

Quantitative Genetic Dissection of Shoot Architecture Traits in Maize: Towards a Functional Genomics Approach

Nick Lauter,* Matthew J. Moscou, Josh Habiger, and Stephen P. Moose

Abstract

Quantitative trait loci (QTL) affecting the total number of leaves made before flowering and the number of leaves below the uppermost ear (NLBE) were mapped and characterized using the intermated B73 × Mo17 recombinant inbred lines (IBMRILs) of maize (*Zea mays* L.). B73 and Mo17 typically make 20 and 17 leaves, 14 and 11 of which are below the ear. Total number of leaves and the number of leaves below the uppermost ear are ~80% heritable in the IBMRILs, which show strongly transgressive phenotypic ranges of 15 to 24 and 10 to 18 leaves for these traits. B73 alleles at loci in chromosome bins 1.06, 3.06, 4.08, 8.04, 8.05, 9.07, and 10.04 increase leaf numbers, with all but the 3.06 QTL affecting both of these highly correlated traits ($r = 0.86$, $p < 0.0001$). Conservative QTL confidence intervals were computed and projected onto the draft maize genome sequence, revealing very narrow localizations (~1 Mb) for four of the seven loci. More than 40% of the heritable variation for both traits is explained by an additive model, squarely accounting for the dramatic parental differences, but leaving the basis of the strong transgression unexplained. In addition, error rate control and confidence interval methods tailored for composite interval mapping are introduced, and their potential for improving QTL reporting is discussed.

THE IDENTIFICATION OF USEFUL GENE TARGETS for breeding and/or transgenic manipulation remains a limiting step in biotechnology-based crop improvement. Quantitative genetic approaches are well suited for detection and characterization of useful natural allelic variation and have emerged as viable gene discovery alternatives to forward and reverse genetics, which may struggle to assign functions to genes due to lethality or complex pleiotropic effects of strong mutations (Bortiri et al., 2006; Hake and Rocheford, 2004; Yu and Buckler, 2006). Simply by scanning the linkage map of a genome for statistical associations between genotype and phenotype, quantitative trait locus (QTL) mapping simultaneously identifies genetic loci affecting a trait of interest and characterizes the relative phenotypic effects of natural alleles (Mackay, 2001). However, the utility of QTL mapping for gene discovery and identification of marker-assisted selection targets depends heavily on how narrowly a QTL can be localized (Salvi and Tuberosa, 2005).

The critical trait-independent components of QTL resolution are the density and abundance of recombination events and the depth and coverage of the genetic markers that delineate them. Both of these are dramatically improved in the intermated B73 × Mo17 recombinant inbred lines (IBMRILs) relative to previous publicly

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Abbreviations: BAC, bacterial artificial chromosome; CIM, composite interval mapping; DTP, days till pollen; IBMRIL, intermated B73 × Mo17 recombinant inbred line; LOD, logarithm of the odds favoring linkage; NLBE, number of leaves below ear; NPCI, nonparametric confidence interval; PLBE, proportion of leaves below ear; QTL, quantitative trait locus (loci); RIL, recombinant inbred line; SIM, standard interval mapping; TISI, two-LOD support interval; TNOL, total number of leaves.

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available maize (*Zea mays* L.) populations (Fu et al., 2006; Lee et al., 2002; Sharopova et al., 2002). In addition, this population was used to genetically anchor the maize physical map, facilitating candidate gene identification through integration of these data types (Coe et al., 2002; Cone et al., 2002). The impact of higher genetic resolution for QTL mapping in the IBMRILs was demonstrated by Balint-Kurti et al. (2007), who showed 5- to 50-fold improvements in QTL localizations compared to results from nonintermated B73 × Mo17 recombinant inbred lines (RILs). The present study demonstrates similarly high levels of genetic resolution and evaluates these gains in the physical context of a nearly sequenced maize B73 genome (<http://www.maizesequence.org>).

The shoot architecture traits under examination in this study, the total number of leaves (TNOL) and the number of leaves below the uppermost ear (NLBE) are agronomically important because they impact flowering time, apical dominance, biomass production, and tolerance of wind stress. Total number of leaves is an excellent measure of the vegetative developmental component of flowering time, which cannot be measured nondestructively in temporal units because it happens inside the whorl of leaves (Vladutu et al., 1999). While variable rates of leaf initiation may uncouple TNOL from the timing of the vegetative to reproductive transition, it probably represents a closer measure of the vegetative developmental component of flowering time than days till pollen or days till silk, the readily observable flowering time traits that also encompass variation contributed by differential rates of inflorescence development. Number of leaves below the uppermost ear allows calculation of the percentage of leaves below the ear (PLBE), an important apical dominance measure associated with the relative timing of pollen and silk viability that governs temporal self-compatibility in maize (Anderson et al., 2004). In addition, TNOL and NLBE affect plant and ear height, respectively, which impact tolerance of wind stress by altering the balance of shoot and root infrastructures and the plant's center of gravity.

Quantitative trait locus reporting practices exercised by the scientific community largely determine the utility of the findings for further investigations (Bernardo, 2004). Here we introduce revised resampling methods for establishing significance thresholds and positional confidence intervals when using QTL Cartographer for composite interval mapping (CIM). Both methods more stringently avoid false reporting by accounting for the use of background markers during the multiple regression step of CIM, which was impractical until a late release of this software package enabled users to limit the number of regression steps during background marker selection (Basten et al., 2003). Hundreds of studies, including Lauter and Doebley (2002) and Lauter et al. (2004), reported CIM results from QTL Cartographer using the embedded permutation test to set significance thresholds for defining what constitutes a QTL. We show that a CIM-specific permutation test that includes background marker reselection

for each permuted dataset is more conservative than what has been widely used. Further, we show that the same set of QTL are reported when we compare our CIM results obtained using this method to standard interval mapping (SIM) results filtered by the conventional permutation test described by Churchill and Doerge (1994). A theoretically similar revision of the Visscher et al. (1996) nonparametric confidence interval (NPCCI) method has been achieved by scripting background marker reselection after each bootstrap sample is selected in CIM. We name this method CIM–NPCCI. When applied to experimental CIM results, this method appears to be accurate and appropriately conservative, both of which are paramount when asking “to clone, or not to clone” a QTL (Salvi and Tuberosa, 2005).

With 95% confidence, we report the localization of four developmental regulatory factors to genomic regions that span an average of 1.1 Mb, with the only cloned maize flowering time QTL among them as proof of concept (Salvi et al., 2007). Of 62 consensus maize flowering time QTL cataloged by Chardon et al. (2004), few have been meaningfully associated with candidate genes, primarily because confidence intervals for QTL tend to be so large. Regions of the rice (*Oryza sativa* L.) genome that are microsyntenous to the narrow physical intervals harboring TNOL and NLBE QTL were mined for candidate genes. Interestingly, homologs of *Arabidopsis* genes that have been shown to have prominent roles in the control of flowering time in dicotyledonous plants were observed, leading us to speculate that natural variation between B73 and Mo17 alleles of these candidate genes may underlie the detected QTL. If true, flowering time regulation in maize may be more similar to that of *Arabidopsis* than previously thought (Chardon et al., 2004). Either way, precision mapping and characterization of natural alleles that incrementally adjust TNOL and NLBE are useful for alteration of flowering time, sexual synchrony, and plant architecture traits in breeding programs.

