Dissection of Leaf Angle Variation in Maize through Genetic Mapping and Meta-Analysis

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ABSTRACT Maize (Zea mays L.) hybrids have transitioned to upright leaf angles (LAs) over the last 50 yr as maize yields and planting densities increased concurrently. Genetic mapping and a meta-analysis were conducted in the present study to dissect genetic factors controlling LA variation. We developed mapping populations using inbred lines B73 (Iowa Stiff Stalk Synthetic), PHW30 (Iodent, expired plant variety protection inbred), and Mo17 (Non-Stiff Stalk) that have distinct LA architectures and represent three important heterotic groups in the United States. These populations were genotyped using genotyping-by-sequencing (GBS), and phenotyped for LA in the F$_2$ and F$_2$\:3 generation. Inclusive composite interval mapping across the two generations of the mapping populations revealed 12 quantitative trait loci (QTL), and a consistent QTL on chromosome 1 explained 10 to 17% of the phenotypic variance. To gain a comprehensive understanding of natural variations underlying LA variation, these detected QTL were compared with results from 19 previous studies. In total, 495 QTL were compiled and mapped into 143 genomic bins. A meta-analysis revealed that 58 genomic bins were associated with LA variation. Thirty-three candidate genes were identified in these genomic bins. Together, these results provide evidence of QTL controlling LA variation from inbred lines representing three important heterotic groups in the United States and a useful resource for future research into the molecular variants underlying specific regions of the genome associated with LA variation.

For the past 50 yr, maize yields and planting densities in the United States have increased concurrently. A comparative analysis of U.S. commercial maize hybrids released since the 1960s revealed that by selecting high yielding hybrids under high planting densities, breeders indirectly selected hybrids with upright LAs (Duvick et al., 2004). Similar results from other maize materials were obtained (Wang et al., 2011; Ma et al., 2014). Simulation (Duncan et al., 1967; Hammer et al., 2009) and empirical evidence (Lee and Tollenaar, 2007; Zhao et al., 2015) suggest that upright LAs combined with higher planting densities improve light distribution within the canopy. For example, modern hybrids intercept 14% more light than older hybrids (Lee and Tollenaar, 2007), and the increased light intercepted from...
leaves near the ear is positively correlated with grain yield (Ma et al., 2014; Zhao et al., 2015). Furthermore, modeling has suggested that LSAs should gradually transition from upright in the upper canopy to less upright in the lower as this configuration would distribute solar energy across multiple leaves while maximizing the total solar energy absorbed by the canopy (Duncan et al., 1967; Zhu et al., 2010). A recent study indicated that LA in the upper canopy was negatively correlated with grain yield, while LA in the lower canopy was positively correlated (Zhang et al., 2017), confirming the earlier suggestion about canopy configuration on grain yield.

Given LA’s role in the adaptation of maize hybrids to high planting densities, it is beneficial to understand the genetic control of LA variation. Five maize genes that modify LA for multiple leaves in the canopy have been cloned: *lgul* (*gll*, Moreno et al., 1997), *lgul* (*gll*, Walsh et al., 1998), *ZmTAC1* (Ku et al., 2011), *CLA4* (Zhang et al., 2014), and *nana plant2* (Best et al., 2016). These genes exhibit different types of dominance; for example, *lgll* (*Becraft et al., 1990*) and *lg2* (*Harper and Freeling, 1996*) are recessive mutations that result in upright LAs, whereas *CLA4* exhibits incomplete dominance (Zhang et al., 2014).

Prior research has identified many QTL controlling LA variation in maize by phenotyping different leaves in the canopy, particularly leaves below the flag leaf (final leaf) and surrounding the ear. However, little research has investigated LA in the lower canopy, specifically below the ear. Previous studies used a meta-analysis to integrate a subset of reported QTL to identify multiple meta–QTL for LA and other canopy architecture traits (Ku et al., 2012; Wang et al., 2016; Zhao et al., 2018). Several known genes physically mapped near these meta–QTL, yet a large-scale meta-analysis for only LA to facilitate separating the genetic control of LA from other canopy architecture traits has not been conducted.

