Natural variation in teosinte at the domestication locus *teosinte branched1* (*tb1*)[1]

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Abstract

Premise of the study: The *teosinte branched1* (*tb1*) gene is a major QTL controlling branching differences between maize and its wild progenitor, teosinte. The insertion of a transposable element (*Hopscotch*) upstream of *tb1* is known to enhance the gene's expression, causing reduced tillering in maize. Observations of the maize *tb1* allele in teosinte and estimates of an insertion age of the *Hopscotch* that predates domestication led us to investigate its prevalence and potential role in teosinte.

Methods: Prevalence of the *Hopscotch* element was assessed across an Americas-wide sample of 1110 maize and teosinte individuals using a co-dominant PCR assay. Population genetic summaries were calculated for a subset of individuals from four teosinte populations in central Mexico. Phenotypic data were also collected from a single teosinte population where *Hopscotch* was found segregating.

Key results: Genotyping results suggest the *Hopscotch* element is at higher than expected frequency in teosinte. Analysis of linkage disequilibrium near *tb1* does not support recent introgression of the *Hopscotch* allele from maize into teosinte. Population genetic signatures are consistent with selection on this locus revealing a potential ecological role for *Hopscotch* in teosinte. Finally, two greenhouse experiments with teosinte do not suggest *tb1* controls tillering in natural populations.

Conclusions: Our findings suggest the role of *Hopscotch* differs between maize and teosinte. Future work should assess *tb1* expression levels in teosinte with and without the *Hopscotch* and more comprehensively phenotype teosinte to assess the ecological significance of the *Hopscotch* insertion and, more broadly, the *tb1* locus in teosinte.

Key words: domestication; maize; teosinte; *teosinte branched1*; transposable element

INTRODUCTION

Domesticated crops and their wild progenitors provide an excellent system in which to study adaptation and genomic changes associated with human-mediated selection (Ross-Ibarra et al., 2007). Perhaps the central focus of the study of domestication has been the identification of genetic variation underlying agronomically important traits such as fruit size and plant architecture (Olsen and Gross, 2010). Additionally, many domesticates show reduced genetic diversity when compared to their wild progenitors, and an understanding of the distribution of diversity in the wild and its phenotypic effects has become increasingly useful to crop improvement (Kovach and McCouch, 2008). But while some effort has been invested into understanding how wild alleles behave in their domesticated relatives (e.g. Bai and Lindhout, 2007), very little is known about the role that alleles found most commonly in domesticates play in natural populations of their wild progenitors (Whitton et al., 1997).

Maize (*Zea mays* ssp. *mays*) was domesticated from the teosinte *Zea mays* ssp. *parviglumis* (hereafter, *parviglumis*) roughly 9,000 B.P. in southwest Mexico (Piperno et al., 2009; Matsuoka et al., 2002). Domesticated maize and the teosintes are an attractive system in which to study domestication due to the abundance of genetic tools developed for maize and well-characterized domestication loci (Hufford et al., 2012a; Doebley, 2004; Hufford et al., 2012b). Additionally, large naturally occurring populations of both *Zea mays* ssp. *parviglumis* (the wild progenitor of maize) and *Zea mays* ssp. *mexicana* (highland teosinte; hereafter *mexicana*) can be found throughout Mexico (Wilkes, 1977; Hufford et al., 2013), and genetic diversity of these taxa is estimated to be high (Ross-Ibarra et al., 2009).

Many morphological changes are associated with maize domestication, and understanding the genetic basis of these changes has been a focus of maize research for a number of years (Doebley, 2004). One of the most dramatic changes is found in plant architecture: domesticated maize is characterized by a central stalk with few tillers and lateral branches terminating in a female inflorescence, while teosinte is highly tillered and bears tassels (male inflorescences) at the end of its lateral branches. The *teosinte branched1* (*tb1*) gene, a repressor of organ growth, was identified as a major QTL involved in branching (Doebley et al., 1995) and tillering (Doebley and Stec, 1991) differences between maize and teosinte. A 4.9 kb retrotransposon (*Hopscotch*) insertion into the upstream control region of *tb1* in maize acts to enhance expression of *tb1*, thus repressing lateral organ growth (Doebley et al., 1997; Studer et al., 2011). Dating of the

Hopscotch retrotransposon suggests that its insertion predates the domestication of maize, leading to the hypothesis that it was segregating as standing variation in ancient populations of teosinte and increased to high frequency in maize due to selection during domestication (Studer et al., 2011). The effects of the *Hopscotch* insertion have been studied in maize (Studer et al., 2011), and analysis of teosinte alleles at *tb1* has identified functionally distinct allelic classes (Studer and Doebley, 2012), but little is known about the role of *tb1* or the *Hopscotch* insertion in natural populations of teosinte.

In teosinte and other plants that grow at high population density, individuals detect competition from neighbors via the ratio of red to far-red light. An increase in far-red relative to red light accompanies shading and triggers the shade avoidance syndrome: a suite of physiological and morphological changes such as reduced tillering, increased plant height and early flowering (Kebrom and Brutnell, 2007). The *tb1* locus appears to play an important role in the shade avoidance pathway in *Zea mays* and other grasses and may therefore be crucial to the ecology of teosinte (Kebrom and Brutnell, 2007; Lukens and Doebley, 1999). In this study we aim to characterize the distribution of the *Hopscotch* insertion in *parviglumis*, *mexicana*, and landrace maize, and to examine the phenotypic effects of the insertion in *parviglumis*. We use a combination of PCR genotyping for the *Hopscotch* element in our full panel and sequencing of two small regions upstream of *tb1* in a subset of teosinte populations to explore patterns of genetic variation at this locus. Finally, we test for an association between the *Hopscotch* element and tillering phenotypes in a population of *parviglumis*.

MATERIALS AND METHODS

Sampling and genotyping—We sampled 1,110 individuals from 350 accessions (247 maize landraces, 17 *mexicana* populations, and 86 *parviglumis* populations) and assessed the presence or absence of the *Hopscotch* insertion (Appendix 1 and Appendix 2, See Supplemental Materials with the online version of this article). DNA was extracted from leaf tissue using a modified CTAB approach (Doyle and Doyle, 1990; Maloof et al., 1984). We designed primers using PRIMER3 (Rozen and Skaletsky, 2000) implemented in Geneious (Kearse et al., 2012) to amplify the entire *Hopscotch* element, as well as an internal primer allowing us to simultaneously check for possible PCR bias between presence and absence of the *Hopscotch* insertion. Two PCRs were performed for each individual, one with primers flanking the *Hopscotch* (HopF/HopR) and one with a flanking primer and an internal primer (HopF/HopIntR). Primer sequences are HopF,

5'-TCGTTGATGCTTTGATGGATGG-3'; HopR, 5'-AACAGTATGATTTCATGGGACCG-3'; and HopIntR, 5'-CCTCCACCTCTCATGAGATCC-3' (Appendix 3 and Appendix 4, See Supplemental Materials with the online version of this article). Homozygotes show a single band for absence of the element (∼300bp) and two bands for presence of the element (∼5kb and ∼1.1kb), whereas heterozygotes are three-banded (Appendix 2, See Supplemental Materials with the online version of this article). When only one PCR resolved well, we scored one allele for the individual. We used Phusion High Fidelity Enzyme (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and the following conditions for amplifications: 98°C for 3 min, 30 cycles of 98°C for 15 s, 65°C for 30 s, and 72°C for 3 min 30 s, with a final extension of 72°C for 10 min. PCR products were visualized on a 1% agarose gel and scored for presence/absence of the *Hopscotch* based on band size.

Sequencing—In addition to genotyping, we chose a subset of *parviglumis* individuals for sequencing. We chose twelve individuals from each of four populations from Jalisco state, Mexico (San Lorenzo, La Mesa, Ejutla A, and Ejutla B). For amplification and sequencing, we selected two regions approximately 600bp in size from within the 5' UTR of *tb1* (Region 1) and from 1,235bp upstream of the start of the *Hopscotch* (66,169bp upstream from the start of the *tb1* ORF; Region 2). We designed the following primers using PRIMER3 (Rozen and Skaletsky, 2000): for the 5' UTR, 5-'GGATAATGTGCACCAGGTGT-3' and 5'-GCGTGCTAGAGACACYTGTTGCT-3'; for the 66kb upstream region, 5'-TGTCCTCGCCGCAACTC-3' and 5'-TGTACGCCCGCCCCTCATCA-3' (Appendix 1, See Supplemental Materials with the online version of this article). We used Taq polymerase (New England Biolabs Inc., Ipswich, Massachusetts, USA) and the following thermal cycler conditions to amplify fragments: 94°C for 3 min, 30 cycles of 92°C for 40 s, annealing for 1 min, 72°C for 40 s, and a final 10 min extension at 72°C. Annealing temperatures for Region 1 and Region 2 were 59.7°C and 58.8°C, respectively. To clean excess primer and dNTPs we added two units of Exonuclease1 and 2.5 units of Antarctic Phosphatase to 8.0 μL of amplification product. This mix was placed on a thermal cycler with the following program: 37°C for 30 min, 80°C for 15 min, and a final cool-down step to 4°C.

