

1 Natural variation in teosinte at the domestication locus

2 *teosinte branched1 (tb1)*

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1 Abstract

2 The *teosinte branched1* (*tb1*) gene, a repressor of lateral organ growth, is a major QTL
3 involved in branching differences between maize and its wild progenitor, teosinte. Further
4 studies have shown that the insertion of a transposable element (*Hopscotch*) upstream of
5 *tb1* enhances its expression, causing the reduction in branching observed in domesticated
6 maize. Observations of the maize *tb1* allele in teosinte individuals, coupled with estimates
7 of the age of insertion of the *Hopscotch* element, led us to investigate the prevalence and
8 potential role of *tb1* in teosinte. Results from genotyping across many natural
9 populations suggest that the *Hopscotch* element is segregating at a higher than expected
10 frequency in a number of populations of two subspecies of teosinte, *Zea mays* ssp.
11 *parviglumis* and *Zea mays* ssp. *mexicana*. Analysis of linkage disequilibrium between the
12 *Hopscotch* element and variation in surrounding regions does not support a hypothesis of
13 recent introgression from maize into teosinte, and we find no evidence of environmental
14 correlations that might suggest recent selection. Finally, two greenhouse experiments
15 with *Zea mays* ssp. *parviglumis* do not suggest an important role for *tb1* in controlling
16 tillering in natural populations of this subspecies. Our findings suggest that the role of
17 the *Hopscotch* in tillering in teosinte is not as straightforward as is in domesticated
18 maize, and that other loci may play a role in observed variation in this trait.

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 (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 J, 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

1 lateral branches terminating in a female inflorescence, while teosinte is highly tillered and
2 bears tassels (male inflorescences) at the end of its lateral branches. The *teosinte*
3 *branched1* (*tb1*) gene, a repressor of organ growth, was identified as a major QTL
4 involved in domestication branching differences achieved through higher expression of the
5 maize allele than the teosinte allele (Doebley et al., 1995, 1997). Further work showed
6 that the insertion of a 4.9 kb retrotransposon (*Hopscotch*) in the upstream control region
7 of *tb1* caused the increased expression of this gene reduction in branching observed in
8 domesticated maize Studer et al. (2011). The effects of this insertion have been observed
9 in tiller number in maize, but little is known about its role, if any, in natural populations
10 of teosinte (Studer et al., 2011). Dating of this element has suggested that its insertion
11 predates the domestication of maize, leading to the hypothesis that it was segregating as
12 standing variation in ancient populations of teosinte and increased to high frequency in
13 maize due to selection during domestication (Studer et al., 2011). Furthermore, Studer
14 and Doebley (2012) investigated the phenotypic effects of nine teosinte *tb1* alleles in an
15 isogenic maize background and found that the introgressions sort into three distinct
16 phenotypic classes, suggesting that variation at the *tb1* locus may play a functional role
17 in teosinte.

18 In high-density species such as teosinte, plants can detect impending competition
19 from their neighbors through detection of the ratio of red to far-red light. An increase in
20 far-red relative to red light accompanies shading and triggers physiological and
21 morphological changes such as reduced tillering, increased plant height and early
22 flowering collectively known as the shade avoidance syndrome (Kebrom and Brutnell,
23 2007). The *tb1* locus appears to play an important role in the shade avoidance pathway
24 in *Zea mays* and other grasses and may therefore be crucial to the ecology of teosinte
25 (Kebrom and Brutnell, 2007; Lukens and Doebley, 1999). In this study we aim to
26 characterize the distribution of the *Hopscotch* insertion in *parviglumis*, *mexicana*, and
27 landrace maize, and to examine the phenotypic effects of the insertion in *parviglumis*. We

1 use a combination of PCR genotyping for the *Hopscotch* element in our full panel and
2 sequencing of two small regions upstream of *tb1* in a subset of teosinte populations to
3 explore patterns of genetic variation at this locus. Finally, we test for an association
4 between the *Hopscotch* element and tillering phenotypes in a population of *parviglumis*.

5 MATERIALS AND METHODS

6 *from AJB formatting instructions: add name, city, spelled-out state (if in USA), and country of*
7 *manufacturers/suppliers after brand names*

8 Sampling and genotyping

9 We sampled 1,110 individuals from 350 accessions (247 maize landraces, 17 *mexicana*
10 populations, and 86 *parviglumis* populations) and assessed the presence or absence of the
11 *Hopscotch* insertion (Table S1, Table S2). DNA was extracted from leaf tissue using a
12 modified CTAB approach (Doyle and Doyle, 1990; Maloof et al., 1984). We designed
13 primers using PRIMER3 (Rozen and Skaletsky, 2000) implemented in Geneious (Kearse
14 et al., 2012) to amplify the entire *Hopscotch* element, as well as an internal primer
15 allowing us to simultaneously check for possible PCR bias between presence and absence
16 of the *Hopscotch* insertion. Two PCRs were performed for each individual, one with
17 primers flanking the *Hopscotch* (HopF/HopR) and one with a flanking primer and an
18 internal primer (HopF/HopIntR). Primer sequences are HopF,
19 5'-TCGTTGATGCTTTGATGGATGG-3'; Hop R, 5'-AACAGTATGATTTTCATGGGACCG-3';
20 and HopIntR, 5'-CCTCCACCTCTCATGAGATCC-3' (Figure S1, Figure S2) *Primers in*
21 *Figure S1 should be labeled* . Homozygotes show a single band for absence of the element
22 (~300bp) and two bands for presence of the element (~5kb and XX *LV, please add the size of*
23 *the second band*), whereas heterozygotes are three-banded (Figure S2). When only one PCR
24 resolved well, we scored one allele for the individual. We used Phusion High Fidelity
25 Enzyme (Finnzymes, Inc.) and the following conditions for amplifications: 98°C for 3

1 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
2 extension of 72°C for 10 min. PCR products were visualized on a 1% agarose gel and
3 scored for presence/absence of the *Hopscotch* based on band size.

4 Sequencing

5 In addition to genotyping, we chose a subset of *parviglumis* individuals for sequencing.
6 We chose twelve individuals from each of four populations from Jalisco state, Mexico
7 (San Lorenzo, La Mesa, Ejutla A, and Ejutla B). For amplification and sequencing, we
8 selected two regions approximately 600bp in size from within the 5' UTR of *tb1*
9 (sequenced region 1) and from 1,235bp upstream of the start of the *Hopscotch* and
10 66,169bp upstream from the start of the *tb1* ORF (sequenced region 2). We designed the
11 following primers using PRIMER3 (Rozen and Skaletsky, 2000): for the 5' UTR,
12 5'-GGATAATGTGCACCAGGTGT-3' and 5'-GCGTGCTAGAGACACYTGTGCT-3'; for the
13 50kb upstream region, 5'-TGTCCTCGCCGCAACTC-3' and
14 5'-TGTCACGCCCCGCCCTCATCA-3' (Figure S1). We used Taq polymerase (New England
15 Biolabs) and the following thermal cycler conditions to amplify fragments: 94°C for 3
16 min, 30 cycles of 92°C for 40 s, annealing for 1 min, 72°C for 40 s, and a final 10 min
17 extension at 72°C. Annealing temperatures for sequenced region 1 and sequenced region 2
18 were 59.7°C and 58.8°C, respectively. To clean excess primer and dNTPs we added two
19 units of Exonuclease1 and 2.5 units of Antarctic Phosphatase to 8.0 μ L of amplification
20 product. This mix was placed on a thermal cycler with the following program: 37°C for
21 30 min, 80°C for 15 min, and a final cool-down step to 4°C.