The US Plant Variety Protection (PVP) Act of 1970 allows private companies to protect the proprietary inbred lines they develop for a set period. As the protection expires for these inbred lines, they become publicly available to explore their genetic diversity. These ex-PVP lines have attracted a lot of attention, with prior research suggesting that today’s germplasm can be traced back to contributions from seven progenitor lines that include public inbred lines B73 and Mo17 and private inbred line PH207 (Mikel and Dudley, 2006; Nelson et al., 2008; Mikel, 2011). These inbred lines represent three important heterotic groups in maize: Iowa Stiff Stalk Synthetic (BSSS), Non-Stiff Stalk (NSS), and Iodent, respectively (Mikel and Dudley, 2006; Mikel, 2011). Investigating LA in lines representing these heterotic groups provides us an opportunity to identify significant regions of the genome that have contributed to modern hybrid’s transition to upright LAs.

In this study, we report the discovery of 12 QTL linked to LA in the lower canopy by genetic linkage mapping. We first developed biparental populations using three different parents (Fig. 1). Selected F2 lines were genotyped using GBS, and both the selected F2 lines and F2:3 families were phenotyped for LA. Inclusive composite interval mapping was conducted to map QTL for LA in both generations. To gain a comprehensive understanding of natural variations underlying LA, we integrated our findings with other reported QTL into genomic bins and conducted a meta-analysis to identify regions of the genome associated with LA variation across a wide range of germplasm.

**MATERIALS AND METHODS**

**Genetic Materials**

Genetic mapping populations were developed using three inbred lines with distinct LA architectures (Fig. 1) that represent three important maize heterotic groups. Inbred line B73 is a BSSS inbred released from Iowa State University in 1972 (Russell, 1972). It has broad leaves, upright LAs in the upper canopy, and slightly less upright LAs below the ear (Fig. 1). Inbred line PHW30 is an ex-PVP Iodent inbred (PVP number 9100102) developed by Pioneer Hi-Bred in the late 1980s. It has a distinct plant architecture with wavy leaves and upright LAs throughout the canopy (Fig. 1), characteristics similar to modern inbred lines. Inbred line Mo17 is a NSS inbred line the University of Missouri released in 1964 (Zuber, 1973). It has broad leaves and relatively flat LAs throughout the canopy (Fig. 1).

In the summer of 2013, we made reciprocal crosses between PHW30 and each of the two other inbreds. The F1 plants were selfed in a 2014 winter nursery, and in the following summer, 500 F2 seeds from each population were planted at 72,000 plants per hectare (~29,000 plants per acre) in ~5.5-m-long plots spaced ~76 cm apart. We combined reciprocal populations, as there were no biologically significant LA differences (Table 1) and herein refer to them as B73 and Mo17 populations.

We applied a modified bidirectional selective genotyping strategy (Lander and Botstein, 1989) by including F2 plants with phenotypic values around the population mean in addition to two trait extreme groups. From the B73 population, we selected 44 F2 plants with the highest phenotypic values, 45 F2 plants with the lowest, and 36 F2 plants equal to the population mean, and from the Mo17 population, we selected 46, 45, and 34 plants, respectively. These three groups are referred to as the upright, flat, and average groups, respectively. In the summer of 2015, 25 F2:3 seeds from selected F2 plants were planted in a single replicate under the same planting conditions. Three representative plants from the middle of the plot were phenotyped for LA, and the calculated mean was recorded as the plot’s phenotypic value. In total, 125 F2:3 families were planted for each population, but only 123 F2:3 families were phenotyped for the B73 population and 120 F2:3 families for the Mo17 population.
Phenotyping for Leaf Angle

The second leaf below the ear leaf was phenotyped for LA on parents, hybrids, F_2 lines, and F_2:3 families starting after all plants completed anthesis. Leaf angle was phenotyped for this leaf because it contrasted the most among the three parents (Fig. 1). A digital image of each plant’s LA was captured with the Field Book Phenotyping app (version 2.3.0; Rife and Poland, 2014) on a Google Nexus 7 Android Tablet. The angle between horizontal and middle of the midrib was measured on each image with ImageJ’s angle tool (Schneider et al., 2012). The R open source statistical programming language and environment (R Core Development Team, 2017) and in-house R scripts were used to analyze the phenotypic data.

Genotyping, Filtering, and Imputation

To map QTL associated with LA variation, DNA was extracted from F_2:3 families. We collected one fresh tissue leaf punch from 10 F_2:3 plants within each F_2:3 family to reconstruct the original F_2 plant genotype (Schon et al., 1994). In total, 125 F_2:3 families from each population were tissue sampled.