Materials and Methods

Plant Materials and Phenotypic Data Collection

A 291-line subset of the 302 IBMRILs (Lee et al., 2002) was obtained from the Maize Genetics Coop Stock Center and grown and phenotyped in 2002, 2003, and 2005 summer nurseries in Urbana, IL. In each year, one row of each line was grown in a randomized design with 75 cm between rows and 23 cm between plants. Single IBMRIL plants were self-pollinated in 2002, and seeds from those ears were planted in 2003 and 2005. Quality control using nine genetic markers (*phi015*, *phi021*, *phi026*, *phi034*, *phi053*, *phi059*, *phi064*, *umc1688*, *umc2092*) was performed on the 288 lines that grew in the 2005 nursery. Of these, 268 lines matched the Maize Mapping Project genotype data exactly (see below). These 268 lines were used for all analyses reported here. Three plants of each IBMRIL were phenotyped in each year, with data missing from only 72 of 2412 plants (3.03%). To reduce

bias, the plants to be phenotyped were randomly chosen by position within the row, excluding end plants and obvious rogues and runts. The plants were chosen at the seedling stage, approximately 3 wk after planting. The fifth leaf, counted from the first to initiate, was marked with athletic field marking paint before senescence of the first leaf. Before senescence of the 5th leaf, the 10th leaf was marked similarly, allowing TNOL and NLBE to be counted just after flowering.

Genotype and Map Data

We obtained IBMRIL genotypes for 2046 loci and their IBM2 map positions from the Maize Mapping Project (<http://www.maizemap.org>; Sharopova et al. 2002). Twenty-seven markers with redundant positions were removed because they failed to reveal any new recombination breakpoints. *mmp195b* was removed due to high levels of missing data and improbably high recombination with adjacent markers, first observed during scrutiny of the 8.05 QTL containing region. The probe sequence (AZ916344) for the five reported *mmp195* restriction fragment length polymorphism markers matches only one position among the 15,502 sequenced maize B73 bacterial artificial chromosomes (BACs). Since the physical contig containing genetic markers that flank *mmp195b* is fully sequenced, the removal is clearly warranted. We also genotyped the full set of IBMRILs at *idp1419* (Fu et al., 2006). This marker reduced the largest gap in the IBM2 map, which happened to contain an important TNOL/NLBE QTL. *idp1419* maps to centimorgan position 622 on chromosome 9 of the IBM2 map (see below), which is between *umc1137* (centimorgan position 603) and *umc1982* (centimorgan position 631). The final data set (2019 markers by 268 lines) used for these QTL analyses had 51,766 missing data points (9.57%). The linkage map is 7090 cM long with an average inter-marker distance of 3.53 cM.

QTL Mapping

The suite of QTL Cartographer v1.17d (Basten et al., 2003) programs was used for all QTL mapping applications. To allow comparisons across methods, both SIM and CIM were performed using models 3 and 6 of Zmapqtl, respectively. Test positions at 1.0-cM intervals throughout the genome were used for both methods. Selection of the best 10 background markers was performed by forward stepwise regression using SRmapqtl. Up to seven background markers were used during CIM, provided that their *F*-statistics exceeded an $\alpha = 0.05$ *p* value threshold. A 10.0-cM blockout window on each side of the CIM test position prevented use of background markers mapping within that sliding window during CIM. Both SIM and CIM were performed using line averages from single-year data. Using the same parameters as used for CIM, Joint CIM (JZmapqtl) was performed with the three separate years of data for each phenotypic trait, achieving summary results (model 10) and allowing QTL \times year interactions to be tested (model 14).

QTL Significance Thresholds

Permutation tests (Churchill and Doerge, 1994) were used to establish significance thresholds for determining whether a peak in the logarithm of the odds favoring linkage (LOD) curve constituted a bonafide QTL. For all three traits in all 3 yr, 1000 permutations of the phenotypic data were analyzed via both SIM and CIM using the genotype and map data described above. The global maximum LOD score was recorded from analysis of each permuted dataset. Logarithm of odds scores from our actual QTL analyses that exceeded the 50th highest score from permuted analyses were deemed significant at the $\alpha = 0.05$ level. Composite interval mapping analyses of the permutations were performed with and without reselection of background markers. *Permute_CIM.pl*, a Perl script that performs a permutation test with reselections, was derived from *Permute.pl* (Basten et al., 2003) and is available on request. In all, 27,000 full genome interval mapping scans were made using permuted data to assess QTL significance for these three traits.

QTL Confidence and Support Intervals

Using a nonparametric approach derived from Visscher et al. (1996), positional confidence intervals for all QTL detected by CIM were established through analysis of 1000 bootstrap-resamplings of the data from each of the 3 yr. Resampling of RILs was performed with replacement until each bootstrap sample contained 268 lines, allowing the significance thresholds established for the actual data to be reasonably applied. To generate CIM-NPCIs, which are nonparametric confidence intervals appropriate for the CIM method, background marker reselection and subsequent genomewide CIM was performed for all three traits on each of the 3000 bootstrap samples using parameters described above. For purposes of comparison, separate CIM analyses were performed on the bootstrap samples without reselection of background markers, which is how bootstrapping has been implemented for CIM in QTL Cartographer until now. We call this the NPCI method, although it should be noted that Visscher et al. (1996) designed this approach for mapping methods akin to SIM, rather than CIM. The NPCIs and CIM-NPCIs for each trait-locus association in each year was computed from CIM results using the following procedures. (i) The CIM data for the genetic region surrounding the QTL in question were extracted and queried for the height and position of their highest LOD peak in each of the 1000 bootstrap samples. (ii) As per the “selective method” of Visscher et al. (1996), bootstrap samples that failed to produce a threshold-exceeding LOD peak in the region of interest were excluded. (iii) The CIM results from the remaining bootstraps were ordered according to the centimorgan position of their LOD peaks, allowing the central 95th quantile to establish the positional bounds of a 95% confidence interval. Automation of the individual steps of the entire procedure was accomplished using Python scripts, which are available on request. For purposes of comparison, we also report a two LOD support interval (TLSI) for each QTL, a widely used positional confidence

estimation that includes the region of the chromosome where the LOD curve remains within two units of the peak value for a QTL.

Integration of Genetic and Physical Maps

Correspondence between the physical map and the IBM2 genetic map was largely achieved by visualizing the overgo hybridization data from Gardiner et al. (2004) using a physical map browser (<http://maizesequence.org>) and a genetic map browser (<http://maizegdb.org>; Lawrence et al., 2007). All data used were current as of 20 June 2008. Centimorgan positions in the tables and text all refer to the IBM2 map, whose framework is maintained for several data types and will continue to be maintained at MaizeGDB (Lawrence et al., 2007). Genbank accession names of BACs and sequences are given in lieu of contig names and megabase positions, which will change as gaps in the physical map are filled. Placement of sequence-based genetic markers was also accomplished using blastn (Altschul et al., 1990), with 15,502 Maize B73 BACs downloaded from GenBank as the database.

Results

Parental Phenotypes

The parents of the F_1 plant from which the IBMRILs were derived differ markedly for TNOL and NLBE. B73 produces ~18% more leaves than Mo17 before the primary shoot apex transitions from leaf to tassel production and ~26% more leaves below the uppermost lateral branch that bears an ear (Table 1). The difference in these percentages reflects the fact that all three of the additional leaves produced by B73 end up below the top ear. Thus, these lines have distinct PLBE yet are similar in that both typically have six leaves between their tassels and uppermost ears (Table 1).