We cloned cleaned fragments into a TOPO-TA vector (Life Technologies, Grand Island, New York, USA) using OneShot TOP10 chemically competent *E. coli* cells, with an extended ligation time of 30 min for a complex target fragment. We plated cells on LB agar plates containing kanamycin, and screened colonies using vector primers M13 Forward and M13 Reverse under the following conditions: 96°C for 5 min; then 35 cycles at 96°C for 30 s, 53°C for 30 s, 72°C for 2

min; and a final extension at 72°C for 4 min. We visualized amplification products for incorporation of our insert on a 1% agarose TAE gel.

Amplification products with successful incorporation of our insert were cleaned using Exonuclease 1 and Antarctic Phosphatase following the procedures detailed above, and sequenced with vector primers M13 Forward and M13 Reverse using Sanger sequencing at the College of Agriculture and Environmental Sciences (CAES) sequencing center at UC Davis. We aligned and trimmed primer sequences from resulting sequences using the software Geneious (Kearse et al., 2012). Following alignment, we verified singleton SNPs by sequencing an additional one to four colonies from each clone. If the singleton was not present in these additional sequences it was considered an amplification or cloning error, and we replaced the base with the base of the additional sequences. If the singleton appeared in at least one of the additional sequences we considered it a real variant and kept it for further analyses.

Genotyping analysis—To calculate differentiation between populations (F_{ST}) and subspecies (F_{CT}) we used HierFstat (Goudet, 2005). These analyses only included populations in which eight or more individuals were sampled. To test the hypothesis that the *Hopscotch* insertion may be adaptive under certain environmental conditions, we looked for significant associations between the *Hopscotch* frequency and environmental variables using BayEnv (Coop et al., 2010). BayEnv creates a covariance matrix of relatedness between populations and then tests a null model that allele frequencies in populations are determined by the covariance matrix of relatedness alone against the alternative model that allele frequencies are determined by a combination of the covariance matrix and an environmental variable, producing a posterior probability (*i.e.,* Bayes Factor; Coop et al. 2010). We used genotyping and covariance data from Pyhäjärvi et al. (2013) for BayEnv, with the *Hopscotch* insertion coded as an additional SNP. Environmental data were obtained from `www.worldclim.org`, the Harmonized World Soil Database (FAO/IIASA/ISRIC/ISSCAS/JRC, 2012) and `www.harvestchoice.org` and summarized by principle component analysis following Pyhäjärvi et al. (2013).

Sequence analysis—For population genetic analyses of sequenced Region 1 and sequenced Region 2 we used the Libsequence package (Thornton, 2003) to calculate pairwise F_{ST} between populations and to calculate standard diversity statistics (number of haplotypes, haplotype diversity, Watterson's estimator $\hat{\theta}_W$, pairwise nucleotide diversity $\hat{\theta}_\pi$, and Tajima's D). To produce a visual representation of differentiation between sequences and examine patterns in sequence clustering by *Hopscotch* genotype we used Phylip

(`http://evolution.genetics.washington.edu/phylip.html`), creating neighbor-joining trees with bootstrap-supported nodes (100 repetitions). For creation of trees we also included homologous sequence data from Maize HapMapV2 (Chia et al., 2012) for teosinte inbred lines (TILs), some of which are known to be homozygous for the *Hopscotch* insertion (TIL03, TIL17, TIL09), as well as 59 lines of domesticated maize.

Introgression analysis—In order to assess patterns of linkage disequilibrium (LD) around the *Hopscotch* element in the context of chromosomal patterns of LD we used Tassel (Bradbury et al., 2007) and calculated LD between SNPs across chromosome 1 using previously published data from twelve plants each of the Ejutla A (EjuA), Ejutla B (EjuB), San Lorenzo (SLO), and La Mesa (MSA) populations (Pyhäjärvi et al., 2013). We chose these populations because we had both genotyping data for the *Hopscotch* as well as chromosome-wide SNP data for chromosome 1. For each population we filtered the initial set of 5,897 SNPs on chromosome 1 to accept only SNPs with a minor allele frequency of at least 0.1, resulting in 1,671, 3,023, 3,122, and 2,167 SNPs for SLO, EjuB, EjuA, and MSA, respectively. We then used Tassel (Bradbury et al., 2007) to calculate linkage disequilibrium (r^2) across chromosome 1 for each population.

We examined evidence of introgression on chromosome 1 in these same four populations (EjuA, EjuB, MSA, SLO) using STRUCTURE (Falush et al., 2003) and phased data from Pyhäjärvi et al. (2013), combined with the corresponding SNP data from a diverse panel of 282 maize lines (Cook et al., 2012). SNPs were anchored in a modified version of the IBM genetic map (Gerke et al., 2013). We created haplotype blocks using a custom Perl script that grouped SNPs separated by less than 5kb into haplotypes. We ran STRUCTURE at K=2 under the linkage model, performing 3 replicates with an MCMC burn-in of 10,000 steps and 50,000 steps post burn-in.

Phenotyping of *parviglumis*—To investigate the phenotypic effects of the *Hopscotch* insertion in teosinte, we conducted an initial phenotyping trial (Phenotyping 1). We germinated 250 seeds of *parviglumis* collected in Jalisco state, Mexico (population San Lorenzo) (Hufford, 2010) where the *Hopscotch* is segregating at highest frequency (0.44) in our initial genotyping sample set. In order to maximize the likelihood of finding the *Hopscotch* in our association population we selected seeds from sites where genotyped individuals were homozygous or heterozygous for the insertion. We chose between 10-13 seeds from each of 23 sampling sites. We treated seeds with Captan fungicide (Southern Agricultural Insecticides Inc., Palmetto, Florida, USA) and germinated them in petri dishes with filter paper. Following germination, 206

successful germinations were planted into one-gallon pots with potting soil and randomly spaced one foot apart on greenhouse benches. Plants were watered three times a day by hand and with an automatic drip containing 10-20-10 fertilizer.

Starting on day 15, we measured tillering index as the ratio of the sum of tiller lengths to the height of the plant (Briggs et al., 2007). Following initial measurements, we phenotyped plants for tillering index every 5 days through day 40, and then on day 50 and day 60. On day 65 we measured culm diameter between the third and fourth nodes of each plant. Culm diameter is not believed to be correlated with tillering index or variation at *tb1*. Following phenotyping we extracted DNA from all plants using a modified SDS extraction protocol. We genotyped individuals for the *Hopscotch* insertion following the protocols listed above. Based on these initial data, we conducted a *post hoc* power analysis using data from day 40 of Phenotyping 1, indicating that a minimum of 71 individuals in each genotypic class would be needed to detect the observed effect of the *Hopscotch* on tillering index.

We performed a second phenotyping experiment (Phenotyping 2) in which we germinated 372 seeds of *parviglumis*, choosing equally between sites previously determined to have or not have the *Hopscotch* insertion. Seeds were germinated and planted on day 7 post fruit-case removal into two gallon pots. Plants were watered twice daily, alternating between fertilized and non-fertilized water. We began phenotyping successful germinations (302 plants) for tillering index on day 15 post fruit-case removal, and phenotyped every five days until day 50. At day 50 we measured culm diameter between the third and fourth nodes. We extracted DNA and genotyped plants following the same guidelines as in Phenotyping 1.

Tillering index data for each genotypic class did not meet the criteria for a repeated measures ANOVA, so we transformed the data using a Box-Cox transformation ($\lambda = 0$) Car Package for R, Fox and Weisberg 2011) to improve the normality and homogeneity of variance among genotype classes. We analyzed relationships between genotype and tillering index and tiller number using a repeated measures ANOVA through a general linear model function implemented in SAS v.9.3 (SAS Institute Inc., Cary, NC, USA). Additionally, in order to compare any association between *Hopscotch* genotype and tillering and associations at other presumably unrelated traits, we performed an ANOVA between culm diameter and genotype using the same general linear model in SAS.

