Identification of Quantitative Trait Loci Affecting Cattle Temperament

BEATRIZ GUTIÉRREZ-GIL, NIA BALL, DEBORAH BURTON, MARIE HASKELL, JOHN L. WILLIAMS, AND PAMELA WIENER

From the Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Roslin, Midlothian EH25 9PS, Scotland, UK (Gutiérrez-Gil, Ball, Burton, Williams, and Wiener); and the Sustainable Livestock Systems Group, Scottish Agricultural College, West Mains Rd, E49 356 Edinburgh, Midlothian, Scotland, UK (Haskell). Beatriz Gutiérrez-Gil is now at the Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, 24071, León, Spain. John L. Williams is now at the Parco Tecnologico Padano, Via Einstein, Polo Universitario, Lodi 26900, Italy.

Address correspondence to P. Wiener at the address above, or e-mail: pam.wiener@roslin.ed.ac.uk.

Abstract

In addition to its potential contribution to improving animal welfare, the study of the genetics of cattle behavior may provide more general insights into the genetic control of such complex traits. We carried out a genome scan in a Holstein × Charolais cross cattle population to identify quantitative trait loci (QTL) influencing temperament-related traits. Individuals belonging to the second-generation of this population (F_2 and backcross individuals) were subjected to 2 behavioral tests. The flight from feeder (FF) test measured the distance at which the animal moved away from an approaching human observer, whereas the social separation (SS) test categorized different activities which the animal engaged in when removed from its penmates. The entire population was genotyped with 165 microsatellite markers. A regression interval mapping analysis identified 29 regions exceeding the 5% chromosome-wide significance level, which individually explained a relatively small fraction of the phenotypic variance of the traits (from 3.8% to 8.4%). One of the significant associations influencing an FF test trait on chromosome 29 reached the 5% genome-wide significance level. Eight other QTL, all associated with an SS test trait, reached the 1% chromosome-wide significance level. The location of some QTL coincided with other previously reported temperament QTL in cattle, whereas those that are reported for the first time here may represent general loci controlling temperament differences between cattle breeds. No overlapping QTL were identified for the traits measured by the 2 different tests, supporting the hypothesis that different genetic factors influence behavioral responses to different situations.

Key words: temperament, fearfulness, animal welfare, QTL, habituation

Temperament can be viewed as the set of behavioral characteristics that contribute to the unique disposition of one animal in contrast to other species members (Kilgour 1975). Individual differences observed in animal temperament have been shown to be influenced by genetics, as well as by age, experience, sex, and environment (Burrow 1997). Animal temperament involves different elements; some of these, such as docility, workability, disposition, and fearfulness, are of particular importance for animal management. Fearful or aggressive animals are more difficult to handle, which increases the time required for routine tasks and the risk of injury for both animals and handlers. Behavior in domestic animals can also be considered as a functional trait as it has practical implications for commercial production. Cattle with poorer temperament scores are associated with decreased daily gains in weight (Voisinet et al. 1997b), lower milk yield (Burrow 1997), and reduced meat quality (Voisinet et al. 1997a).

In cattle, evidence for genetic effects on responses to human contact is provided by differences between animals of different breeds reared under the same management conditions (Murphey et al. 1980) and by heritability estimates reported for temperament traits (Morris et al. 1994; Burrow 1997; Boissy et al. 2005). Therefore, breeding might be used to improve cattle behavior traits, although because of the low-to-moderate heritabilities of these traits, additional information such as that provided by molecular markers might be needed to improve the efficiency of classical breeding.

The study of the genetics of behavior in mammals has been mainly focused on mice, where the existence of inbred lines has increased the power to detect quantitative trait loci (QTL) (Flint 2003; Willis-Owen and Flint 2006) and to identify candidate genes related to behavior (e.g., *Rgs2*; Yalcin et al. 2004). Although findings in mice may give new insights into our understanding of temperament or fear in

mammals in general, some gene effects may be species-specific, and therefore, QTL identified in mice may not be relevant in livestock species and vice versa.

The development of genetic maps for livestock species and the greater attention to welfare standards in farm animals has led to an increasing number of behavioral studies in these species, especially in poultry (reviewed by Buitenhuis et al. 2005). In cattle, there have been only a handful of QTL studies focused on behavioral traits (i.e., Fisher et al. 2001; Schmutz et al. 2001; Wegenhoft 2005) as well as a few studies on dairy production traits in which temperament during milking was also measured (Spelman et al. 1999; Schrooten et al. 2000; Hiendleder et al. 2003).