DNA was extracted with a Qiagen DNA extraction kit and then digested with ApeKI and barcoded for 288-plex GBS (Elshire et al., 2011). Single nucleotide polymorphisms (SNPs) were called from the sequencing reads with the Tassel5-GBS Production Pipeline using the ZeaGBSv2.7 Production TOPM and reported as B73 RefGen_v2 physical positions (Glaubitz et al., 2014). FIL-LIN and maize donor haplotypes (V5) were used for SNP imputation (Swarts et al., 2014).

The SNPs were identified in the parents and then filtered and corrected for allelic dropouts in the progeny using in-house java scripts. The parental lines for each population were first compared to identify SNPs. These SNPs were selected in the progeny, and SNPs with >15% missing data and segregation distortion (by testing the Mendelian segregation ratio with a p-value <0.001) were removed. A sliding window algorithm with a 15-SNP window and an 1-SNP step (Su et al., 2017) was applied to address heterozygous genotypes called as homozygous because of low read coverage associated with the GBS method (Elshire et al., 2011).

Quantitative Trait Loci Mapping

IciMapping software (version 4.0.6.0; Meng et al., 2015) was used to map QTL. PHW30 was labeled as parent A for both populations and coded appropriately. Markers in linkage disequilibrium were binned using the following software options: missing rate percentage was set to 100,
A modified selective genotyping method was used to select 125 F₂ plants from each population, and DNA was collected from 10 F₂,3 plants to reconstruct the F₂ genotype. Genotyping-by-sequencing was used to generate the genotypic data for both populations. Using the filtered progeny genotypic data, two genetic maps were developed and combined into a consensus map. We identified 94,078 SNP sites segregating in the B73 population and 46,724 segregating in the Mo17 population. After filtering, imputation, and binning, 2710 bins developed the B73 population linkage map, and 2129 bins developed the Mo17 population linkage map. Genetic map lengths were 1726.5 cM for the B73 population and 1628.88 cM for the Mo17 population. Combining the two genetic maps resulted in a consensus map of 1808.9 cM.

Inclusive composite interval mapping with the consensus map was conducted separately for each population.
and generation. In total, 12 QTL were detected for LA (Fig. 2). Six QTL were detected across both generations of the B73 population (Fig. 2A,B), and all six were nonoverlapping according to the QTL interval's physical position (Table 2). We detected three QTL in each of the two generations (Fig. 2A,B). Six QTL were detected across both generations of the Mo17 population (Fig. 2C,D), and five were nonoverlapping according to the QTL interval's physical positions (Table 2). Five QTL were detected in the F2 generation, and one was detected in the F2:3 generation (Fig. 2C,D). A single-marker scan with physical mapping of unprocessed genotypic data provided supporting evidence for these results (Supplemental Fig. S2).

Table 2. Quantitative trait loci detected across two combined populations and two generations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Generation</th>
<th>Chromosome</th>
<th>Position</th>
<th>Physical interval (RefGen_v3)</th>
<th>LOD</th>
<th>Phenotypic variance explained %</th>
<th>Additive effect</th>
<th>Dominance effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>B73</td>
<td>F2</td>
<td>1</td>
<td>191.00</td>
<td>275,062,657–275,835,496</td>
<td>6.25</td>
<td>16.60</td>
<td>5.19</td>
<td>0.03</td>
</tr>
<tr>
<td>Mo17</td>
<td>F2</td>
<td>1</td>
<td>192.91</td>
<td>275,843,509–276,767,207</td>
<td>4.74</td>
<td>10.44</td>
<td>3.32</td>
<td>-0.02</td>
</tr>
<tr>
<td>Mo17</td>
<td>F2:3</td>
<td>1</td>
<td>192.91</td>
<td>275,843,509–276,767,207</td>
<td>3.91</td>
<td>14.43</td>
<td>3.66</td>
<td>1.06</td>
</tr>
<tr>
<td>B73</td>
<td>F2:3</td>
<td>2</td>
<td>159.00</td>
<td>180,806,386–182,824,212</td>
<td>4.82</td>
<td>13.01</td>
<td>2.19</td>
<td>1.28</td>
</tr>
<tr>
<td>Mo17</td>
<td>F2</td>
<td>2</td>
<td>240.84</td>
<td>227,218,624–227,302,619</td>
<td>6.03</td>
<td>13.29</td>
<td>3.60</td>
<td>1.16</td>
</tr>
<tr>
<td>Mo17</td>
<td>F2:3</td>
<td>2</td>
<td>240.84</td>
<td>227,218,624–227,302,619</td>
<td>7.43</td>
<td>17.17</td>
<td>4.12</td>
<td>-0.66</td>
</tr>
<tr>
<td>B73</td>
<td>F2:3</td>
<td>3</td>
<td>91.74</td>
<td>29,175,464–29,212,960</td>
<td>4.88</td>
<td>13.78</td>
<td>2.40</td>
<td>0.48</td>
</tr>
<tr>
<td>B73</td>
<td>F2</td>
<td>3</td>
<td>92.00</td>
<td>36,470,328–37,674,938</td>
<td>5.04</td>
<td>8.77</td>
<td>0.72</td>
<td>0.26</td>
</tr>
<tr>
<td>Mo17</td>
<td>F2:3</td>
<td>3</td>
<td>131.00</td>
<td>186,407,816–186,516,273</td>
<td>3.41</td>
<td>7.54</td>
<td>1.41</td>
<td>4.77</td>
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<tr>
<td>B73</td>
<td>F2</td>
<td>8</td>
<td>39.00</td>
<td>102,435,103–102,836,776</td>
<td>3.04</td>
<td>7.54</td>
<td>1.41</td>
<td>4.77</td>
</tr>
</tbody>
</table>

