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Epistatic and environmental interactions for quantitative trait loci involved in maize evolution

LEWIS N. LUKENS AND JOHN DOEBLEY*
Department of Plant Biology, University of Minnesota, St Paul, Minnesota 55108, USA

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Summary

To test for epistasis and allele-specific environmental responses among quantitative trait loci (QTL) involved in the evolution of maize from its ancestor (teosinte), teosinte alleles of two QTL previously shown to control much of the morphological difference between these plants were introgressed into an isogenic maize background. Plants of each of the four two-locus homozygous classes for the two QTL were grown in two environments. Three morphological traits and the level of mRNA accumulation for one QTL (*teosinte branched1*, *tb1*) were measured. *tb1* has a large additive effect on morphology that was correlated with its message level. The second QTL had only negligible effects on morphology when isolated in an isogenic background, but exhibited a strong interaction effect on morphology in combination with *tb1*. This interaction is also evident in *tb1* message levels, suggesting that this second QTL may act as an upstream regulator of *tb1*. The combined effect of the maize alleles at the two QTL makes *tb1* message levels over fourfold higher. Plants homozygous for the teosinte allele at *tb1* showed greater phenotypic plasticity across environments than plants homozygous for the maize allele. Our results support two hypotheses. First, maize plant architecture may have evolved by selection for a gene complex rather than the additive effects of individual loci alone. Secondly, selection during maize domestication for an allele of *tb1* which lacks environmental plasticity may have led to the fixation of a morphological form that can be induced in teosinte by environmental conditions.

1. Introduction

The inheritance of quantitative traits has been described as a 'moving target' since these traits are affected not only by the actions of multiple individual genes, but also by the interactions between genes (epistasis) and between genes and environmental factors. Thus, quantitative trait loci (QTL) characterized in one genetic background or environment may behave rather differently when these factors are altered. The extent to which environmental and epistatic interactions influence the inheritance and evolution of quantitative traits is not well understood. While epistasis was once considered to make a small contribution to quantitative variation (Crow, 1987), there is growing evidence, or at least enthusiasm, that it plays a more central role (see Cheverud & Routman, 1995). Although environment has long been known to influence quantitative traits, exactly how this influence is exercised at the genetic and molecular levels and its significance for quantitative trait evolution remain unclear (Via, 1993; West-Eberhard, 1986).

QTL mapping with molecular markers catalysed a major advance in understanding the inheritance of quantitative traits (Tanksley, 1993). Nevertheless, this methodology has known limitations (Beavis, 1994). One probable weakness is a lack of power to detect non-additive or epistatic interactions between QTL. Most QTL studies reveal little or no evidence for epistasis (Stuber *et al.*, 1992; Xiao *et al.*, 1995; Liu *et al.*, 1996), yet, when individual QTL are isolated in isogenic backgrounds, epistasis is commonly observed (Doebley *et al.*, 1995; Long *et al.*, 1995; Eshed & Zamir, 1996; but see Laurie *et al.*, 1997). QTL mapping may underestimate the number of non-additive interactions for three reasons. First, even large mapping populations contain few individuals in the two-locus double homozygous classes, limiting

* Corresponding author. Department of Genetics, 445 Henry Mall, University of Wisconsin, Madison, WI 53706, USA. Tel: +1 (608) 262 3112. Fax: +1 (608) 262 2976. e-mail: jdoebley@facstaff.wisc.edu

statistical power to detect non-additive deviations for these genotypes. Secondly, mapping populations may segregate for many QTL. Thus, when testing for an interaction between any two QTL, there will be additional segregating QTL that may interfere with the detection of an interaction between the pair under consideration. Finally, searching for epistatic interactions involves many statistical tests, so significance thresholds must be increased accordingly.

QTL effects are environmentally sensitive (Gupta & Lewontin, 1982; Gurganus *et al*., 1998) and this sensitivity results in phenotypic plasticity or the ability of organisms to take on alternative developmental fates depending on environmental cues. Phenotypic plasticity can help maintain genetic variation within populations (Via & Lande, 1985). It is likely to have particular importance for plants since their sedentary nature dictates that they adjust to their local environment. Species with great phenotypic plasticity have been seen as likely progenitors for novel species that express only one of the possible developmental fates of their ancestors (West-Eberhard, 1986).

Our group has been studying the morphological evolution of cultivated maize (*Zea mays* ssp. *mays*) from its wild ancestor, teosinte (*Zea mays* ssp. *parviglumis*), using a quantitative genetic approach (Doebley & Stec, 1991, 1993). As a result of human selection for higher yield and greater harvestability, maize and teosinte differ radically in plant and inflorescence architecture. Most notably, teosinte possesses long lateral branches that are tipped by tassels (staminate inflorescences), while maize has short lateral branches tipped by ears (pistillate inflorescences). We have shown that these differences in branch length and inflorescence sex are controlled by multiple QTL (Doebley & Stec, 1993). However, among these QTL there is one of distinctly large effect that has been identified as the *teosinte branched1* (*tb1*) gene (Doebley, *et al*., 1995). Recently, molecular cloning and analysis of *tb1* suggests that the *tb1* protein functions as a repressor of organ growth (Doebley *et al*., 1997). The maize allele of *tb1* shows higher message levels in branch primordia (greater repression of branch elongation and shorter branches) while the teosinte allele shows lower message levels (less repression of branch elongation and longer branches).