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

1 30 s, 72°C for 2 min; and a final extension at 72°C for 4 min. We visualized amplification
2 products for incorporation of our insert on a 1% agarose TAE gel.
3 Amplification products with successful incorporation of our insert were cleaned using
4 Exonuclease 1 and Antarctic Phosphatase following the procedures detailed above, and
5 sequenced with vector primers M13 Forward and M13 Reverse using Sanger sequencing at
6 the College of Agriculture and Environmental Sciences (CAES) sequencing center at UC
7 Davis. We aligned and trimmed primer sequences from resulting sequences using the
8 software Geneious (Kearse et al., 2012). Following alignment, we verified singleton SNPs
9 by sequencing an additional one to four colonies from each clone. If the singleton was not
10 present in these additional sequences it was considered an amplification or cloning error,
11 and we replaced the base with the base of the additional sequences. If the singleton
12 appeared in at least one of the additional sequences we considered it a real variant and
13 kept it for further analyses.

14 Genotyping analysis

15 We examined discrepancies between observed and expected genotype frequencies by
16 calculating Hardy-Weinberg Equilibrium (HWE). To calculate differentiation between
17 populations (F_{ST}) and subspecies (F_{CT}) we used HierFstat (Goudet, 2005). These
18 analyses only included populations in which 8 or more individuals were sampled. To test
19 the hypothesis that the *Hopscotch* insertion may be adaptive under certain environmental
20 conditions, we looked for significant associations between the *Hopscotch* frequency and
21 environmental variables using BayEnv (Coop et al., 2010). BayEnv creates a covariance
22 matrix of relatedness between populations, and then tests a null model that allele
23 frequencies in populations are determined by the covariance matrix of relatedness alone
24 against the alternative model that allele frequencies are determined by a combination of
25 the covariance matrix and an environmental variable, producing a posterior probability
26 (Bayes Factor)(Coop et al., 2010). We used genotyping and covariance data from

1 Pyhäjärvi et al. (2013) for BayEnv, with the *Hopscotch* insertion coded as an additional
2 SNP (). Environmental data were obtained from www.worldclim.org, the Harmonized
3 World Soil Database and www.harvestchoice.org, and summarized by principle
4 component analysis (Pyhäjärvi et al., 2013).

5 Sequence analysis

6 For population genetic analyses of sequenced region 1 and sequenced region 2 we used the
7 analysis package of Libsequence (Thornton, 2003) to calculate pairwise F_{ST} between
8 populations, and to calculate standard diversity statistics (number of haplotypes;
9 haplotype diversity; Watterson’s estimator $\hat{\theta}_W$; pairwise nucleotide diversity $\hat{\theta}_\pi$; and
10 Tajima’s D). To produce a visual representation of differentiation between sequences and
11 to examine patterns in sequence clustering by *Hopscotch* genotype we used Phylip
12 (<http://evolution.genetics.washington.edu/phylip.html>) to create
13 neighbor-joining trees with bootstrapping (100 repetitions) to examine the support of
14 nodes in our trees. For creation of trees we also included homologous sequence data from
15 teosinte inbred lines (TILs), some of which are known to be homozygous for the
16 *Hopscotch* insertion (TIL03, TIL17, TIL09), as well as 59 lines of domesticated maize and
17 landraces (data from Maize HapMapV2, (Chia et al., 2012)).

18 Introgression analysis

19 In order to assess patterns of linkage disequilibrium (LD) around the *Hopscotch* element
20 in the context of chromosomal patterns of LD we used Tassel (Bradbury et al., 2007) and
21 calculated LD between SNPs across chromosome 1 using previously published data from
22 twelve plants each of the Ejutla A (EjuA), Ejutla B (EjuB), San Lorenzo (SLO), and La
23 Mesa (MSA) populations (Pyhäjärvi et al., 2013). We chose these populations because we
24 had both genotyping data for the *Hopscotch* as well as chromosome-wide SNP data for
25 chromosome 1. For each population we filtered the initial set of 5,897 SNPs on

1 chromosome 1 to accept only SNPs with a minor allele frequency of at least 0.1, resulting
2 in 1,671, 3,023, 3,122, and 2,167 SNPs for SLO, EjuB, EjuA, and MSA, respectively. We
3 then used Tassel (Bradbury et al., 2007) to calculate linkage disequilibrium (r^2) across
4 chromosome 1 for each population.

5 We examined evidence of introgression on chromosome 1 in these same four
6 populations (EjuA, EjuB, MSA, SLO) using STRUCTURE (Falush et al., 2003) and the
7 same phased 55K SNP data from (Pyhäjärvi et al., 2013) that we used for LD analysis,
8 combined with the corresponding SNP data from a diverse panel of 282 maize lines (Cook
9 et al., 2012). SNPs were anchored in a modified version of the IBM genetic map ((Gerke
10 et al., 2013), <http://arxiv.org/abs/1307.7313>). We created haplotype blocks using a
11 custom Perl script that grouped SNPs separated by less than 5kb into haplotypes. We ran
12 STRUCTURE at K=2 under the linkage model, performing 3 replicates with an MCMC
13 burn-in of 10,000 steps and 50,000 steps post burn-in. *i'd like this perl script on github, maybe in
14 this repo or as a gist. also structure input file too. all the stuff we'd need to redo this. See above note..not all of
15 this or the BayEnv was script'ified sure, even command line info should be included where possible. idea is to
16 maximize reproducibility – either other people or subsequent students. for example, matt has a student who wants
17 to work on tb1 in natural pops, and she might want to try/redo some of these analyses with the same or new data.
18 okay so I should just put my command line stuff with good commenting as to what is what in repository?*

19 Phenotyping of *Zea mays*. ssp. *parviglumis*

20 To investigate the phenotypic effects of the *Hopscotch* insertion in teosinte, we conducted
21 an initial phenotyping trial (Phenotyping 1). We germinated 250 seeds of *parviglumis*
22 collected in Jalisco state, Mexico (population San Lorenzo) (Hufford, 2010) where the
23 *Hopscotch* is segregating at highest frequency (0.44) in our initial genotyping sample set.
24 In order to maximize the likelihood of finding the *Hopscotch* in our association
25 population we selected seeds from sites where genotyped individuals were homozygous or
26 heterozygous for the insertion. We chose between 10-13 seeds from each of 23 sampling

1 sites. We treated seeds with fungicide and germinated them in petri dishes with filter
2 paper. Following germination, 206 successful germinations were then planted into one
3 gallon size pots with potting soil and randomly spaced one foot apart on greenhouse
4 benches. Plants were watered three times a day with an automatic drip containing
5 10-20-10 fertilizer. *it ended up being a combination of drip and hand watering because they dried out so much*
6 *and did better when they had water on the leaves as well as in the soil*

7 To investigate the phenotypic effects of the *Hopscotch* insertion in teosinte, we
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10 where the *Hopscotch* is segregating at highest frequency (0.44) in our initial genotyping
11 sample set. In order to maximize the likelihood of finding the *Hopscotch* in our
12 association population we selected seeds from sites where genotyped individuals were
13 homozygous or heterozygous for the insertion. We chose between 10-13 seeds from each of
14 23 sampling sites. We treated seeds with fungicide and germinated them in petri dishes
15 with filter paper. Successful germinations (206 individuals) were then planted into one
16 gallon size pots with potting soil and randomly spaced one foot apart on greenhouse
17 benches. Plants were watered three times a day.

