Natural Variation at sympathy for the ligule Controls Penetration of the Semidominant Liguleless narrow-R Mutation in Zea mays

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ABSTRACT Leaf architecture determines plant structural integrity, light harvesting, and economic considerations such as plant density. Ligules, junctions at the leaf sheath and blade in grasses, protect stalks from environmental stresses and, in conjunction with auricles, controls leaf angle. Previous studies in mutants have revealed recessive liguleless mutants (lg1 and lg2) and dominant mutations in knotted1-like homeobox genes (Lg3-O, Lg4, and Kn1) involved in ligule development. Recently, a new semidominant liguleless mutant, Liguleless narrow (Lgn-R), has been characterized in maize that affects ligule and auricle development and results in a narrow leaf phenotype. We show that quantitative genetic variation affects penetration of Lgn-R. To examine the genetic architecture underlying Lgn-R expressivity, crosses between Lgn-R/+ mutants in a B73 background and inbred B73 × Mo17 recombinant inbred lines were evaluated in multiple years and locations. A single main-effect quantitative trait locus (QTL) on chromosome 1 (sympathy for the ligule; sol) was discovered with a Mo17-contributed allele that suppressed Lgn-R mutant phenotypes. This QTL has a genetic interaction with a locus on chromosome 7 (lucifer; lcf) for which the B73-contributed allele increases the ability of the sol×lcf allele to suppress Lgn-R. Neither of the genetic intervals likely to contain sol or lcf overlap with any current liguleless genes nor with previously identified genome-wide association QTL connected to leaf architecture. Analysis of phenotypes across environments further identified a genotype by environment interaction determining the strength of the sol×lcf interaction.

The ligule is an important architectural feature of grass leaves that protects the stalk from disease and environmental insults. In maize, the ligule and auricles allow the leaf blade to lean away from the sheath, which remains wrapped around the stalk (Foster and Timmermans 1949). Study of liguleless mutants has identified genes with roles in meristem maintenance, leaf polarity, and leaf angle (Moreno et al. 1997; Walsh et al. 1998). The latter is a trait of critical importance to plant density and maize agriculture (Duvick 2005). In maize and other grass species, mutant studies, and quantitative trait loci (QTL) mapping have identified the genomic locations of loci affecting leaf architecture (Mickelson et al. 2002; Tian et al. 2011; Yu et al. 2012; Zeng et al. 2009).

Classical and contemporary mutant studies have identified genes required for ligule development: lg1 is located on the short arm of chromosome 2; and lg2 is located on the long arm of chromosome 3 (Beckett 1975). Maize homozygous for recessive loss-of-function alleles at liguleless1 or liguleless2 lack a ligule and auricles (Becraft and Freeling 1991; Harper and Freeling 1996; Moreno et al. 1997), resulting in more erect leaves. lg1 encodes a protein similar to SQUAMOSA PROMOTER-BINDING (Moreno et al. 1997) and lg2 encodes a basic leucine zipper protein transcription factor (Walsh et al. 1998). In addition to these, lg3, located on 3S (Kerstetter et al. 1994), and lg4, located on 8L (Fowler and Freeling 1991), were identified from dominant mutations that altered ligule formation. The semidominant Liguleless3 alleles cause the leaf blade, ligule, and auricle tissues to adopt a sheath-like phenotype (Fowler and Freeling 1996). The Liguleless4 dominant mutants, much like Liguleless3, show no auricle or ligule tissue, with blade tissue appearing to be sheath-like; however, the tissue disruption occurs near the leaf margins (Fowler and Freeling 1998).
interaction between were analyzed for shared regions of homozygosity, which, along with of fertile, successfully suppressed IBM RIL x 2298 in increased fertility, leaf length, and leaf width. The RIL genotypes suppresses mutant phenotype expression in lucifer (lcf) on the long arm of chromosome 1, called Lgn-R. Plant growth and architecture with environmental determinants of development. We propose that this genetic network helps to integrate genotypes are called in unless a recombination event is predicted to occur. R/qtl package manual, Broman et al. 2010). One-dimensional genome scans were performed using scanone by the Expectation Maximization method. Scanone, using method Expectation Maximization, assumes a single QTL model and follows standard interval mapping (R/qtl package manual; Broman et al. 2010). Experiment-wide permutation thresholds (alpha < 0.05) were determined separately for each trait with 1000 permutations. Two-dimensional genome scan and tests for epistasis were performed by marker-pair regression analysis using the scan2two function (Broman et al. 2003). Scan2two tests a two-QTL model and result summaries provide multiple assessments of interaction. Scan2two results were summarized using the “int” flag for interaction summary and by the “best” method to look for additional QTL by two dimensional scanning. Experiment-wide significance for the two dimensional scans (alpha < 0.05) were determined for each trait and procedure (“int” and “best”)
with 1000 permutations. The complete mapping procedure, scripts, primary data, and PCA outputs are all available as a supplement to this manuscript or from the authors by request (Supporting Information, File S1).