Progeny Phenotypes

The IBMRILs consistently showed wide-ranging phenotypes for TNOL and NLBE, making 15 to 24 leaves, with 10 to 18 of them below the top ear (Fig. 1). Among 793 within-year line averages for TNOL and NLBE respectively, 21% and 33% of the IBMRIL phenotypes were transgressive, or statistically outside the range of the parental phenotypes. Only 17% of the line averages showed transgression for both TNOL and NLBE, meaning that 37% were transgressive for at least one of the two traits. For both traits, transgression above B73 levels was more common and more severe than that seen below

Mo17 levels (Table 1, Fig. 1). Consistent with this imbalance, the difference in the proportions of transgressive line averages for TNOL versus NLBE is predominantly accounted for by lines that were not transgressive for TNOL but were transgressive above B73 levels for NLBE. Among the 157 (20% of 793) cases of transgression for only a single trait, 122 were of this class. This upward shift in NLBE values among IBMRILs is also reflected by the PLBE phenotypes, which range from 60.0 to 82.5%. Strikingly, the mean PLBE values for the 3 yr (71.4, 72.7, and 72.9%) were all higher than the high parent levels (Table 1), a rare case among populations of cereal inbreds.

Heritability

To assess the relative contributions of genotype and environment, broad-sense heritabilities (line effects) and year effects were estimated by linear mixed model analysis (JMP 6.0, SAS Institute, Cary, NC) of phenotype data collected from 2340 plants (three plants per line for 268 IBMRILs in each of 3 yr with 3% missing data). The heritabilities of TNOL and NLBE are very high, indicating that these phenotypes are largely controlled by genetic factors (Table 2). Nevertheless, TNOL and NLBE variances attributable to year effects were statistically significant and not insubstantial (Table 2). One basis for this effect can be seen in the histograms for TNOL and NLBE values plotted by year, wherein the distributions in both years two and three are shifted upward relative to year 1 due to average mean differences of 0.62 and 0.70 leaves for the two traits, respectively (Fig. 1). Indeed, when the same statistical model is applied to the three partial data sets that use data from only 2 of the 3 yr, only the pairing of years 2 and 3 shows no significant effect of year (not shown). This trend is also seen in the year to year correlations for these traits. For TNOL, r values are 0.801, 0.847 and 0.807 for year 1 \times year 2, year 2 \times year 3, and year 3 \times year 1 correlations, respectively. For NLBE, these values are 0.745, 0.779, and 0.691. For all six correlations, $p < 0.0001$.

The heritability and year effect estimates for PLBE are much lower than those for TNOL and NLBE (Table 2). However, the ratio between PLBE phenotypic variances attributable to line and year effects remains similar, suggesting that error variances of TNOL and NLBE are compounded in the derivation of this calculated trait.

Consistency of QTL Detection

Using stringent method-specific type I error rate thresholds, SIM and CIM approaches identified largely, but not exclusively overlapping sets of trait–locus associations (Tables 3 and 4). So that consistency of detection can be evaluated for TNOL, NLBE and PLBE QTL, SIM and CIM results from single-year analyses are presented. For both methods, all of the QTL were detected in at least two of the three single-year analyses, although this was not a reporting requirement for either method (Tables 3 and 4). The proportions of QTL detected in all 3 yr were 8/12ths and 4/8ths from SIM and CIM, respectively. The

Table 1. Parental phenotypes.[†]

Trait	B73, $n = 20$	Mo17, $n = 20$	MPD
Total no. of leaves	20.00 \pm 0.15	16.95 \pm 0.09	3.05
No. of leaves below ear	14.20 \pm 0.12	11.25 \pm 0.12	2.95
Percentage of leaves below ear	71.0 \pm 0.5	66.7 \pm 0.6	4.3

[†]Means \pm SE and mean parental differences (MPD) are reported.

difference in total numbers of QTL detected by the two methods is easily accounted for. Standard interval mapping detected five QTL effects that were not detected by CIM (bins 8.04 and 10.04 for TNOL and 1.08, 8.04, and 10.04 for NLBE), while CIM detected a single TNOL QTL in bin 4.08 that was not detected by SIM. Thus, 7 of the 13 statistically significant trait–locus associations were detected in common by the two methods. Notably, differential detection according to method was consistent across years and traits.

QTL Effects

Collectively, SIM and CIM identified six TNOL QTL with estimated effects ranging from mild to moderately strong (Tables 3 and 4). The 8.05 and 9.07 QTL consistently had the strongest effects, each adding approximately one leaf to lines homozygous for the B73 allele relative to those homozygous for the Mo17 allele. By the same measure, the 1.06 and 10.04 QTL each add approximately a half leaf. Since both SIM and CIM report results from a separate statistical model for each QTL, we performed multiple regression to estimate the combined effects of all reported QTL on these traits (JMP 6.0, SAS Institute, Cary, NC). The six QTL explain 33% of the phenotypic variance (adjusted $r^2 = 0.328$), which is about 41% of the heritable variation (Table 2). Lines homozygous for Mo17 (low) alleles at these six QTL averaged 17.4 ± 0.2 leaves, while lines carrying only the high alleles averaged 20.8 ± 0.2 leaves. The difference of 3.4 leaves well exceeds the mean parental difference but spans less than half of the range between the phenotypic extremes of the IBMRIL population (Fig. 1).

The combined effect of the six NLBE QTL (Tables 3 and 4) explains 29% of the phenotypic variance (adjusted $r^2 = 0.293$), which is about 40% of the heritable variation (Table 2). Lines homozygous for Mo17 alleles at these six QTL had average NLBE values of 12.4 ± 0.3 , while lines with the opposite alleles had 14.7 ± 0.3 leaves below their ears. These QTL collectively account for 78% of the mean parental difference but span less than one-third of the range between the IBMRIL phenotypic extremes (Fig. 1).

Since the year effect was significant for both TNOL and NLBE (Table 2), genotype \times environment effects were estimated for each QTL by Joint CIM mapping. No significant QTL \times year interactions were observed (not shown).