18 Starting on day 15, we measured tillering index, the ratio of the sum of tiller lengths
19 to the height of the plant (Briggs et al., 2007). Tillering index has been shown to be the
20 most effective way to observe the phenotypic effects of the *Hopscotch* insertion on plant
21 architecture in maize (Clark et al., 2006). Following initial measurements, we phenotyped
22 plants for tillering index every 5 days through day 40, and then on day 50 and day 60.
23 On day 65 we measured culm diameter between the third and fourth nodes of each plant.
24 Culm diameter is not believed to be correlated with tillering index, or variation at *tb1*
25 (e.g. *Hopscotch* genotype). Following phenotyping we extracted DNA from all plants
26 using a modified SDS extraction protocol (<http://www.ars.usda.gov>). We genotyped
27 individuals for the *Hopscotch* insertion following the protocols listed above. Based on

1 these initial data, we conducted a post hoc power analysis using data from day 40 of
2 phenotyping 1, indicating that a minimum of 71 individuals in each genotype class are
3 needed to detect the observed effect of the *Hopscotch* on tillering index. *do you still have these*
4 *posthoc calculations? I believe I do, otherwise they are likely in a lab meeting slide on Dropbox – do you want*
5 *them in here? would be good to include in a github, again so we could go back and reassess how we do things in*
6 *case, for example, we decide to give the greenhouse experiment a 3rd try (yes, i'm a masochist) I would do it a*
7 *3rd time, I'm convinced something went wrong....third time with expression analyses :)*

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

17 Resulting tillering index data for each genotype class did not meet the criteria for a
18 repeated measures ANOVA, so we transformed the data using a Box-Cox transformation
19 ($\alpha = 0$) implemented in the car package in R (Fox and Weisberg, 2011) to improve the
20 normality and homogeneity of variance among genotype classes. We analyzed
21 relationships between genotype and tillering index and tiller number using a repeated
22 measures ANOVA through a general linear model function implemented in SAS v.9.3
23 (SAS Institute Inc., Cary, NC, USA). Additionally, in order to compare any association
24 between *Hopscotch* genotype and tillering and associations at other presumably unrelated
25 traits, we performed an ANOVA between culm diameter and genotype using the same
26 general linear model in SAS. *please add SAS scripts/code to a gist or something.*

RESULTS

2 Genotyping

3 Genotype of the *Hopscotch* insertion was confirmed with two PCRs for 837 individuals.
4 Among the 247 maize landrace accessions genotyped, all but 8 were homozygous for the
5 presence of the insertion (??, ??). *please fix table/figure references to say table/figure* Within our
6 *parviglumis* and *mexicana* samples we found the *Hopscotch* insertion segregating in 37
7 and 4 populations *this is confusing as the map shows 37 populations. we should be consistent about reporting*
8 *for all pops, or all pops with $n \geq 8$, etc. or at least be explicit what cutoff we are using for each result* ,
9 respectively, and at highest frequency in the states of Jalisco, Colima, and Michoacán in
10 central-western Mexico in both subspecies (1) *the map only shows parviglumis. can we add mexicana?*
11 *the text makes it seem as if both should be on the map* . We examined Hardy-Weinberg equilibrium in
12 a total of 14 populations (10 *parviglumis* and 4 *mexicana*) with more than 8 individuals
13 sampled per population. Three populations (RIMPA0073, RIMPA0093, and
14 RIMPA0158) show evidence of deviations from expected genotype frequencies under the
15 assumptions of HWE ($p < 0.05$). *in what direction? too many hets? what's the F ? they weren't all in the*
16 *same direction, do you still want me to list out? no need to list all, but if there were sig. deviations in multiple*
17 *directions in different pops, that is worth saying. maybe could list them all out in supp. table?*
18 *please fix whitespace and black border on figure*

19 Using our *Hopscotch* genotyping data, we calculated differentiation between
20 populations (F_{ST}) and subspecies (F_{CT}) for populations in which we sampled 8 or more
21 alleles. F_{CT} is 0 within our dataset, and we found similar levels of F_{ST} among
22 populations within each subspecies (0.22) *is this an average? this comes from libsequence?* and
23 among all populations (0.23), to those reported in genome-wide estimates from previous
24 studies Pyhäjärvi et al. (2013) (1).

25 Although we found large variation in *Hopscotch* allele frequency among our
26 populations, BayEnv analysis did not indicate a correlation between the *Hopscotch*

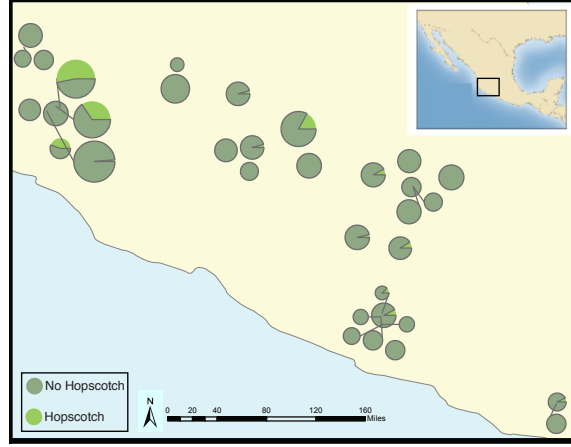


Figure 1: Map showing the frequency of the *Hopscotch* allele in populations of *Zea mays* ssp. *parviglumis* where we sampled more than 6 individuals. Size of circles reflects number of alleles sampled.

Table 1: Pairwise F_{CT} values from sequence and *Hopscotch* genotyping data

Comparison	Seq. Region 2	Seq. Region 1	<i>Hopscotch</i>
EjuA & EJuB	0	0	0
EjuA & MSA	0.328	0.326	0.186
EjuA & SLO	0.258	0.416	0.28
EjuB & MSA	0.365	0.397	0.188
EjuB & SLO	0.29	0.512	0.28
MSA & SLO	0	0.007	0.016

1 insertion and environmental variables (all Bayes Factors < 1;).