We present here the results of a genome scan for behavior-related traits carried out in an experimental population produced by crossing Holstein (dairy breed) and Charolais (beef breed) founders using a combined F_2 and backcross breeding design. Although the primary aim of the experiment was to characterize QTL influencing traits for which the breeds are known to differ (e.g., beef and dairy production traits), the second-generation animals of this herd were also assessed using behavioral tests designed to measure fearfulness of humans and of social separation (SS). The scores for these tests were used as phenotypic measures to detect QTL affecting temperament-related traits in cattle.

Materials and Methods

Resource Population

A 3-generation resource population, bred by mating 7 Charolais bulls with pure-bred Holstein cows, was established at the Roslin Institute (UK) with the aim of mapping QTL affecting economically interesting traits in cattle. A total of 137 F_1 animals were produced and used to generate 2 types of second-generation crossbred individuals: F_2 individuals (315) were obtained by mating together F_1 animals, and reciprocal backcross individuals (186) were produced by crossing F_1 cows with Charolais bulls (88 Charolais backcrosses [CB1]) and F_1 bulls with additional Holstein cows (98 Holstein backcrosses [HB1]). In addition to various dairy- and beef-production–related traits, other traits of interest in cattle (e.g., coat color, behavior, vaccine responses, and general health scoring) were recorded from the second-generation individuals of this population, which were born in 4 consecutive cohorts (from 1998 to 2001).

The behavioral tests were carried out in the female animals born during the first 3 years (years 1 to 3). A partial set of data was also recorded from the male calves of this herd; however, those results were not analyzed further because they were incomplete. Animals were born in the spring and were reared, as far as possible, under the same management conditions in each year. Female calves were removed from their dams 24 h after birth and placed in individual pens to be reared on a dairy calf "bucket-rearing" system. Between 2 and 10 weeks of age, the calves were housed in groups of 3–5. At 10 weeks, animals of similar weight and age were housed in groups of 11–14 (in 10 × 6-m

pens). After weaning from milk substitute (at approximately 6 weeks), the animals were fed on a silage and concentrate diet. Hay and straw were also offered ad libitum. The animals were regularly handled for procedures such as weighing, measuring, and blood sampling. Thus, they received close human contact during rearing.

Behavioral Tests

Behavioral tests were carried out when individuals were housed in the 11–14 animal groups. Over the 3 years, the heifer calves were tested in the spring when they were approximately 10–12 months of age, having spent most of their life housed in groups. In order to measure different fearfulness traits, 4 behavioral tests were carried out. The tests were carried out 3 times in year 1 animals to examine the consistency of the response of an individual across test repeats. The repeats of the same test were carried out within a few days of each other. Two of the 4 tests, a flight from feeder (FF) test and a social separation (SS) test, showed substantial interanimal variability and intraanimal repeatability ($r \geq 0.5$) according to standard guidelines for behavioral measures (Martin and Bateson 1993) and therefore were carried out in the following 2 years, where the 2 tests were repeated twice on each animal. A detailed description of the behavioral tests is reported elsewhere (Ball 2004). Here we describe only the 2 tests considered in this article.

The FF Test

The FF test was carried out while the animal was standing at one of a series of automatic feeders across the front of the home pen and was intended to measure the reaction of an animal when actively approached by a human being. When the animal put its head into one of the feeders, an observer stood stationary for 20 s in the corridor in front of it and then approached slowly with the aim of touching the animal. Animals were given a score on a linear scale from 1 to 6, depending on the distance remaining between the observer and the feeder when the animal moved away, with higher scores for shorter distances. Animals that would not enter the feeder because of the human presence received a null score and were eliminated from further analysis. If the animal did not move away even when touched, it received the maximum score.

The SS Test

This test measured the responses of an animal when separated from its penmates. To avoid excessive distress and factors associated with entry into a novel pen unduly affecting the behavior shown, the test was carried out in the "home pen" and with 2 "companion" animals in the next pen. The group to be tested was first moved to a different pen, and after allowing the animals to settle for 10 min, each animal was moved one at a time back to the home pen, where a video system was used to record the responses of the test animal over a 5-min test period. The behaviors observed during the test period were classified as states (walking, running, gambolling, lying, kneeling, escape

Table I. Basic statistics, data range, and total number of individuals with available data for the traits considered in the analysis

Test (total individuals tested) Year (number of animals tested by year)	Trait description	Trait	Mean	SD	Minimum	Maximum	Number
FF test (193 tested individuals)	Flight distance Test 1	FL1	4.782	0.832	2	6	193
Y1 (53), Y2 (72), Y3 (68)	Flight distance Test 2	FL2	4.393	1.253	1	6	191
	FL1–FL2	FL habituation	0.393	1.055	2	4	191
SS test (196 tested individuals)	Walking + Escaping + Running Test 1[a]	WER1	113.885	71.297	0	262.6	191
Y1 (55), Y2 (72), Y3 (69)	Walking + Escaping + Running Test 2[a]	WER2	113.483	78.569	0	262.8	189
	WER1–WER2	WER habituation	−0.860	53.194	−150.6	198.8	184
	Standing in alert Test 1[a]	SA1	65.450	35.820	0	190.2	191
	Standing in alert Test 2[a]	SA2	60.744	39.282	0	206.6	189
	SA1–SA2	SA habituation	4.292	45.014	−136.2	127.9	184
	Vocalization Test 1[b]	V1	10.832	8.807	0	56	191
	Vocalization Test 2[b]	V2	10.259	9.111	0	52	189
	V1–V2	V habituation	0.484	5.993	−24	19	184

[a] States (duration) of the SS test.

