FAT	AI	56	451	1358	386	0.6361	0.0198	0.0001	0.0546
	HS	56	592	1541	528	0.3541	0.0082	0.0001	0.0304
MARB	AI	69	548	1368	470	0.7066	0.0251	0.0001	0.0917
	HS	69	718	1544	638	0.5603	0.0148	0.0005	0.0519
MILK	AI	48	375	1454	321	0.5629	0.0383	0.0024	0.1135
MH	AI	38	291	1113	253	0.6629	0.0621	0.0016	0.1501
MW	AI	52	372	1392	321	0.6308	0.0492	0.0006	0.1405
REA	AI	59	487	1358	418	0.6837	0.0237	0.0002	0.1089
	HS	59	668	1531	601	0.4719	0.0115	0.0010	0.0505
SC	AI	41	329	1520	286	0.4576	0.0217	0.0017	0.0583
WW	AI	54	418	1439	354	0.6489	0.0542	0.0007	0.1745
	HS	54	576	1617	519	0.4331	0.0107	0.0017	0.0317
YH	AI	24	202	1383	175	0.5099	0.0573	0.0054	0.1604
YW	AI	51	390	1555	335	0.6434	0.0534	0.0008	0.1689

[1]Traits measured in half-sibs are phenotypes, but in AI sires are Expected Progeny Differences (EPDs).
[2]HS, half-sib families; AI, artificial insemination sires.
[3]Total number of unique alleles across all loci included in the model.
[4]Total number of sires with an EPD or steers with a phenotype.
[5]Rank of the coefficient matrix for the fitted additive model. Rank = No. alleles–No. markers +1 if there are no other singularities.
[6]Genetic or phenotypic variation explained when markers most closely associated with each identified QTL were simultaneously included in the additive model.
[7]Average proportion of variation explained when only one marker was included in the additive model.
[8]Variation explained by the marker with the smallest effect on the trait.
[9]Variation explained by the marker with the largest effect on the trait.

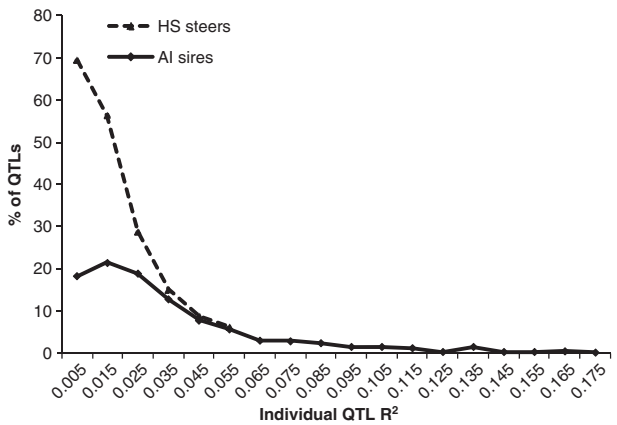

Figure 2 Distributions of QTL by the amount of genetic (AI sires) or phenotypic (HS Steers) variance explained by the nearest flanking microsatellite locus under an additive model.

upwards, as the validation analyses were performed in the QTL discovery populations. Independent validation populations are required to obtain unbiased estimates of the extent of variation explained by these QTL, which will lead to smaller estimates than found here (Xu 1998; Luo *et al.* 2003; Van Eenennaam *et al.* 2007). Despite this, we show that there are a large number of QTL of large effect underlying economically important traits in beef cattle (Fig. 2), as has recently been predicted by Chamberlain *et al.* (2007) and demonstrated by Cole *et al.* (2009) in dairy cattle.

By analysing SNP × trait associations within the perspective of a linkage analysis, we were able to localize SNPs with a significant trait effect relative to the positions of detected QTL (Table S8). In the absence of other closely linked QTL, causal polymorphisms should be detected in families that segregate for the polymorphism, and the strongest QTL signal should occur at the polymorphism's map position on the chromosome. In this case, the approach can reject causality for polymorphisms that do not generate a segregation signal or that do not map to the position of strongest QTL signal. However, no QTL may be detected, or a detected QTL may not be centred over a causal polymorphism, if there are multiple closely linked QTL in the vicinity of a tested causal polymorphism, and we have shown that a

doi: 10.1111/j.1365-2052.2010.02063.x

large number of large-effect QTL underlie variation in quantitative traits in Angus cattle. For example, the *DGAT1* p.LysK232Ala SNP genotype has been established as being causal for variation in milk component yields, and as alternate *DGAT1* genotypes produce milk which varies in energy content (Grisart *et al.* 2004; calculations not shown), we expected to detect a *DGAT1* effect on the growth of calves from dams sired by bulls with differing genotypes. In fact, only 6 of the 240 association tests performed produced a stronger signal than that between *DGAT1* and MILK in the AI sires ($-\log_{10}P_{nominal} = 13.42$ and $R^2 = 0.0338$; Table S8). However, only one of the 10 AI sires whose families were analysed by GRIDQTL was heterozygous for the *DGAT1* p.Lys232Ala polymorphism. The GRIDQTL analysis for this sire (61936) indicated a suggestive QTL for MILK ($-\log_{10}P_{nominal} = 1.71$) towards the centromere of BTA14, but not at the position of *DGAT1*. Presumably this reflects the fact that other milk QTL are located in this region of chromosome 14 (Bennewitz *et al.* 2004). Interestingly, the frequency of the K (p.Lys232) allele in Angus (0.148) is similar to the frequency in Swedish Red (0.09) and Swedish Holstein (0.12) cattle (Naslund *et al.* 2008), but lower than in Dutch Holstein-Friesian (0.20) (Grisart *et al.* 2002) and German Holsteins (0.53) (Bennewitz *et al.* 2004).