RESULTS

Genotyping—Genotype of the *Hopscotch* insertion was confirmed with two PCRs for 837 individuals. Among the 247 maize landrace accessions genotyped, all but eight were homozygous for the presence of the insertion (Appendix 1 and Appendix 2, See Supplemental Materials with the online version of this article). Within our *parviglumis* and *mexicana* samples we found the *Hopscotch* insertion segregating in 37 and four populations, respectively, and at highest frequency in the states of Jalisco, Colima, and Michoacán in central-western Mexico (Fig. 1).Using our *Hopscotch* genotyping, we calculated differentiation between populations (F_{ST}) and subspecies (F_{CT}) for populations in which we sampled eight or more alleles. We found that $F_{CT} = 0$, and levels of F_{ST} among populations within each subspecies (0.22) and among all populations (0.23) are similar to those reported genome-wide in previous studies (Pyhäjärvi et al. 2013; Table 1). Although we found large variation in *Hopscotch* allele frequency among our populations, BayEnv analysis did not indicate a correlation between the *Hopscotch* insertion and environmental variables (all Bayes Factors < 1).

Sequencing—To investigate patterns of sequence diversity and linkage disequilibrium (LD) in the *tb1* region, we sequenced two small (<1kb) regions upstream of the *tb1* ORF in four populations. After alignment and singleton checking we recovered 48 and 40 segregating sites for the 5' UTR region (Region 1) and the 66kb upstream region (Region 2), respectively. For Region 1, Ejutla A has the highest values of haplotype diversity and θ_π, while Ejutla B and La Mesa have comparable values of these summary statistics, and San Lorenzo has much lower values. Additionally, Tajima's D is strongly negative in the two Ejutla populations and La Mesa, but is less negative in San Lorenzo (Table 2, Appendix 2, See Supplemental Materials with the online version of this article). For Region 2, haplotype diversity and θ_π, are similar for Ejutla A and Ejutla B, while La Mesa and San Lorenzo have slightly lower values for these statistics (Table 2). Tajima's D is positive in all populations except La Mesa, indicating an excess of low frequency variants in this population (Table 2). Pairwise values of F_{ST} within population pairs Ejutla A/Ejutla B and San Lorenzo/La Mesa are close to zero for both sequenced regions as well as for the *Hopscotch*, while they are high for other population pairs (Table 1). Neighbor joining trees of our sequence data and data from the teosinte inbred lines (TILs; data from Maize HapMapV2, Chia et al. 2012) do not reveal any clear clustering pattern with respect to population or *Hopscotch* genotype (Appendix 5, See Supplemental Materials with the online version of this

article); individuals within our sample that have the *Hopscotch* insertion do not group with the teosinte inbred lines or domesticated maize that have the *Hopscotch* insertion.

Evidence of introgression—The highest frequency of the *Hopscotch* insertion in teosinte was found in *parviglumis* sympatric with cultivated maize. Our initial hypothesis was that the high frequency of the *Hopscotch* element in these populations could be attributed to introgression from maize into teosinte. To investigate this possibility we examined overall patterns of linkage disequilibrium across chromosome one and specifically in the *tb1* region. If the *Hopscotch* is found in these populations due to recent introgression we would expect to find large blocks of linked markers near this element. We find no evidence of elevated linkage disequilibrium between the *Hopscotch* and SNPs surrounding the *tb1* region in our resequenced populations (Figure 2), and r^2 in the *tb1* region does not differ significantly between populations with (average r^2 of 0.085) and without (average $r^2 = 0.082$) the *Hopscotch* insertion. In fact, average r^2 is lower in the *tb1* region ($r^2 = 0.056$) than across the rest of chromosome 1 ($r^2 = 0.083$; Table 3).

The lack of clustering of *Hopscotch* genotypes in our NJ tree as well as the lack of LD around *tb1* do not support the hypothesis that the *Hopscotch* insertion in these populations of *parviglumis* is the result of recent introgression. However, to further explore this hypothesis we performed a STRUCTURE analysis using Illumina MaizeSNP50 data from four of our *parviglumis* populations (EjuA, EjuB, MSA, and SLO) and the maize 282 diversity panel (Cook et al., 2012; Pyhäjärvi et al., 2013). The linkage model implemented in STRUCTURE can be used to identify ancestry of blocks of linked variants which would arise as the result of recent admixture between populations. If the *Hopscotch* insertion is present in populations of *parviglumis* as a result of recent admixture with domesticated maize, we would expect the insertion and linked variants in surrounding sites to be assigned to the "maize" cluster in our STRUCTURE runs, not the "teosinte" cluster. In all runs, assignment to maize in the *tb1* region across all four *parviglumis* populations is low (average 0.017) and much below the chromosome-wide average (0.20; Table 4; Fig. 3).

Phenotyping—To assess the contribution of *tb1* to phenotypic variation in tillering in a natural population, we grew plants from seed sampled from the San Lorenzo population of *parviglumis*, which had a high mean frequency (0.44) of the *Hopscotch* insertion based on our initial genotyping. We measured tillering index (TI), the ratio of the sum of tiller lengths to plant height, for 216 plants (Phenotyping 1) from within the San Lorenzo population, and genotyped plants for the *Hopscotch* insertion. We found the *Hopscotch* segregating at a frequency of 0.65 with no significant deviations from expected frequencies under Hardy-Weinberg equilibrium.

After performing a repeated measures ANOVA between our transformed tillering index data and *Hopscotch* genotype we find no correlation between genotype at the *Hopscotch* insertion and tillering index (Fig. 4), tiller number, or culm diameter.

We performed a second grow-out of *parviglumis* from San Lorenzo (Phenotyping 2) to assess whether lighting conditions or sample size may have affected our ability to detect an effect of *tb1*. For the second grow-out we measured tillering index every five days through day 50 for 302 plants. We found the *Hopscotch* allele segregating at a frequency of 0.69, with a 0.6 frequency of *Hopscotch* homozygotes, and a 0.2 frequency of both heterozygotes and homozygotes for the teosinte allele. Results were similar to Phenotyping 1, with no significant correlation between *Hopscotch* and any of the three phenotypes measured.

DISCUSSION

Adaptation occurs due to selection on standing variation or *de novo* mutations. Adaptation from standing variation has been well-described in a number of systems; for example, selection for lactose tolerance in humans (Plantinga et al., 2012; Tishkoff et al., 2007), variation at the *Eda* locus in three-spined stickleback (Kitano et al., 2008; Colosimo et al., 2005), and pupal diapause in the Apple Maggot fly (Feder et al., 2003). Although the adaptive role of standing variation has been described in many systems, its importance in domestication is not as well studied.

In maize, alleles at domestication loci (*RAMOSA1*, Sigmon and Vollbrecht 2010; *barren stalk1*, Gallavotti et al. 2004; and *grassy tillers1*, Whipple et al. 2011) are thought to have been selected from standing variation, suggesting that diversity already present in teosinte may have played an important role in maize domestication. The *teosinte branched1* gene is one of the best characterized domestication loci, and, while previous studies have suggested that differences in plant architecture between maize and teosinte are a result of selection on standing variation at this locus (Clark et al., 2006; Studer et al., 2011), much remains to be discovered regarding natural variation at this locus and its ecological role in teosinte.

Studer et al. (2011) genotyped 90 accessions of teosinte (inbred and outbred), providing the first evidence that the *Hopscotch* insertion is segregating in teosinte (Studer et al., 2011). Given that the *Hopscotch* insertion has been estimated to predate the domestication of maize, it is not surprising that it can be found segregating in populations of teosinte. However, by widely sampling across teosinte populations our study provides greater insight into the distribution and prevalence of the *Hopscotch* in teosinte. While our findings are consistent with Studer et al.

(2011) in that we identify the *Hopscotch* allele segregating in teosinte, we find it at higher frequency than previously suggested. Many of our populations with a high frequency of the *Hopscotch* allele fall in the Jalisco cluster identified by Fukunaga et al. (2005), perhaps suggesting a different history of the *tb1* locus in this region than in the Balsas River Basin where maize was domesticated (Matsuoka et al., 2002). Potential explanations for the high frequency of the *Hopscotch* element in *parviglumis* from the Jalisco cluster include gene flow from maize, genetic drift, and natural selection.

While gene flow from crops into their wild relatives is well-known, (Ellstrand et al., 1999; Zhang et al., 2009; Thurber et al., 2010; Baack et al., 2008; Hubner et al., 2012; Wilkes, 1977; van Heerwaarden et al., 2011; Barrett, 1983), our results are more consistent with Hufford et al. (2013) who found resistance to introgression from maize into teosinte around domestication loci. We find no evidence of recent introgression in our analyses. Clustering in our NJ trees do not reflect the pattern expected if maize alleles at the *tb1* locus had introgressed into populations of teosinte. Moreover, there is no signature of elevated LD in the *tb1* region relative to the rest of chromosome 1, and Bayesian assignment to a maize cluster in this region is both low and below the chromosome-wide average (Fig. 3, Table 4). Together, these data point to an explanation other than recent introgression for the high observed frequency of *Hopscotch* in a subset of our *parviglumis* populations.