2 **Sequencing**

3 To investigate patterns of sequence diversity and linkage disequilibrium (LD) in the *tb1*
4 region, we sequenced two small (<1kb) regions upstream of the *tb1* ORF in four
5 populations. After alignment and singleton checking we recovered 40 and 48 segregating
6 sites for the 50kb upstream region and the 5' UTR region, respectively. For region 1,
7 Ejutla A has the highest values of haplotype diversity, and θ_π , while Ejutla B and La
8 Mesa have comparable values of these summary statistics, and San Lorenzo has much
9 lower values. Additionally, Tajima's D is strongly negative in the two Ejutla populations
10 and La Mesa, but is more positive in San Lorenzo (2). *can drop theta W from table and text. we*
11 *show pi and D, which is sufficient. fix caption in table. also add Hopscotch allele frequencies to table or list*
12 *somewhere – i think it's useful for comparison of seq stats the frequencies are in supplemental table 1, do you*
want them put in this table too, or just me to refer people to supp table 1? yeah refer to table is fine

Table 2: Add caption

Population	# Haplotypes	Hap. Diversity	$\hat{\theta}_W$	$\hat{\theta}_\pi$	Tajima's D
<i>Seq. region 2 (50kb upstream)</i>					
EJUA	8	0.89394	0.01548	0.01763	0.6231
EJUB	8	0.89394	0.01493	0.01591	0.29504
MSA	3	0.68182	0.01111	0.01055	-0.22212
SLO	4	0.74242	0.01167	0.01413	0.93185
<i>Seq. region 1(5' UTR)</i>					
EJUA	8	0.85897	0.00874	0.00527	-1.64955
EJUB	5	0.70909	0.00663	0.00378	-1.83123
MSA	6	0.68182	0.00646	0.00373	-1.75506
SLO	3	0.31818	0.00176	0.00137	-0.72873

13

1 For region 2, haplotype diversity, θ_W , and θ_π , are similar for Ejutla A and Ejutla B,
 2 while La Mesa and San Lorenzo have slightly lower values for these statistics (2).
 3 Tajima's D is positive in all populations except San Lorenzo, indicating an excess of low
 4 frequency variants in this population (2). Pairwise values of F_{ST} within population pairs
 5 Ejutla A/Ejutla B and San Lorenzo/La Mesa are 0 for both sequenced regions as well as
 6 for the *Hopscotch*, while they are high for other population pairs (1). Neighbor joining
 7 trees of our sequence data and data from the teosinte inbred lines (TILs; data from Maize
 8 HapMapV2, (Chia et al., 2012)) do not reveal any clear clustering pattern with respect to
 9 population or *Hopscotch* genotype (??); individuals within our sample that have the
 10 *Hopscotch* insertion do not group with the teosinte inbred lines or the lines of
 11 domesticated maize that have the *Hopscotch* insertion.

12 Evidence of introgression

13 The teosinte populations *which?* with the highest frequency of the *Hopscotch* insertion in
 14 this study were sympatric with cultivated maize. Our initial hypothesis was that the high
 15 frequency of the *Hopscotch* element in these populations could be attributed to
 16 introgression from maize into teosinte. To investigate this possibility we examined overall
 17 patterns of linkage disequilibrium across chromosome one, and specifically in the *tb1*
 18 region. If the *Hopscotch* is found in these populations due to recent introgression we
 19 would expect to find large blocks of linked markers near this element. We find no
 20 evidence of elevated linkage disequilibrium between the *Hopscotch* and SNPs surrounding
 21 the *tb1* region in our resequenced populations (2), and r^2 in the *tb1* region does not differ
 22 significantly between populations with (average r^2 of 0.085) and without the *Hopscotch*
 23 genotype (average $r^2 = 0.082$). In fact, average r^2 is lower in the *tb1* region ($r^2 = 0.056$)
 24 than across the rest of chromosome 1 ($r^2 = 0.083$) (3). *table is too wide, need to round numbers,*
 25 *and column headers are messed up.*

26 The lack of clustering of *Hopscotch* genotypes in our NJ tree as well as the lack of LD

Table 3: r^2 values between SNPs in the *tb1* region (positions 264,596,664-265,891,456 on chromosome 1 of the maize AGPv2 genome) and the rest of chromosome 1, within the 5' UTR (Sequenced region 1), and within the 66,169 bp upstream region (Sequenced region 2).

Population	Chromosome 1	tb1 region	Seq. region 1	Seq. region 2
Ejutla A	0.095426101	0.050304	0.747295	0.214933
Ejutla B	0.068681837	0.051295	0.660354	0.186395
La Mesa	0.069500533	0.053306	0.914286	0.766234
San Lorenzo	0.100536784	0.067251	0.912281	0.636364

1 around *tb1* does not support the hypothesis that the *Hopscotch* insertion in these
2 populations of *parviglumis* is the result of recent introgression. However, to further
3 explore this hypothesis we performed a STRUCTURE analysis using Illumina
4 MaizeSNP50 data from four of our *parviglumis* populations (EjuA, EjuB, MSA, and
5 SLO) and the maize 282 diversity panel (Cook et al., 2012; Pyhäjärvi et al., 2013). The
6 linkage model implemented in STRUCTURE can be used to identify ancestry of blocks of
7 linked variants, which would arise as a result of recent admixture between populations. If
8 the *Hopscotch* insertion is present in populations of *parviglumis* as a result of recent
9 admixture with domesticated maize, we would expect the insertion and linked variants in
10 surrounding sites to be assigned to the "maize" cluster in our STRUCTURE runs, not
11 the "teosinte" cluster. In all runs, assignment to maize in the *tb1* region across all four
12 *parviglumis* populations is low (average 0.017) *is this really 0.017 or 0.17? Yes really 0.017*
13 *assignment to maize in the tb1 region, and avg assignment across chr1 is 0.2 I also have a table of assignment*
14 *values for SLO individuals based on genotype. Though we had decided this wasn't super informative because sample*
15 *size was low* and much below the chromosome-wide average (0.20; 3).
16 *please put figures in the text rather than at the end. I can't figure out why the figures are going at the end. I*