Fig. 2. Linkage mapping of leaf angle. Logarithm of odds (LOD) plots for selected chromosomes from the inclusive interval mapping. Results for LA in the B73 population using selected F2 plants (A), F2:3 families (B), Mo17 population using selected F2 plants (C), and F2:3 families (D). Chromosomes were selected for plotting if it contained a significant QTL among all results. The horizontal red line indicates the significance threshold, and vertical dotted lines separate the selected chromosomes.
The QTL near ~275 Mb on chromosome 1 was consistently detected in both generations of the Mo17 population and in the F2 generation of the B73 population (Table 2). A peak in this region was also detected in the F2.3 generation of the B73 population, but its LOD score (2.41) was below the significance threshold (Fig. 2B). Additionally, the QTL on chromosome 3 that was detected in the F2.3 generation of the B73 population and the F2 generation of the Mo17 population maps to the same genetic position (~92 cM) but physically map ~7 Mb apart (Table 2). The detected QTL were located on chromosomes 1, 2, 3, 4, and 8 (Table 2).

**Phenotype, Population, and Quantitative Trait Loci Summary**

Different leaves were phenotyped in dissecting LA variation in previous genetic mapping studies (Fig. 3, Table 3). Phenotyped leaves varied from one leaf below the flag leaf to the second leaf below the ear, with most of the studies phenotyping the leaves near the main ear (Fig. 3, Table 3). Eight studies phenotyped multiple leaves but used the mean phenotypic value of all leaves measured as the final trait value (Table 3). Nine studies, including this one, phenotyped a single leaf, while three studies phenotyped consecutive leaves and mapped them as separate traits (Table 3).

In addition to the phenotyped leaves, the parents and genetic mapping populations used by the different studies were summarized (Supplemental Table S2, S3). The populations were developed from genetic crosses using 133 inbred lines, 71 of which were unique (Supplemental Table S2). Inbred line B73 was used 32 times in a cross (~24% of all inbred lines used), while 50 parents were only used once (Supplemental Table S2). Additionally, the number of genetic mapping populations per study ranged from 1 to 25, and the size of those populations ranged from 94 to 397 (Table 3).

Overall, 495 QTL associated with LA were reported through linkage mapping within individual populations or joint linkage mapping across populations (Supplemental Table S3). Reported QTL per study ranged from 3 to 149 (Table 3), and the phenotypic variance explained by each QTL ranged from 0.41 and 85.05% (Supplemental Table S3). Furthermore, the estimated additive effect for the reported QTL ranged from −8.51 to 6.67, and the estimated dominance effect ranged from −2.88 to 10.77 (Supplemental Table S3).