Although recent introgression seems unlikely, we cannot rule out ancient introgression as an explanation for the presence of the *Hopscotch* in these populations. If the *Hopscotch* allele was introgressed in the distant past, recombination may have broken up LD, a process that would be consistent with our data. We find this scenario less plausible, however, as there is no reason why gene flow should have been high in the past but absent in present-day sympatric populations. In fact, early generation maize-teosinte hybrids are common in these populations today (MB Hufford, pers. observation), and genetic data support ongoing gene flow between domesticated maize and both *mexicana* and *parviglumis* in a number of sympatric populations (Hufford et al., 2013; Ellstrand et al., 2007; van Heerwaarden et al., 2011).

Remaining explanations for differential frequencies of the *Hopscotch* among teosinte populations include both genetic drift and natural selection. Previous studies using both SSRs and genome-wide SNP data have found evidence for a population bottleneck in the San Lorenzo population (Hufford, 2010; Pyhäjärvi et al., 2013), and the lower levels of sequence diversity in this population in the 5' UTR (Region 1) coupled with more positive values of Tajima's D are

consistent with these earlier findings. Such population bottlenecks can exaggerate the effects of genetic drift through which the *Hopscotch* allele may have risen to high frequency entirely by chance. A bottleneck in San Lorenzo, however, does not explain the high frequency of the *Hopscotch* in multiple populations in the Jalisco cluster. Moreover, available information on diversity and population structure among Jaliscan populations (Hufford, 2010; Pyhäjärvi et al., 2013) is not suggestive of recent colonization or other demographic events that would predict a high frequency of the allele across populations. Finally, diversity values in the 5' UTR of *tb1* are suggestive of natural selection acting upon the gene in natural populations of *parviglumis*. Overall nucleotide diversity is 76% less than seen in the sequences from the 66kb upstream region, and Tajima's D is considerably lower and consistently negative. In fact, values of Tajima's D in the 5' UTR are toward the extreme negative end of the distribution of this statistic previously calculated across loci sequenced in *parviglumis* (Wright et al., 2005; Moeller et al., 2007). Though not definitive, these results are consistent with the action of selection on the upstream region of *tb1*, perhaps suggesting an ecological role for the gene in *parviglumis*.

Significant effects of the *Hopscotch* insertion on lateral branch length, number of cupules, and tillering index in domesticated maize have been well documented (Studer et al., 2011). Weber et al. (2007) described significant phenotypic associations between markers in and around *tb1* and lateral branch length and female ear length in a sample from 74 natural populations of *parviglumis* (Weber et al., 2007); however, these data did not include markers from the *Hopscotch* region 66kb upstream of *tb1*. Our study is the first to explicitly examine the phenotypic effects of the *Hopscotch* insertion across a wide collection of individuals sampled from natural populations of teosinte. We have found no significant effect of the *Hopscotch* insertion on tillering index or tiller number, a result that is discordant with its clear phenotypic effects in maize. One interpretation of this result would be that the *Hopscotch* controls tillering in maize (Studer et al., 2011), but tillering in teosinte is affected by variation at other loci. Consistent with this interpretation, *tb1* is thought to be part of a complex pathway controlling branching, tillering and other phenotypic traits (Kebrom and Brutnell, 2007; Clark et al., 2006). A recent study by Studer and Doebley (2012) examined variation across traits in a three-taxa allelic series at the *tb1* locus. Studer and Doebley (2012) introgressed nine unique teosinte *tb1* segments (one from *Zea diploperennis*, and four each from *mexicana* and *parviglumis*) into an inbred maize background and investigated their phenotypic effects. Phenotypes were shown to cluster by taxon, indicating *tb1* may underlie morphological diversification of *Zea*. Additional analysis in Studer and Doebley

(2012) suggested tillering index was controlled both by *tb1* and loci elsewhere in the genome. Clues to the identity of these loci may be found in QTL studies that have identified loci controlling branching architecture (*e.g.,* Doebley and Stec 1991, 1993). Many of these loci (*grassy tillers*, *gt1*; *tassel-replaces-upper-ears1*, *tru1*; *terminal ear1*, *te1*) have been shown to interact with *tb1* (Whipple et al., 2011; Li, 2012), and both *tru1* and *te1* affect the same phenotypic traits as *tb1* (Doebley et al., 1995). *tru1*, for example, has been shown to act either epistatically or downstream of *tb1*, affecting both branching architecture (decreased apical dominance) and tassel phenotypes (shortened tassel and shank length and reduced tassel number; Li 2012). Variation in these additional loci may have affected tillering in our collections and contributed to the lack of correlation we see between *Hopscotch* genotype and tillering. Finally, although photoperiod for Phenotyping 2 reasonably approximated that of the normal *parviglumis* growing season, greenhouse-specific environmental conditions (plant density, light regime, etc...) may have contributed to tillering responses different from those found in nature, obscuring the effect of the *Hopscotch* insertion on variation.

In conclusion, our findings demonstrate that the *Hopscotch* allele is more widespread in populations of *parviglumis* and *mexicana* than previously thought. Analysis of linkage using SNPs from across chromosome 1 does not suggest that the *Hopscotch* allele is present in these populations due to recent introgression; however, it seems unlikely that the insertion would have drifted to high frequency in multiple populations. We do, however, find preliminary evidence of selection on the *tb1* locus in *parviglumis*; this coupled with our observation of high frequency of the *Hopscotch* insertion in a number of populations suggests that the locus may play an ecological role in teosinte. In contrast to domesticated maize, the *Hopscotch* insertion does not appear to have a large effect on tillering in *parviglumis*. Future studies should examine expression levels of *tb1* in teosinte with and without the *Hopscotch* insertion and further characterize the effects of additional loci involved in branching architecture (e.g. *gt1*, *tru1*, and *te1*). These data, in conjunction with more exhaustive phenotyping, should help reveal the ecological significance of the domesticated *tb1* allele in natural populations of teosinte.

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Table 1. Pairwise F_{ST} values from sequence and *Hopscotch* genotyping data

Comparison	**Region 1**	**Region 2**	***Hopscotch***
EjuA & EjuB	0	0	0
EjuA & MSA	0.326	0.328	0.186
EjuA & SLO	0.416	0.258	0.280
EjuB & MSA	0.397	0.365	0.188
EjuB & SLO	0.512	0.290	0.280
MSA & SLO	0.007	0	0.016

Table 2. Population genetic statistics from resequenced regions near the *tb1* locus

Population	**# Haplotypes**	**Hap. Diversity**	$\hat{\theta}_\pi$	**Tajima's D**
Region 1(5' UTR)				
EJUA	8	0.859	0.005	-1.650
EJUB	5	0.709	0.004	-1.831
MSA	6	0.682	0.004	-1.755
SLO	3	0.318	0.001	-0.729
Region 2 (66kb upstream)				
EJUA	8	0.894	0.018	0.623
EJUB	8	0.894	0.016	0.295
MSA	3	0.682	0.011	-0.222
SLO	4	0.742	0.014	0.932

Table 3. mean r^2 values between SNPs on chromosome 1, in the broad *tb1* region, within the 5' UTR of *tb1* (Region 1), and 66kb upstream of *tb1* (Region 2).

Population	**Chr. 1**	***tb1* region**	**Region 1**	**Region 2**
Ejutla A	0.095	0.050	0.747	0.215
Ejutla B	0.069	0.051	0.660	0.186
La Mesa	0.070	0.053	0.914	0.766
San Lorenzo	0.101	0.067	0.912	0.636

Table 4. Assignments to maize and teosinte in the *tb1* and chromosome 1 regions from STRUCTURE