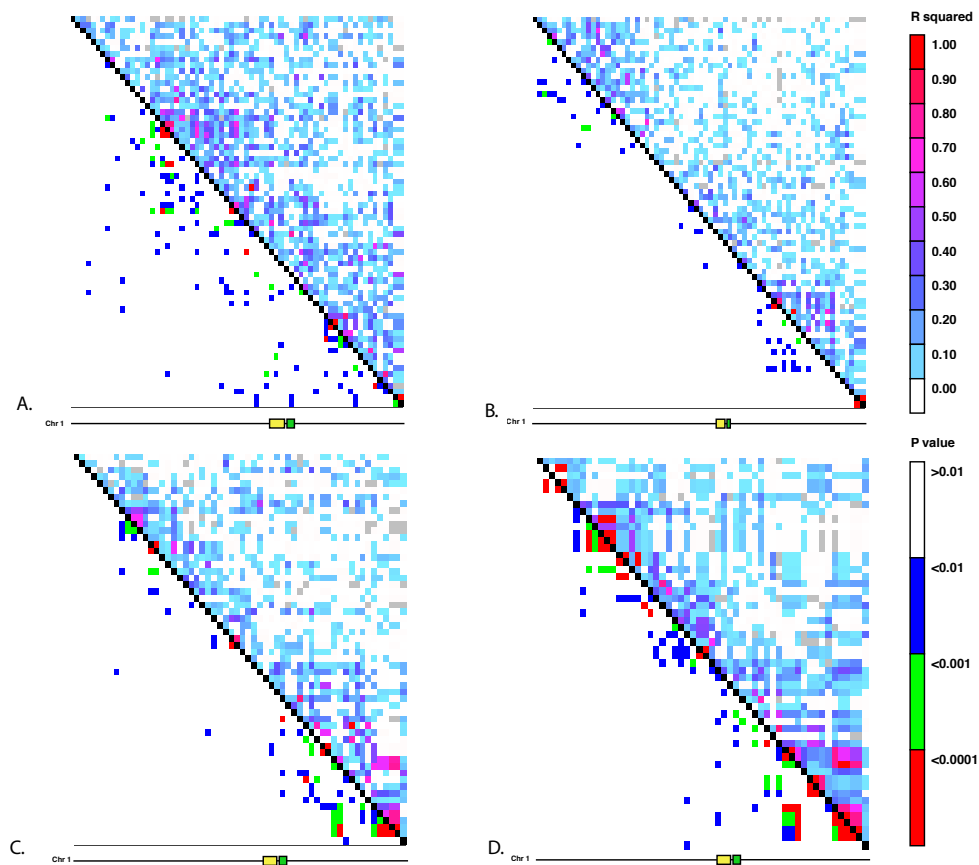


Figure 2: Linkage disequilibrium for SNPs in Mb 261-268 on chromosome 1. The yellow rectangle indicates the location of the *Hopscotch* insertion and the green represents the *tb1* ORF. A) Ejutla A; B) Ejutla B; C) La Mesa; D). San Lorenzo

1 *did them following the format in Sofiane's 282 paper*

2 Phenotyping

3 To assess the contribution of *tb1* to phenotypic variation in tillering in a natural
 4 population, we grew plants from seed sampled from the San Lorenzo population of
 5 *parviglumis*, which had a high mean frequency (0.44) of the *Hopscotch* insertion from our

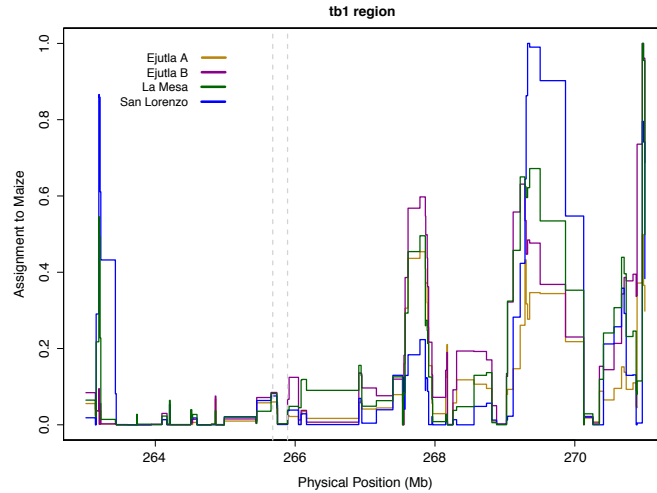


Figure 3: STRUCTURE assignment to maize across a section of chromosome 1. The dotted lines mark the beginning of the sequenced region 50kb upstream (Sequenced region 2) and the end of the *tb1* ORF.

1 initial genotyping. We measured tillering index (TI), the ratio of the sum of tiller lengths
2 to plant height, for 216 plants from within the San Lorenzo population, and genotyped
3 plants for the *Hopscotch* insertion. We found the *Hopscotch* segregating at a frequency of
4 0.65 with no significant deviations from expected frequencies under Hardy-Weinberg
5 equilibrium. After performing a repeated measures ANOVA between our transformed
6 tillering index data and *Hopscotch* genotype we find a weak positive correlation between
7 presence of the *Hopscotch* and tillering index on day 40 ($p=0.0848$), but no correlation
8 between tillering index and genotype on any other day (4). Additionally we find no
9 significant correlation between tiller number and *Hopscotch* genotype, or culm diameter
10 and *Hopscotch* genotype in phenotyping 1. *shouldn't we expect a negative correlation between Hop and*
11 *TI on day 40? need to have an A and B in the figure and explain one is for pheno1 and one is for pheno2. please*
12 *explain whiskers and dots on figure too. sure, I mean, presumably we would expect things with Hop to have a*

1 *smaller TI yup, but we should mention that the expectation is negative/*
2 *lots of white space in fig 4 and fig. s1 too.* We performed a second grow-out of teosinte to
3 assess whether lighting conditions or sample size may have affected our ability to detect
4 and effect of *tb1*. For the second grow-out we measured tillering index every five days
5 through day 50 for 302 plants. We found the *Hopscotch* allele segregating at a frequency
6 of 0.69, *is it in HWE in this pop?* with a 0.6 frequency of *Hopscotch* homozygotes, and a 0.2
7 frequency of both heterozygotes and homozygotes for the teosinte allele. We found
8 similar patterns, with a weak positive correlation between tillering index and *Hopscotch*
9 genotype at day 40 ($p=0.0611$), with no significant correlation on any day. Similarly,
10 relationships between *Hopscotch* genotype and tiller number, and *Hopscotch* genotype
11 and culm diameter are not significant.

12 DISCUSSION

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

21 In maize, alleles at important domestication loci (*RAMOSA1*, (?); *barren stalk1*,
22 (Gallavotti et al., 2004); and *grassy tillers1*, (Whipple et al., 2011)) have been shown to
23 have been selected from standing variation, suggesting that diversity already present in
24 teosinte may have played an important role in the domestication of maize. The *teosinte*
25 *branched1* gene has long been a central focus of research concerning maize domestication,
26 and, while previous studies have suggested that differences in plant architecture between

1 domesticated maize and teosinte are a result of selection on standing variation, little is
2 known about variation at this locus in teosinte (Clark et al., 2006; Studer et al., 2011).
3 Studer et al. (2011) genotyped 90 accessions of teosinte (inbred and outbred), providing
4 the first evidence that the *Hopscotch* insertion is segregating in teosinte (Studer et al.,
5 2011).

6 Given that the *Hopscotch* insertion has been estimated to predate the domestication
7 of maize, it is not surprising that it can be found segregating in populations of teosinte.
8 However, in sampling numerous individuals from many teosinte populations our study
9 provides greater insight into the distribution and prevalence of the *Hopscotch* in teosinte.
10 While our findings are consistent with a previous study by Studer et al. (2011) in that we
11 identified the *Hopscotch* allele segregating in teosinte, we find it at higher frequency than
12 previously suggested (Studer et al., 2011). Many of our populations with high frequency
13 of the *Hopscotch* allele fall in the Jalisco cluster identified by Fukunaga (????), possibly
14 suggesting a different history of the *tb1* locus than in the Balsas region where maize was
15 domesticated (Matsuoka et al., 2002). While gene flow from crops into their wild relatives
16 is well-known, ((Ellstrand et al., 1999; Zhang et al., 2009; Thurber et al., 2010; Baack
17 et al., 2008; Hubner et al., 2012; Wilkes, 1977; van Heerwaarden et al., 2011; Barrett,
18 1983)), our results are more consistent with Hufford et al. (2013) who found resistance to
19 introgression from maize into teosinte (Hufford et al., 2013). Furthermore, Hufford et al.
20 (2013) showed that domestication loci, such as *tb1*, are particularly resistant to
21 introgression in both directions of gene flow (i.e., maize to teosinte and teosinte to maize)
22 (Hufford et al., 2013).

