QTL Controlling Masculinization of Ear Tips in a Maize (*Zea mays* L.) Intraspecific Cross

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ABSTRACT Maize is unique among cereal grasses because of its monoecious flowering habit. Male flowers are normally restricted to the tassel that terminates the primary shoot, whereas female flowers occur as ears at the terminal nodes of lateral branches. We observed Ki14, a tropical maize inbred that produces an ear tipped by a staminate (male) spike under certain environmental conditions, such as long daylengths. Recombinant inbred lines derived from the cross between temperate line B97, which was never observed to produce a staminate ear tip, and Ki14 segregated for the trait under long daylengths. Some progeny lines that had even longer staminate tips than Ki14 were male fertile. We mapped three QTL controlling staminate ear tip using a two-part (binomial plus normal) model. A major QTL on chromosome 3 had a large effect on penetrance of the trait (whether a line would produce staminate ear tips or not) as well as its severity (the length of the staminate tip). This QTL seems to be linked to, but at a distinct position from, a previously mapped QTL controlling the proportion of staminate florets in ears in progeny from crosses between maize and teosinte. Two additional QTL affecting staminate ear tip severity overlapped with QTL controlling photoperiod response previously mapped in this population. Alleles conferring photoperiod sensitivity for delayed flowering at these QTL seem to enhance the production of staminate ear tips under long daylengths.

Maize (*Zea mays* L. subsp. *mays*) is monoecious, with staminate (male) flowers located at the tip of the primary shoot in a structure referred to as the tassel and pistillate (female) flowers located at the tip of one or more lateral branches in the ear. The restriction of male flowers to the tassel occurs due to the abortion of pistils in the florets in the early development of the tassel; similarly, stamens are aborted during the development of ears (Irish 1996). Unusual environmental conditions or mutations at a few key developmental loci can result in feminization of the tassel or masculinization of the ear (Chuck *et al.* 2007; Dellaporta and Calderon-Urrea 1994; Emerson and Emerson 1922; Heslop-Harrison 1961; Iltis 1983; Irish 1996).

Teosinte (*Zea mays* L. subsp. *parviglumis*), the closest relative and presumed ancestor of maize, has a distinct growth habit and ear morphology compared with maize (Iltis 1983; Doebley 2004). Teosinte has numerous long lateral branches, which bear ears at interme-

diate nodes and tassels at the terminal node; in contrast, maize has very short lateral branches (compressed into a shank) that terminate in a female ear (Doebley 2004; Iltis 1983; Mangelsdorf 1974). Doebley and colleagues (Doebley 2004; Doebley and Stec 1991; Doebley and Stec 1993; Doebley *et al.* 1995) mapped quantitative trait loci (QTL) conditioning the key morphological differences between maize and teosinte and identified two of the underlying genes, *tb1* and *tga1* (Clark *et al.* 2006; Doebley *et al.* 1997; Wang *et al.* 2005). *tb1* has a major effect on the lateral branching pattern and inflorescence placement in segregating populations derived from crosses between maize and teosinte. Another QTL on chromosome 3L also has a major effect on these and other domestication traits, but its effect disappears in predominantly maize genetic backgrounds due to epistatic interaction with *tb1* (Doebley 2004; Doebley *et al.* 1995).

Plasticity for sexual determination of florets in ears exists in some maize varieties (Mangelsdorf 1974). We previously reported remarkable plasticity for vegetative and sexual organ determination in the tropical maize inbred line Ki14 grown under long daylengths in a controlled environment condition (Figure S4 in Coles *et al.* 2010). In addition, we observed that Ki14 often produced a spike of staminate florets at the tips of its ears when grown under long daylengths in field environments. Under short daylengths, this abnormality was not observed. Recombinant inbred lines (RILs) derived from the cross of

temperate line B97 and Ki14 that were used to study the inheritance of photoperiod sensitivity also segregated for the staminate ear tip trait under long daylengths, and we were able to score these lines for this phenotype in one long daylength environment, permitting mapping of QTL affecting this trait.