**Meta-Quantitative Trait Loci Analysis**

After dividing the genome into 210 genomic bins ~10 Mb in size, the 495 reported LA QTL were mapped into 143 genomic bins across all 10 chromosomes. Within a genomic bin, multiple QTL from the same study and canopy level were removed, leaving 325 nonredundant QTL (~66%). After calculating the grouped p-value for each genomic bin using a weighted Z-test, there was evidence of linkage ($P < 7.1 \times 10^{-10}$) of LA to 58 genomic bins distributed across all 10 chromosomes (Fig. 4, Supplemental Table S4). These genomic bins contained 203 nonredundant LA QTL (~41%), representing a moderate enrichment (~2.3-fold) of LA QTL in these regions. To identify any pattern that connected canopy position with the linked genomic bins, each study’s phenotyped leaves were partitioned into six canopy levels (Table 3). When the 58 linked genomic bins were compared with the canopy level used to detect the QTL, no clear pattern was observed (Fig. 4).

**Meta-Analysis Candidate Genes**

From the 58 genomic bins that were associated with LA, we focused on summarizing the 15 most prominent genomic bins (Fig. 4, Table 4). These 15 genomic bins contained 82 of the 325 nonredundant QTL (~25%) and represented a ~3.5-fold enrichment of LA QTL in these regions. All chromosomes except chromosome 6 contained a prominent genomic bin (Table 4) and chromosomes 1, 2, 3, 7, and 10 contained multiple (Table 4).

We identified 12 candidate genes in 10 of the 15 prominent genomic bins: seven genes from maize and five maize orthologs from rice genes (Fig. 4, Table 4). Prominent genomic bins contained two maize genes known to alter LA: *lg1* and *lg2*. Twelve studies reported 28 QTL in the same genomic bin as *lg1* (Supplemental Table S4). Two other maize genes also known to alter LA, *droopy leaf1* (Strable et al., 2017) and *droopy leaf2* (Strable et al., 2017) were identified. Other prominent genomic bins...
Table 3. Leaf angle quantitative trait loci (QTL) studies in maize.

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of populations</th>
<th>Population size</th>
<th>No. of QTL</th>
<th>Leaves measured</th>
<th>Canopy level</th>
<th>Fig. 3 reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mickelson et al., 2002</td>
<td>1</td>
<td>180</td>
<td>10</td>
<td>Average of all leaves above ear leaf</td>
<td>Mid-upper</td>
<td>B</td>
</tr>
<tr>
<td>Yu et al., 2006</td>
<td>2</td>
<td>120; 114</td>
<td>9</td>
<td>Third leaf below flag leaf</td>
<td>Upper</td>
<td>H</td>
</tr>
<tr>
<td>Lu et al., 2007</td>
<td>1</td>
<td>397</td>
<td>6</td>
<td>Average of first through third leaf above ear leaf</td>
<td>Mid-upper</td>
<td>D</td>
</tr>
<tr>
<td>Ku et al., 2010</td>
<td>1</td>
<td>229</td>
<td>3</td>
<td>Average of first leaf above ear leaf, ear leaf, and first leaf below ear leaf</td>
<td>Middle</td>
<td>E</td>
</tr>
<tr>
<td>Tian et al., 2011</td>
<td>25</td>
<td>~195 each</td>
<td>30</td>
<td>One leaf below flag leaf</td>
<td>Upper</td>
<td>G</td>
</tr>
<tr>
<td>Ku et al., 2012</td>
<td>1</td>
<td>256</td>
<td>5</td>
<td>Average of first leaf above ear leaf, ear leaf, and first leaf below ear leaf</td>
<td>Middle</td>
<td>E</td>
</tr>
<tr>
<td>Wassom, 2013</td>
<td>1</td>
<td>93</td>
<td>3</td>
<td>Mid-upper</td>
<td>K</td>
<td></td>
</tr>
<tr>
<td>Liu et al., 2014</td>
<td>1</td>
<td>144</td>
<td>5</td>
<td>Mid-upper</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Potts, 2014</td>
<td>9</td>
<td>~35 each</td>
<td>12</td>
<td>Mid-upper</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Chen et al., 2015</td>
<td>1</td>
<td>144</td>
<td>5</td>
<td>Mid-upper</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Ding et al., 2015</td>
<td>1</td>
<td>305</td>
<td>14</td>
<td>Average of first through fourth leaf above ear leaf</td>
<td>Upper</td>
<td>C</td>
</tr>
<tr>
<td>Hou et al., 2015</td>
<td>1</td>
<td>266</td>
<td>14</td>
<td>First leaf above ear leaf</td>
<td>Middle</td>
<td>J</td>
</tr>
<tr>
<td>Li et al., 2015</td>
<td>3</td>
<td>183; 172; 183</td>
<td>17</td>
<td>Average of first leaf above ear leaf, ear leaf, and first leaf below ear leaf</td>
<td>Middle</td>
<td>E</td>
</tr>
<tr>
<td>Chang et al., 2016</td>
<td>1</td>
<td>150</td>
<td>17</td>
<td>First leaf above ear leaf</td>
<td>Middle</td>
<td>J</td>
</tr>
<tr>
<td>Ku et al., 2016</td>
<td>4</td>
<td>215; 208; 212; 223</td>
<td>21</td>
<td>Average of first through fourth leaf above ear leaf</td>
<td>Upper</td>
<td>C</td>
</tr>
<tr>
<td>Pan et al., 2017</td>
<td>10</td>
<td>~188 each</td>
<td>149</td>
<td>First leaf above ear leaf</td>
<td>Middle</td>
<td>J</td>
</tr>
<tr>
<td>Wang et al., 2017</td>
<td>1</td>
<td>220</td>
<td>17</td>
<td>Ear leaf</td>
<td>Middle</td>
<td>K</td>
</tr>
<tr>
<td>Zhang et al., 2017</td>
<td>1</td>
<td>196</td>
<td>24</td>
<td>Average of all leaves in canopy</td>
<td>All</td>
<td>A</td>
</tr>
<tr>
<td>Zhao et al., 2018</td>
<td>2</td>
<td>202;218</td>
<td>33</td>
<td>Ear leaf</td>
<td>Middle</td>
<td>K</td>
</tr>
<tr>
<td>This study</td>
<td>2</td>
<td>125;125</td>
<td>12</td>
<td>Second leaf below ear leaf</td>
<td>Lower</td>
<td>L</td>
</tr>
</tbody>
</table>