In this report, we will extend our prior QTL analyses in two directions. First, we will use nearly isogenic lines (NILs) that possess the maize and teosinte alleles of two QTL, one of which is *tb1*, to examine the importance of QTL by QTL interactions. Previously, we demonstrated a strong epistatic interaction between these two QTL when assayed in a teosinte genetic background (Doebley *et al*., 1995). Here, we analyse their interaction in a maize genetic background. Secondly, for these same two QTL, we examine QTL by environment interactions in the context of the morphological evolution of maize from teosinte. Specifically, we test the hypothesis that the evolution of maize involved fixation of a phenotypic state (short lateral branches) in maize that can be induced environmentally in teosinte (Doebley *et al*., 1995). We do this by determining whether the teosinte allele of *tb1* provides greater phenotypic plasticity for plant architecture than does the maize allele. We assay both the epistatic and environmental interactions, not only at the phenotypic level but also at the molecular level by determining the interaction effects on the level of *tb1* messenger RNA (mRNA).

2. Materials and methods

(i) *Plant materials*

Prior research identified QTL on the long arms of chromosomes 1 (1L) and 3 (3L) with large effects on the differences in plant and inflorescence architecture between maize and teosinte (Doebley & Stec, 1993). Subsequently, the segments of these chromosome arms harbouring the QTL were transferred into the maize inbred line, W22, with four generations of backcrossing to the recurrent parent (W22) (Doebley *et al*., 1995). We extended the backcrossing for two more generations for a total of six generations of backcrossing. This produced two NILs – W22-T1L and W22-T3L – homozygous for the teosinte alleles in the regions of interest on chromosome arms 1L and 3L, respectively. We also crossed W22-T1L and W22-T3L to each other and selfed their hybrid to produce a population in which the two teosinte chromosome segments were segregating simultaneously in the W22 background. One individual from this population that was homozygous for both teosinte segments was selfed to produce the line W22-T1L/T3L.

(ii) *Experimental design*

W22, W22-T3L, W22-T1L and W22-T1L/T3L seedlings were grown in the same field at the Minnesota Agricultural Experiment station in St Paul in the summer of 1998 under either a high- or low-density planting regime. Seedlings in the low-density group were spaced 36 inches (91 cm) apart within rows, and rows were 30 inches (76 cm) apart. Seedlings in the high-density group were spaced 12 inches (30 cm) apart within rows that were 30 inches (76 cm) apart. To further increase plant density in the high-density group, sunflowers (*Helianthus annuus*) were planted in two rows approximately 12 inches (30 cm) from either side of the maize seedlings. The sunflowers grew so quickly relative to the maize plants that it was necessary to prune the sunflowers several times during the growing season to allow the maize plants sufficient light to survive.

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A total of four plots with 100 plants each were grown: two plots under the high-density and two under the low-density treatment. In each plot, 25 seedlings each of the four NILs (W22, W22-T3L, W22-T1L and W22-T1L/T3L) were grown in a completely randomized design. For one plot of each treatment group, plants were allowed to grow until fully mature and were measured for several morphological traits. For the second plot, axillary inflorescence primordia were dissected from the immature plants and frozen for subsequent RNA extraction. Care was taken to harvest primordium from both treatment groups that were at the same stage of development, using primordia length and silk initiation as development markers. The stage for these markers was similar in both treatment groups. The mean length of the primordia in the high-density treatment plot was 2·31 cm versus 2·47 cm in the low-density treatment plot, and 32% (31/97) of primordia in the low-density plot had begun to develop silks versus 36% (35/98) of primordia in the high-density treatment group. To obtain inflorescences at a similar developmental stage, it was necessary to dissect primordia from plants in the high-density plot approximately 1 week later than from plants in the low-density plot.

(iii) *Quantitative trait analysis*

The QTL under study affect several aspects of plant and inflorescence architecture. To analyse the morphological effects of the QTL we measured three traits: the average length of the internodes in the uppermost primary lateral branch (LBIL); the percentage of staminate (male) spikelets in the uppermost primary lateral inflorescence (STAM); and the extent of basal, lateral branch growth or tillering (TILL). TILL was measured as the sum of the lengths of the individual tillers expressed as a percentage of the length of the main stalk. Thus, a plant with two tillers, each 75% as long as the main stalk, would have a tillering score of 150%. STAM was measured on fully mature inflorescences that were harvested and dried approximately 6 weeks after pollination. LBIL and TILL were measured after the plants had fully matured, and plants that were noticeably diseased or stunted were excluded (8/100 in the high-density plot and 9/100 in the low-density plot). All measurements were made in masked fashion (without knowledge of the genotype of the plant).