23 We find no evidence of recent introgression in our analyses. Clustering patterns in
24 our NJ trees do not reflect a pattern expected if maize alleles at the *tb1* locus had
25 introgressed into populations of teosinte. Moreover, analysis of linkage in the *tb1* region
26 does not reveal patterns of high LD relative to the rest of chromosome 1, and assignment
27 to maize in this region in our STRUCTURE analysis is lower than the average across

1 chromosome 1 (3, 4). Together, these data point to an explanation other than recent
2 introgression for the high observed frequency of *Hopscotch* in some of our *parviglumis*
3 populations.

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

Population	<i>tb1</i> region		Chr 1	
	Maize assignment	Teosinte assignment	Maize assignment	Teosinte assignment
Ejutla A	0.02158681	0.9784132	0.2026814	0.7973186
Ejutla B	0.01888194	0.9811181	0.1872131	0.8127869
La Mesa	0.0118675	0.9881333	0.8068998	0.1931017
San Lorenzo	0.01551389	0.9844861	0.2048252	0.7951748

4 Although recent introgression seems unlikely, we cannot rule out ancient introgression
5 as an explanation for the presence of the *Hopscotch* in these populations. If the *Hopscotch*
6 allele was introgressed in the distant past, they could have been sufficient recombination
7 to break up any initial LD, leading to observations similar to those obtained here. We
8 find this scenario less plausible, however, as there is no reason why gene flow should have
9 been high in the past but absent in present-day sympatric populations. In fact, early
10 generation maize-teosinte hybrids are easy to find in these populations today (MB
11 Hufford, pers. observation), and genetic data support ongoing gene flow between
12 domesticated maize and both *Zea mays* ssp. *mexicana* and *Zea mays* ssp. *parviglumis* in
13 a number of sympatric populations (Hufford et al., 2013; Ellstrand et al., 2007; ?).

14 Other explanation for differential frequencies of the *Hopscotch* among teosinte
15 populations include both drift and natural selection. Previous studies using both SSRs
16 and genome-wide SNP data have found evidence for a population bottleneck in the San
17 Lorenzo population (Hufford, 2010; Pyhäjärvi et al., 2013), and the lower levels of
18 sequence diversity in the 5' UTR region and the more positive values of Tajima's D we

1 present here are consistent with these findings. *deviations from HWE may be consistent too if we see*
2 *excess of homozygotes. do we?* . This bottleneck, however, does not explain differences in
3 *Hopscotch* allele frequency among populations, and the available information on diversity
4 and population structure among these populations (Hufford, 2010; Pyhäjärvi et al., 2013)
5 is not suggestive of colonization or other demographic events that might predict a high
6 frequency of the allele in multiple populations. *here we need a few sentences on selection. the 5'*
7 *UTR has much more negative D than the upstream. do we know the Hop genotype for sequenced lines? can we*
8 *separate the sequences into hop/no hop and look for differences? it wasn't until we did this that gt1 stuff really*
9 *popped out. we should know for some of them, i will check*

10 The phenotypic effects of the *Hopscotch* insertion in domesticated maize have been
11 well documented (Clark et al., 2006; Studer et al., 2011), and Weber et al. (2007) have
12 described its effects in partially inbred lines of teosinte (Weber et al., 2007) *i don't think these*
13 *were inbred. please doublecheck.* . Our study is the first to explicitly examine the phenotypic
14 effects of the *Hopscotch* insertion in individuals sampled from a natural population of
15 teosinte. *isn't this what weber did?? for 70+ populations!?* However, we found no significant effect
16 of the *Hopscotch* on tillering index or tiller number in our phenotyping experiments, and
17 the effect of the *Hopscotch* insertion in teosinte is discordant with that of maize. The lack
18 of correlation between *Hopscotch* genotype and tillering index or tiller number is
19 surprising given its effects in maize. It is certainly possible that even though previous
20 data demonstrate an effect of the *Hopscotch* on tillering in maize (Studer et al., 2011),
21 that the effect of the *Hopscotch* in teosinte is more complicated and may be more difficult
22 to observe. Moreover, *tb1* is a single gene in a complex pathway that affects branching
23 and tillering traits, and perhaps in combination with alleles at other loci the phenotypic
24 effects of the *Hopscotch* on tillering may not be consistent. *this section still needs work. i think we*
25 *have to do more here. weber shows an association between SNPs in tb1 and branch length. we need to discuss*
26 *that!*

27 *MBH todo* Variation at *tb1* has also been shown to contribute to phenotypes other

1 than tillering (Clark et al., 2006), and a recent study by Studer and Doebley (2012)
2 examined the possibility of an allelic series at the *tb1* locus in teosinte. Studer and
3 Doebley (2012) introgressed 9 separate teosinte segments (one from *Zea diploperennis*,
4 and four from both *Zea mays* ssp. *mexicana* and *Zea mays* ssp. *parviglumis*) spanning
5 the *tb1* locus into an isogenic maize background and investigated their effects on
6 previously associated phenotypes. They found that plants with teosinte chromosomal
7 segments had greater tillering than their maize isogenic lines, and that different
8 chromosomal segments of *tb1* confer different amounts of tillering, suggesting that there
9 are multiple genetic factors in this region that affect tillering. However, in addition to
10 elucidating variance in tillering among *tb1* teosinte segments, Studer and Doebley (2012)
11 found significant variance among W22 control lines, suggesting that there are other
12 genetic factors aside from alleles at the *tb1* locus that affect tillering in maize. Doebley
13 and Stec (1991, 1993) first attempted to map QTL controlling many of the phenotypic
14 differences between domesticated maize and teosinte, and demonstrated the existence of
15 numerous QTL that contribute to the differences in branching architecture between the
16 two. Many of these loci (*grassy tillers*, *gt1*; *tassel-replaces-upper-ears1*, *tru1*; *terminal*
17 *ear1*, *ter1*) have been shown to interact with *tb1* (Whipple et al., 2011; Li, 2012), and
18 both *tru1* and *ter1* have been shown to affect the same phenotypic traits as *tb1* (Doebley
19 et al., 1995). *tassel-replaces-upper-ears1* (*tru1*), for example, has been shown to act
20 either epistatically or downstream of *tb1*, affecting both branching architecture (decreased
21 apical dominance) and tassel phenotypes (shortened tassel and shank length and reduced
22 tassel number) (Li, 2012). It seems plausible that variation in some of these other loci
23 could have affected tillering in our greenhouse population, and contributed to the lack of
24 correlation we see between *Hopscotch* genotype and tillering.