The meta-analysis integrated the 12 newly identified QTL into 10 genomic bins, and we explored those that contain candidate genes (Table 2). Four of these genomic bins contain candidate genes, and lg2 was included in the 15 prominent genomic bins (Table 4). The three QTL located on chromosome 1 physically map near one of the six maize phytochrome genes, *phytochrome C1* (*PhyC1*; Sheehan, 2004). Additionally, the cloned maize gene *ZmTAC1* (Ku et al., 2011) was identified as a candidate gene. Finally, another maize ortholog of a rice gene that modifies the bending of the leaf’s lamina joint, *TLD1* (Zhang et al., 2009), was identified as a candidate gene.

**DISCUSSION**

Research has shown that commercial hybrids released over the last 50 yr have increasingly more upright LAs (Duvick et al., 2004; Wang et al., 2011; Ma et al., 2014). This study investigated three maize inbred lines with distinct LA architectures from important US heterotic groups. Inbred line B73 has slightly less upright LAs in the lower canopy than PHW30, while Mo17 has the least upright. The LA for F$_1$ plants from the B73 crosses was about equal to the average of the parents, while LA for F$_1$ plants from the Mo17 crosses was greater than the average of the parents. Other studies reporting LA phenotypes on F$_1$ plants and the corresponding parents reported F$_1$ plants about equal to the midparent value (Ku et al., 2010) and F$_1$ plants greater than both parents (Hou et al., 2015). Transgressive segregation in both the F$_2$ and F$_{2:3}$ generations was observed in all populations, which is consistent with previous studies (Mickelson et al., 2002; Ku et al., 2010; Chen et al., 2015; Hou et al., 2015). Slight differences in the distributions of the F$_2$ and F$_{2:3}$ generations could be explained by the decrease in heterozygosity between the F$_2$ and F$_{2:3}$ generation that can result in less vigorous plants therefore causing the plants to become less upright.

All 12 QTL detected across both populations and generations in this study were supported by at least one prior study. These 12 QTL were detected on chromosomes 1, 2, 3, 4, and 8 by phenotyping a leaf in the lower canopy. The QTL at ~227 Mb on chromosome 2, ~29 Mb on chromosome 3, and ~176 Mb on chromosome 3 were near or within QTL intervals detected from other studies that phenotyped leaves in different parts of the canopy (Yu et al., 2006; Ku et al., 2011; Tian et al., 2011; Potts, 2014).
QTL in these three regions all have positive additive effects indicating PHW30 as the source of the allele and explain substantial amounts of phenotypic variance (~9–17%). Furthermore, two of the three QTL are near cloned maize genes, \( \lg 2 \) (chromosome 3, ~176 Mb) and \( \text{ZmTAC1} \) (Chromosome 2, ~221 Mb) that alter LA in multiple leaves throughout the canopy. In a separate experiment, we developed a backcross family with PHW30 and Mo17 as the recurrent parent that appears to be segregating for a single gene causing upright LAs for all leaves in the canopy. We are currently genotyping and phenotyping this family to help determine the underlying molecular variant that explains the phenotype observed in this family.