(iv) *Northern blots and hybridization signal quantification*

Total RNA was isolated from inflorescence primordia by grinding the tissue in liquid nitrogen and extracting with Tri-Reagent (Molecular Research Center). The RNA samples (12 μg of each) were electrophoresed in 1·5% formaldehyde agarose gels for 4 h at 70 V and 46 mA and transferred to nylon membranes (MSI) to make the northern blots (Sambrook *et al*., 1989). The blots were probed with cloned DNA fragments of three genes: (1) NH780, a 780 bp genomic fragment from the coding region of the maize *tb1* gene (Doebley *et al*., 1997), (2) cycIaZm, a *cyclinB*-like cDNA isolated from maize meristems (Renaudin *et al*., 1994), and a maize *ubiquitin* cDNA fragment (Christiansen & Quail, 1989). For all probes, the insert of a plasmid clone was separated from the plasmid vector in low-melting-point agarose electrophoretic gels and labelled with [^{32}P]dCTP as described by Feinberg & Volgelstein (1983) except that the labelling reactions were allowed to proceed for 5 h at 37 °C. Unincorporated nucleotides were separated from the labelled probe in spin columns (Sambrook *et al*., 1989). Nylon filters were prehybridized for 45 min at 68 °C with Quickhyb hybridization solution (Stratagene), then the heat-denatured labelled probe was added to the hybridization vessel. Hybridization proceeded for 1·5 h at 68 °C. Following hybridization, the filters were washed twice for 15 min at room temperature in $2 \times$ SSC/0·1% SDS and once for 30 min at 60 °C in $0{\cdot}1 \times$ SSC/0·1% SDS. The filters were then wrapped in plastic and exposed to phosphor imaging screens for 12–18 h. A phosphor imager (STORM scanner, Molecular Dynamics) and Imagequant software (Molecular Dynamics) were used to quantify the amounts of *tb1*, *cyclinB* and *ubiquitin* message in the RNA samples.

We measured amounts of both *cyclinB* (CYC-RNA) and *tb1* (TB-RNA) mRNA while controlling for the amount of RNA loaded in each lane and for variation among the blots. To control for variation among blots, a control sample was run in two lanes on each gel (blot) and used to normalize the intensity of the hybridization signals across blots. To control for variation in the amount of RNA added to each lane, we measured the intensity of the *ubiquitin* hybridization signal for each sample and calculated the TB-RNA and CYC-RNA amounts as a proportion of the *ubiquitin* signal. TB-RNA was measured for a total of 159 RNA samples from both treatment groups representing the four genotypic classes. CYC-RNA was measured for 64 samples of all four genotypes from the low-density treatment group.

(v) *Statistical analysis*

Analysis of variance (ANOVA) was used to determine whether the teosinte chromosome segments have significant effects on the traits. This analysis involved only the low-density treatment since plants for this treatment reflect a more normal state, while plants under the high-density treatment are noticeably

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stressed. ANOVAs for each morphological and molecular character were performed with the following model:

$$y = \mu + Q1_i + Q3_j + (Q1_i \times Q3_j) + e,$$

where y is the phenotype (or RNA amount) for a plant, μ is the overall mean, $Q1_i$ and $Q3_j$ are the effects of the QTL on chromosomes 1 and 3, $Q1_i \times Q3_j$ is their interaction effect, e is the random error effect, and i and j are indicator variables with two levels corresponding to the presence or absence of the teosinte chromosome segment.

Phenotypic plasticity is the change in the average phenotype expressed by a genotype in different environments. To estimate the extent and variation of phenotypic plasticity of different genotypes between the high- and low-density environments, ANOVAs were performed with the following model:

$$y = \mu + Trt_k + Q1_i + Q3_j + (Trt_k \times Q1_i) + (Trt_k \times Q3_j) + e,$$

where Trt_k is the environmental treatment with two levels (k) corresponding to high-density or low-density growth conditions. The variation among genotypes in phenotypic plasticity was estimated from the observed genotype–environment interaction. Tests of significance of F ratios and estimates of variance components were obtained using JMP (SAS Institute).

3. Results

(i) *Phenotypic effects of the T1L segment*

We first assess the phenotypic differences among the NILs in the low-density environment, which reflects more normal conditions, as opposed to the high-density environment under which the plants are stressed. In the low-density environment there are striking visual differences in both inflorescence and plant architecture between the W22 and W22-T1L NILs which reflect the effects of the T1L segment. W22-T1L plants produce several tillers (basal branches), whereas W22 plants are tillerless or produce only one tiller (Fig. 1*a*, *b*). At upper nodes on the main stem, W22-T1L plants bear long, lateral branches, whereas W22 plants bear shorter lateral branches (Fig. 1*a*, *b*). W22-T1L plants also produce more lateral branches at upper nodes as compared with the W22 plants (data not shown). For W22-T1L plants the inflorescence at the tip of the uppermost branch has a mixture of staminate and pistillate spikelets, whereas for W22 plants this inflorescence is purely pistillate (Fig. 2*a*, *b*). In addition, the inflorescence of W22-T1L plants is narrower and has fewer kernels in each row as compared with those of W22 plants (Fig. 2*a*, *b*). In all respects, the effects of segment T1L are to make the plants more teosinte-like in appearance.