25 In summary, our findings demonstrate that the *Hopscotch* allele is more widespread
26 in populations of *parviglumis* and *mexicana* than previously thought. Analysis of linkage
27 using SNPs from across chromosome 1 does not suggest that the *Hopscotch* allele is

1 present in these populations due to recent introgression; however, it seems unlikely that it
 2 would have drifted to high frequency in multiple populations and there may be another
 3 explanation for the high frequency we observe in some of our populations. The *Hopscotch*
 4 does not appear to have a strong effect reducing tillering in teosinte as it does in maize,
 5 and other loci involved in branching architecture may play also play roles in the
 6 regulation of tillering in teosinte. Finally, although we see no clear evidence of recent
 7 strong selection, the high frequency of the *Hopscotch* insertion in a number of
 8 populations continues to suggest to us that it plays an ecological role in teosinte. In the
 9 future, additional experiments will be needed to examine expression levels of *tb1* and
 10 additional loci involved in branching architecture (e.g. *gt1*, *tru1*, and *ter1*) in conjunction
 11 with a more exhaustive phenotyping and genotyping assay. *why not Phyb and phya? Are they*
 12 *necessary to include? I'd had them in before in a paragraph but had been voted out I'd ditch gt1 tru1 ter1 and*
 13 *maybe just cite some people including phyb etc.*
 14 *please check format of supp figs and tables; some are running off the page. you can use "longtable" to fix that*
 15 *(ask Paul for example). check fig/table references, bibliography, etc. what does "rotation" mean in supp. table 3?*
 16 *it isn't mentioned in methods. please check that all the tables and figs (including supplement) are referenced in the*
 17 *text.*

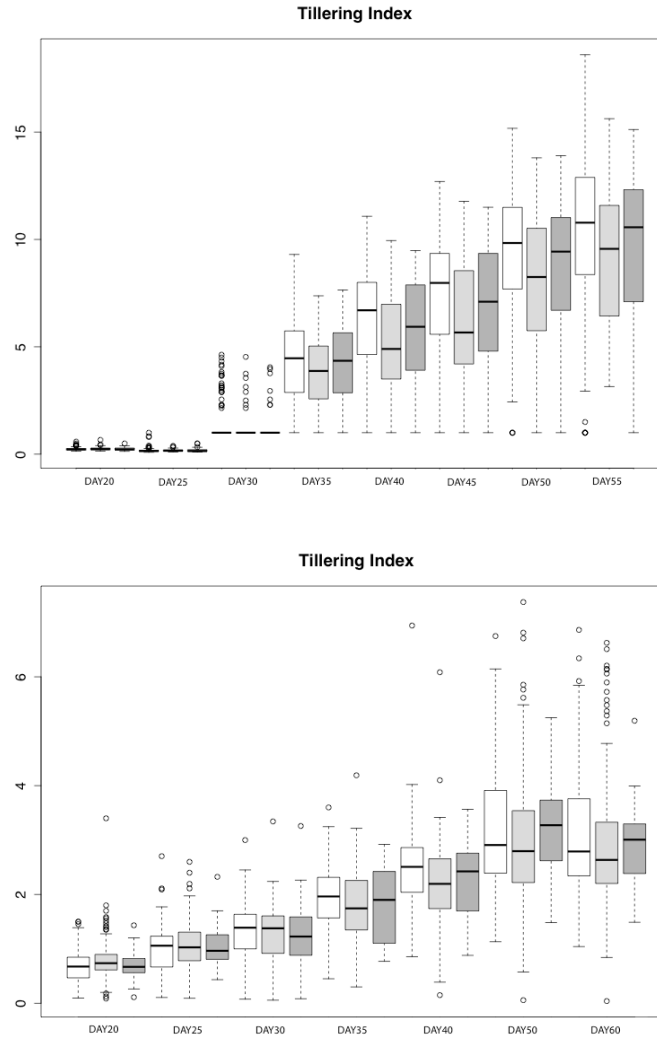


Figure 4: Box-plot showing tillering index in our greenhouse population from day 20-60. White indicates individuals homozygous for the *Hopsotch*, light grey represents heterozygotes, and dark grey represents homozygotes for the teosinte (No *Hopsotch*) allele. Within boxes, dark black lines represent the median, and the edges of the boxes are the first and third quartiles.

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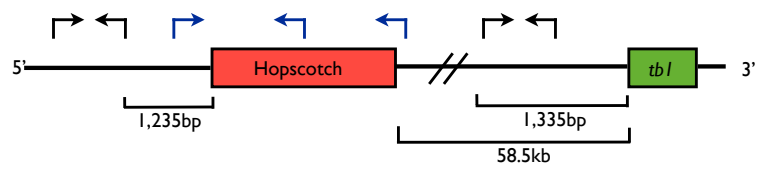
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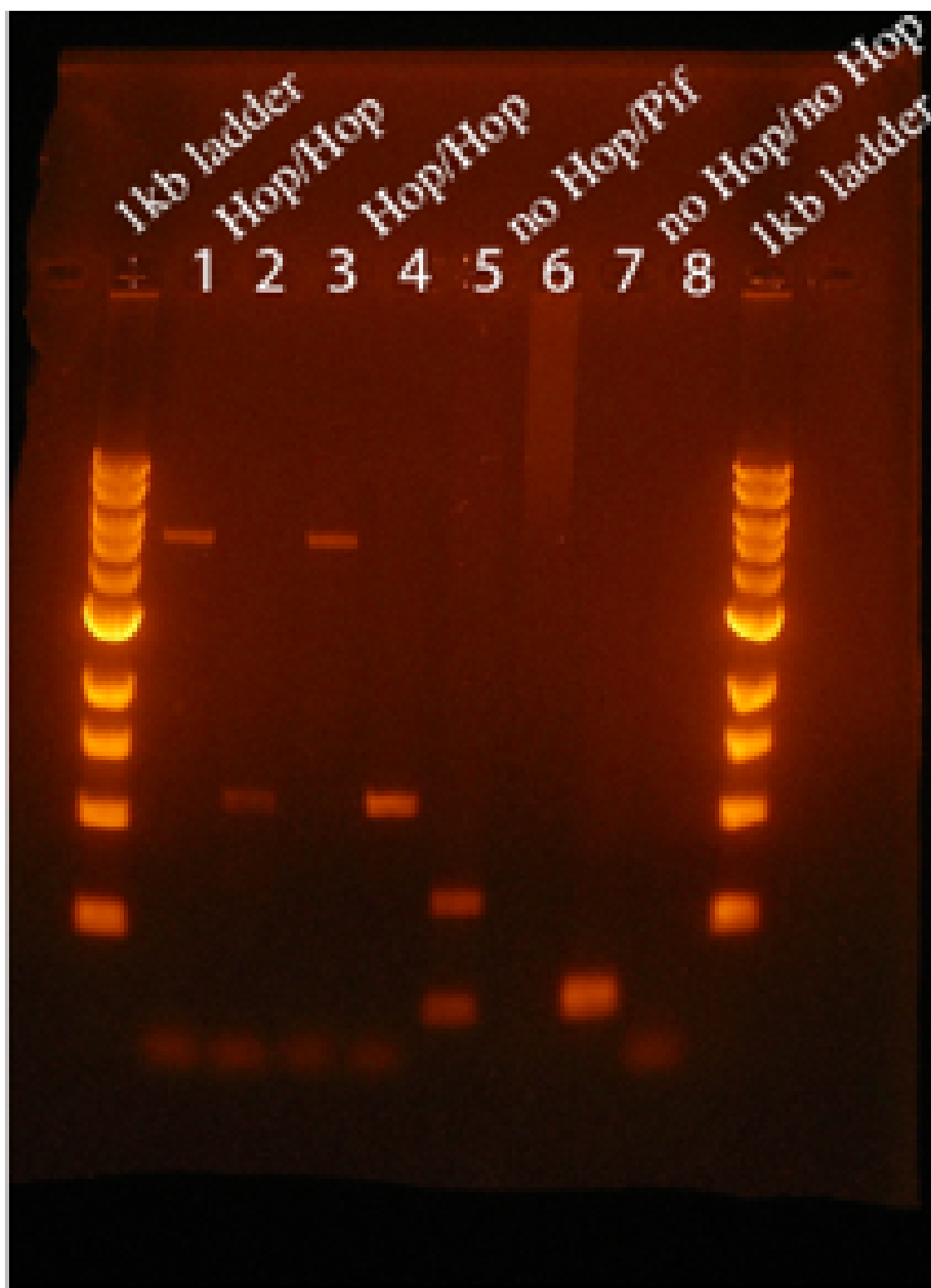
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18 Supplementary Materials