[b] Event (frequency) of the SS test.

activities, standing alert, standing occupied, and interacting with parts of the pen) or events (vocalize, urinate, and defecate). Based on a principal component analysis presented by Ball (2004), we selected traits to be included in the genetic analysis as measures of the stress response to SS. Briefly, this analysis showed that the states walk (W), escape (E), and run (R) were highly correlated and strongly influenced the first principal component (PC1), which explained 26% of the total variation observed in the animals' responses. The sum of these 3 behaviors (WER) was therefore defined as a measure of sociality in our analysis. We also considered in the analysis the standing alert (SA) state, which had a strong loading on the second principal component (PC2), and the vocalize event (V), which showed a strong loading on both PC1 and PC2, as traits measuring the animals' stress response to SS.

Treatment and Analysis of Phenotypic Data

The first- and second-test repeats carried out with each animal were treated as separate traits for the genetic analyses because the repeatabilities for these measures were only moderately high (FF test: 0.58 ± 0.09; SS test: 0.59 ± 0.09) and because of the possible novelty inherent in Test 1. We also defined a "habituation" measure (Mackintosh 1974) for each trait as the difference between the scores obtained in the first- and second-test repeats (Test 1 value − Test 2 value). The genetic analysis thus included 12 traits in total (see Table 1).

Phenotypic correlations among the traits assessed by the different tests (except the habituation measures) were calculated in MINITAB (Minitab Inc. 2003, State College, PA). Correlation coefficients were considered significant if they differed from zero in a two-tailed test ($P < 0.05$).

Prior to further analysis, the distributions of data were assessed. The FF test showed a unimodal and symmetric distribution, whereas the distribution values for the traits scored in the SS test were positively skewed. Therefore, the V trait, which had been recorded as a frequency, was transformed using a square-root transformation, and a logit transformation was used to normalize the WER and SA durations (expressed as percentages of the total SS test time). These transformations resulted in more symmetrical distributions for the SS traits. For habituation scores of both the FF and SS tests, the raw data were used directly as they had fairly symmetric distributions.

Genotyping and Linkage Maps

Standard protocols were used for DNA extraction from blood samples (Sambrook et al. 1989) and microsatellite genotyping. A total of 165 markers were included in the linkage analysis. Linkage maps constructed for each autosome using CRIMAP 2.4 software (Green et al. 1990) were previously presented in Gutiérrez-Gil et al. (2008), together with the average information content (AIC) per chromosome. For the overall genome linkage map, AIC was approximately 0.6.

QTL Analysis

The combined F_2–backcross option of the web-based QTL Express software (Seaton et al. 2002) was used to perform a QTL analysis using a linear regression method (Haley et al. 1994). The 12 behavioral traits were analyzed across the 29 bovine autosomes under a single QTL model with additive and dominance effects. The test statistics were calculated as an F-ratio at every centimorgan (cM) interval across the chromosome. Fixed effects considered in the QTL analysis of all the traits were cohort, age at testing, and genetic background (F_2, CB1, HB1). Test order was also used as a covariate in the QTL analysis of the SS test measurements.

Table 2. QTLs affecting temperament traits in the Charolais × Holstein population considered in this study