To quantify the magnitude of these differences on plant and inflorescence architecture, we measured the three traits – LBIL, STAM and TILL – for the plants of each NIL. W22-T1L plants have internodes that are 2·4 cm longer (LBIL) than those of W22 plants (Table 1*a*). Since there are approximately nine internodes in the branch, the T1L segment lengthens the branch by about 20 cm. W22-T1L plants typically produce two or more tillers that are as tall as the main stalk, while W22 plants typically produce only one tiller (or none) that is only 65% of the height of the main stalk. Finally, W22-T1L plants have inflorescences with 20% staminate spikelets as compared with 0 for W22. The effects of the T1L segment are highly significant on all three traits (Table 2).

Several lines of evidence have shown that the QTL on chromosome 1L is *tb1* (Doebley & Wang, 1997). Thus, we consider the effects of T1L (i.e. the difference between W22 and W22-T1L) to represent principally the action of this single gene with alleles *tb1-maize* and *tb1-teosinte* in W22 and W22-T1L, respectively.

(ii) *Phenotypic effects of segment T3L*

The visual and metric differences between W22 and W22-T3L are more modest than those between W22 and W22-T1L. While W22-T3L plants, like teosinte, have slightly longer branch internodes and more tillers than W22 plants (Table 1*a*; Fig. 1*a*, *c*), these differences are not statistically significant (Table 2). The axillary inflorescences of W22-T3L plants have a significantly greater percentage of staminate spikelets than W22 but the difference is small (0·5%), as there are usually just a few staminate spikelets near the tip of the ear (Tables 1a, 2). Otherwise, the ears of W22-T3L plants are more slender than those of W22 plants and their kernels are somewhat pointed (Fig. 2*a*, *c*). W22-T3L plants produce a significantly larger number of lateral branches than W22 plants (data not shown). In all respects the effects of the T3L segment are to make the plants more teosinte-like in appearance; however, W22-T3L is not strongly differentiated from W22 and it does not appear to possess a QTL of large effect on the traits analysed. This result contrasts with prior QTL mapping experiments which indicated that the long arm of chromosome 3 possesses a QTL (called QTL-3L) of large effect on the differences in plant and inflorescence architecture that distinguish maize and teosinte (Doebley & Stec, 1993).

(iii) *Epistasis*

In addition to examining the effects of the individual teosinte segments in W22 background, we analysed the interaction between these two segments for the low-density treatment. Plants possessing both teosinte segments are only slightly more tillered than plants

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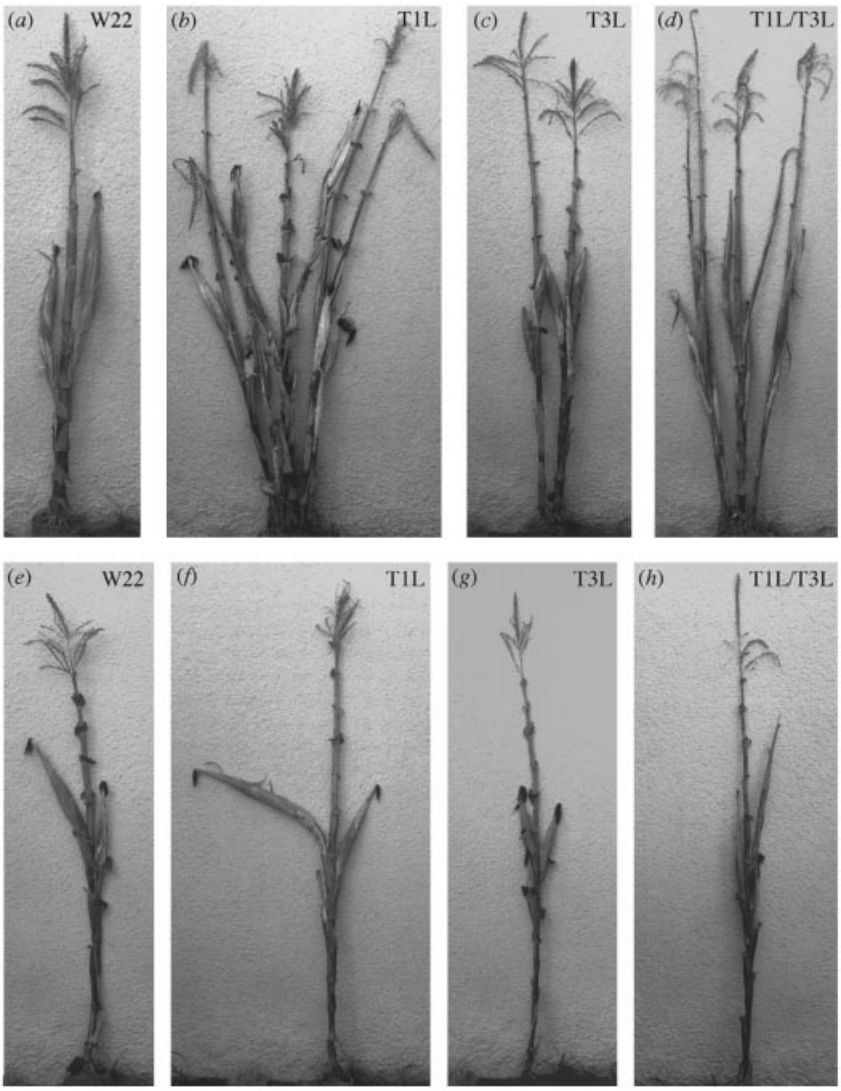