A Standard Least Squares model implemented in the JMP statistical software package (version 8.0.1; SAS Institute Inc. 2009) examined the genotype by environment interactions for data from 2009 from both locations. The genotypes of both sol, estimated with marker umc2145, and lcf, estimated with marker nbp1, were compared with the leaf area traits for each location and across both locations.

Validation of sol and lcf effect
At the Gill Tract farm in 2011, a subset of IBM RIL x Lgn-R/+ F1 plants (IBM18, IBM30, IBM69, and IBM72) as well as BC1 IBM RIL x Lgn-R (IBM RIL x Lgn-R F1 backcrossed to B73) families segregating for the B73 and Mo17 alleles at sol were measured for leaf length and width of the 6th leaf from the tassel as well as plant height. A total of 187 individuals were measured. DNA was isolated from leaf samples for each individual and genotyped using the polymerase chain reaction markers umc2145 at chromosome one 94 cM for sol and bnlg1792 at chromosome seven 66 cM for lcf. Individuals were scored as either “B73,” “Mo17,” or heterozygous (B73/Mo17) for each marker. Because the RILs that effectively rescued Lgn-R plants were fixed for lcf B73 and the recurrent parent was B73, these BC1 plants were not segregating for lcf. To examine the effect of sol on mutant and wild-type leaf development, a comparison of leaf length and width data as well as plant height and the sol genotype was estimated by a Standard Least Squares model using the JMP software package (version 8.0.1, SAS Institute Inc. 2009).

RESULTS
The dominant Lgn-R mutation results in narrower and shorter leaves, reduced ligule, fewer leaves, failure of ear development, and reduced tassel branches compared with recessive wild-type alleles (Figure 1). Introggression of Lgn-R into the Mo17 background partially suppresses the mutant phenotype (Table 1). These observations suggest the presence of genetic modifiers of Lgn-R that are polymorphic between Mo17 and B73. To identify and localize modifying loci, we used the intercrossed RIL population developed from a B73 x Mo17 cross (referred to as “rescuing” RIL x Lgn-R F1 lines) and the Mo17 parent to identify QTL for leaf morphology traits from both the IN and CA 2009 data.

Table 1 “Rescuing” RIL x Lgn-R F1 lines and the Mo17 parent leaf morphology traits from both the IN and CA 2009 data

| Lgn-R F1 Genotypea | Locationb | Lengthc | Width | Leaf Area | LbW |
|-------------------|-----------|---------|-------|-----------|-----|
| RIL x Lgn-R+/+ (IBM4) | IN1 | 54.00d | 5.17 | 279.25 | 10.44 |
| RIL x Lgn-R+/+ (IBM25) | IN1 | 56.14 | 3.97 | 222.96 | 14.14 |
| RIL x Lgn-R+/+ (IBM47) | IN1 | 55.63 | 4.09 | 227.37 | 13.61 |
| RIL x Lgn-R+/+ (IBM55) | IN1 | 54.43 | 3.77 | 205.27 | 14.43 |
| RIL x Lgn-R+/+ (IBM65) | IN1 | 48.88 | 3.59 | 175.33 | 13.62 |
| wild-type Mo17 | IN1 | 91.40 | 11.30 | 1032.82 | 8.09 |
| wild-type Mo17 | IN2 | 87.40 | 11.22 | 980.63 | 7.79 |
| wild-type Mo17 | CA | 97.60 | 12.32 | 1202.43 | 7.92 |

Figure 1 The effect of the Mo17 background on the Lgn-R+/+ phenotype. (A) Ligular region of wild-type (left) and Lgn-R+/+ (right) in B73 grown in Albany, CA. (B) Ligular region of wild type (left) and Lgn-R+/+ (right) in Mo17 in West Lafayette, IN. (C) Whole plants grown in West Lafayette, IN. Lgn-R+/+ in B73 (left) is compared to rescued Lgn-R+/+ F1s (center and right) generated by crosses to IBM lines. Scale bars: 10 cm.

1. LIL, recombinant inbred line; QTL, quantitative trait loci.
2. RIL x Lgn-R F1 genotypes were identified as partially rescued phenotypes by the 2009 IBM RIL QTL mapping data.
3. Three locations are listed, two measurements made in West Lafayette, IN, and one made in Gill Tract, CA.
4. All phenotype measurements were made on the 6th leaf from the tassel. Length and width measurements are the mean value for four to eight individuals and leaf area (length x width) and LbW (length by width) measurements were calculated using length and width measurements.
5. All measurements are in cm units.
Crosses were made using Lgn-R pollen in the B73 background onto IBM lines to give rise to IBM RIL x Lgn-R/+ F1s. Stand counts, leaf number, leaf length, and leaf width measurements were made on the 6th leaf from the tassel of each F1 individual grown in 2 locations: Gill Tract, CA, and West Lafayette, IN, in the summer of 2009.