The objectives of this study were to map QTL controlling staminate ear tips in this maize population. Determination of QTL positions allows us to address the hypothesis that the gene(s) controlling this trait are known mutants of sex determination in maize (*e.g.*, *anther ear 1*) or represent standing variation for domestication traits remaining in maize after the domestication bottleneck and more than 5000 years of selection for fully pistillate ears.

MATERIALS AND METHODS

From the cross of B97 and Ki14, we derived $F_{5:6}$ or $F_{6:7}$ RILs, whereby each RIL was derived from a unique F_2 plant. Selfing generations alternated between long-day summer nurseries in Clayton, NC, and short-day winter nurseries in Homestead, FL. RIL seed was increased via sib mating and bulking of up to 20 plants per line; this bulked seed was then used in the experiment. We evaluated 217 RILs from this cross, plus the parental lines B97 and Ki14 and the check inbred line B73, in three long daylength and three short daylength environments (Coles *et al.* 2010). The staminate ear tip trait was consistently observed only in the long daylength environment of summer season 2006 in Clayton, NC. In that environment, we employed an 11 × 20 alpha lattice design with two complete replications. The design was augmented by including B73 as an additional check within each incomplete block to help control spatial variation, resulting in 480 plots. Each plot consisted of 12 plants sown in a 1.8-m row with 0.6-m alleys between the front and back of each row and 1-m spacing on either side of each row. Each plot was given a single rating of 0–4 based on the median extent of masculinization of ear tips: 0, normal ear with no staminate florets; 1, ear with a very small tassel-like formation at the tip; 2, ear tipped by a substantial staminate tassel–like formation that did not extend outside of the husk; 3, ear tipped by a staminate tassel–like formation long enough to extend outside of the husk; 4, ear tipped by a staminate tassel spike that extended out of the husk and had anthers that shed pollen. Raw phenotypic data are provided in Table S1.

Phenotypic data analysis

Data from the RIL populations were analyzed using SAS version 9.1.3 Proc Mixed (SAS Institute Inc. 2002–2008). An initial analysis was performed in which incomplete blocks that showed no evidence of significant variation in the models examined were eliminated from the model. The resulting model was used to compute RIL and parental line least square means for QTL mapping: $Y_{ij} = \mu + R_i + G_j + \in_{ij}$, where μ is the overall mean, R_i is the effect of the ith complete replication, G_j is the effect of the jth RIL or check line genotype, and $\in_{ij}$ is the residual error effect for the jth genotype in the ith replication. In this model, RIL lines were considered fixed and replications considered random. We observed that residual values were not independent of predicted values from this model, because the variation among residual values maximum at predicted scores near two and minimum at predicted scores near zero or four. Natural log and square root transformations did not ameliorate this problem; therefore, we analyzed untransformed data. Estimated RIL means are in Table S2. The variation among RILs was used to estimate heritability (Holland *et al.* 2003) and was estimated separately from parental variation with a modification of the previous model, treating RIL effect as a random variable (Piepho *et al.* 2006).

Genotypic data

Genomic DNA was extracted from the bulked leaf tissue of 10 plants representing each of the RILs using the Invitrogen Charge Switch Genomic DNA Extraction kit (Invitrogen Corporation, Carlsbad, CA). We used simple sequence repeat (SSR) markers from the Maize Genetics and Genomics Database (Lawrence *et al.* 2007), SNP markers from the Panzea project (www.panzea.org; Zhao *et al.* 2006), and PCR-based photoperiod response candidate gene markers to define linkage groups (Coles *et al.* 2010). SSR and candidate gene markers were genotyped using gel electrophoresis on Agarose SFR (Amresco Incorporated, Solon, OH). A total of 1536 SNP markers were genotyped using the Illumina Golden Gate SNP genotyping assay (Yan *et al.* 2009). Four candidate gene markers and 79 SSR markers were polymorphic in the B97×Ki14 population and were assayed on all RILs. Because of limited resources, only a subset of the 15 earliest flowering, 15 latest flowering, and a random sample of 98 RILs from the B97×Ki14 population were genotyped with the 1536 SNPs, of which 476 were polymorphic and reliably segregating in this population. After removing 3 RILs that seemed to be contaminated and 3 RILs that did not produce ears, we had phenotypic and genotypic data on 211 RILs. Data are included in Table S2.