Fig. 1. Plants of the four NILs under low-density (*a*, *b*, *c*, *d*) and high-density (*e*, *f*, *g*, *h*) growth. (*a*, *e*) W22 maize inbred; (*b*, *f*) W22-T1L; (*c*, *g*) W22-T3L; and (*d*, *h*) W22-T1L/T3L.

possessing the T1L segment alone (Table 1 *a*), and the effects of the two segments are purely additive with a non-significant interaction term (Table 2). Similarly, plants possessing both teosinte segments have internodes that are only slightly longer than those of plants possessing the T1L segment alone (Table 1 *a*), and again the effects of the two segments on LBIL are purely additive (Table 2). Accordingly, for plant architecture, W22-T1L and W22-T1L/T3L plants are not visually distinct (Fig. 1 *b*, *d*).

The situation for STAM is notably different. Plants possessing both teosinte segments have inflorescences that are 90% staminate as compared with 21% for W22-T1L and 0·5% for W22-T3L plants (Table 1 *a*). The interaction between the two segments is synergistic and highly significant (Table 2). The effect of this interaction is visually striking when one compares inflorescences of the four NILs (Fig. 2 *a–d*). The effect of the interaction is greater than the effect of either segment alone since T1L adds 21% staminate spikelets and T3L adds 0·5%, while the interaction between these segments adds 69% staminate spikelets [90%–(21% + 0·5%)] (Table 1). Thus, together these two teosinte chromosome segments, potentially just two QTL (*tb1* and QTL-3L), produce the nearly complete conversion of the ear on the tip of the branch into a tassel.

(iv) *Effects on* tb1 *transcript accumulation*

To begin to investigate the molecular basis for the morphological effects of chromosome segments T1L and T3L, we measured the level of *tb1* RNA in inflorescence primordia (TB-RNA) of 77 samples representing the four NILs – W22, W22-T1L, W22-T3L, and W22-T1L/T3L – grown in the low-density environment. W22 plants exhibit about twice as much TB-RNA as W22-T1L plants (Table 3), and the effect

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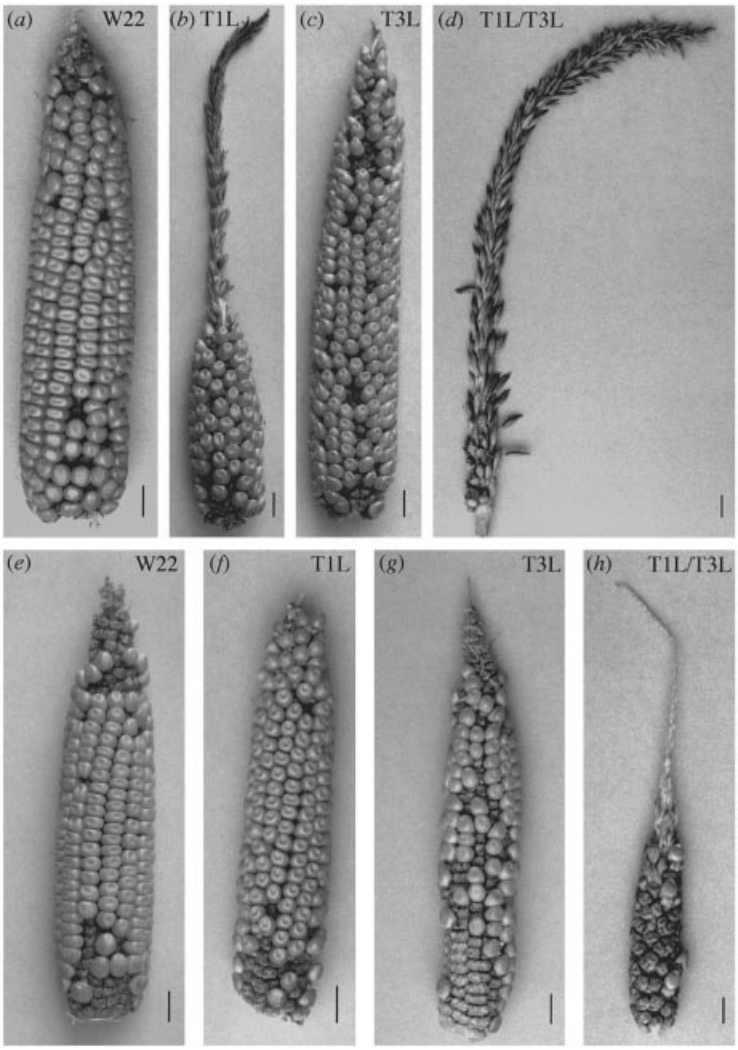