Light sensitivity reduction contributed to modern maize’s transition to upright LAs (Fellner et al., 2003, 2006). Maize gene \( \text{PhyC1} \) (Chromosome 1, ~227 Mb; Sheehan, 2004) physically maps within the three QTL intervals detected on chromosome 1. A previous study phenotyped a leaf near the ear and used ex-PVP lines and B73 to detect a QTL ~4 Mb upstream of this gene (Potts, 2014). Of the 20 studies summarized, these are the only two targeted genetic mapping studies to use modern inbred lines to identify QTL for LA. The \( \text{PhyC1} \) gene has been cloned in maize (Sheehan, 2004), and previous results in maize and rice suggested that phyC can perceive far-red light signals (Takano et al., 2005; Dubois et al., 2010). High planting densities reduce red light and increase far-red light, resulting in a decrease in the red to far-red ratio. This decrease can trigger shade-avoidance responses in plants that can include altering LA and other architectural modifications to increase the amount of unfiltered light reaching their leaves (Franklin and Whitelam, 2005). Positive additive effects for all three QTL from this study indicated that the more modern and upright LA inbred, PHW30, is the source of the allele.
Table 4. Candidate genes identified in the 15 prominent genomic bins from the meta-analysis using the nonredundant set of reported quantitative trait loci (QTL).

<table>
<thead>
<tr>
<th>Rank</th>
<th>Chromosome</th>
<th>Genomic bin</th>
<th>No. of QTL</th>
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<th>Candidate genes</th>
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thus suggesting PHW30 might have a different PhyC1 allele with decreased sensitivity to red to far-red light ratios.

Under high planting densities, the ideal LA architecture for the maize canopy should gradually move from upright LAs in the upper canopy to less upright LAs in the lower (Duncan, 1971; Long et al., 2006; Zhang et al., 2017). Previous research dissecting the genetic control of LA in maize has focused on phenotyping a single leaf or averaging the phenotypes from multiple leaves. Without phenotyping and genetically mapping multiple individual leaves in the canopy, it is difficult to differentiate QTL controlling LA specifically in one part of the canopy from QTL controlling LA in multiple parts. For example, leaves from the upper canopy to the mid-lower canopy were phenotyped in the studies that detected QTL within the genomic bin on chromosome 2 (0–10 Mb) that contains lg1 (Moreno et al., 1997). Even though this particular mutant is known to affect LA in all parts of the canopy (Becraft et al., 1990), for other QTL, without phenotyping and genetically mapping for LA in multiple leaves in the canopy, it is difficult to make this differentiation. Previous research has genetically mapped LA for individual leaves and identified leaf specific QTL, but consecutive leaves were phenotyped rather than leaves in different parts of the canopy (Liu et al., 2014; Chen et al., 2015; Chang et al., 2016). To differentiate leaf-specific from canopy-wide QTL that control LA, we are currently genotyping doubled haploid lines developed from the selected F₂ plants from this study and phenotyping four leaves throughout the canopy for LA.

In addition to differentiating leaf-specific from canopy-wide QTL, parents with contrasting LAs in the canopy should be used for developing genetic mapping populations. Past LA studies in maize, including this one, have developed genetic mapping populations by crossing inbreds with upright LAs throughout the canopy by inbreds with flat LAs (Ku et al., 2010, 2012; Zhang et al., 2014; Chen et al., 2015; Ding et al., 2015; Li et al., 2015). These types of crosses resulted in 12 studies reporting QTL in the same genomic bin as lg1.