Chr[a]	Trait	cM[b]	F value[c]	P_c value,[d] (P_g value)[e]	a[f]	d[f]	V[g]	Flanking markers[h]
BTA1	SA habituation	0	5.77	0.0285	15.07*	20.08*	5.17	**BM6438**
BTA1	V habituation	142	6.37	0.0146	1.85*	2.08*	5.79	**BMS4044**
BTA4	V habituation	68	7.44	0.0055	−2.83***	−2.50*	6.86	MAF50-DIK26
BTA4	SA habituation	69	5.69	0.0286	−6.77	−29.13***	5.09	MAF50-DIK26
BTA6	WER habituation	3	5.34	0.0492	−6.31	28.45**	4.72	DIK5076-BM1329
BTA7	V1	41	4.59	0.0499	0.22	1.04**	3.77	RM6-BM1853
BTA7	V habituation	93	4.95	0.038	2.22**	1.98	4.31	ILSTS006-**INRA53**
BTA8	WER habituation	115	5.15	0.0394	5.33	−45.98***	4.53	DIK75-CSSM47
BTA9	V2	31	5.1	0.0299	−0.65 ***	−0.24	4.34	BM2504-UWCA9
BTA9	WER habituation	69	4.81	0.0379	−7.66***	30.16**	4.18	BM888-CSRM60
BTA10	V habituation	43	5.55	0.0348	2.80	−1.04	4.94	BMS528-TGLA378
BTA11	SA habituation	44	5.27	0.0262	−12.78	31.07**	4.65	ILSTS100-IDVGA3
BTA16	WER2	0	5.48	0.0277	−0.11	−0.68***	4.71	**BM121**
BTA16	V1	49	7.56	0.005	0.62***	−0.31	6.69	ETH11-BM719
BTA16	SA1	87	6.59	0.0061	0.25	0.61**	5.76	HUJ625-**DIK4011**
BTA18	V1	21	7.09	0.0051	0.58***	−0.40	6.24	IDVGA31-ABS13
BTA19	WER habituation	40	5.5	0.0205	26.86***	15.39	4.89	BMS2142-CSSM65
BTA19	SA2	72	6.3	0.0098	0.06	0.57***	5.52	CSSM65-**ETH3**
BTA19	V2	72	5.11	0.036	−0.10	0.72**	4.10	CSSM65-**ETH3**
BTA20	FL1	64	4.53	0.0403	0.03	0.44**	3.70	DIK15-BM5004
BTA21	WER habituation	65	4.72	0.0315	−8.67	−32.22**	4.08	HEL10-TGLA337
BTA25	FL1	30	4.53	0.024	−0.02	0.41**	3.85	BM737-INRA222
BTA25	V1	27	4.71	0.0284	0.42**	−0.32	3.89	BM737-INRA222
BTA25	V2	33	7.63	0.0016	0.56***	−0.34	6.82	BM737-INRA222
BTA26	V2	6	4.65	0.0315	−0.19	0.84**	3.88	ABS12-HEL11
BTA28	FL2	0	5.53	0.0137	−0.242	−0.617**	4.69	BP23
BTA29	V habituation	31	6.57	0.0087	1.25	−2.81**	5.98	RM44-**MNB166**
BTA29	FL1	65	9.45	0.0004 (0.014)	−0.34**	0.48**	8.41	DIK94-MNB101
BTA29	FL2	66	6.09	0.0102	−0.42*	0.63*	5.24	DIK94-MNB101

[a] Chr, chromosome.

[b] cM, relative position in cM Kosambi, from the beginning of the sex-averaged linkage map, for the maximum F-statistic value in the chromosome.

[c] Maximum F-statistic value for the chromosome.

[d] P_c value = chromosome-wide P value obtained by permutation test for that position (Churchill and Doerge 1994).

[e] P_g value = genome-wide P value for that position by applying the following Bonferroni correction: $P_{genomewide} = 1 - (1 - P_{chromosomewise})^{(1/r)}$, where r indicates the contribution of the chromosome to the total genome length (de Koning et al. 1999). Only indicated for P_g value < 0.05.

[f] Additive and dominance effects, respectively (in units of the analyzed trait). $a > 0$, Holstein allele is associated with higher values of the trait; $a < 0$, Charolais allele is associated with higher values of the trait. Same sign of a and d, dominance of the Holstein allele; opposite sign of a and d, dominance of the Charolais allele. Significance level: *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

[g] Percentage of variance explained by the QTL (adapted from Knott et al. 1996).

[h] Markers flanking the position of the maximum F-statistic. Markers in bold caps are <1 cM from the maximum F-statistic.

For each trait/chromosome combination, the position showing the highest F value was considered the most likely position of the QTL. At that position, the additive and dominance QTL effects were estimated. The additive effect was estimated as half the difference between the phenotypic values for homozygotes for the Charolais and Holstein alleles at the QTL, with a positive or a negative sign indicating that the Holstein or the Charolais allele, respectively, increased the value of the trait score. The dominance effect was estimated as the deviation of the heterozygotes from the mean of the 2 homozygotes. If the sign of the dominance effect is the same as the additive effect, this indicates dominance of the Holstein allele; where

the signs are different, the Charolais allele was dominant. The proportion of the phenotypic variance due to the QTL was calculated as the reduction in the residual variance due to the inclusion of the QTL in the model (adapted from Knott et al. 1996).