QTL mapping

We mapped QTL for the staminate ear tip phenotype using the consensus linkage map for four populations (including B97×Ki14) created by Coles *et al.* (2010) because this map is based on a very large sample size and is more reliable than the individual population map. We mapped QTL using R/QTL software (Broman *et al.* 2003). The R/QTL script used is included as supporting information (File S1). Because the distribution of genotype mean values exhibited a substantial peak at zero (Figure 1), we employed the two-part binary plus normal analysis method (Broman and Sen 2009). This models the phenotype in two parts: it analyzes lines with trait values above zero using a normal phenotype model and separately analyzes the trait across all lines as a binary trait (0 or >0). The two-part model produces three LOD scores for each genomic position tested: one for the hypothesis that a QTL at that position increases the probability that the phenotype score is greater than zero; one for the hypothesis that a QTL at that position changes the mean phenotype score among lines that have a nonzero phenotype; and a third LOD score that is the sum of the scores for the individual parts of the model (Broman and Sen 2009).

We used 1000 permutations to identify genome-wide thresholds for declaring the presence of a QTL (Doerge and Churchill 1996). After identifying QTL with the two-part model, we estimated QTL effects and associated variation for both the two-part model and a standard normal model. The normal model was fit using 128 simulated genotype draws to account for missing data and interval positions between markers (Broman and Sen 2009). Approximate 95% Bayes credible intervals were estimated by rescaling the LOD scores from the initial QTL scan of a chromosome to a distribution and identifying the interval that contained 95% of the LOD distribution (Broman and Sen 2009). To further investigate the effect of trait scale on QTL detection, we transformed the original scores into four different binary scales, where RILs were classified as zero if their mean score $\leq$ 0, 1, 2, or 3; otherwise, RILs were classified as one. In each case, we used a genome scan with a binary model version of the Haley-Knott method and identified significant QTL as those with LOD scores > 3.0.
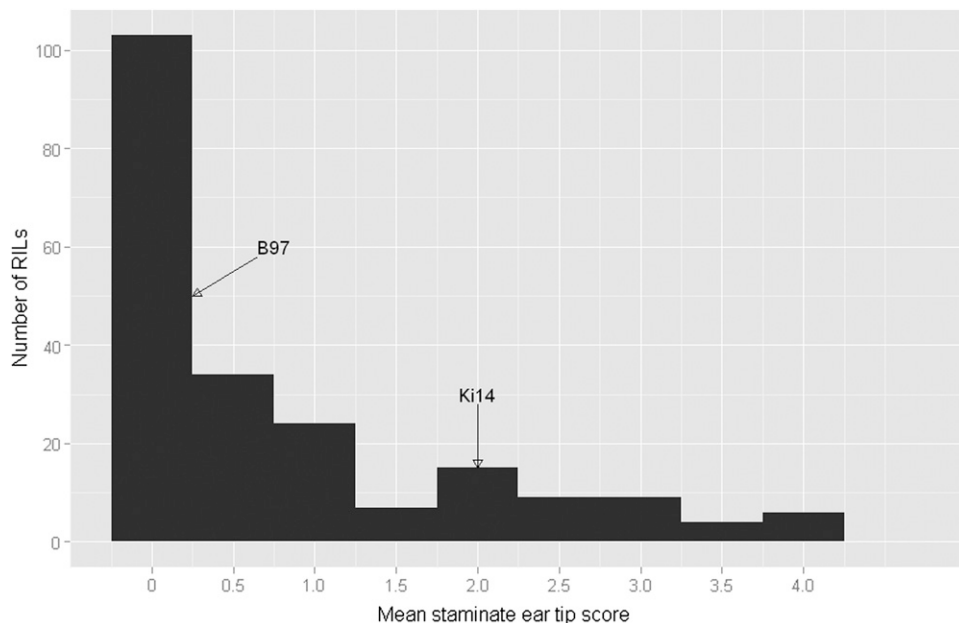
Figure 1 Histogram representing the distribution of mean staminate ear tip score among RILs derived from the cross of B97 and Ki14. RIL, recombinant inbred line.