Pleiotropic Inferences

Total number of leaves and NLBE are closely related and highly correlated traits ($r = 0.86$, $p < 0.0001$, $n = 2340$). Five of the six chromosomal positions harboring QTL that affect TNOL are also detected as NLBE QTL. For the allelic differences mapped to bins 1.06, 8.04, 8.05, 9.07, and 10.04, evidence supporting inferences of pleiotropic action exists in several forms: coincident LOD peak locations and generally congruent LOD curves (Fig. 2), tightly overlapping confidence intervals across methods and years (Tables 3 and 4), and consistent directions and relative magnitudes of effect for these QTL (Tables 3 and

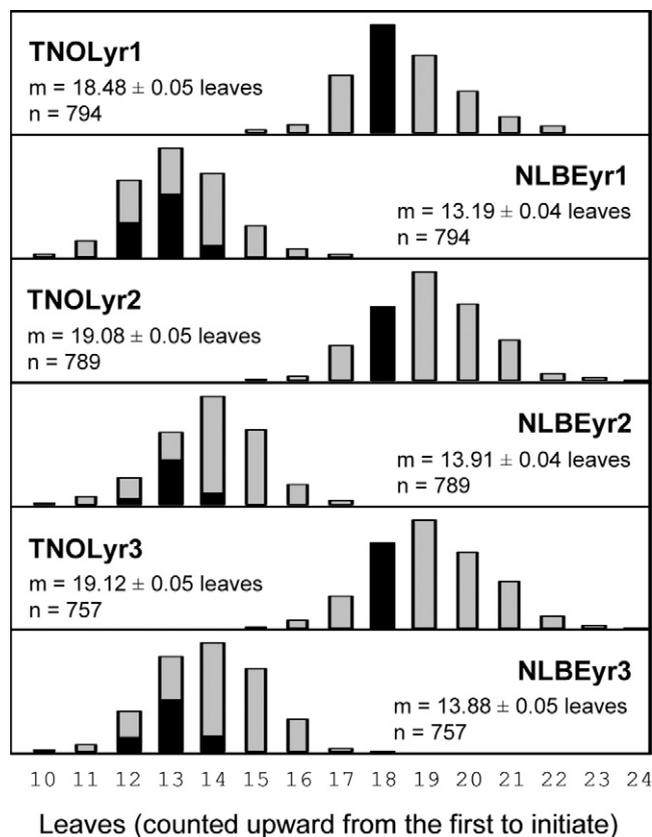


Figure 1. Phenotypic ranges, trait relationships and variation among years in maize. Count histograms depict the intermated B73 \times Mo17 recombinant inbred line (IBMRIL) phenotypic distributions for total number of leaves (TNOL) and number of leaves below ear (NLBE) in three separate years. Means, standard errors, and numbers of plants are reported. The black NLBE histograms show the phenotypic distributions of the IBMRILs that had 18 leaves, providing a visual measure of the extent to which these two traits are phenotypically correlated.

4). For the purposes of discussion, we consider these putative cases of pleiotropic action by the QTL.

Given the extent of apparent genetic coregulation between TNOL and NLBE, the fact that the 4.08 and 3.06 QTL appear to affect only one of these two traits provokes both interest and suspicion. Are these loci

Table 2. Trait heritabilities in the intermated B73 \times Mo17 recombinant inbred line (IBMRIL) maize population. For each trait, a linear model was used to estimate broad-sense heritability (H^2) and environmental effect (year) using nine plants measured for each of 268 IBMRILs. The partial R^2 values and their related p values are given for both components of each model.

Trait	H^2	year	n
Total no. of leaves	0.793 [†]	0.047 [‡]	2340
No. of leaves below ear	0.729 [†]	0.070 [‡]	2340
Percentage of leaves below ear	0.355 [†]	0.017 [‡]	2340

[†] $p < 0.00001$.

[‡] $p < 0.0001$.

Table 3. Quantitative trait loci (QTL) estimates from standard interval mapping (SIM).[†]

Trait	Chr	cM	EWT	LOD	α	PVE	TLSI
TNOLyr1	1.06	523.9	3.65	3.67	0.31	0.060	506.4–543.3
	8.04	333.4	3.65	7.77	0.52	0.146	317.2–346.6
	8.05	362.5	3.65	8.60	0.53	0.150	356.6–364.5
	9.07	608.5	3.65	9.06	0.59	0.221	591.9–615.2
	10.04	244.3	3.65	4.47	0.35	0.079	238.3–250.9
NLBEyr1	3.06	444.3	3.74	6.32	0.38	0.112	431.2–473.3
	8.04	344.0	3.74	6.62	0.43	0.122	337.3–347.5
	8.05	362.5	3.74	7.17	0.44	0.129	356.6–363.4
	9.07	609.5	3.74	7.56	0.50	0.189	591.2–615.8
	10.04	244.6	3.74	4.06	0.28	0.062	237.8–257.2
PLBEyr1	3.06	453.7	3.64	5.71	1.04	0.105	435.8–457.1
TNOLyr2	8.04	344.0	3.63	7.19	0.53	0.133	316.7–347.9
	8.05	362.5	3.63	9.47	0.58	0.166	359.1–363.4
	9.07	610.5	3.63	8.53	0.61	0.213	590.6–615.4
NLBEyr2	1.06	541.3	3.67	3.87	0.31	0.065	521.0–554.6
	3.06	463.1	3.67	5.26	0.38	0.100	437.1–473.2
	8.04	320.6	3.67	6.58	0.41	0.108	316.7–347.9
	8.05	362.5	3.67	7.49	0.47	0.135	359.7–365.9
	9.07	612.5	3.67	6.47	0.49	0.165	603.9–625.6
PLBEyr2	3.06	463.1	3.77	7.77	1.06	0.150	453.1–470.0
TNOLyr3	1.06	523.9	3.59	4.52	0.40	0.078	509.7–542.5
	8.04	326.8	3.59	6.10	0.50	0.114	317.4–347.9
	8.05	362.5	3.59	5.12	0.46	0.093	356.6–365.9
	9.07	611.5	3.59	10.47	0.73	0.267	605.4–617.2
	10.04	244.6	3.59	4.22	0.30	0.068	237.8–250.9
NLBEyr3	1.06	540.1	3.62	3.54	0.32	0.067	508.4–549.3
	3.06	463.1	3.62	4.99	0.38	0.101	451.9–472.6
	8.04	325.8	3.62	4.50	0.37	0.084	314.9–336.6
	8.05	362.5	3.62	4.50	0.35	0.071	357.2–365.9
	9.07	611.5	3.62	9.58	0.62	0.263	605.4–616.4
PLBEyr3	3.06	465.1	3.68	8.30	1.19	0.176	459.5–470.9

[†]The chromosomal bin (Chr) and centimorgan (cM) position on the IBM2 map (see <http://www.maizegdb.org/>) are reported for each QTL peak where the LOD curve exceeds the experiment-wise threshold (EWT) for significance at the $\alpha = 0.05$ level. The additive genetic effect (α) expresses the estimated phenotypic consequence of substituting one Mo17 allele with one B73 allele at a QTL; negative values of α would indicate that the high allele came from Mo17. The phenotypic variance explained (PVE) is the partial R^2 estimate for the QTL. A two LOD support interval (TLSI) reports the centimorgan boundaries of the contiguous chromosomal segment wherein the LOD score remains within two units of its peak.

really acting in ways that uniquely impact just one of the traits? For the case of the 3.06 QTL, detection as a locus that also affects PLBE suggests that it does not simply add more leaves to the plant (Tables 3 and 4). The effect estimates for the 3.06 QTL further indicate that this locus directly impacts the position of the uppermost ear; it accounts for only 13 to 15% of the heritable variation for NLBE but could be described as a single, major-effect locus affecting PLBE, for which it explains 30 to 57% of the heritable variation (Tables 3 and 4). Significantly, the failure to detect loci affecting PLBE in bins 1.06, 8.04,

8.05, 9.07, and 10.04 supports the inferences of pleiotropy described above.

The effects of the 4.08 locus were also investigated further. In both years that this locus was detected for its effect on TNOL using CIM (Table 4, Fig. 2), there were also mild effects on NLBE in the CIM reports, with LOD scores of 3.2 and 4.4, and with proportion of the phenotypic variance explained values of 0.038 and 0.045, respectively. Since no such effect was detected for PLBE, it appears that the 4.08 QTL simply adds leaves to the plant, contributing incrementally to NLBE values.