Leaf length and width measurements were highly correlated across environments within and across locations (Figure 2). Correlation of length and width traits for location GT (CA) had an r = 0.7397 (Figure 2A). Both length and width measurement correlations in location IN and location GT were statistically significant at P < 0.001 (data not shown). We calculated two other traits by using the leaf length and width measurements: leaf area (length × width) and a length width ratio (length/width). The calculated leaf area for each individual was also highly correlated across environments (r = 0.6839 and P < 0.001) as well as within location IN (r = 0.9346 and P < 0.001) (Figure 2B). The correlation of leaf measurements allowed a PCA to generate single values that better represented the genetic effect on leaf development of each IBM RIL. Thus, use of the PCA should increase both the sensitivity and accuracy of QTL mapping.

To create values for the multiple traits measured, PCA of covariances defined a single trait for length and width measurements across both locations (LW_all) as well as locations separately (LW_IN and LW_GT); and finally, leaf area across all locations (LA_all) and leaf area in IN only (LA_IN). Principal component values for LW_all, LW_IN, LW_GT, LA_all, and LA_IN explained much of the variation in the measured traits (87.06%, 94.08%, 99.01%, 83.83%, and 96.74%, respectively).

QTL mapping

One-dimensional genome scans identified a single QTL, sympathy for the ligule (sol), on the short arm of chromosome 1 (Table 2). Multiple traits shared this QTL, and it was detected with: count, location IN2 (count_IN2); leaf area, location CA (LA_GT); leaf area, location IN (LA_IN); location IN (length and width PCA value; LW_IN); and length and width for all locations (LW_all) (Figure 3 and Table 2).

Localization of sol by a two LOD drop-off for the QTL affecting LW_all mapped sol to likely be between 92.9 and 121.6 cM on chromosome 1 in the IBM ISU v4 map. A 95% Bayesian credible interval was substantially less specific, estimating sol to be between 5.6 cM and 133.9 cM on chromosome 1.

Validation of extreme rescue phenotypes in IBM x Lgn-R detects the lcf locus

In 2010, a subset of the IBM RIL x Lgn-R F1 crosses (IBM18, IBM30, IBM69, and IBM72), which had previously exhibited suppression of the mutant phenotype, was grown at the Purdue Agronomy Center for Research and Education (Table 3 and Figure S1). This subset of IBM RILs rescued much of the Lgn-R phenotype, displaying leaf length and width measurements approaching wild-type siblings (Table 3 and Figure S1). In 2011, the same subset of IBM RILs was grown as IBM RIL x Lgn-R/+ F1 and (IBM RIL x Lgn-R) x B73 F1 (a B73 recurrent parent BC1 of the IBM RIL carrying Lgn-R) plants were grown at the Gill Tract farm in Albany, CA. Phenotypic rescue was again observed. Thus, over 3 years and in all locations, these RIL were able to rescue the Lgn-R mutant phenotype (data not shown). On close examination of the genotypes among these “rescuing” IBM RILs (Table 3 and Figure S1) as well as those RILs identified in Table 1 (IBM4, IBM25, IBM47, IBM55, and IBM65), a region on chromosome 1 between 97.5 cM and 99.7 cM was invariantly Mo17 in these RILs (Figure 4 and Table S2). This region is a subset of the previously identified sol interval, which mapped to 94 and 135.3 cM. In addition, a region of chromosome 7 between 65.7 cM and 69 cM was invariantly B73 in the rescuing RILs (Figure 4 and Table S2).

We tested for the effect of the region on chromosome 7 on suppression of Lgn-R expression while controlling for the effect of segregation at sol. Phenotypic data from the full 63 RIL mapping population (Figure 4 and Table S2) were analyzed in pairwise marker regression tests using the marker umc2145 as a proxy for sol and each marker in the chromosome 7 segment (Figure 4 and Table S2). Each regression included the umc2145 genotype, the genotype of a marker from chromosome 7, and the pairwise interaction.
between these two markers. The inclusion of a marker from the chromosome 7 region increased the fit of the model to the data and returned significant \( \text{sol} \times \text{lcf} \) interaction terms indicating an epistatic relationship between the \( \text{sol} \) and \( \text{lcf} \) variants. Thus, this region contains a second QTL interacting with \( \text{sol} \), which we name \( \text{lucifer} \) (\( \text{lcf} \)).