RESULTS

Neither parental line B97 nor temperate check inbred line B73 was observed to have any staminate florets in the ear, but tropical parental line Ki14 had a score of two in both replications. The distribution of the staminate ear tip phenotype in the RIL population was distinctly non-normal, with most lines having a phenotype score of zero (no staminate florets in the ear; Figure 1). The estimate of heritability on a line mean basis was 89% (approximate standard error = 2%). Although the asymmetrical distribution and heterogeneous residual variation of the trait scale violate some assumptions of heritability estimation, the high value indicates that staminate ear tip was scored reliably in this environment. Of the 211 RILs evaluated, 28 (13%) had staminate ear tip scores greater than Ki14. Five RILs had mean scores of four, indicating that their ears had staminate tassel spikes protruding from the husks and shedding pollen.

At the genome-wise $\alpha = 0.05$ threshold, we detected two QTL with the two-part model (Figure 2). The QTL with largest effect was on chromosome 3L, with a peak at marker PZA02402.1 (133 cM on

the combined genetic map, 172 Mbp on the B73 RefGen_v2 chromosome 3 sequence; Maize Genetics and Genomics Database). This position had a large effect on ear tip masculinization when the scores were treated as binomial (0 vs. $>$0), indicating that that Ki14 alleles at this QTL increase the probability that a line will express masculinized ear tips. This observation may be interpreted as a QTL affecting the penetrance of the staminate ear tip.

A second QTL was identified on chromosome 9, near marker PZ02648.8 (87.5 cM on the combined map and 26–33 Mbp on the B73 RefGen_v2 chromosome 9 sequence). This QTL only affected the severity of the trait, among those lines that expressed staminate florets. A third QTL was identified on chromosome 10, just below the $\alpha = 0.05$ genome-wise threshold and significant at the $\alpha = 0.10$ genome-wise threshold for the joint two-part model LOD score. This QTL was located near marker PZA00647.9 (78.3 cM on the combined map, 127 to 128 Mbp on the B73 RefGen_v2 chromosome 10 sequence). The chromosome 3 QTL was associated with 15% of the variation for staminate ear spike treated as a binary
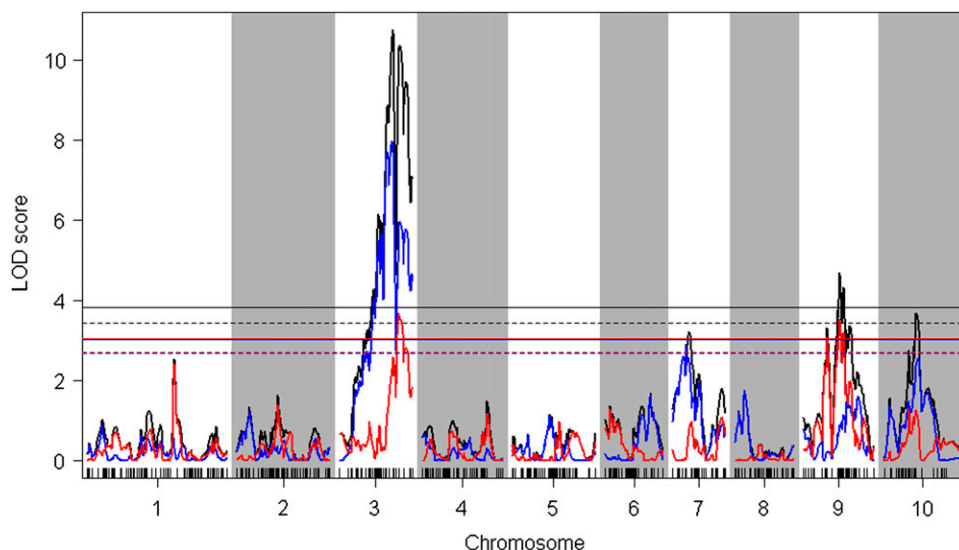

Figure 2 LOD scores from full genome scan across 10 chromosome pairs of maize using two-part model for staminate ear tip score. Black curve represents total two-part model LOD score, blue curve represents LOD score for penetrance, and red curve represents LOD score for severity at each genome position. Dashed horizontal lines represent genome-wise $\alpha = 0.10$ LOD threshold, and solid horizontal lines represent genome-wise $\alpha = 0.05$ LOD threshold for declaring a significant QTL based on 1000 permutations.