Fig. 2. Axillary inflorescences of the four NILs under low-density (*a*, *b*, *c*, *d*) and high-density (*e*, *f*, *g*, *h*) growth. (*a*, *e*) W22 maize inbred; (*b*, *f*) W22-T1L; (*c*, *g*) W22-T3L; and (*d*, *h*) W22-T1L/T3L. Scale bar represents 1 cm.

Table 1. *Trait means* (±*SE*) *for NILs under low- and high-density growth conditions*

	W22	W22-T1L	W22-T3L	W22-T1L/T3L
(a) *Low-density treatment*				
LBIL (cm)	4·19±0·16	6·54±0·47	4·85±0·32	7·41±0·59
STAM (%)	0·00±0·00	20·61±3·48	0·52±0·51	90·03±2·12
TILL (%)	65·33±12·54	243·21±24·54	91·17±14·54	284·29±19·13
(b) *High-density treatment*				
LBIL (cm)	3·11±0·07	5·90±0·29	3·22±0·19	5·98±0·35
STAM (%)	0·00±0·00	4·78±1·38	6·05±1·07	52·96±4·38
TILL (%)	7·83±5·42	0·40±0·40	18·04±6·94	0·00±0·00

of segment T1L (actually *tb1* itself) is highly significant (Table 4). This difference is highly consistent among samples and can be readily detected by visual inspection of the northern blots (Fig. 3). This result is consistent with our previous estimate that *tb1* message accumulation for the maize allele is about twice that of the teosinte allele (Doebley *et al*., 1997).

The T3L segment did not affect TB-RNA (Table 4). Plants with this teosinte segment produced slightly higher values for TB-RNA than pure W22 plants, although not significantly higher (Table 3). This result is consistent with the phenotypic analysis (above), which indicated that segment T3L has little or no effect on morphology as compared with pure W22.

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Table 2. *ANOVA showing the F-ratios for the effects of the teosinte chromosome segments on the morphometric traits*

	Effects			
Trait	T1L	T3L	T1L × T3L	d.f. (error)
TILL	111·44***	3·60	0·18	83
LBIL	37·38***	3·61	0·67	83
STAM	880·41***	355·10***	344·72***	83

*** $P < 0{\cdot}001$.

Again, the similarity in TB-RNA between W22 and W22-T3L was consistent among samples as shown by visual inspection of the northern blots (Fig. 3).

Since the two teosinte chromosome segments showed a strong epistatic interaction for STAM, we expected this interaction to be seen at the molecular level, as measured by TB-RNA. The mean TB-RNA values for the NILs suggest that this may be the case, since T1L reduces TB-RNA by 50%, T3L has no effect, but the two segments together reduce TB-RNA to only 21% of the W22 level (Table 3). This interaction effect is significant (Table 4), and the northern blots typically showed the weakest signal for W22-T1L/T3L samples. Thus, parallel to the synergistic interaction that was seen for STAM, we see a similar epistatic interaction for the level of *tb1* message accumulation in ear primordia. This result suggests that QTL-3L alters the level of *tb1* message, perhaps by regulating *tb1* expression.

(v) *Effect on* cyclinB *message levels*

tb1 belongs to the TCP family of putative basic helix–loop–helix transcription factors that are expressed in actively dividing cells (Cubas *et al.*, 1999). It is thought to function as a repressor of the growth of organs in which its message accumulates (Doebley *et al.*, 1997). A reasonable hypothesis for how *tb1* represses organ growth would be that it suppresses cell division. Consistent with this hypothesis, rice PCF genes, which are TCP family members, have a known role in cell cycle regulation (Kosugi & Ohashi, 1997). To assay whether *tb1* controls organ growth via suppression of cell division, we measured *cyclinB* message levels in ear primordia of the four NILs.

Table 4. *ANOVA showing the F-ratios for the effects of the teosinte chromosome segments on messenger RNA levels for* teosinte branched1 (*TB-RNA*) *and* cyclinB (*CYC-RNA*)

	Effects			
Trait	T1L	T3L	T1L × T3L	d.f. (error)
TB-RNA	48·70***	0·22	4·4*	74
CYC-RNA	0·01	6·60*	1·85	62

* $P < 0{\cdot}05$; *** $P < 0{\cdot}001$.

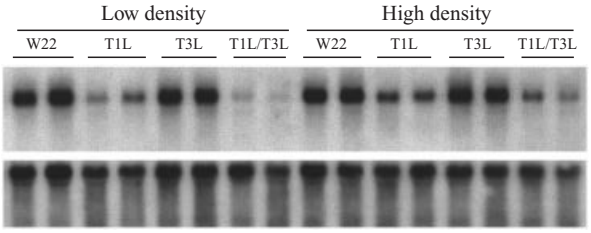

Fig. 3. Autoradiographs of a northern blot probed with *tb1* (above) and *ubiquitin* (below). RNA samples were obtained from all four NILs and both environmental treatments.