Of all the markers tested within the invariantly B73 region of the rescuing RILs, the marker npb1 had the strongest interaction with \( \text{sol} \). Figure 5 shows the effect of genotype at \( \text{sol} \) (marker umc2145) and \( \text{lcf} \) (marker npb1) on the first principal component of leaf measurements. The \( \text{sol}^{\text{Mo17}} \) allele partially suppresses the effect of \( \text{Lgn-R} \) (Figure 5) and occurs as an invariant region within the rescuing RIL (Figure 4 and Table S2). Consistent with the occurrence of a B73 segment from chromosome 7 in the rescuing RIL (Figure 4), the \( \text{lcf}^{\text{B73}} \) allele enhances rescue of \( \text{Lgn-R} \) by \( \text{sol}^{\text{Mo17}} \) (Figure 5). For all phenotypic traits examined, except for stand count data, \( \text{lcf} \) (marker npb1) modifies \( \text{sol} \) (marker umc2145) such that when \( \text{sol}^{\text{Mo17}} \) and \( \text{lcf}^{\text{B73}} \) are found together, the \( \text{Lgn-R} \) phenotype is substantially suppressed (Figure 5 and Figure S2).

### Table 2 Interval mapping QTL summary for each trait

| Trait   | LOD score | Marker   | Chr | Position | CI.low | CI.high | 5% LOD |
|---------|-----------|----------|-----|----------|--------|---------|---------|
| LA_GT   | 4.1*      | umc2145  | 1   | 94       | 91.7   | 99.4    | 3.66    |
| LW_GT   | –         | –        | –   | –        | –      | –       | 3.64    |
| Count GT| –         | –        | –   | –        | –      | –       | 4.01    |
| Count IN1| –       | –        | –   | –        | –      | –       | 4.04    |
| Count IN2| 4.242*  | bnl5.59a | 1   | 133.5    | 97.5   | 135     | 4.11    |
| LA_IN   | 6.02*     | IDP1423  | 1   | 27.1     | 109    | 128     | 4.27    |
| LW_IN   | 4.726*    | umc2229  | 1   | 109.6    | 93.4   | 111     | 4.08    |
| LW_all  | 4.826*    | umc2229  | 1   | 109.6    | 93.4   | 111     | 4.08    |
| LA_all  | –         | –        | –   | –        | –      | –       | –       |

QTL, quantitative trait loci; LOD, xxx.

- The LOD score is indicated if a QTL was detected for that trait and noted with a * if the LOD score is above the experiment-wide permutation threshold at \( \alpha = 0.05 \).
- Markers, chr (chromosome) and position are from the Illinois State University IBM map (version 4, http://www.maizegdb.org).
- Confidence intervals (CI.low and CI.high) are indicated for each QTL, with lower CI indicated as CI.low and the higher CI indicated as CI.high.
- The experiment-wide permutation threshold at \( \alpha = 0.05 \) for each QTL, as calculated using R/qtl (see the section Materials and Methods).

* Denotes statistical significance at \( \alpha = 0.05 \).

Figure 3: Interval mapping QTL results for leaf morphology traits. All figures show the LOD score on the x-axis and map position for chromosome 1 only on the y-axis. An experiment-wide permutation threshold was calculated for each trait and the \( \alpha = 0.05 \) threshold is represented by the line on each figure. (A) Count, location IN2 trait. (B) Leaf Area, location CA trait. (C) Leaf area, location IN trait. (D) Location IN trait. (E) Length and width for all locations trait.
Table 3 Mean values of leaf morphology traits for “rescuing IBM” RIL x Lgn-R F1 deemed “rescued lines” as well as parent lines measured in West Lafayette, IN (2010)

| Lgn-R F1 Genotype* | Lengthb | Width  | Leaf Area | LbW  |
|--------------------|----------|--------|-----------|------|
| RIL x Lgn-R/+ (IBM18) | 43.47    | 4.19   | 192.45    | 12.07|
| RIL x Lgn-R/+ (IBM30) | 41.90    | 3.86   | 198.00    | 12.43|
| RIL x Lgn-R/+ (IBM69) | 39.53    | 3.91   | 168.87    | 11.67|
| RIL x Lgn-R/+ (IBM72) | 42.63    | 3.59   | 166.65    | 14.18|
| B73 (wild type)     | 77.0     | 8.92   | 687.50    | 8.65 |
| Mo17 (wild type)    | 65.83    | 8.33   | 550.42    | 7.95 |

*RIL, recombinant inbred line.
*a RIL x Lgn-R/+ F1 genotypes were identified as rescued phenotypes in the 2010 West Lafayette, IN, growing season.
b Length, width, leaf area, and length by width (LbW) were phenotypic measurements made on the 6th leaf from the tassel. Leaf area (length x width) and LbW (length by width) measurements were calculated using length and width measurements.

Two-dimensional genome-wide scan for genetic interactions

The detection of a single pairwise interaction and positive transgressive epistasis demonstrated that we missed QTL due to epistasis in our single-dimension QTL scan. The `scantwo` function (R/qtl, method marker regression) was used to examine all pairwise combinations of markers by regression (Broman et al. 2003). Experiment-wide permutation thresholds (1000 permutations, threshold of alpha < 0.05) were calculated from the `scantwo` results for each phenotype. To simplify the output, summaries display only the best QTL-pair for each chromosome pair. Putative interactions were detected two ways. First, the data were summarized to display differences between the full model (two QTL model with interactions) and reduced models (e.g., a two QTL model with no interaction; Broman et al. 2006). The second method maximized the interaction LOD for a pair of positions on each chromosome, thus returning the pair with the greatest synergistic effect (Broman et al. 2006). LODs were calculated for each summary type for each trait. Significant interactions are summarized in Table 4. Almost all interactions above their respective experiment-wise permutation thresholds included the sol region on chromosome 1. This finding is not surprising, given the dramatic impact of sol and the observation that it was the only main effect QTL identified, thereby returning greater LOD scores for models that include this QTL.