Epistatic Interactions

Two-way ANOVAs were used to test for interaction effects among these QTL in a pairwise manner. Three two-locus interactions were statistically significant after Bonferroni correction for multiple tests. Two of these involve the 4.08 locus, which was found to interact synergistically with both the 8.04 and 8.05 QTL (Table 5). The more-than-additive effects of these interactions are quite strong, with estimated phenotypic contributions of 0.34 and 0.41 leaves, respectively (Table 5). These substantial gains in leaf number represent 25 and 26% of the total phenotypic effects that can be explained by the respective two-locus models. Since the 8.04 and 8.05 loci are loosely linked (Table 3) and appear to have a modest more-than-additive interaction with one another (Table 5), we investigated their interaction effects with the 4.08 locus in a full factorial three-locus model. The interactions between 8.04 and each of the other two QTL remain significant ($p < 0.007$), but the 8.05 by 4.08 effect does not ($p = 0.53$).

A significant less-than-additive interaction was detected between the 1.06 and 10.04 QTL (Table 5). Such an interaction effect suggests that the allelic differences at these loci may cause functionally overlapping alterations during development that ultimately affect leaf number. Consistent with this hypothesis, SIM estimates of effects for these two QTL are nearly equivalent, yet only the 1.06 QTL is detected by CIM (Tables 3 and 4). Their epistatic interaction and/or differential interactions with other loci likely account for this method-associated discrepancy (Zeng, 1994).

Consistency of Positional Estimates

The positions of the LOD peaks for the QTL in bins 4.08, 8.05, 9.07, and 10.04 were highly consistent across years and mapping methods, as well as among traits for which pleiotropic action has been inferred (Tables 3 and 4, Fig. 2). By contrast, the QTL in bins 1.06, 3.06, and 8.04 did not localize to consistent positions (Tables 3 and 4, Fig. 2). For these loci, variation in peak position is equally well represented by within-trait-between-year and within-year-between-trait contrasts (Table 3). Thus, the aforementioned inferences of pleiotropic action on TNOL and NLBE for the 1.06 QTL and on NLBE and PLBE for the 3.06 QTL remain appropriate.

Consistency of LOD peak position is predictive of the breadth of the TLSI, a measure that approximates a 95%

confidence interval (Mangin et al. 1994; van Ooijen 1992). Loci with consistent peak positions had an average SIM TLSI width of 17.6 cM, compared with 27.9 cM for loci with inconsistent peak positions ($p < 0.001$; Table 3). Nevertheless, the TLSIs for the 1.06, 3.06, and 8.04 loci do not always encompass the peak LOD position for what we infer is the same effect detected in a different year or for a related trait in the same year (Table 3). Composite interval mapping TLSIs, as well as the less-conservative CIM–NPCIs, also failed in several cases to encompass all of the LOD peak positions associated with the locus of interest (Table 4). Low LOD scores, locus complexity, linkage to a major-effect QTL, and trait complexity are possible explanations for why these QTL localize poorly and could individually or collectively explain why all three methods examined do not capture the disparate peak positions observed. These results demonstrate the value of experimental replication and caution against relying solely on statistical inference (TLSI) or reinterrogation (NPCI).

Discussion

Precision mapping of QTL that affect leaf number and ear position was accomplished using the IBMRIL maize population, which has an abundance of recombination events and a marker density that is sufficient to cover, but not saturate, this extensive map (Lee et al., 2002; Sharopova et al., 2002; Fu et al., 2006; Fig. 2). B73 alleles at seven loci were shown to increase NLBE, with six of them appearing to act more generally to increase TNOL made before flowering. We discuss the use of CIM-specific nonparametric methods that are appropriate for QTL reporting in a functional genomics context, correspondence with known loci, functional and candidate gene hypotheses, and cloning prospects for these agronomically relevant QTL.

Stringent Control of Type I Errors

Setting the experiment-wise type I error rate dramatically affects QTL reporting and interpretations of trait inheritance. Permuted versions of the phenotype data are repeatedly analyzed to establish the strengths of chance statistical associations between genotype and phenotype (Churchill and Doerge, 1994). Composite interval mapping differs from SIM in that it fits additional genetic factors in the model while testing a given interval. Thus, to properly set the type I error rate threshold for CIM, we wrote a script for QTL Cartographer that reselects the cofactors for each permutation of the phenotype data before conducting CIM.

Averaged across three replicate experiments on three traits, the $\alpha = 0.05$ experiment-wise type I error rate thresholds were 5.46 ± 0.03 when background markers were reselected (Table 4), and 4.12 ± 0.03 when they

Table 4. Quantitative trait loci (QTL) estimates from composite interval mapping (CIM).[†]

Trait	Chr	cM	EWT	LOD	<i>a</i>	PVE	TLSI	CIM–NPCI
TNOLyr1	1.06	529.0	5.62	5.66	0.35	0.074	517.0–553.6	522.1–547.9
	4.08	473.6	5.62	7.90	0.39	0.091	450.4–491.7	466.2–475.0
	8.05	362.5	5.62	9.40	0.48	0.119	357.9–369.6	359.7–364.7
	9.07	609.5	5.62	9.77	0.50	0.156	592.9–623.5	587.9–616.5
NLBEyr1	3.06	445.0	5.52	8.10	0.37	0.101	441.6–470.0	438.1–459.1
	8.05	362.5	5.52	7.40	0.39	0.098	357.9–369.6	359.1–365.3
	9.07	615.5	5.52	5.99	0.38	0.112	591.9–627.7	606.5–631.7
PLBEyr1	3.06	453.7	5.45	9.77	1.29	0.155	432.6–472.1	444.8–457.3
TNOLyr2	1.06	539.1	5.59	5.75	0.35	0.068	517.0–588.2	524.5–547.9
	4.08	473.6	5.59	7.73	0.41	0.091	462.5–489.7	467.7–475.0
	8.05	361.5	5.59	10.90	0.55	0.145	356.6–369.6	359.7–364.0
	9.07	608.5	5.59	7.25	0.48	0.125	589.9–629.7	594.9–615.5
NLBEyr2	3.06	445.0	5.21	5.82	0.31	0.071	443.3–473.1	438.1–469.1
	8.05	362.5	5.21	8.53	0.43	0.112	357.9–369.6	359.1–365.1
PLBEyr2	3.06	463.1	5.29	9.51	1.10	0.154	449.0–469.1	459.1–469.3
TNOLyr3	1.06	529.0	5.53	6.28	0.39	0.077	517.0–542.3	522.1–542.3
	9.07	612.5	5.53	9.27	0.67	0.225	592.9–625.7	606.5–617.8
NLBEyr3	3.06	461.1	5.57	5.60	0.41	0.067	443.3–469.1	444.6–471.0
	9.07	612.5	5.57	8.51	0.59	0.233	592.9–626.7	606.5–618.2
PLBEyr3	3.06	464.1	5.35	11.36	1.30	0.201	451.1–471.1	459.1–469.3