The anticipated result was that the teosinte chromosome segments should be associated with higher levels of *cyclinB* expression, and thus more actively dividing cells and longer branches. All the NILs possessing teosinte chromosome segments do indeed show greater amounts of *cyclinB* message than W22 (Table 3), but the difference is small and significant only for the T3L segment (Table 4). Thus, there is no good evidence that *tb1* and/or QTL-3L regulate organ growth through an effect on the cell cycle.

(vi) *Environmental effects*

While wild plants typically exhibit different phenotypes in response to their local environment, many

Table 3. *Relative levels of* teosinte branched1 *and* cyclinB *messenger RNAs* (±*SE*) *for the NILs under low- and high-density growth conditions*

	W22	W22-T1L	W22-T3L	W22-T1L/T3L
(a) *Low-density treatment*				
TB-RNA	11·22 ± 1·02	5·44 ± 0·92	13·16 ± 1·81	2·40 ± 0·53
CYC-RNA	24·28 ± 1·18	27·14 ± 1·75	32·04 ± 2·93	29·52 ± 1·54
(b) *High-density treatment*				
TB-RNA	12·33 ± 1·46	5·55 ± 0·79	11·06 ± 1·14	3·21 ± 0·94

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Table 5. *ANOVA showing the F-ratios for the effects of the teosinte chromosome segments and environmental treatment* (*Trt*) *on the morphometric traits and messenger RNA level for* teosinte branched1 (*TB-RNA*)

	Effects					
Trait	Trt	T1L	T3L	Trt × TL	Trt × T3L	d.f. (error)
LBIL	26·95***	128·26***	3·38	0·50	2·04	173
STAM	24·91***	241·55***	130·63***	35·12***	0·98	173
TILL	350·52***	97·11***	4·60*	127·17***	2·51	173
TB-RNA	0·00	90·06***	1·94	0·34	0·63	151

* $P < 0{\cdot}05$; *** $P < 0{\cdot}001$.

crop plants show less phenotypic plasticity across environments (Harlan, 1992). For example, wild plants can be branched or unbranched depending on whether they experience little or much competition from surrounding vegetation, but crops tend to remain unbranched (a high degree of apical dominance) whether grown in the open or in competition with other plants. Teosinte and maize fit this pattern with maize being essentially unbranched in all environments and teosinte being typically branched but capable of exhibiting an unbranched phenotype when grown under high density. Doebley *et al.* (1995) hypothesized that maize evolution involved selection for an allele of *tb1* that was insensitive to the environmental signal (high versus low density growth), causing maize to have an unbranched (high-density) phenotype under both high- and low-density growth.

Since our NILs isolate the effects of *tb1-maize* and *tb1-teosinte* in an otherwise essentially uniform background, they can be used to test this hypothesis. This was done by growing all four NILs under high- and low-density growth conditions. The plants of some lines showed a visible environmental response. Under low-density growth, W22-T1L and W22-T1L/T3L are strongly tillered, while under high density they are much less tillered (Fig. 1 b, d, f, h). In contrast, W22 and W22-T3L show less of a difference in plant architectures between these two environments. Similarly, under low-density growth, W22-T1L and W22-T1L/T3L have inflorescences with many male spikelets, although they produce far fewer male spikelets under high-density growth (Fig. 2*b*, *d*, *f*, *h*). For W22 and W22-T3L, the number of male spikelets appears more stable across environments. Thus, as anticipated, lines possessing the T1L segment (or *tb1-teosinte*) show greater phenotypic plasticity than lines lacking this segment (Table 1).

Analysis of variance (ANOVA) was used to test for interactions between the chromosome segments and the environmental treatments (Table 5). Environmental treatment and segment T1L had highly significant effects on all three morphological traits. Segment T3L had significant or nearly significant effects on these traits. Most interestingly, as hypothesized there are significant interaction effects between treatment and segment T1L for tillering (TILL) and the number of staminate spikelets (STAM). The nature of this interaction is that the mean trait values for W22-T1L vary greatly across treatments, while the mean trait values for W22 vary much less. Segment T3L did not show a significant interaction with environmental treatment. These results are consistent with the hypothesis that *tb1-maize* is less capable of responding to environmental signals than is *tb1-teosinte*.

If the maize versus teosinte alleles of *tb1* control the differential phenotypic response of the NILs to the environmental treatment, this might be reflected by a change in the level of *tb1* message across environments. *tb1* message levels should be higher under high-density growth. From the autoradiographs it appears that both W22-T1L and W22-T1L/T3L lines show somewhat more *tb1* message in the high-density than the low-density environment (Fig. 3). However, analysis of TB-RNA using ANOVA failed to show a significant interaction between segment T1L and treatment as would be expected if the environmental response in phenotype were controlled by changes in the expression of *tb1* (Table 5).