Genome-wide multiple testing correction via permutation was used and significant interaction was detected between chromosome 1 at the sol QTL and chromosome 7 at lcf (Table 4), confirming our observation within the suppressing RIL. lcf was detected using the PCA value for length and width using either CA or the PCA from all locations (IN and CA). Additional two-QTL models were significant at unlinked locations on chromosome 1 (Table 4) affecting PCA value for length and width (IN only) and PCA value for leaf area (CA and IN). Lastly, a two-QTL model identified loci on chromosome 2 and chromosome 5 affecting the stand counts for location IN_1 (Table 4). A paralog of lgn, sister of liguleless narrow (sbn; GRMZM2G009506), is also located on chromosome 5 but at an unlinked position at the opposite end of the chromosome, excluding the possibility that sbn encodes this QTL.

Confirmation of sol effects on leaf development in segregating material

In the 2011 field season at Gill Tract Farms (Albany, CA), an evaluation of sol and lcf effects on leaf and plant morphology was performed in segregating families. Four IBM lines that provided rescue of Lgn-R in the aforementioned experiments (IBM18, IBM30, IBM69, and IBM72;
effect the genotype at lgn was also included, as these families are segregating 1:1 for Lgn-R/+ and wild-type lgn homozygotes. All three primary parameter estimates as well as the interaction of sol with Lgn-R were statistically significant (Table 5). The interaction between the sol genotype and mutant phenotype indicate that sol impacts phenotype within the IBM x Lgn-R/+ individuals to a greater degree than in lgn wild-type siblings.

**Genotype x environment interaction**

Genotype by environment (GxE) interactions were estimated in the 63 IBM RIL mapping crosses using a Standard Least Squares model. We determined the effects of sol and lcf and their interaction (using markers umc2145 and nbp1, respectively) while also considering the growth environments as factors. Across all environments (CA and IN), we determined GxE and (GxG) x E interaction (Table 6). The interactions between sol and lcf ($P < 0.0001$) as well as sol x lcf x environment ($P < 0.0003$) were both statistically significant (Table 6). If we examine the leaf area phenotype within each location, sol was significant in both environments ($P = 0.0223$, CA and IN both needed) but the interaction between sol and lcf was only detected in IN ($P < 0.001$; Table 7). It appears that some aspect of the environment or cultural practice that differs between the IN and CA field sites influences the expression of Lgn-R phenotypes and with it lcf and the interaction effect of lcf and sol.

**Leaf morphology QTL comparison**

Tian and Co-Workers (2011) previously carried out a genome-wide QTL mapping experiment for leaf morphology using the nested-association panel, which includes a Mo17-B73 contrast. We compared our sol QTL region (94 cM to 133.5 cM on the IBM ISU v4 map; Table S1) with the Tian et al. (2011) genome-wide association study single-nucleotide polymorphism (SNP)/Indel collection. For clarity, both the Tian et al. (2011) SNP positions within the sol QTL and IBM ISU v4 map positions defining the sol locus are provided as maize assembly AGPv1 coordinates in Table 8 (B73 v1 map; http://www.maizegdb.org; Schnable et al. 2009). All SNPs with significant associations to leaf traits in Tian et al. (2011) within the broad sol interval on chromosome 1, 54,036,789–183,652,422 bp (94–133.5 cM; Table S1), are listed in Table 8. The narrowed invariant Mo17 region within sol identified by the rescuing RILs (Table 3, Figure 4, and Table S2) from 97.5 cM and 99.7 cM was converted to 62,995,272 bp to 66,027,760 bp on chromosome 1 in the AGPv1 build. Not all SNPs could be mapped to AGPv2; those SNPs that were unambiguously mapped to locations in the AGPv2 maize assembly (http://www.maizesequence.org) also are presented in Table 8. In total, four joint-linkage analysis QTL, 21 significant GWA SNPs, and 1 indel fell within the Bayesian credible interval for sol for leaf angle, leaf length, or leaf width. The narrowed QTL region did not include any QTL or SNP/indels from the work of Tian and Co-Workers (2011) genome-wide association study (2011) for leaf morphology traits overlapped with sol. Likewise, of the leaf morphology QTL identified in the IBM population by Mickelson et al. (2002), none were linked to sol. Thus, by using natural variation to search for mutant suppressors and genetic interaction, we have detected the gene, sol, for leaf morphology. In addition to detecting sol, we have also detected an interacting locus, lcf, opening up the possibility of constructing a genetic pathway including