[†]The chromosomal bin (Chr) and centimorgan (cM) position on the IBM2 map (see <http://www.maizegdb.org/>) are reported for each QTL peak where the LOD curve exceeds the experiment-wise threshold (EWT) for significance at the $\alpha = 0.05$ level. The additive genetic effect (*a*) expresses the estimated phenotypic consequence of substituting one Mo17 allele with one B73 allele at a QTL; negative values of *a* would indicate that the high allele came from Mo17. The phenotypic variance explained (PVE) is the partial R^2 estimate for the QTL. A two LOD support interval (TLSI) reports the centimorgan boundaries of the contiguous chromosomal segment wherein the LOD score remains within two units of its peak. The CIM-specific nonparametric confidence intervals (CIM–NPCI) are reported in centimorgans and delineate the bounds of $\alpha = 0.05$ positional confidence intervals.

weren't. The widely used “without reselection” approach produces a substantially lower threshold, which likely leads to false discovery and contributes to QTL results appearing inconsistent (Bernardo, 2004). If we had implemented this method to establish the threshold for CIM results, several additional QTL would have appeared significant in one of the three single-year analyses. Using the “background markers reselected” method, every locus reported was deemed a QTL in at least two of the years, demonstrating that this method is meaningfully more conservative. In addition, SIM results filtered by conventional permutation tests (Churchill and Doerge, 1994) reported essentially the same set of loci (Tables 3 and 4), with exceptions required only for loci found to have epistatic interactions with other QTL (Table 5). Similarly, none of these were detected in fewer than 2 of the 3 yr (Table 3), providing further evidence that the “background markers reselected” method for CIM threshold determination is appropriate, as well as statistically more correct.

Confidence Intervals for QTL Positions

Determining statistical confidence for QTL positions poses a particularly significant challenge for CIM, which

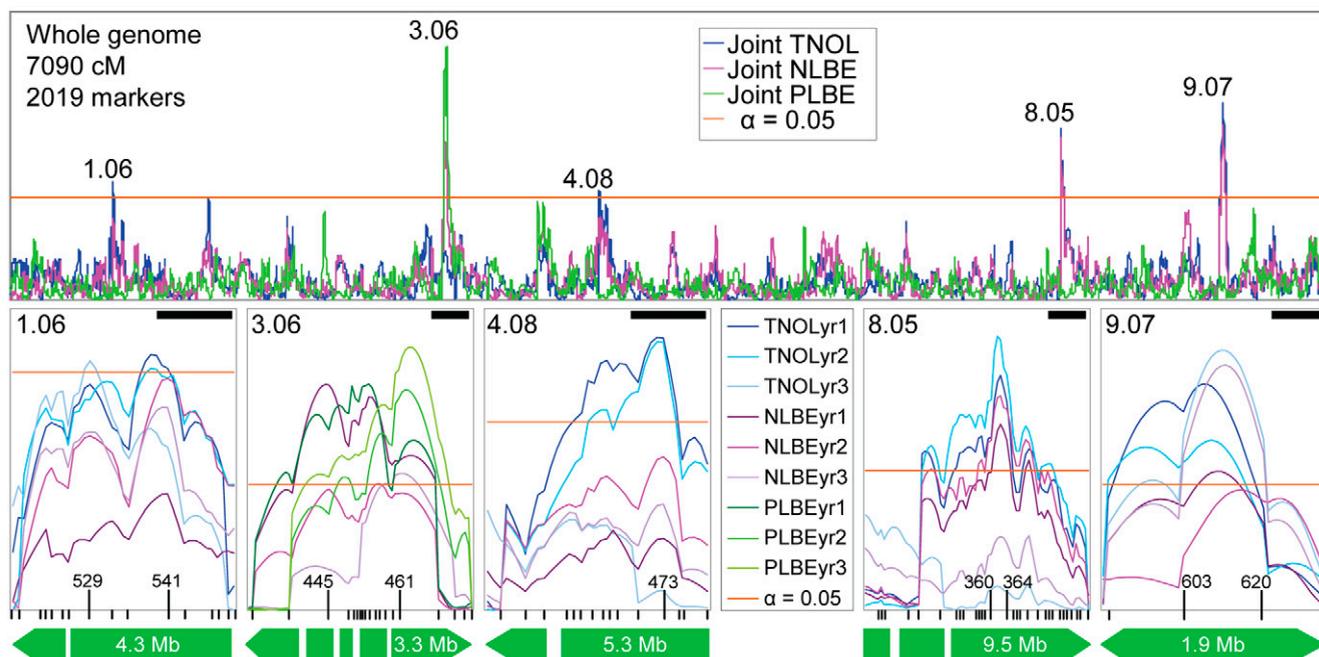


Figure 2. Threshold-normalized plots of logarithm of odds (LOD) curves indicating quantitative trait loci (QTL). No Y-scales are given, as the LOD curves are each normalized to the $\alpha = 0.05$ experiment-wise threshold of the respective trait (see Table 4). The whole genome plot shows Joint-composite interval mapping (CIM) results. The narrow region plots show CIM results and are labeled with the chromosomal bin of the QTL they depict. Black scale bars represent 10 cM. Hatch marks indicate relative positions of markers, with key centimorgan positions labeled. Physical map contigs are drawn as green boxes, with arrows indicating extension beyond view. The sizes of the depicted portion of the most relevant contigs are given. TNOL, total number of leaves; NLBE, number of leaves below ear; PLBE, proportion of leaves below ear.

is known to produce positionally inaccurate LOD curves at unacceptably high frequencies (Li et al., 2007; A. Crosset, N. Lauter, and T. Love, unpublished simulation results). Such errors can be attributed to epistasis, linked effects, and overfitting the model, although LOD curve shape can also change dramatically according to how the user sets the walking speed and window size parameters (Balint-Kurti et al., 2007; Basten et al., 2003; Li et al., 2007). Most QTL papers report support intervals based on how the LOD curve drops away from its peak. This class of methods has been shown to perform well for SIM across a variety of population types, sample sizes, marker densities, and QTL effect magnitudes (Manichaikul et al., 2006). Support interval methods have only been evaluated for CIM in a limited context, where their performance was compared only to the NPCI approach (Kim et al., 2002). The fact that LOD curve shape and peak position depend in part on CIM user parameters prompted development of a nonparametric reinterrogation method appropriate for CIM.

Nonparametric methods have been applied extensively to the positional confidence problem (Lebreton and Visscher, 1998; Visscher et al., 1996; Walling et al., 2002; Walling et al., 1998). However, they have not yet been tailored to specifically treat the CIM procedure. The NPCI method used by Kim et al. (2002) to evaluate CIM results could be viewed as a misapplication of the Visscher et al. (1996) method, which was designed for SIM.

In the present study, we observed that the NPCIs were much narrower than the CIM-NPCIs, indicating the bias introduced by repeated use of the original background marker set (Fig. 3).

The CIM-NPCI method appears to provide accurate, moderately conservative confidence intervals; the QTL peaks were captured in all 20 cases tested (Table 4), and the CIM-NPCIs were twofold less conservative than the CIM TLSIs (14.2 vs. 28.5 cM; Table 4). A recent study indicates that the CIM-NPCI ($\alpha = 0.05$) method has a higher probability of capturing simulated QTL peaks than the CIM TLSI method does, especially when marker density is as high as it is in the IBMRIL population (A. Crosset, N. Lauter, and T. Love, unpublished results). Thus, this method effectively deals with observed inconsistencies associated with effects of marker position relative to the QTL position, which has previously been shown to be an issue for the maximum likelihood estimation procedure used for interval mapping (Manichaikul et al., 2006).