Accession	USDA Accession ID	Locality	Number alleles sampled	<i>Hopscotch</i>
RIHY0009	N/A	N/A	2	
RIMME0006	566673	Durango, Mexico	2	
RIMME0007	566680	Guanajuato, Mexico	2	
RIMME0008	566681	Michoacan, Mexico	2	
RIMME0009	566682	Distrito Federal, Mexico	2	
RIMME0011	566685	Mexico, Mexico	2	
RIMME0014	714151	Breeders line; Puga: 11066	6	
RIMME0017	699874	Ayotlan, Mexico	8	
RIMME0021	N/A	El Porvenir, Mexico	69	0
RIMME0026	N/A	Opopeo, Mexico	42	0
RIMME0028	N/A	Puruandiro, Mexico	28	0
RIMME0029	N/A	Ixtlan, Mexico	35	
RIMME0030	N/A	San Pedro, Mexico	27	
RIMME0031	N/A	Tenango del Aire, Mexico	25	
RIMME0032	N/A	Nabogame, Mexico	24	
RIMME0033	N/A	Puerta Encantada, Mexico	25	
RIMME0034	N/A	Santa Clara, Mexico	23	
RIMME0035	N/A	Xochimilco, Mexico	25	
RIMPA0001	87168	El Salado, Mexico	4	
RIMPA0003	87171	Mazatlan, Mexico	8	
RIMPA0017	87200	N/A	4	
RIMPA0019	87213	El Salado, Mexico	2	
RIMPA0029	87244	N/A	2	
RIMPA0031	87249	N/A	2	
RIMPA0035	87288	Jalisco, Mexico	4	
RIMPA0040	288185	Mexico, Mexico	4	
RIMPA0042	288187	Guerrero, Mexico	4	
RIMPA0043	288188	36 Guerrero, Mexico	4	
RIMPA0045	288193	Guerrero, Mexico	4	
RIMPA0055	714152	Breeders line	2	
RIMPA0056	714153	Breeders line	2	
RIMPA0057	714154	Breeders line	2	
RIMPA0058	N/A	N/A	4	

Accession	Number of alleles sampled	<i>Hopscotch</i> Frequency
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

PC1		PC2		PC3		PC4		PC5		PC6	
Var	Rot	Var	Rot	Var	Rot	Var	Rot	Var	Rot	Var	Rot
bio1	0.146	bio4	0.244	prec7	0.287	ts_clay	0.41	bio2	0.38	bio10	0.086
tmean11	0.146	bio3	0.241	prec8	0.276	v_mod	0.359	sq4	0.328	x_mod	0.111
tmean12	0.145	bio7	0.241	prec11	0.262	ts_sand	0.329	ts_loam	0.289	sq3	0.207
bio11	0.145	prec6	0.237	bio13	0.247	bio15	0.272	ts_sand	0.266	bio1	0.146
tmax12	0.145	sq7	0.218	prec1	0.246	prec4	0.259	sq7	0.231	v_mod	0.359
tmin5	0.145	prec9	0.217	bio16	0.242	x_mod	0.244	bio18	0.213	prec1	0.287
tmean1	0.145	sq3	0.207	prec12	0.24	prec3	0.226	bio13	0.207	bio1	0.146
tmean2	0.145	prec12	0.207	bio19	0.238	sq3	0.21	prec11	0.183	sq4	0.328
tmin4	0.145	bio12	0.204	bio12	0.231	prec5	0.21	bio7	0.17	sq3	0.207
tmax1	0.145	bio19	0.196	prec2	0.222	prec7	0.19	bio16	0.163	ts_sand	0.329
tmean4	0.145	prec2	0.188	bio18	0.221	sq4	0.186	bio4	0.157	bio1	0.146
tmin11	0.144	prec1	0.185	sq4	0.2	bio3	0.185	bio12	0.156	prec1	0.287
tmax11	0.144	prec10	0.184	prec9	0.18	bio18	0.178	bio3	0.155	tmax1	0.145
tmin12	0.144	bio16	0.183	prec10	0.171	sq7	0.132	prec6	0.154	tmax1	0.145
tmin2	0.144	prec8	0.17	prec5	0.161	bio14	0.116	x_mod	0.152	bio1	0.146
tmean5	0.144	prec5	0.165	prec4	0.154	bio13	0.099	prec9	0.144	tmax1	0.145
tmean10	0.144	bio14	0.158	sq3	0.147	bio16	0.095	prec8	0.143	bio1	0.146
bio6	0.144	bio13	0.151	bio2	0.143	prec8	0.09	v_mod	0.142	ts_loam	0.289
tmax2	0.144	bio17	0.149	bio17	0.129	bio7	0.077	bio15	0.136	ts_clay	0.41
tmean3	0.144	prec3	0.144	ts_loam	0.127	bio4	0.075	prec7	0.112	tmin1	0.143
tmin1	0.143	ts_clay	0.141	v_mod	0.123	bio2	0.074	prec4	0.108	tmin1	0.143
tmin10	0.143	bio2	0.129	prec3	0.113	prec2	0.074	bio14	0.096	prec1	0.287
Altitude	0.143	prec7	0.108	x_mod	0.111	bio19	0.068	tmax7	0.093	tmin1	0.143
bio9	0.143	tmax6	0.107	bio14	0.099	prec12	0.056	tmax8	0.092	tmin1	0.143
tmin3	0.143	x_mod	0.106	bio4	0.07	ts_loam	0.053	prec1	0.091	tmin1	0.143
bio10	0.142	bio15	0.098	tmax3	0.067	tmax12	0.047	prec2	0.086	tmean1	0.145
tmax10	0.142	ts_loam	0.088	ts_clay	0.065	bio17	0.047	tmin11	0.086	tmax1	0.145
tmax3	0.142	tmean6	0.085	bio15	0.056	bio9	0.043	prec5	0.082	tmax1	0.145
tmax4	0.142	tmin7	0.082	tmax2	0.055	tmax8	0.042	bio17	0.082	tmean1	0.145
tmin6	0.142	bio5	0.082	tmean3	0.052	tmax1	0.041	tmin12	0.08	bio1	0.146
tmean9	0.141	tmean7	0.081	ts_sand	0.05	tmax5	0.039	prec3	0.078	tmin1	0.143
tmin9	0.141	prec4	0.08	prec6	0.048	tmax7	0.039	tmax9	0.078	prec1	0.287

Ejutla A	4	0.15217	0.11902	0.76191
Ejutla B	5	0.15258	0.14877	0.07412
La Mesa	3	0.12802	0.08926	1.09209
San Lorenzo	3	0.09098	0.08926	0.04845