**Table 4** A summary of the maximum interaction LOD scores above an experiment-wide permutation threshold for a two-QTL model (scantwo, R/qt)

| Interactiona | pos1b | pos2c | lod.fulld | lod.fv1e | lod.intf |
|--------------|-------|-------|-----------|---------|---------|
| Count data, IN1 location | c2:c5 | 63.8 | 83.2 | 9.86* | 7.167 | 7.46* |
| PCA value for length and width, IN only | c1:c1 | 119 | 134 | 10.82* | 5.11 | 5.95* |
| PCA value for length and width, GT only | c1:c4 | 122 | 80.9 | 8.38* | 4.87 | 6.11* |
| PCA value for length and width, IN only | c1:c7 | 122 | 81.4 | 8.38* | 4.87 | 6.11* |
| PCA value for length and width, IN only | c1:c3 | 93.9 | 189.3 | 10.6* | 5.633 | 6.29* |
| PCA value for length and width, IN only | c1:c7 | 93.4 | 70.3 | 11.3* | 6.138 | 8.23* |
| PCA value for leaf area, all locations | c1:c1 | 63.3 | 110 | 10.3* | 6.53 | 6.53* |

GT, xxx; LOD, xxx; QTL, quantitative trait loci; PCA, principal component analysis.

a Shows the two chromosomes with positions that interact in a two-QTL model (scantwo, R/qt).

b Indicates the genome position on the first chromosome listed in the “interaction” column.

c Indicates the genome position on the second chromosome listed in the “interaction” column.

d Lod full represents values from the full model with QTL (mu+pos1+pos2+(1x2)+error).

e Lod.fv1 represents a comparison of the full model with QTL on chromosome j and k (assume only 1 QTL on each chromosome).

f Lod.int compares the full model with QTL on chromosome j and k and indicated interaction between the QTL.

* Denotes statistically significant at the experiment-wide permutation threshold at $a = 0.05$. One thousand permutations were conducted for each phenotype using scantwo (two-QTL model, R/qt).
Table 5 Parameter estimates for B73, IBM x Lgn-R F1 and IBM x Lgn-R BC1 individuals using the Standard Least Squares model

| Model Parameters | Leaf Width* | Leaf Length* | Plant Height* |
|------------------|-------------|-------------|--------------|
|                  | DFb | SS    | F Ratio | P Value | SS    | F Ratio | P Value | SS    | F Ratio | P Value |
| Background       | 7   | 217.76 | 19.18   | <0.001*** | 5624.89 | 12.62   | <0.001*** | 16933.45 | 27.2039 | <0.001*** |
| sol             | 1   | 39.26  | 24.21   | <0.001*** | 553.381 | 8.69    | 0.0036**  | 14577.19 | 16.39    | <0.001*** |
| Mutant           | 1   | 747.73 | 461.06  | <0.001*** | 16772.33 | 263.32  | <0.001*** | 92337.60 | 103.84   | <0.001*** |
| Mutant x sol     | 1   | 54.17  | 33.40   | <0.001*** | 917.84  | 14.41   | 0.0002**  | 5003.19  | 5.63     | 0.0188**  |
| Whole model      | 10  | 1498.64 | 92.41  | <0.001*** | 29971.55 | 47.05   | <0.001*** | 480587.93 | 54.05    | <0.001*** |
| Error            | 172 | 278.94 | 10.9957 |           | 40927.26 |         |           | 153836.84 |          |           |
| Total            | 182 | 1777.58 |         |           | 634424.78 |         |           |          |          |           |

DF, degrees of freedom; REML, restricted maximum likelihood; SS, xxx.

a Leaf length and width, as well as and plant height measurements, were made in 2011 (Gill Tract Farm; Albany, CA) for IBM x Lgn-R/+ individuals.
b DF calculated from a REML model. DFs are the same for each phenotype and are thus listed once.
c Different backgrounds were defined in 2011 data: B73, B73/Mo17, and IBM x Lgn-R/+ F1 individuals.
d sol is defined as the genotype of IBM x Lgn-R/+ individuals using marker umc2145. Genotypes are defined as B73, Mo17 or heterozygous B73/Mo17.
e BC1 IBM x Lgn-R/+ individuals were observed to be mutant (Lgn-R-like) or wild type (IBM sibling-like).
** Denotes statistical significance at α = 0.01.
*** Denotes statistical significance at α = 0.001.