■ **Table 1 Summary of binary and normal model QTL positions, effects, and associated phenotypic variation for staminate ear tip score in B97×Ki14 RILs**

Chromosome	QTL Position (cM)			Binary Model			Normal Model		
	95% Bayes Credible Interval Start	LOD Peak	95% Bayes Credible Interval End	Additive Effect	% Variation	P (χ^2)	Additive Effect	% Variation	P (F-test)
3	133.2	133.2	146.2	0.62	15.1	4.0×10^{-9}	0.44	12.4	8.5×10^{-9}
9	55.7	87.5	115.6	–	–	–	0.25	5.5	9.1×10^{-5}
10	59.6	78.3	78.3	–	–	–	0.26	5.4	9.9×10^{-5}
Combined model					15.1			29.0	

RIL, recombinant inbred line.

character and with 12% of the variation for the score on the original scale (Table 1). The QTL on chromosomes 9 and 10 each were associated with about 5% of the variation individually for variation in the original score scale. The three QTL combined were associated with 29% of the variation for the original score variation (Table 1). Tests for epistasis among these three QTL indicated that there was no significant epistatic variation.

Genome scans of alternate binary classifications revealed that the chromosome 3 QTL was significant (LOD > 3.0) for all cases, except for the classification into ≤3.0 vs. >3.0 groups. The chromosome 9 QTL was significant for classification into ≤1.0 vs. >1.0, the chromosome 10 QTL was significant for classification into ≤2.0 vs. >2.0, and both chromosome 9 and 10 QTL were significant for classification into ≤3.0 vs. >3.0 group. These results are congruent with the chromosome 3 QTL acting as the primary determinant of masculinization of ear tips and the chromosomes 9 and 10 QTL acting as minor effect QTL that increase the expression of the trait.

DISCUSSION

Inspection of the three-locus genotype values reveals that most, but not all, RILs homozygous for B97 alleles at all three QTL positions had scores of zero (Figure 3). A few of those RILs had mean scores up to 1.5, however. RILs homozygous for Ki14 alleles at all three loci had a mean score of about two, equal to Ki14 itself. This three-locus model cannot explain the transgressive RIL segregants with scores up to four; perhaps B97 alleles at QTL with small effects that were not detected

promote higher scores in combination with Ki14 alleles at the three major QTL loci. Indeed, the LOD peak observed on chromosome 7 (Figure 2), which was below the threshold for declaring a significant QTL, had a small (2%) effect on variation when fit in a model with the other three QTL, but it was unusual in that the B97 alleles at this region increased staminate ear tip score (Figure S1).

Emerson and Emerson (1922) described the *anther ear* (*an1*) mutation that results in staminate ear tips, which we observed in the B97×Ki14 RIL population. The *an1* mutation, however, also produces stamens throughout the ear, including in florets that produce seeds (Emerson and Emerson 1922). Furthermore, *an1* and its paralog *an2* map to chromosome 1, where no QTL for staminate ear tip was found. Therefore, we conclude that the genes controlling staminate ear tip in this population are distinct from *an1* and *an2*.