The Inheritance of TNOL and NLBE in the IBMRILs

Overall, the inheritance of these traits seems quite simple. They are highly heritable (Table 2) and a large amount of the phenotypic variance can be accounted for by just a handful of loci with significant effects on the phenotype (Tables 3 and 4). However, an anomalous

result was encountered in the course of dissecting how variation in shoot architecture is controlled among progeny of the cross between B73 and Mo17. Significant transgressive segregation was observed (Table 1, Fig. 1), yet only QTL alleles from B73 were found to increase leaf number. The simple explanation for this unexplained transgression is that Mo17 alleles at certain loci contribute to the inheritance of TNOL and NLBE but are not detected as QTL. Masking due to tight linkage of opposite effects should not explain such a parent-specific effect, so we could presume that the QTL effects are simply too small to detect. This could be a consequence of the stringency of the thresholds applied, but it seems doubtful, since LOD congruence across years was used to scan for small effects (data not shown). Environmental sensitivity of loci with small effects could have allowed them to escape detection by both approaches, in which case it would be interesting that one parent carries the more sensitive alleles. Alternatively, an intriguing possibility is that widespread nonallelic complementation effects may be playing a role, similar to what could underlie heterotic effects. In such a case, presence of Mo17 alleles in IBM-RILs at certain loci would ameliorate the effects of B73 alleles at too many loci, producing inbred plants with more leaves by a heritable, but difficult-to-detect mechanism. Perhaps intensive experimental replication and better delineation of recombination breakpoints could provide a conclusive test of this hypothesis.

Correspondence with Known QTL and Functional Hypotheses

Although TNOL and NLBE have not been studied very widely in maize, six of the detected QTL correspond closely with previously identified QTL. This overlap is largely due to a wealth of flowering time and ear height studies in maize, many of which utilized B73 and Mo17 parentage. The functional sources of the correspondence are simply that more leaves are made when flowering is delayed, and that apical dominance is exerted from above, such that NLBE typically increases as TNOL increases. In a meta-analysis of 22 QTL studies, Chardon et al. (2004) plotted 313 flowering time QTL that occupy 62 consensus positions on a comparative map. Six of these were deemed to be major meta-QTL, and map to 1.06, 8.04, 8.05, 9.03, 10.04, and 10.06. Four of these meta-loci overlap closely with the peaks of QTL from the present study that pleiotropically affect TNOL and NLBE (Tables 3 and 4). Of these, the linked *Vegetative-generative transition* QTL in bins 8.05 (*Vgt1*) and 8.04 (*Vgt2*) are best known (Koester et al., 1993; Salvi et al., 2002, 2007; Vladutu et al., 1999).

The 9.07 TNOL/NLBE QTL is coincident with a days till pollen (DTP) QTL detected in the IBM-RILs during an investigation of the relationship between flowering time and southern leaf blight resistance (Balint-Kurti et al., 2007). The TLSI was slightly wider (cM position 591–616) but largely overlaps with the 9.07 support and

Table 5. Two-way ANOVA tests for epistatic interactions between total number of leaves (TNOL) quantitative trait loci (QTL).[†]

	1.06	4.08	8.04	8.05	9.07
4.08	0.1186, 0.180				
8.04	0.4908, -0.084	0.0031, 0.340 [‡]			
8.05	0.2756, -0.140	0.0006, 0.408 [‡]	0.0156, 0.360		
9.07	0.3446, 0.100	0.1900, 0.108	0.0717, -0.216	0.9788, 0.012	
10.04	0.0006, -0.420 [‡]	0.1072, 0.192	0.7605, 0.036	0.0390, 0.272	0.2976, -0.120

[†]For all 15 of the possible pairwise interactions among the six TNOL QTL, a *p* value and genotype × genotype (G×G) interaction effect estimate are reported; $G \times G = (TNOL_{BB} - TNOL_{MM}) - (TNOL_{BM} - TNOL_{MM}) - (TNOL_{MB} - TNOL_{MM})$, where *B* and *M* subscripts indicate homozygosity for B73 and Mo17 alleles at the pair of loci being tested. Since B73 donates the high allele at all six of these QTL, the more generalized equation that specifies high and low alleles was bypassed.

[‡]Indicates statistical significance after Bonferroni correction for multiple tests at the $\alpha = 0.05$ level ($0.05/15 = 0.0033$).

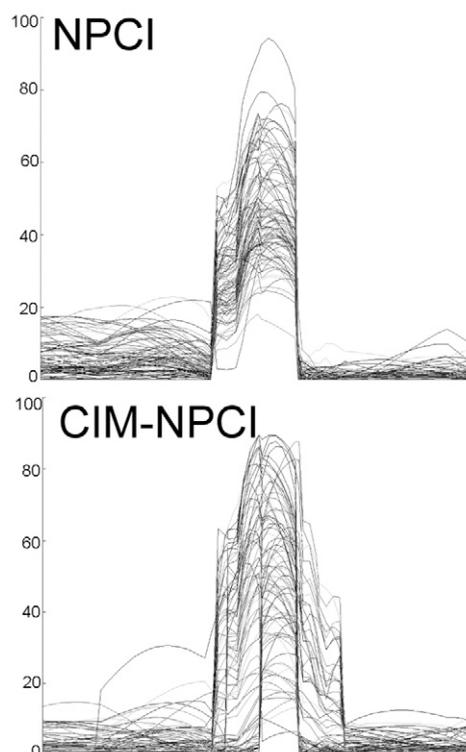


Figure 3. Comparison of two protocols for establishing confidence intervals for quantitative trait loci (QTL) detected using composite interval mapping (CIM). The same 100 bootstrap resamplings of one original intermated B73 × Mo17 recombinant inbred line dataset were analyzed using the nonparametric confidence interval (NPCI) and CIM–NPCI methods. The plots are 25 cM wide, depict a single QTL region, and are typical of the NPCI vs. CIM–NPCI comparison. The QTL likelihood curves produced using the NPCI method are generally taller and narrower than those produced by the CIM–NPCI method, demonstrating the bias arising from predetermination of background markers for CIM.

confidence intervals reported here (Tables 3 and 4, Fig. 2). B73 alleles at the 9.07 locus increase DTP, which is consistent with the effect direction seen for TNOL and NLBE (Tables 3 and 4). Notably, this major effect QTL was not previously detected in flowering time studies in

previous populations derived from crosses between B73 and Mo17, perhaps due to inadequate linkage with the distal-most 9L marker on previous maps (Abler et al., 1991; Beavis et al., 1994; Stuber et al., 1992).

The failure to detect the 4.08 QTL in any of the flowering time studies (Chardon et al., 2004) suggests a potential role in accelerating the rate of leaf initiation. Such a role could explain the synergistic epistatic interactions with loci that delay flowering (Table 5), as rate times duration is a multiplicative function that creates a more-than-additive effect. This hypothesis is further supported by the detection of a QTL at the same position in bin 4.08 that affects the number of juvenile leaves in the IBMRILs but not the proportion of total leaves that are juvenile (N. Lauter and S. Moose, unpublished results).