Chromosome-wide significance levels, which only take account of multiple tests on a specific chromosome, were calculated by permutation testing individually for each trait (Churchill and Doerge 1994). Genome-wide P values were obtained by applying the following Bonferroni correction: $P_{genomewide} = 1 - (1 - P_{chromosomewise})^{(1/r)}$, where r indicates the contribution of the chromosome to the total genome length (de Koning et al. 1999). Because this was an

initial scan, and also for ease of comparison of our results with those of other studies (as suggested by de Koning et al. 1999), we did not perform a correction for multiple traits. Empirical 95% confidence intervals (CIs) were calculated by the bootstrapping method (Visscher et al. 1996).

Results

Phenotypic Analysis

The number of animals with data for each test in each year and summary statistics for each trait are detailed in Table 1. For the 3 years that the FF test was carried out, flight distance test 1 (FL1) was significantly greater than flight distance test 2 (FL2) ($P < 0.001$) and FL habituation was positive, showing that the heifers tended to move away more quickly in the second test than in the first one. In the SS test, the duration for WER was significantly longer than for SA ($P < 0.001$) for both test repeats. This indicates that, in general, the animals spent more time in the active states included in the WER measure (walk, escape, and run) than in the SA state. For all SS test traits (WER, SA, and V), mean values of the first test were greater than the second test; however, these differences were not significant.

For most of the FF and SS traits, there were high and significant correlations between the scores of the first- and second-test repeats (results not shown). For the SS test, the SA durations of each test repetition showed a significant negative correlation with the corresponding WER-combined duration (-0.19 and -0.27 for Tests 1 and 2, respectively), due in part to the inherent correlation between measures whose sum is fixed (300 s). Vocalization (V) was positively and significantly correlated with WER (0.38 and 0.44 for Tests 1 and 2, respectively). Some moderate and significant correlations were observed between traits measured in the 2 behavioral tests, for example, both FL1 and FL2 were significantly negatively correlated with WER1 and V2. These measures were also negatively correlated with WER2 and V1, but this was only significant for FL1–V1.

QTL Analysis Results

A total of 29 QTL distributed across 17 chromosomes were identified at the 5% chromosome-wide level, as shown in Table 2. Of them, 5 QTL were associated with FF traits and 24 influenced SS test traits. The QTL for FL1 on chromosome 29 reached the genome-wide significance level (see Figure 1). Eight other QTL reached the chromosome-wide 1% significance level, all affecting SS test traits and mainly with influence on the vocalization frequency (V habituation on chromosomes 4 and 29, V1 on chromosomes 16 and 18, V2 on chromosome 25, and SA1 on chromosome 16). A substantial proportion of the significant QTL (13 out of 29) affected habituation scores. The traits for which the highest number of QTL was found were WER habituation and those related to the vocalization frequency (V1, V2, and V habituation). There was only a single QTL for WER (WER2 on BTA16).

Eight chromosomes showed more than one significant linkage association, although only on chromosome 29 were QTL found for traits scored in the 2 different tests. Overlapping or close QTL influencing the different traits assessed by the SS test were uncommon, although there were QTL for SA and V close together on chromosomes 4 and 19. Overlapping QTL were found for the same measure scored in the 2 test replicates on chromosomes 25 (for V1 and V2, at 27–33 cM) and 29 (for FL1 and FL2, at 65–66 cM). Hence the genome-wide significant QTL for FL1 on the distal end of chromosome 29, between markers DIK94 and MNB101, was accompanied by a 5% chromosome-wide significant QTL for FL2 (Figure 1). These 2 QTL for the FF test scores showed the same mode of action, with both significant additive and dominance effects, and the Charolais allele associated with less fearful animals in the FF test and showing dominance relative to the Holstein allele. Another QTL for V habituation was detected close to the middle of this chromosome (at 31 cM). The 2 QTL detected on chromosome 25, between markers BM737 and INRA222, showed an additive mode of inheritance, where the Holstein allele was associated with an increased frequency of vocalization.

Considering the QTL influencing individual test scores (rather than habituation measures), the Holstein allele was associated with greater distress responses than the Charolais allele (6 out of the 7 associations with significant additive effects). For the QTL affecting habituation scores, there was no clear pattern in the direction of additive effect; sometimes the Holstein allele was associated with a greater habituation to the test and sometimes it was the Charolais allele.

For the 8 significant QTL that showed only significant additive effects (7 of them with effects on the frequency of vocalization, Table 2), the range of the additive effects expressed in standard deviation (SD) units varied from 0.29 SD (V1 on chromosome 25) to 0.51 SD (WER habituation on chromosome 19). Five other QTL showed both significant additive and dominance effects, and for 15 QTL, only the dominance effects were significant. The percentage of the variance explained by the identified QTL ranged from 3.7%, for FL1 on chromosome 20, to 8.41%, for FL1 on chromosome 29, which was the only genome-wide significant QTL. The 95% CI obtained by boot-strapping included, in all the cases, a very large fragment along the chromosome map. Hence, the shortest 95% CI (36-cM length), which was obtained for the genome-wide significant QTL on chromosome 29, spanned about half of the chromosomal length.