We found a strong QTL for the staminate ear tip phenotype on chromosome 3 in a region where several floral development genes have previously been mapped, including *terminal ear1* (*te1*) and *tassel seed4* (*ts4*) (Chuck *et al.* 2007; Veit *et al.* 1998). The phenotypic effects of known mutations at these genes are distinct from the staminate ear tip phenotype we observed in this population, however. *te1* and *ts4* may produce feminized tassels rather than masculinized ears, and they affect leaf initiation and meristem branching in ways not observed in this study. The 95% Bayes credible interval containing this QTL corresponds to positions 171.4 to 176.3 Mbp on the B73 RefGen_V2 sequence. *te1* is located at 165.1 Mbp and *ts4* is at 136 Mbp, outside of the QTL interval.
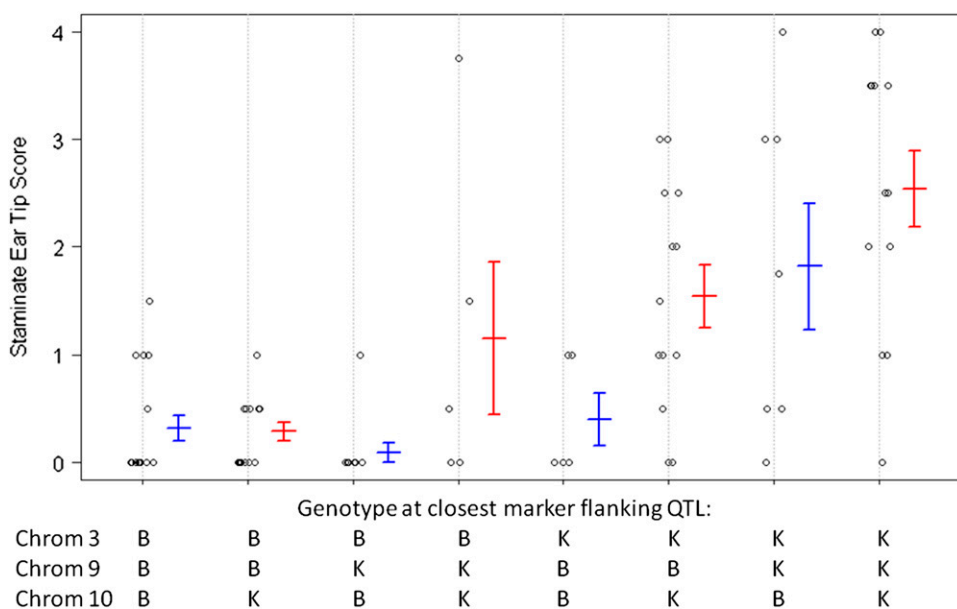

Figure 3 Three-locus genotype values for staminate ear tip score for RILs with complete genotype scores at markers at peak of three QTL. Y-axis is mean staminate ear tip score for each RIL, and X-axis indicates two-allele genotypes at each of three markers. Genotype classes at the marker closest to the LOD peak of the three QTL (PZA02402.1 on chromosome 3, PZA02648.2 on chromosome 9, and PZA00647.9 on chromosome 10) are given as "B" for lines homozygous B97 allele and "K" for lines homozygous for the Ki14 allele. Vertical bars represent standard errors for three-locus genotype means, where the central hash mark represents the mean. RIL, recombinant inbred line.

Genotype at closest marker flanking QTL:								
Chrom 3	B	B	B	B	K	K	K	K
Chrom 9	B	B	K	K	B	B	K	K
Chrom 10	B	K	B	K	B	K	B	K

A similar region of chromosome 3 was shown to have a strong effect on the fraction of primary lateral inflorescence spikelets that were male in multiple crosses between maize and teosinte (Briggs *et al.* 2007; Doebley and Stec 1991; Doebley and Stec 1993; Doebley *et al.* 1995). However, these teosinte QTL intervals end near a marker located at 163 Mbp on chromosome 3, just before the start of the approximate 95% Bayes credible interval of the staminate ear tip QTL on chromosome 3. Therefore, we have evidence that the staminate ear tip QTL segregating in maize is linked to, but distinct from, a major QTL controlling proportion of staminate florets in the terminal lateral inflorescence in maize-teosinte hybrid populations. Imprecision in QTL location due to environmental and experimental error affecting phenotypic scoring and a 10-cM gap in our map immediately adjacent to the proximal side of our QTL interval prevent drawing a definite conclusion about the relationship between the chromosome 3 QTL mapped in this study and that mapped previously by Doebley and colleagues (Briggs *et al.* 2007; Doebley *et al.* 1995).

The smaller effect QTL for staminate ear tip that we identified on chromosomes 9 and 10 did not overlap previously identified domestication QTL or known floral sex determination genes. However, the 95% Bayes credible support intervals for both of these regions overlapped QTL identified for photoperiod response for silking date and other developmental traits (*ZmPR3* and *ZmPR4*) in this population by Coles *et al.* (2010). Staminate ear tips were observed in this population only under long daylengths. The overlap of QTL for photoperiod response and staminate ear tips suggests that staminate ear tips may be produced as a result of photoperiod-induced flowering delay specifically in genotypes most sensitive to photoperiod under long daylengths.

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