The 3.06 NLBE/PLBE QTL is functionally distinguished from the other six QTL because it does not affect TNOL, which suggests a role in apical dominance control or response. Doebley and colleagues (Doebley and Stec, 1991, 1993; Doebley et al., 1995) reported a major maize domestication QTL affecting lateral branch outgrowth and lateral inflorescence branching that maps adjacent to *umc60* (centimorgan position 452.7), which is contained within the 3.06 locus described here (Tables 3 and 4, Fig. 2). Ragot et al. (1995) used Central and South American germplasm crossed with Mo17 and B73 to identify a QTL just distal to *umc60* that affected both ear height and number of leaves above the ear. The support intervals for the domestication and ear position QTL encompass ~25% of the recombinational space on chromosome 3 in each case, compared with less than 4% in the present study.

Candidate Gene Hypotheses and Positional Cloning Prospects

The high genetic resolution of the IBMRIL population results in QTL being localized to smaller physical regions than in nonintermated maize populations (Balint-Kurti et al., 2007). This gain is demonstrated again by the precise detection and localization of *Vgt1*, the Gaspé Flint allele of which has been shown to harbor upstream regulatory elements that cause low expression of an *Apetala2*-like gene, *ZmRap2.7* (Salvi et al., 2007). *ZmRap2.7* (EF659467) physically maps between *ufg80* (BH418312) and *ufg74* (BH418266), the markers that immediately flank the LOD peak of the 8.05 QTL (Fig. 2). These markers are only 1.2 Mb apart, yet their genetic interval harbors 15 recombination events among the 268 IBMRILs used, yielding resolution similar to that of the introgression library used to clone the *Vgt1* (Salvi et al., 2002, 2007).

A recent review of positional accuracy for mapping major-effect QTL showed that the original LOD peaks typically map within 0.7 cM of the eventually cloned QTL, with this distance rising to 1.2 cM for moderate-effect QTL (Price, 2006). This bodes well for positional cloning the 4.08 and 9.07 QTL, especially since the IBM2 map averages 0.35 Mb per cM (2500 Mb/7090 cM). In the 9.07 region, more genetic markers are required to exploit

a favorably high recombination rate per megabase. Addition of *idp1419* in the present study resulted in a twofold reduction of TLSI and CIM–NPCI widths for the 9.07 QTL (not shown), but wide flanking marker intervals of 16.5 and 13.5 cM remain compressed in a physical region of just 1.6 Mb (Fig. 2). The average CIM–NPCI for this QTL occupies less than half of the recombinational space between centimorgan positions 603 and 633 (Table 4). A prime candidate gene, *ZmMADS1* (AF112148; Heuer et al., 2001), resides in the genetic and physical center (AC150630) of this narrow QTL, not in bin 1.01 as previously concluded from overgo hybridization results (Gardiner et al., 2004). Across embryonic, vegetative, and reproductive tissues, *ZmMADS1* is coexpressed with *ZmMADS3*, which was shown to decrease leaf number when transgenically overexpressed (Heuer et al., 2001). Moreover, *ZmMADS1* is the closest maize homolog of the *Arabidopsis* gene, *SOC1* (O64645; Becker and Theißen, 2003; Tadege et al., 2003), whose expression in the facultative long-day pathway is known to promote flowering in (Borner et al., 2000; Lee et al., 2000; Samach et al., 2000; Yoo et al., 2005; Yu et al., 2002). Addition of molecular markers surrounding *ZmMADS1* will likely be sufficient to positionally test this candidate hypothesis. Alternatively, an association mapping approach could be taken (Yu and Buckler, 2006).

The CIM–NPCI of the 4.08 QTL is contained within a 0.9-Mb region between *umc2384* and *bnlg2162*, the markers at centimorgan positions 467.1 and 475.7 (Table 4). This region has strong microsynteny with the short arm tips of rice chromosomes 11 and 12 (Odland et al., 2006). These co-orthologous rice regions contain ~60 genes with conserved order (Ouyang et al., 2007), and each includes a *GAI1*-like DELLA domain-containing protein (Q2RB59 = Os11 g03110, ABA95687 = Os12 g02870). *GAI1* orthologs in wheat (*Triticum aestivum* L.) and maize are known to affect leaf number (Peng et al., 1999). Thus, the maize *GAI1*-like gene (Q2RB59) on a BAC (AC215507) mapping directly under the LOD peak of the 4.08 QTL is a reasonable candidate. Molecular marker saturation together with progeny testing of IBMRILs with informative recombination events offer a clear path to positionally testing this hypothesis.

The average TLSI for the 1.06 TNOL/NLBE QTL covers <5 Mb of physically mapped BACs but includes an intervening gap of unknown size (Tables 3 and 4, Fig. 2). A 1.3-Mb contiguous region spanning the entire Joint CIM TLSI (centimorgan positions 532.8 to 544.2) for this QTL is microsyntenous with a portion of rice chromosome 8 that contains 54 genes (Odland et al., 2006). One of these encodes a SQUAMOSA PROMOTER BINDING-LIKE (SPL) protein similar to SPL3, which is known to affect flowering time in *Arabidopsis* (Cardon et al., 1997; Gandikota et al., 2007; Wu and Poethig, 2006). This maize *SPL* gene (AJ011618) resides on a BAC (AC205546) between markers at centimorgan positions 535.1 and 541.3 on the IBM2 map. Positional testing of this hypothesis would best be accomplished by analysis

of near-isogenic lines, which would make the mild effect magnitude and apparent complexity of this locus more tractable (Salvi and Tuberosa, 2005).

Due to wider TLSIs, lower per megabase recombination rates, and gaps in the physical map, no candidate gene hypotheses were made for the 8.04 and 10.04 QTL. The ~15 cM TLSI for the 10.04 QTL spans 16 Mb across five BAC contigs. The ~33 cM TLSI for the 8.04 QTL spans 8 Mb across five BAC contigs.

While the 3.06 locus does not localize to a narrow interval or single defined contig, we echo the Gallavotti et al. (2004) hypothesis that *barren stalk1* (*bal*) is a good candidate for the well-known apical dominance effects mapped to bin 3.06. Pursuant to functional and positional QTL correspondence, we placed *bal* (AY683001) on BAC (AC195348), which resides in a region of the physical map that corresponds to centimorgan 463, which is squarely beneath the PLBE LOD peak in several cases (Tables 3 and 4, Fig. 2). Thus, we consider *bal* a viable candidate gene to explain the majority of the natural variation in apical dominance phenotypes observed among the IBMRILs.

Conclusions

Quantitative trait locus dissection of complex traits in an intermated RIL population has sufficient genetic resolution to make positional cloning of major and moderate effect QTL a routine next step. Dramatically higher resolution also makes inference of function based on apparent pleiotropic action much more reasonable, improving prospects for more routine use of QTL mapping as a tool for functional characterization of natural allelic variation. The resolution per line in the IBMRILs makes this population ideal for experiments where phenotyping is expensive, such as quantitative genetic analysis of transcriptomic or metabolomic datasets. As use of high resolution public populations supported by physical maps and genome sequences becomes widespread, database utilization will become increasingly important for maximization of dataset utility (Jaiswal et al., 2006; Lawrence et al., 2007; Wan and Pavlidis, 2007). To this end, standardization of methods must occur. As a means, we have reinitiated development and testing of method-appropriate nonparametric methods that affect the central practices of QTL reporting for composite interval mapping.

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