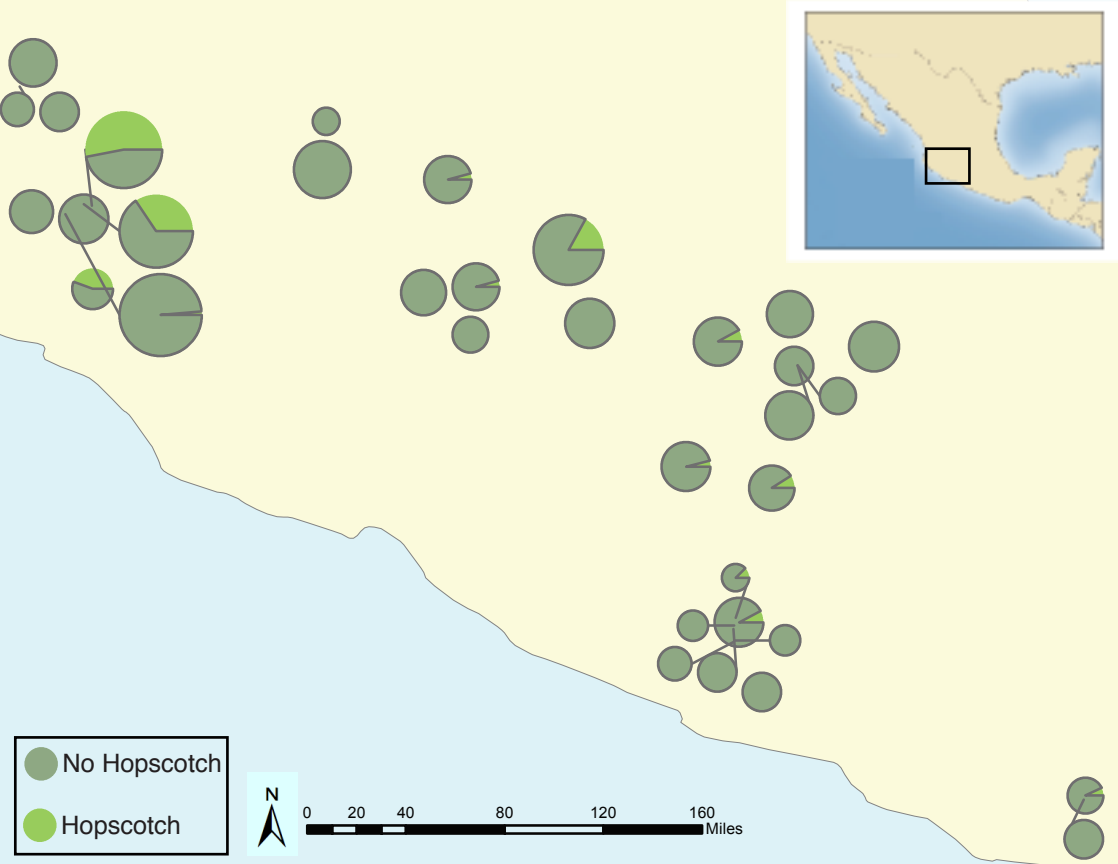
	***tb1* region**		**Chr 1**	
Population	**Maize**	**Teosinte**	**Maize**	**Teosinte**
Ejutla A	0.022	0.978	0.203	0.797
Ejutla B	0.019	0.981	0.187	0.813
La Mesa	0.012	0.988	0.193	0.807
San Lorenzo	0.016	0.984	0.205	0.795

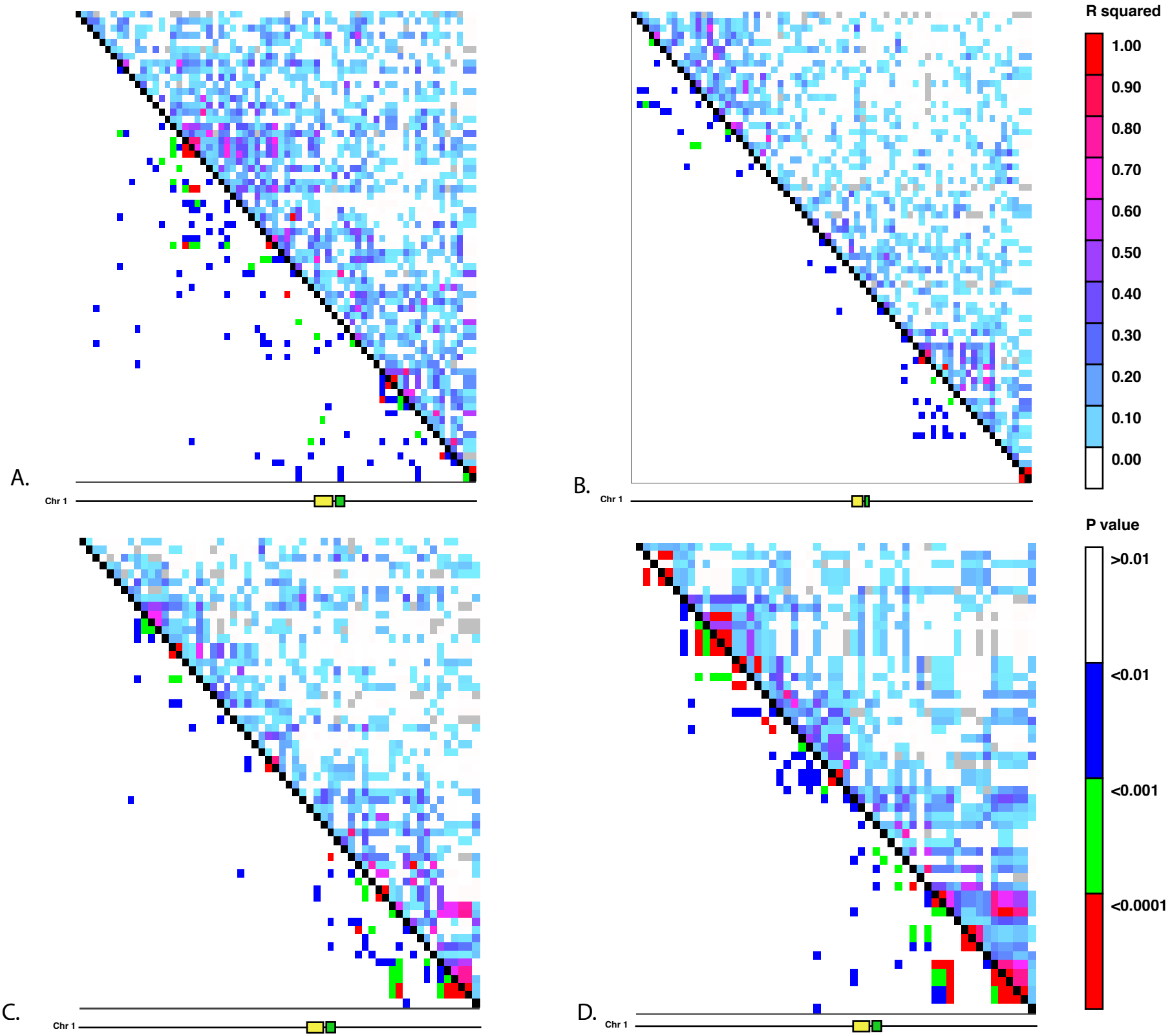
Figure 1. Map showing the frequency of the *Hopscotch* allele in populations of *parviglumis* where we sampled more than 6 individuals. Size of circles reflects number of alleles sampled.

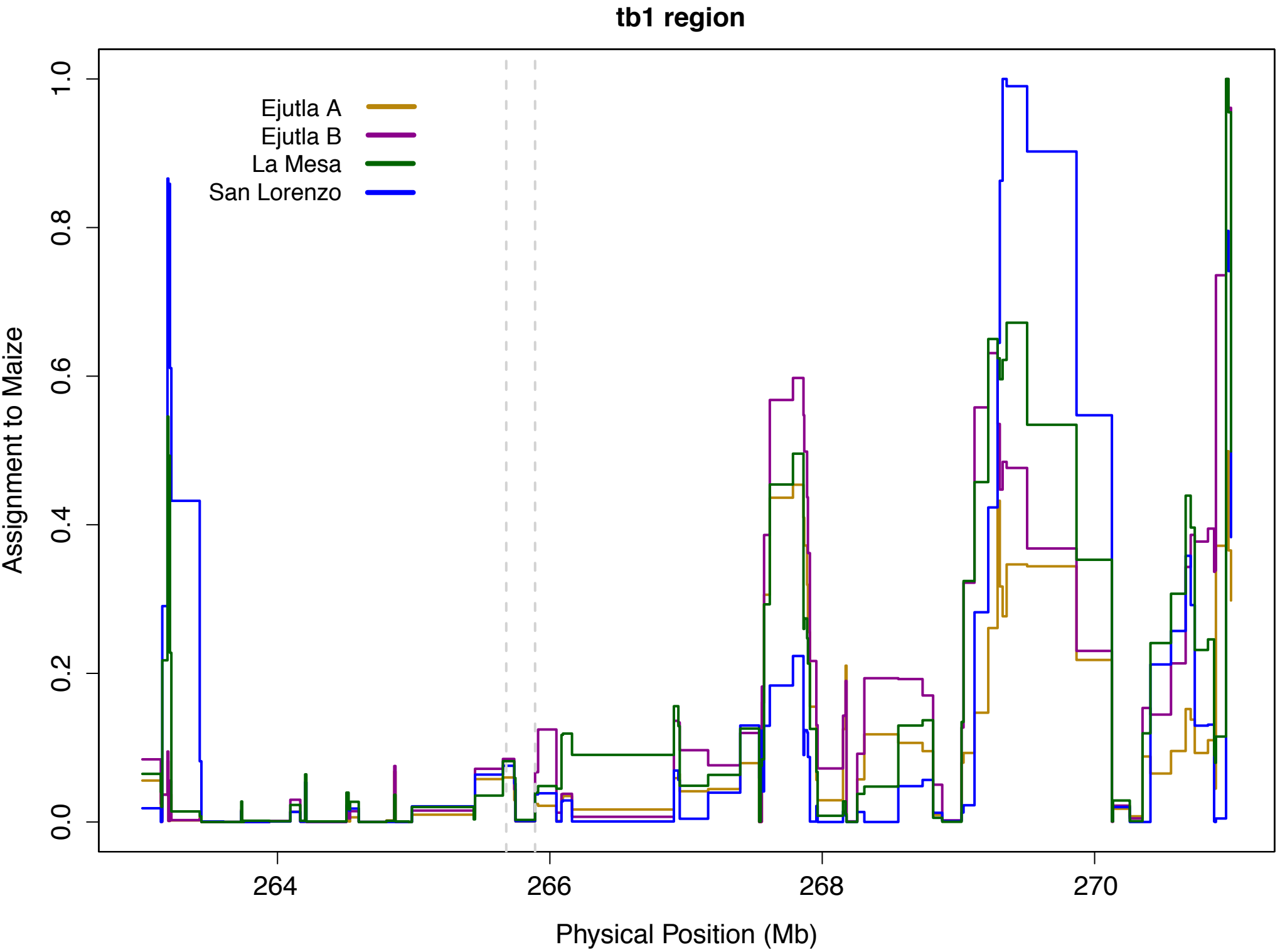
Figure 2. Linkage disequilibrium for SNPs in Mb 261-268 on chromosome 1. The yellow rectangle indicates the location of the *Hopscotch* insertion and the green represents the *tb1* ORF. A) Ejutla A; B) Ejutla B; C) La Mesa; D) San Lorenzo. The upper triangle above the black diagonal is colored based on the r^2 value between SNPs while the bottom triangle is colored based on p-value for the corresponding r^2 value.