Discussion

Genetic Architecture of Cattle Temperament

In the current study, we mapped QTL for temperament in cattle using a resource population obtained by crossing 2 cattle breeds, Holstein and Charolais, which differ
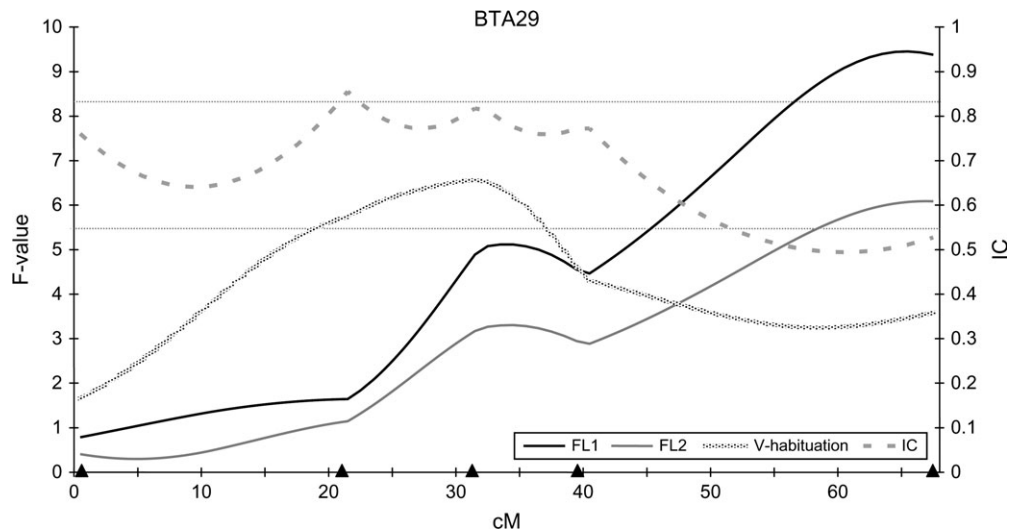
Figure 1. *F*-statistic profiles for the significant QTL identified on chromosome 29 for temperament traits on the left, *y* axis. Marker information content is shown as a dashed line on the right, *y* axis. The horizontal lines indicate the approximate 5% chromosome-wide and 5% genome-wide significance thresholds, as average of the individual thresholds calculated for the represented traits. Triangles on the *x* axis indicate the relative position of markers TGLA86, RM44, MNB-166, DIK94, and MNB-101.

extensively in appearance and use. The second-generation crossbred individuals of the herd were subject to an FF test and an SS test.

For the 12 traits considered, we detected 29 QTL significant at the 5% chromosome-wide level (Table 2), with 5 of them showing effects on FF test traits and the rest controlling the scoring variation of SS test variables. Taking into account the total number of tests carried out (12 traits × 29 chromosomes), 17 QTL would have been expected by chance alone for that level of significance, and therefore, some of our significant results are likely to be genuine QTL. Only one of the significant associations reached the 5% genome-wide threshold, and the fraction of phenotypic variance explained by the QTL reported here ranged from 3.7% (for FL1 on chromosome 20) to 8.4% (for the genome-wide significant QTL affecting FL1 on chromosome 29).

Other QTL may have been missed by the analysis as data from murine QTL studies indicate that most QTL affecting behavioral traits explain less than 10% of the variance (Flint 2003), and therefore, many of them may be undetectable by genome scans such as the study presented here (Otto and Jones 2000). Other factors have also influenced the power of our study. For example, the likely possibility that the 2 founder breeds were not fixed for different alleles at these QTL (as there is no evidence of divergent selection for temperament-related traits between the Charolais and Holstein breeds) would also have reduced the power of our study (Alfonso and Haley 1998). Furthermore, the limited number of individuals analyzed and the nature of the traits examined, which are strongly influenced by environmental factors, may have had a negative influence on the power of the analysis.

Many genome-wide significant QTL, which explain a higher proportion of the phenotypic variance, have been identified in this experimental cattle population for production-related traits (e.g., meat quality traits; growth and carcass traits, B. Gutiérrez-Gil et al. 2008, unpublished data). These traits also tended to have higher heritabilities than the behavior traits. A similar difference in the number of genome-wide significant associations and size of effects was observed for QTL affecting growth traits and behavioral traits measured in a comparable crossbred chicken population (Schütz et al. 2002). Therefore, supporting general observations from other species, temperament traits in cattle appear to be controlled by genes of smaller effect than those influencing classical production traits.