A goal in understanding quantitative trait variation is to determine the underlying molecular variation (Mackay, 2001). Integrating detected QTL from many independent studies through a meta-analysis increases the resolution of those QTL and brings us closer to determining the causal molecular variations. From our meta-analysis results, we identified cloned genes, lg1 and lg2, as candidate genes that were corroborated by other LA meta-analyses (Ku et al., 2012; Wang et al., 2016; Zhao et al., 2018) providing evidence to the power these methods have in detecting candidate genes. The recently proposed omnigenic model postulates that there are a moderate number of genes that directly affect a particular trait, or core genes, and any variant in the numerous genes that compose the highly connected cell regulatory networks can affect these core genes (Boyle et al., 2017). For example, genes like lg1 and lg2 may be core genes since they directly affect LA, and any variants in the numerous genes involved in leaf development, such as the other genomic bins detected in the meta-analysis, may affect the regulation or function of lg1, lg2, or other core genes associated with LA.

Many candidate genes detected from the meta-analysis were maize orthologs of rice genes that modify LA through changes in cell size and bending in the lamina joint (Supplemental Table S1). While both are members of the grass family, maize and rice have slightly different leaf structures. In maize, the ligule and auricle are structures that separate the blade and sheath. The auricle can act as a hinge, allowing the leaf to bend away from the stem and help support the weight of maize’s wider and heavier leaf. Loss of ligule and auricle (Becraft et al., 1990; Harper and Freeling, 1996), variations in the auricle size (Kong et al., 2017; Strable et al., 2017), and
proteomic differences and changes in midrib cells (Wang et al., 2015; Strable et al., 2017) are known biological mechanisms that can alter maize LA. On the other hand, rice’s ligules and auricles are outside of the leaf and act more as appendages, and the lamina joint separates the blade and sheath. There are cloned rice genes that alter LA by modifying leaf bending through changing cell size in the lamina joint (Duan et al., 2006; Je et al., 2010; Jiang et al., 2012; Wu et al., 2013; Gao et al., 2014; Feng et al., 2016). Changes in cell size near the leaf collar in maize have been reported in near isogenic lines differing only by LA related gene CLA4 (Zhang et al., 2014). Combined with the results of our meta-analysis results, cell size near the abaxial boundary between the blade and sheath near the midrib may also contribute to LA variation in maize.

The results from our study provide additional knowledge on QTL associated with LA in the lower canopy and a stronger framework for determining the molecular variations underlying LA variation in maize. Our genetic mapping data provide evidence of QTL associated with maize LA variation in the second leaf below the ear. Questions remain on differentiating QTL controlling LA in one part of the canopy from QTL controlling LA in multiple parts. Current research is underway to address these remaining questions by phenotyping and genetically mapping LA for multiple leaves in the canopy using material developed from this study.

CONCLUSION
Understanding the genetic control of complex traits like LA involves linking observed phenotypic variation to regions of the genome. Our study investigated LA variation in three inbred lines with distinct LA architectures that represent important US heterotic groups. We have identified unique regions of the genome linked to LA variation, with a major effect QTL consistently detected on chromosome 1. Integration of these results with other reported QTL through a meta-analysis identified 58 regions of the genome associated with LA variation. While some of these regions contain genes known to affect LA in maize, other regions contain maize orthologs from rice genes that are associated with modifying cell size and bending in the leaf’s lamina joint. These could be useful regions of the genome to target for future comparative genomic research into identifying additional molecular variants associated with LA variation. Together, these results advance our knowledge about LA variation in maize to assist breeders in developing hybrids with a canopy-wide LA architecture that are adapted to future increases in planting density.

Supplemental Information Available
Supplemental Fig. S1. Histograms of phenotypic values for LA across the F2 and F2:3 generation for the B73 and Mo17 populations.

Supplemental Fig. S2. Linkage mapping compared with single marker scan results for LA. Scans were done with the uncorrected genotypic data for LA across B73 population using selected F2 plants (A), F2:3 families (B), Mo17 population using selected F2 plants (C), and F2:3 families (D). Top sections represent the linkage mapping results, while the bottom sections represent the single marker scan results.

Supplemental Table S1. List of candidate genes known to affect LA from maize, rice, and sorghum, and the corresponding maize gene model.

Supplemental Table S2. Summary of the parents the 20 studies used to develop genetic mapping populations.

Supplemental Table S3. Summary of the 495 reported QTL reported from 20 studies including the present study.

Supplemental Table S4. Details about the 58 genomic bins, candidate genes, and reported QTL linked to LA variation detected from the meta-analysis.

Data and code used in this study was uploaded in an online public repository: https://github.com/mdzievitz/Meta_QTL-LA-Mapping-Paper.

Conflict of Interest Disclosure
The authors declare that there is no conflict of interest.

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