While linked QTL were not detected within 8 cM, several SNP/duplication loci × trait associations with $-\log_{10}P_{nominal}$ values >7.0 were identified: *ADIPOQ_DQ156119:c.199A>G, c.1436-1506dup, c.1596C>T* and *c.10245T>C* for CW, FAT, MH, MW, WW and YW; *ADIPOQ_DQ156119:c.199A>G* and *c.10245T>C* for YH; *LEP_AB070368:c.1759C>G* and *TG_X05380:c.422C>T* for MILK; and *SST_DG156121:c.362A>G* for MARB, MH, REA and YH (Table S8). The strength of these associations suggests the existence of QTL in these regions of the genome that were not detected in the linkage analyses either because there are multiple closely linked QTL in these regions or because the QTL are at low allele frequencies. The peak signal for the location of QTL influencing REA and CED was identified at the chromosomal locations of *SST_DG156121:c.362A>G* and *TG_X05380:c.422C>T* respectively. However, all other SNP/duplication loci tested, including an association between *TG_X05380:c.422C>T* and MARB, can be rejected in Angus cattle.

Conclusion

While the phenotypic selection of cattle has been practised since domestication, selection on many economically important traits has recently been accomplished through the use of EPDs. Despite the strong historical selection on phenotypes and EPDs, there remains ample variation in quantitative traits, and the frequency of many trait-enhancing QTL alleles in American Angus is intermediate (Table 3). While on average > 50% of a trait's genetic variance was explained when all of the detected QTL for a trait were analysed, individually each QTL explained a small proportion of the genetic variance in each trait (Fig. 2), which is consistent with the large number of detected QTL (Chamberlain *et al.* 2007) and the infinitesimal genetic model (Barton & Keightley 2002). However, the distribution of QTL variation in Fig. 2 represents detected variation rather than the actual variation present within the bovine genome. For a fixed sample size, the power to detect a QTL is positively related to the size of the QTL effect (R^2), and the majority of large-effect QTL but only a minority of small-effect QTL will be detected. Thus, we expect the actual bovine QTL variation distribution to have an exponential shape.

For a MAS programme to be effective, information from multiple QTL must be used simultaneously, as tests for individual QTL are unlikely to explain more than 5% of the genetic variation within a trait. As a result of the random genomic distribution of these QTL, MAS will apply selection to the majority of the genome within each generation. Clearly, the number of QTL underlying each trait is so large that significant numbers of causal mutations will not be identified until whole genome resequencing and mutation discovery becomes inexpensive and analytically practical. Thus, the future of MAS in livestock must be based upon the simultaneous discovery of markers linked to all QTL, which can be cost-effectively assayed in commercial applications. The former has already been accomplished in dairy cattle through a process known as genomic selection (VanRaden *et al.* 2009), where statistical prediction models for genetic merit are developed from populations that have been genotyped for high-density single nucleotide polymorphisms using approaches such as the Illumina BovineSNP50 assay (Matukumalli *et al.* 2009). However, the cost of delivery of these assays is sufficiently high that the beef cow-calf sector cannot afford their use for estimating the merit of yearling bulls or replacement heifers. Increasing genetic merit via MAS by implementing tests based upon reduced panels of SNPs that predict the contributions of the QTL reported here are likely to be the short-term solution to this problem. Producers must also balance selection for increased production with overall health, or risk a reduction in disease resistance (Rauw *et al.* 1998; Heringstad *et al.* 2000). Treating for diseases is one of the largest production costs (Kossaibati & Esslemont 1997; Snowder *et al.* 2006) and a successful MAS programme will select for both increased disease resistance and productivity.

Acknowledgements

We are indebted to numerous breeders of registered Angus cattle and to the AI companies that provided semen. In particular, we are grateful to Dr Harvey Blackburn, the National Animal Germplasm Program and to the University of Maryland for providing samples from a large number of older bulls. This project was supported by the University of

doi: 10.1111/j.1365-2052.2010.02063.x

Missouri and by National Research Initiative grants number 2005-35205-15448 and 2008-35205-18864 from the USDA Cooperative State Research, Education and Extension Service.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Number of half-sib progeny for each of the Circle A steer families and the artificial insemination sires with large sib-ships of sons also used in artificial insemination.

Table S2 Summary of microsatellite, single nucleotide polymorphism and duplication marker information.

Table S3 Multiplex PCR reagent concentrations and annealing temperature for 5 μl total volume.

Table S4 Multiplex PCR conditions.

Table S5 Single nucleotide polymorphism and duplication locus allele-specific primer sequences.

Table S6 Single nucleotide polymorphism or duplication locus allele counts.

Table S7 Marker coverage information.

Table S8 Summary of SNP by trait association test results.

Table S9 QTL data summary.

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doi: 10.1111/j.1365-2052.2010.02063.x