Table 6 Examination of main effects of sol, lcf, and location as well as genotype x environment (GxE) interactions calculated for leaf area across locations (IN and CA) using the 2009 dataset

| Model Parameters | DFa | Leaf Area, CA and IN |
|------------------|-----|----------------------|
|                  |     | SS      | F Ratio | P Value |
| lcf (nbp1)       | 1   | 34059.67 | 19.6343 | <0.0001*** |
| sol (umc2145)    | 1   | 127130.14 | 73.2865 | <0.0001*** |
| lcf x sol        | 1   | 60314.35 | 34.9521 | <0.0001*** |
| Location         | 2   | 26093.90 | 75.2043 | <0.0001*** |
| lcf x location   | 2   | 17149.37 | 4.9430  | 0.0087**  |
| sol x location   | 2   | 66084.31 | 19.0478 | <0.0001*** |
| lcf x sol location| 2   | 30825.07 | 8.8848  | 0.0003*** |
| Whole model      | 11  | 439921.61 | 23.05   | <0.0001*** |
| Error            | 117 | 202960.01 |         |          |
| Total            | 128 | 642881.63 |         |          |

a DF is the degrees of freedom for each parameter in the model. The full model and error DFs are included.
b x indicates interaction between two or more parameters within the model.
- Genotype of sol and lcf were used for GxE; IN and CA define the location parameter. Main effects and interactions were calculated using a Standard Least Squares model (JMP, version 8.0.1, SAS Institute Inc., 2009).

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Table 8 QTL identified for leaf architecture located within the Bayesian credible interval for the Lgn QTL

| QTL Type | SNP/Indel | AGPv1 Position, bp<sup>a</sup> | B73v2 Position<sup>b</sup> | Chr<sup>c</sup> | Pos, cM<sup>d</sup> | Allele<sup>e</sup> | P Value<sup>f</sup> | Effect<sup>g</sup> | Polymorphic between B73 and Mo17<sup>h</sup> |
|----------|-----------|-------------------------------|---------------------------|-----------|----------------|----------------|----------------|----------------|-------------------|
| Leaf angle<sup>i</sup> | m82 | PZE01114045567 | 114045567 | 115217353 | 1 | 89.088 | A/G | 1.30E−11 | 0.59 | A/G (yes) |
| m82 | PZE0118507354 | 118507354 | na | 1 | 89.279 | G/− | 4.00E−13 | 0.70 | G/− (?) |
| m82 | PZE01148006384 | 148006384 | na | 1 | 89.595 | T/C | 2.10E−11 | 0.90 | T/C (yes) |
| m82 | PZE01173899899 | 173899899 | 174030908 | 1 | 94.380 | G/A | 3.00E−17 | 0.69 | G/A (yes) |
| Leaf length | m52 | PZE0151533532 | 51533532 | 51403200 | 1 | 66.023 | G/T | 5.40E−13 | 0.56 | G/T (yes) |
| m52 | PZE0151575729 | 51575729 | na | 1 | 66.074 | C/T | 1.90E−12 | 0.56 | C/C (no) |
| m52 | PZE0152025282 | 52025282 | na | 1 | 66.624 | G/C | 4.50E−11 | 0.51 | G/− (?) |
| m69 | PZE0182570732 | 82570732 | 83787495 | 1 | 82.402 | T/G | 1.40E−19 | 0.57 | T/G (yes) |
| m69 | PZE0182570902 | 82570902 | na | 1 | 82.402 | −/ACGT | 3.40E−15 | 0.53 | −/ (?) |
| m69 | PZE0192531865 | 92531865 | na | 1 | 85.471 | T/C | 1.40E−24 | 6.69 | T/T (no) |
| na<sup>h</sup> | PZE01101787357 | 101787357 | na | 1 | 87.956 | C/T | 5.00E−08 | 6.25 | C/C (no) |
| na | PZE01102256886 | 102256886 | na | 1 | 88.027 | −/A | 9.00E−08 | 0.76 | −/ (?) |
| na | PZE01136166765 | 136166765 | na | 1 | 89.474 | T/C | 1.10E−06 | 7.58 | T/C (yes) |
| Leaf width | m56 | PZE0152655271 | 52655271 | na | 1 | 67.395 | G/C | 1.90E−14 | 1.29 | G/N (?) |
| m56 | PZE015309826 | 5309826 | 53422803 | 1 | 68.440 | G/A | 6.60E−08 | 0.59 | G/G (v1) OR G/A (v2) |
| m56 | PZE0159424192 | 59424192 | 59358936 | 1 | 71.602 | C/G | 4.90E−09 | 0.95 | C/C (no) |
| na | PZE01109523099 | 109523099 | na | 1 | 88.813 | T/C | 9.10E−08 | 0.96 | T/N (?) |
| na | PZE01135358038 | 135358038 | na | 1 | 89.509 | A/C | 6.10E−12 | 1.05 | N/N (?) |
| na | PZE01146105232 | 146105232 | 147197048 | 1 | 89.575 | C/A | 2.20E−08 | 1.25 | C/A (yes) |
| na | PZE01148471044 | 148471044 | 148404129 | 1 | 89.599 | T/C | 8.10E−10 | 1.07 | T/C (yes) |
| na | PZE01150965828 | 150965828 | 150898913 | 1 | 90.012 | G/C | 3.70E−13 | 1.72 | G/G (no) |
| na | PZE01153739401 | 153739401 | 153696642 | 1 | 90.393 | A/G | 8.10E−17 | 1.95 | A/A (no) |

QTL, quantitative trait loci; SNP, single-nucleotide polymorphism.