Figure 3. STRUCTURE assignment to maize across a section of chromosome 1. The dotted lines mark the beginning of the sequenced region 66kb upstream (Region 2) and the end of the *tb1* ORF.

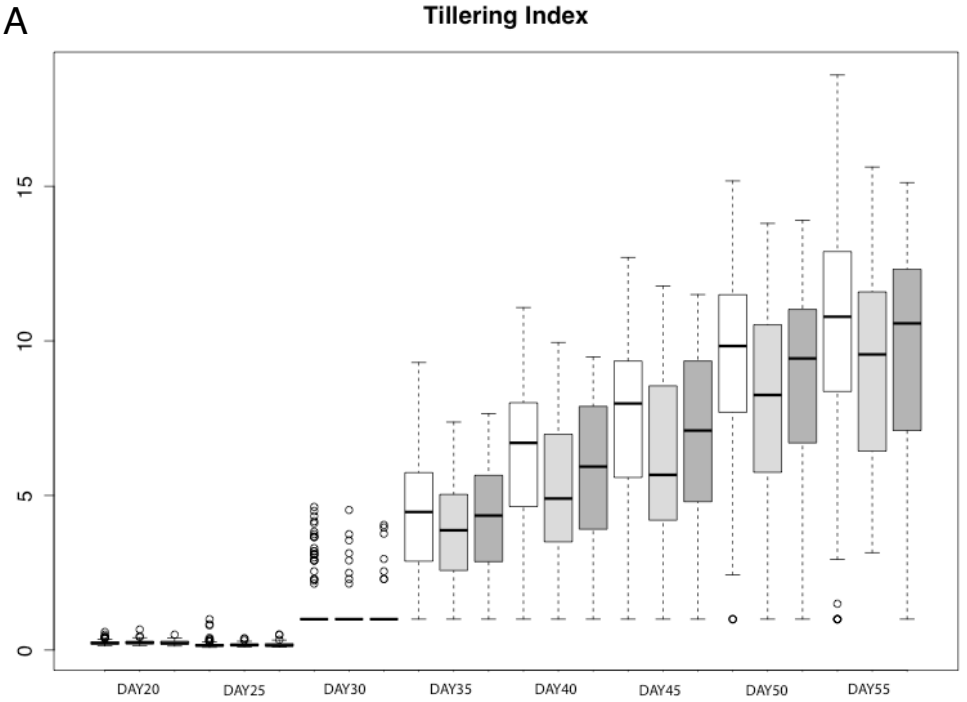
Figure 4. Box-plots showing tillering index in greenhouse grow-outs for Phenotyping 1 (A) and Phenotyping 2 (B). White indicates individuals homozygous for the *Hopscotch*, light grey represents heterozygotes, and dark grey represents homozygotes for the teosinte (No *Hopscotch*) allele. Within boxes, dark black lines represent the median, and the edges of the boxes are the first and third quartiles. Outliers are displayed as dots, the maximum value excluding outliers is shown with the top whisker, while the minimum excluding outliers is shown with the bottom whisker.


No Hopscotch
Hopscotch
N
0
20
40
80
120
160
Miles

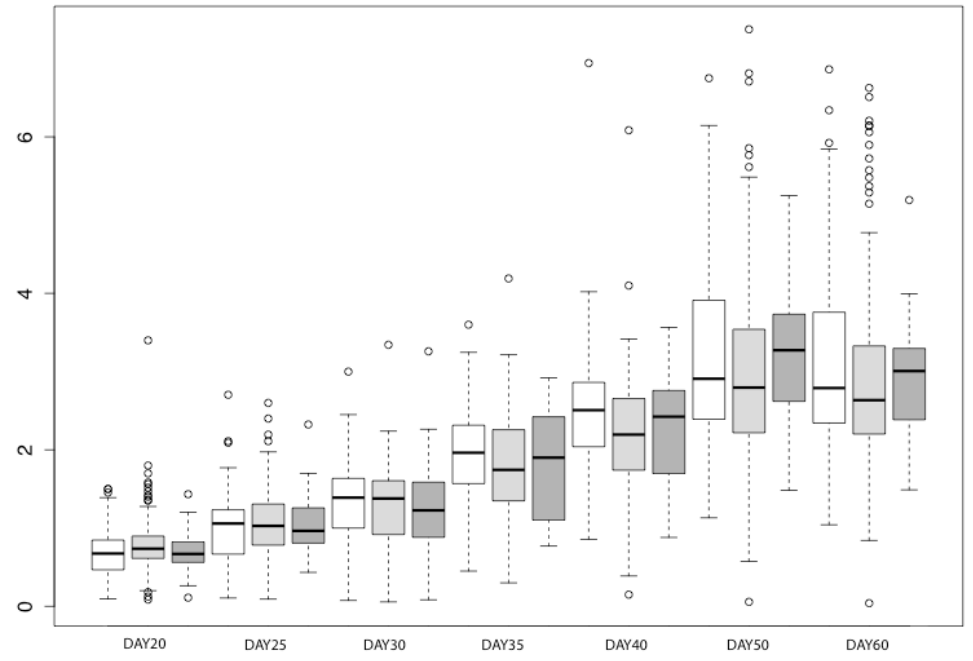

A.
Chr 1
B.
Chr 1
R squared
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
C.
Chr 1
D.
Chr 1
P value
>0.01
<0.01
<0.001
<0.0001


tb1 region
Ejutla A
Ejutla B
La Mesa
San Lorenzo
Assignment to Maize
0.0
0.2
0.4
0.6
0.8
1.0
264
266
268
270
Physical Position (Mb)

A


Tillering Index
0
5
10
15
DAY20
DAY25
DAY30
DAY35
DAY40
DAY45
DAY50
DAY55

B


Tillering Index
0
2
4
6
DAY20
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DAY30
DAY35
DAY40
DAY50
DAY60

Appendix S1: Accessions of *Zea mays* ssp. *mexicana* (RIMME) and *Zea mays* ssp. *parviglumis* (RIMPA) sampled. RIHY is a *Z. mays* ssp. *parviglumis* and *Zea mays* ssp. *mays* hybrid.