Additional genetic complexity is contributed by the high dominance levels of some QTL identified in this study. This finding is consistent with data on behavioral traits reviewed by Meffert et al. (2002), where 40% of compiled studies demonstrated dominance effects. Substantial dominance effects have previously been reported for behavioral traits, including those studied in mice (Dudek et al. 1983; Peripato et al. 2002), birds (van Oers et al. 2004), bees (Guzmán-Novoa et al. 2002), and cattle (Wegenhoft 2005). As marker coverage was not even across the 29 autosomes, we examined whether significant dominance effects were associated with chromosomes with low information content, as suggested by Buitenhuis et al. (2004). However, no significant correlation between these 2 parameters was found.

The QTL reported in this article influenced at least 2 different aspects of temperament: fearfulness of human contact as measured in the FF test and response to the SS mimicked in the SS test. In general, the QTL associated with traits assessed by these 2 different tests did not overlap, and

therefore, different loci appear to govern the fearfulness responses. The idea that fear cannot be regarded as one simple trait was first discussed comprehensively by Archer (1973) when reviewing studies in mice and rats and has been supported by many other authors (reviewed by Ramos and Mormède 1998). However, this theory has not been widely accepted, and other authors have suggested that an animal that is fearful in one context will show the same reaction in other fear-inducing situations (Broadhurst 1976; Gray 1979; Jones 1996). At the genetic level, our results would support the former hypothesis and suggest that a fearful response in different contexts has different underlying genetic causes. The control of different aspects of behavior by different genetic factors (multidimensionality) has also been suggested in relation to anxiety-related behavior in mice (Ramos and Mormède 1998; Turri et al. 2001). In our study, the stimuli that triggered the responses differed between tests. In the FF test, animals demonstrated fearfulness of human contact, whereas the fearfulness or anxiety experienced during SS in the SS test is related to the natural gregariousness that characterizes domestic ruminants (Bouissou et al. 2001). Studies in other species, such as rats (File et al. 1991) and pigs (Lawrence et al. 1991), have shown that an individual that is highly anxious in a social situation might not experience the same level of anxiety when exposed to a nonsocial challenge.

Although fixation of different alleles in the founder breeds is unlikely to hold true for most of the QTL detected in this study, it is noteworthy that the Holstein allele was associated with a higher level of distress response for both FF and SS test QTL. This is not consistent with previously documented differences in responses to humans demonstrated between beef and dairy breeds, where dairy cows are generally more approachable (i.e., predicted to have higher FF scores) than beef cows, independent of the rearing system (Murphey et al. 1980). However, other work shows that Holsteins are more sound and touch sensitive than beef cattle to sudden, intermittent stimuli (Lanier et al. 2000). Further studies assessing the effects of breed on behavior are needed to clarify these trends.

Correspondence with Other Studies

The significant QTL regions detected in this study were compared with QTL for temperament-related traits previously reported for cattle. For ease of comparison, we have provided the location of the flanking or linked markers based on the latest published version of the bovine linkage map (Ihara et al. 2004). However, caution must be taken when comparing these results as different ethological tests were used to assess temperament, and therefore, they may have considered different aspects of this complex trait. Additionally, there were many differences between the structure of the tests and the genetics of the animals tested. Schmutz et al. (2001) measured the responses of animals to isolation during handling objectively using an electronic device, and the observations used to calculate habituation were separated by 2–6 months, whereas in our study, tests

Table 3. Comparative study of QTL results

Present study				Other studies				
Chromosome	Trait	Linked markers	Position (cM)[a]	Trait	Linked markers	Position (cM)[a]	Population type	Reference
1	SA habituation	BM6438	2	Temperament + habituation	BMS574	15	Beef cattle	Schmutz et al. (2001)
4	SA habituation, V habituation	MAF50-DIK26	51–86	Disposition	TEXAN17-MAF50	28–51	Bos taurus × Bos indicus	Wegenhoft (2005)
9	V2	BM2504-UWCA9	31–50	Temperament + habituation	ILSTS013	48	Beef cattle	Schmutz et al. (2001)
16	SA1	HUJ625-DIK4011	89–99	Disposition	INRA013-BMS462	87–94	Bos taurus × Bos indicus	Wegenhoft (2005)
18	V1	ABS13-INRA121	16–30	Disposition	BL1016-BM8151	25–40	Bos taurus × Bos indicus	Wegenhoft (2005)
29	V habituation	RM044-MNB166	24–33	Temperament	BMS764-BMC8012	11–21	Dairy cattle	Hiendleder et al. (2003)

The identified QTL that may be related to previously reported temperament QTL in cattle are shown.