<sup>a</sup> QTL in which the SNP or indel falls within the supportive interval based on the eQTL position, as defined by Tian et al. (2011).

<sup>b</sup> The AGPv1 position, allele, P value, and effect are all taken from Tian et al. (2011).

<sup>c</sup> Each SNP or indel listed falls within the Bayesian credible interval for AGPv1 map.

<sup>d</sup> The SNP location is also listed from the B73 v2 position from http://www.maizegdb.org.

<sup>e</sup> B73 and Mo17 genotypes at indicated SNP position was determined from the Hap map v1 or v2 (http://www.panzea.org). SNPs polymorphic between B73 and Mo17 are indicated as yes.*.

<sup>f</sup> QTL were previously identified by Tian et al. (2011) and are partitioned in the three different phenotypes: leaf angle, length, and width.

<sup>g</sup> Indicates that SNPs have yet to be mapped to B73v2 map.

<sup>h</sup> Indicates the SNP or indel listed falls outside the QTL supporting interval position (cm).

(http://qteller.com/). Consistent with the penetrance of the Lgn-R phenotypes in B73 and Mo17, Lgn mRNA is accumulated at a greater level in the apices of B73 as compared with Mo17 (Figure S3). The opposite expression difference was observed for sln, which exhibited a greater accumulation of mRNA in RNA isolated from Mo17 shoots than was observed for B73 (Figure S4).

Integration of QTL, genetic interaction, and mRNA expression data provide a potential molecular mechanism for the modulation of leaf morphology and shoot development by sol and lcf. The sol QTL on chromosome one could be explained by an allele at a regulatory gene in Mo17 that suppresses the expression of lgn, resulting in suppression of the lgn phenotype in the presence of the dominant mutant allele. The presence of multiple splice forms of lgn (http://www.maizegdb.org) make this a possible candidate mechanism for the sol QTL, as well. Alternatively, expression difference need not underlie the sol or lcf QTL. Coding sequence changes to known genes or the presence of coding sequence in the Mo17 chromosome not present in the B73 reference, and therefore unannotated for expression, could be responsible for the suppression of Lgn-R. The expression of sln is greater in shoots of Mo17 and suppression of sln expression by the
B73 allele at lcf could provide the mechanism for \textit{sol}Lig4-dependent mutant phenotype suppression by that QTL. The hypothesis of a \textit{lgn}-affecting regulatory QTL at \textit{sol} is consistent with the requirement of \textit{sol}Lig4 for the expression of \textit{Lg}B73 and the previous proposal that \textit{lgn} phenotype manifestation is due to up regulation of \textit{sln} in the shoot apex by the dominant \textit{Lgn-R} allele (Moon et al. 2013). Testing of candidates genes for \textit{sol} and \textit{lcf}, and the roles they may play in the expression regulation and alternative splicing of \textit{lgn} and the unusual effect of the 8bp UTR insertion allele \textit{lgn-dAc} (Moon et al. 2013), await future experiments.

Neither \textit{lcf} nor the interaction between the \textit{sol} and \textit{lcf} QTL were detected in CA (Table 1 and Figure 3). Multiple leaf morphology measures differed between the growing areas used throughout the 4 years of experimentation. According to the National Oceanic and Atmospheric Administration, the mean maximum temperatures during the summer of 2009 indicate that of the two sites used, IN was 6–13°F warmer (Figure S5) than Gill Tract, CA. The expressivity of the \textit{lgn} phenotype was strongest in West Lafayette, IN, than any of the other locations. The penetrance of the \textit{Lgn-R} allele was so strong that gross morphological and quantitative measures of phenotype expression in IN look similar to the \textit{Lgn-R} homozygotes in other areas. For example, heterozygous \textit{Lgn-R+} plants exhibit a complete failure of all reproductive structures in the B73 background in West Lafayette, IN. Although any differences in these two locations including soil characteristics and management techniques could also explain the lack of \textit{lcf} detection in this CA location. As compared with the Gill Tract, CA site, stronger expression of \textit{Lgn-R} suppression was observed in the 2010 growing season in IN (West Lafayette), during the 2012 season in Davis, CA, and in Valle de Banderas, Nayarit, MX during the winter of 2012 (data not shown). All three of these sites were substantially warmer than the Gill Tract Farm in Albany, CA. Regardless of the cause, the elucidation of a novel genetic pathway affecting leaf morphology and plant architecture including \textit{Lgn-R}, \textit{lcf}, and \textit{sol}, and multiple epistatic and environmental interactions was identified in this study of natural variation affecting expression of a dominant mutant phenotype.

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