Accession	USDA ID	Locality	Alleles Sampled	*Hopscotch* Freq.	No *Hopscotch* Freq.
RIHY0009	N/A	N/A	2	0.5	0.5
RIMME0006	566673	Durango	2	0	1
RIMME0007	566680	Guanajuato	2	0	1
RIMME0008	566681	Michoacan	2	0	1
RIMME0009	566682	Distrito Federal	2	0	1
RIMME0011	566685	Mexico	2	0	1
RIMME0014	714151	Breeders line	6	0	1
RIMME0017	699874	Ayotlan	8	0	1
RIMME0021	N/A	El Porvenir	69	0.17	0.83
RIMME0026	N/A	Opopeo	42	0.07	0.93
RIMME0028	N/A	Puruandiro	28	0.04	0.96
RIMME0029	N/A	Ixtlan	35	0	1
RIMME0030	N/A	San Pedro	27	0	1
RIMME0031	N/A	Tenango del Aire	25	0.08	0.92
RIMME0032	N/A	Nabogame	24	0	1
RIMME0033	N/A	Puerta Encantada	25	0	1
RIMME0034	N/A	Santa Clara	23	0	1
RIMME0035	N/A	Xochimilco	25	0	1
RIMPA0001	87168	El Salado	4	0	1
RIMPA0003	87171	Mazatlan	8	0.13	0.87
RIMPA0017	87200	N/A	4	0	1
RIMPA0019	87213	El Salado	2	0.50	0.50
RIMPA0029	87244	N/A	2	0.50	0.50
RIMPA0031	87249	N/A	2	0.5	0.5
RIMPA0035	87288	Jalisco	4	0	1
RIMPA0040	288185	Mexico	4	0	1
RIMPA0042	288187	Guerrero	4	0.25	0.75
RIMPA0043	288188	Guerrero	4	0	1
RIMPA0045	288193	Guerrero	4	0	1
RIMPA0055	714152	Breeders line	2	0	1
RIMPA0056	714153	Breeders line	2	0.50	0.50
RIMPA0057	714154	Breeders line	2	0.50	0.50
RIMPA0058	N/A	N/A	4	0.50	0.50
RIMPA0059	N/A	N/A	4	1	0
RIMPA0060	714157	Breeders line	2	0	1
RIMPA0061	714158	Breeders line	4	0.5	0.5
RIMPA0062	714159	Breeders line	4	0.5	0.5
RIMPA0063	714160	Breeders line	4	0	1
RIMPA0064	714161	Breeders line	3	0	1
RIMPA0065	714162	Breeders line	4	0.25	0.75
RIMPA0068	699861	Jalisco, Mexico	16	0	1
RIMPA0069	699862	Ixtlan	14	0.14	0.86
RIMPA0070	699863	Benito Jaurez	16	0	1
RIMPA0071	699864	Tuzantla	28	0	1
RIMPA0072	699865	Tiquicheo	16	0	1
RIMPA0073	699866	Tiquicheo	16	0.12	0.88
RIMPA0074	699867	Huetamo	12	0	1
RIMPA0075	699868	Huetamo	2	0	1
RIMPA0076	699869	Huetamo	4	0	1
RIMPA0077	699870	Caracuaro	2	0	1
RIMPA0078	699871	Caracuaro	2	0.5	0.5
RIMPA0079	699872	Villa Madero	14	0	1
RIMPA0080	699873	Guachinango	12	0	1
RIMPA0081	699875	Ameca	16	0	1
RIMPA0083	699877	Tepoztlan	14	0	1
RIMPA0084	699878	Tepoztlan	16	0	1
RIMPA0085	699879	Miahuatlan	16	0	1
RIMPA0086	699880	Miahuatlan	16	0.06	0.94
RIMPA0087	699881	Tecoanapa	24	0	1
RIMPA0089	699883	Guerrero	12	0	1
RIMPA0090	699884	Guerrero	10	0	1
RIMPA0091	699885	Guerrero	16	0	1
RIMPA0092	699886	Guerrero	10	0	1

Accession	USDA ID	Locality	Alleles Sampled	*Hopscotch* Freq.	No *Hopscotch* Freq.
RIMPA0093	699887	Guerrero	26	0.08	0.92
RIMPA0094	699888	Guerrero	2	0	1
RIMPA0095	699889	Guerrero	4	0	1
RIMPA0096	699890	Guerrero	26	0.04	0.96
RIMPA0097	699891	Guerrero	6	0	1
RIMPA0098	699892	Guerrero	4	0	1
RIMPA0099	699893	Guerrero	4	0	1
RIMPA0100	699894	Guerrero	6	0	1
RIMPA0101	699895	Guerrero	2	0	1
RIMPA0103	699897	Guerrero	2	0	1
RIMPA0104	699898	Guerrero	22	0.09	0.91
RIMPA0105	699899	Guerrero	6	0	1
RIMPA0106	699900	Guerrero	6	0.33	0.67
RIMPA0107	699901	Guerrero	4	0	1
RIMPA0108	699902	Guerrero	6	0	1
RIMPA0109	699903	Michoacan	4	0.25	0.75
RIMPA0110	699904	Michoacan	2	0	1
RIMPA0111	699905	Michoacan	4	0	1
RIMPA0112	699906	Michoacan	4	0.25	0.75
RIMPA0114	699908	Michoacan	6	0.17	0.83
RIMPA0116	699910	Mexico	2	0	1
RIMPA0117	699911	Mexico	4	0	1
RIMPA0118	699912	Mexico	6	0.17	0.83
RIMPA0119	699913	Mexico	2	0	1
RIMPA0120	699914	Mexico	1	1	0
RIMPA0121	699915	Mexico	2	0	1
RIMPA0128	699922	Mexico	2	0.5	0.5
RIMPA0129	699923	Michoacan	2	0.5	0.5
RIMPA0135	699929	Nayarit	24	0	1
RIMPA0138	699932	Jalisco	2	0.5	0.5
RIMPA0139	699933	Jalisco	1	1	0
RIMPA0142	699936	Colima	18	0.44	0.56
RIMPA0144	699938	Jalisco	2	1	0
RIMPA0145	699939	Michoacan	1	1	0
RIMPA0147	699941	Jalisco	1	1	0
RIMPA0155	N/A	Jalisco	73	0.01	0.99
RIMPA0156	N/A	Jalisco	20	0	1
RIMPA0157	N/A	Jalisco	58	0.34	0.66
RIMPA0158	N/A	Jalisco	64	0.53	0.47
RIMPA0159	N/A	Jalisco	26	0	1
RIMPA0162	21785	N/A	4	0	1

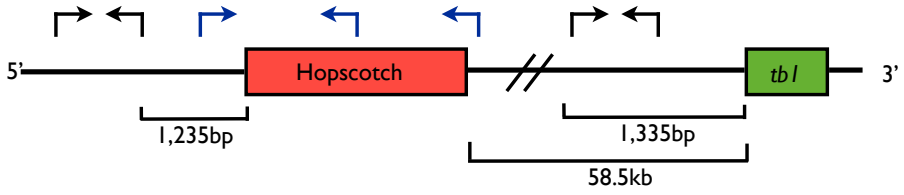
Appendix S2: *Hopscotch* frequency in sampled *Zea mays* ssp. *mays* (RIMMA).

Accession	Alleles Sampled	*Hopscotch* Freq.
RIMMA0066	2	1
RIMMA0075	2	1
RIMMA0077	2	1
RIMMA0079	2	1
RIMMA0081	2	1
RIMMA0084	2	1
RIMMA0086	2	1
RIMMA0088	2	1
RIMMA0089	2	1
RIMMA0090	2	1
RIMMA0092	4	1
RIMMA0094	4	1
RIMMA0097	2	1
RIMMA0099	2	1
RIMMA0100	2	1
RIMMA0101	2	1
RIMMA0104	2	1
RIMMA0108	2	1
RIMMA0111	6	1
RIMMA0115	2	1
RIMMA0117	2	1
RIMMA0130	2	1
RIMMA0133	2	1
RIMMA0134	2	1
RIMMA0135	2	1
RIMMA0142	2	0.5
RIMMA0143	4	1
RIMMA0146	4	1
RIMMA0149	2	1
RIMMA0152	2	1
RIMMA0153	2	1
RIMMA0154	2	1
RIMMA0155	2	1
RIMMA0156	2	1
RIMMA0157	2	1
RIMMA0158	2	1
RIMMA0159	2	1
RIMMA0160	2	1
RIMMA0162	2	1
RIMMA0166	2	1
RIMMA0167	2	1
RIMMA0168	2	1
RIMMA0169	2	1
RIMMA0172	2	1
RIMMA0174	4	1
RIMMA0177	2	1
RIMMA0178	2	1
RIMMA0179	2	1
RIMMA0181	2	1
RIMMA0183	2	1
RIMMA0184	2	1
RIMMA0186	2	1
RIMMA0187	1	1
RIMMA0188	2	1
RIMMA0195	2	1
RIMMA0196	2	1
RIMMA0197	2	1
RIMMA0198	2	1
RIMMA0199	2	1
RIMMA0200	2	1
RIMMA0202	2	1
RIMMA0203	2	1
RIMMA0206	2	1
RIMMA0208	2	1
RIMMA0209	2	1