[a] Markers linked to or flanking the QTL are given together with their position in the latest version of the bovine linkage map (Ihara et al. 2004).

were scored objectively by the observer and repeats were only a few days apart. In other behavioral studies, different parameters and breed crosses have been used. Hiendleder et al. (2003) assessed temperament in dairy cows by considering the behavior of animals during milking, whereas Wegenhoft (2005) scored disposition (ease of handling) in a *Bos taurus* × *Bos indicus* population. Despite the different methodologies implemented to assess behavioral responses, an overlap between our results and those of others was observed (see Table 3). For example, the QTL identified for SS traits on chromosomes 1 and 9 in this study show correspondence with linkage associations reported by Schmutz et al. (2001) in relation to responses of animals to isolation during handling. Further studies would be needed to assess whether these 2 effects are controlled by the same loci. Those regions independently identified by different experiments may provide evidence of QTL segregating in several cattle breeds and also may contain genes with a general, rather than specific, effect on temperament. Some of the QTL identified by our analysis, including the genome-wide significant QTL on chromosome 29, did not show a correspondence with previous studies. Because our study is the first genome scan to examine behavior traits in a cattle population created by crossing divergently specialized breeds, these newly discovered associations may represent some of the genes underlying the temperament differences between breeds shaped by artificial breeding.

Genes Underlying Temperament Traits

Despite the preliminary nature of the results obtained in a genome scan and the need for an increased marker density to effectively conduct a candidate gene search, it is worth commenting on genes that may contribute to the effects reported here. Following a bioinformatics approach, we looked for possible coincidence on the bovine genome (http://www.hgsc.bcm.tmc.edu/projects/bovine/; http://www.ensembl.org/index.html) between the flanking intervals of the QTL identified here and the location of genes that have been associated with stress responses and temperament traits in a variety of mammals, including cattle, or with anxiety disorders and related personality traits in humans or mouse models. These include genes related to the regulation of the levels of stress hormones, such as the corticotrophin-releasing hormone (Sapolsky et al. 2000; Curley et al. 2006, 2008; Kadarmideen and Janss 2007), and to neurotransmitter or neuropeptide pathways (reviewed by Hovatta and Barlow 2008).

The most notable candidate gene found in a QTL region is the *DRD4* (type 4 dopamine receptor) gene. This gene maps to human chromosomal region HSA11p15.5 and, according physical mapping studies, is located at the distal end of bovine chromosome 29 (Amarante et al. 2000; Hayes et al. 2003; Everts-van der Wind et al. 2005), within the CI for the genome-wide QTL we detected for cattle fearfulness to human approach (FL trait). In mouse, the absence of dopamine D4 receptors results in enhanced reactivity to

unconditioned fear-evoking stimuli (Falzone et al. 2002), which may suggest a relationship with the trait measured by the FL test. In humans, *DRD4* is associated with novelty seeking behavior (Benjamin et al. 1996; Ebstein et al. 1996) and certain physical disorders such as compulsive and addictive behaviors in adults (Comings et al. 1999) and attention-deficit hyperactivity disorder in children (Swanson et al. 1998). In birds, this gene has also been shown to influence personality variation (Fidler et al. 2007).

Other genes associated with temperament traits are also found within QTL regions identified in our study. The proximal end of chromosome 16, where a QTL for WER2 was identified, includes the gene *RGS2*, a regulator of G-protein signaling, which has been associated with anxiety in mice and humans (Yalcin et al. 2004; Smoller et al. 2008). In addition, a candidate gene for schizophrenia in humans (Mah et al. 2006), *PLXNA2*, maps to the flanking interval of the QTL for SA1, located at the distal end of chromosome 16. During development, *PLXNA2* plays a key role in the formation of complex circuits required for neural function as it works as a guidance molecule that directs the growth of axons along specific pathways (Dickson 2002). Finally, the flanking interval of the QTL for FL1 on chromosome 20 harbors the gene encoding the prolactin precursor receptor (*PRL-R*). This gene is associated with maternal and social behavior in rodents (Leckman and Herman 2002), and has recently been associated with autism in humans (Yrigollen et al. 2008). Further research is needed to assess the possible relationship of these genes with the QTL identified in the Holstein × Charolais cross-population studied here.

Conclusions

The work presented here has identified QTL regions controlling temperament traits in cattle. Although only one of the significant associations reached the genome-wide level, the correspondence between QTL regions detected in our study and those found in other studies lends support to several of these associations. Other new QTL reported here may represent loci influencing distress responses that differ across cattle breeds.

Ultimately, the identification of the genes responsible for the variation will contribute to the general understanding of both animal and human behavior. In the meantime, if strong marker-trait associations can be confirmed, these markers could be used in marker-assisted selection, which has an advantage over traditional breeding schemes for traits that are difficult to measure and have low heritabilities (Lande and Thompson 1990), as is the case for temperament-related traits.

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