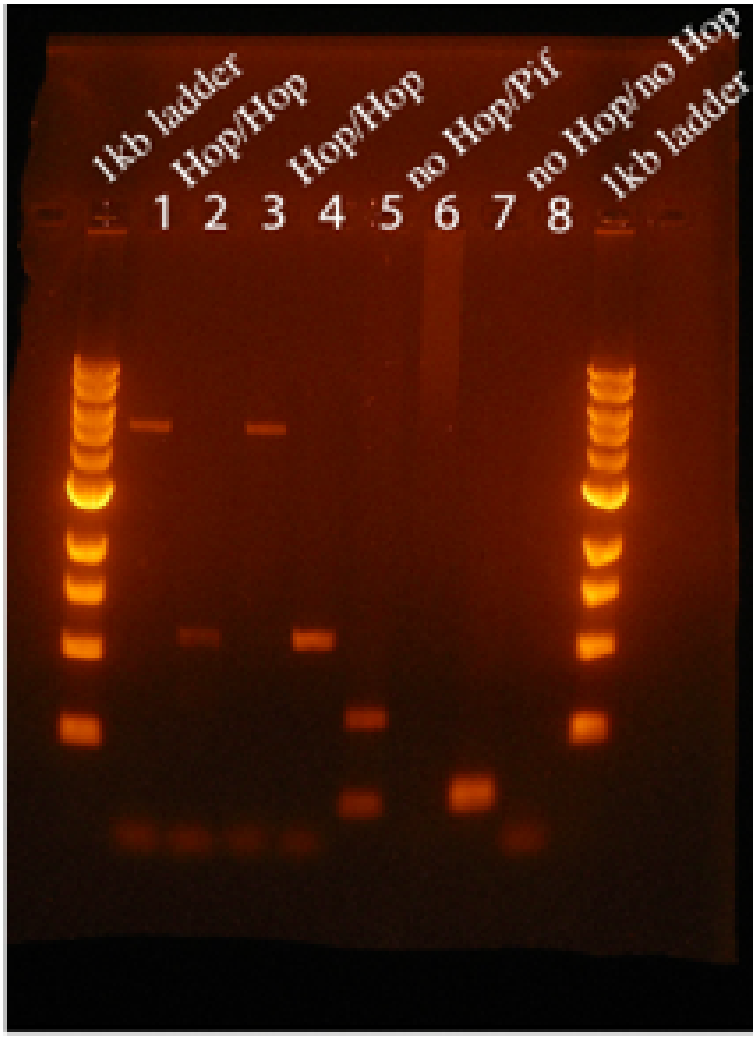
Accession	Alleles Sampled	*Hopscotch* Freq.
RIMMA0210	2	1
RIMMA0212	2	1
RIMMA0213	2	1
RIMMA0214	2	1
RIMMA0217	2	1
RIMMA0218	2	1
RIMMA0220	2	1
RIMMA0221	2	1
RIMMA0222	2	1
RIMMA0223	2	1
RIMMA0226	2	1
RIMMA0227	2	1
RIMMA0228	2	1
RIMMA0229	2	1
RIMMA0230	2	1
RIMMA0232	2	1
RIMMA0233	2	1
RIMMA0235	2	0.5
RIMMA0242	2	1
RIMMA0243	2	1
RIMMA0247	4	1
RIMMA0248	2	1
RIMMA0249	2	1
RIMMA0252	2	1
RIMMA0253	2	1
RIMMA0254	2	1
RIMMA0256	2	1
RIMMA0257	2	1
RIMMA0258	2	1
RIMMA0259	2	1
RIMMA0260	2	1
RIMMA0262	2	1
RIMMA0263	2	1
RIMMA0264	2	1
RIMMA0265	2	1
RIMMA0268	2	1
RIMMA0269	2	1
RIMMA0270	2	1
RIMMA0272	2	1
RIMMA0275	2	1
RIMMA0276	2	1
RIMMA0277	2	1
RIMMA0279	2	1
RIMMA0280	2	1
RIMMA0283	2	1
RIMMA0285	2	1
RIMMA0288	2	1
RIMMA0290	2	1
RIMMA0291	2	1
RIMMA0292	2	1
RIMMA0293	2	1
RIMMA0298	2	1
RIMMA0302	2	1
RIMMA0305	2	1
RIMMA0310	2	1
RIMMA0312	2	1
RIMMA0320	2	1
RIMMA0322	2	1
RIMMA0324	2	1
RIMMA0334	2	1
RIMMA0336	2	1
RIMMA0337	2	1
RIMMA0338	2	1
RIMMA0339	2	1
RIMMA0340	2	1
RIMMA0341	2	1
RIMMA0342	2	1

Accession	Alleles Sampled	*Hopscotch* Freq.
RIMMA0344	2	1
RIMMA0346	2	1
RIMMA0347	2	1
RIMMA0350	2	1
RIMMA0351	2	1
RIMMA0352	2	1
RIMMA0357	2	1
RIMMA0360	18	1
RIMMA0362	8	1
RIMMA0363	16	1
RIMMA0370	17	1
RIMMA0372	24	1
RIMMA0373	22	1
RIMMA0408	2	1
RIMMA0411	1	1
RIMMA0413	1	1
RIMMA0414	1	1
RIMMA0424	8	0.9
RIMMA0432	1	1
RIMMA0434	2	0.5
RIMMA0435	1	1
RIMMA0442	2	1
RIMMA0443	2	1
RIMMA0444	2	1
RIMMA0445	2	1
RIMMA0446	2	1
RIMMA0447	2	1
RIMMA0448	2	1
RIMMA0449	2	1
RIMMA0450	2	1
RIMMA0451	2	1
RIMMA0452	2	1
RIMMA0453	2	1
RIMMA0454	2	1
RIMMA0455	2	1
RIMMA0456	2	1
RIMMA0457	2	1
RIMMA0458	2	1
RIMMA0459	2	1
RIMMA0483	2	1
RIMMA0490	2	1
RIMMA0515	2	1
RIMMA0537	2	1
RIMMA0550	2	1
RIMMA0553	2	1
RIMMA0559	2	1
RIMMA0561	2	1
RIMMA0562	2	1
RIMMA0571	2	1
RIMMA0572	2	1
RIMMA0577	2	1
RIMMA0579	2	1
RIMMA0590	2	1
RIMMA0591	2	1
RIMMA0592	2	1
RIMMA0593	2	1
RIMMA0594	2	1
RIMMA0595	2	1
RIMMA0596	2	1
RIMMA0597	2	1
RIMMA0598	2	1
RIMMA0599	2	1
RIMMA0600	2	1
RIMMA0601	2	1
RIMMA0602	2	1
RIMMA0603	2	1
RIMMA0604	2	1

Accession	Alleles Sampled	*Hopscotch* Freq.
RIMMA0605	2	1
RIMMA0606	2	1
RIMMA0607	2	1
RIMMA0608	2	1
RIMMA0609	2	1
RIMMA0610	2	1
RIMMA0611	2	1
RIMMA0612	2	1
RIMMA0613	2	1
RIMMA0622	2	0.5
RIMMA0624	1	1
RIMMA0629	2	0.5
RIMMA0631	2	0.5
RIMMA0659	2	1
RIMMA0660	2	1
RIMMA0678	1	1
RIMMA0681	2	1
RIMMA0683	2	1
RIMMA0684	2	1
RIMMA0685	4	1
RIMMA0693	2	1
RIMMA0694	2	1
RIMMA0695	1	1
RIMMA0699	2	1
RIMMA0704	2	1
RIMMA0706	2	1
RIMMA0711	1	1
RIMMA0713	2	1
RIMMA0715	2	1
RIMMA0717	2	1
RIMMA0718	2	1
RIMMA0723	2	1
RIMMA0724	2	1
RIMMA0725	1	1
RIMMA0728	1	1
RIMMA0732	2	1
RIMMA0734	2	1
RIMMA0738	2	1
RIMMA0739	2	1
RIMMA0744	2	1
RIMMA0747	1	1
RIMMA0748	2	1
RIMMA0749	2	1
RIMMA0750	2	0.5
RIMMA0751	1	1
RIMMA0752	1	1
RIMMA0753	2	1
RIMMA0755	2	1



Appendix S3: Representation of the upstream regulatory region of *tb1*, showing the *tb1* coding region (green) and the *Hopscotch* insertion (red). Arrows show the location of primer sets; in black, primers used for amplification and sequencing (Region 1; within the 5' UTR, and Region 2; 66,169 bp upstream from the tb1 ORF); in blue, primers used to genotype the *Hopscotch* insertion.



Appendix S4: Agarose gel image of amplification products using our primer sets. Genotypes are indicated at the top of the gel.

Appendix S5: Neighbor-joining tree of the sequenced region in the 5' UTR (right; Region 1) and the 66,169 bp upstream region (left; Region 2) of *tb1*. Individuals with genotype data are colored: Homozygous for the teosinte (no *Hopscotch*) allele (red), homozygous for the maize (*Hopscotch*) allele (blue), heterozygotes (purple). TILs (teosinte inbred lines) are colored in green, with stars indicating the 3 TILs known to have the *Hopscotch* insertion. Black indicates individuals not genotyped for the *Hopscotch* insertion.