4. Discussion

(i) *Additive effects of teosinte chromosome segments*

One goal of QTL mapping is to measure accurately the QTL effects on quantitative traits (Tanksley, 1993). Although new statistical approaches for QTL mapping have enabled greater precision in identifying both the positions and effects of QTL (Zeng, 1994), empirical studies in which different QTL alleles are introgressed into an otherwise isogenic background are desirable because they allow one to measure single QTL effects in the absence of the confounding influence of other segregating QTL. In this study, the teosinte alleles of two previously identified QTL were introgressed into a maize inbred line. The resulting NILs enabled us to extend prior work on these QTL

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by using a more uniform genetic background. Since one of these two QTL (*tb1*) has been cloned, we could also extend prior work by examining *tb1* mRNA levels in the NILs.

The QTL located on chromosome 1L corresponds to *tb1*, with alleles *tb1-teosinte* and *tb1-maize* (Doebley *et al*., 1995). *tb1-teosinte* alone has a major effect on both plant and inflorescence architecture, rendering the W22 inbred line much more teosinte-like. In comparison with the maize inbred line W22, W22-T1L plants are on average fourfold more tillered, have 21% more staminate spikelets in their ears, and have lateral branch internodes that are 2·4 cm longer (Table 1). In the original maize-teosinte F2 population, the effects of *tb1-teosinte* were a 35% increase in staminate spikelets and 2·1 cm longer internodes (Doebley *et al*., 1995). When assayed in teosinte genetic background, the effects were larger: 41% increase in staminate spikelets and 5·7 cm longer internodes (Doebley *et al*., 1995). Thus, the effects of *tb1* appear to be influenced by genetic background, with teosinte background providing the larger effect. However, in all backgrounds *tb1* has a sizable additive effect and would seem to represent a major QTL.

QTL-3L behaves quite differently. When introgressed into W22, the teosinte allele of this QTL had little, if any, additive effect on the three morphometric traits (Tables 1, 2). Similarly, when assayed in teosinte genetic background, QTL-3L had no significant effects on either branch internode length or the percentage of stiminate spikelets (Doebley *et al*., 1995). These results contrast sharply with the original maize-teosinte F2 population in which the teosinte allele of QTL-3L was associated with a 24% increase in the percentage of staminate spikelets and branch internodes that were 2·2 cm longer. Thus, this QTL appears to be a 'moving target' with effects that are highly background-dependent.

In addition to the morphometric traits, we assayed QTL effects at the molecular level by measuring the level of *tb1* mRNA in inflorescence primordia. Previously, it was shown that homozygous *tb1-maize* plants have twice as much *tb1* message as homozygous *tb1-teosinte* plants (Doebley *et al*., 1997). For the low-density treatment, we observed this same relative difference (Table 3). This suggests that the functional difference between *tb1-teosinte* and *tb1-maize* may be in their regulation. This interpretation is supported by a population genetic analysis of *tb1* which revealed the signature of a past selective sweep in maize on the *tb1* promoter region but not on its protein coding region (Wang *et al*., 1999).

(ii) *Epistasis*

In this study we observed a strong synergistic, epistatic interaction between QTL-3L and *tb1* for the percentage of staminate spikelets in the inflorescence (Table 2). The epistatic effect is larger than the combined additive effects of these two QTL. In a previous study, an interaction between these two QTL was also observed (Doebley *et al*., 1995). In that study, the QTL were analysed in a teosinte genetic background, and, curiously, epistasis was observed but for different morphological traits. In the teosinte background, the percentage of staminate spikelets showed no interaction, but the presence/absence of the pedicellate spikelet and the arrangement of the cupulate fruitcases did. The latter two traits are invariant (or nearly so) among the NILs in W22 background, and thus could not be studied. The fact that the QTL affect different traits in these two backgrounds suggests that there are even higher-level epistatic interactions among the QTL that differentiate maize and teosinte. Thus, although *tb1* and QTl-3L interact to regulate a common developmental process (the rate of organ growth), the effect on the phenotypic level depends on genetic background.

In addition to a phenotypic epistatic interaction, we observed an interaction for *tb1* message levels. As with the phenotype, the teosinte allele of QTL-3L has no (or minimal) effect on its own, but, when combined with *tb1-teosinte*, there is a detectable negative synergistic interaction (Tables 3, 4). For message level, the interaction effect is small and only marginally significant ($P = 0{\cdot}04$). The reason that we detect only a small interaction effect with low statistical significance may be the result of error variance among the multiple northern blots that were needed to assay all the RNA samples. Although corrected by including two control lanes per blot, our sense is that among-blot error probably weakened the results.

The epistatic interaction between *tb1* and QTL-3L explains why we observed large phenotypic effects for QTL-3L in the original F2 population but only weak effects when this QTL was transferred into the homogeneous background of a NIL. In the F2, both *tb1* and QTL-3L were segregating, allowing the effects of QTL-3L to be expressed through its interaction with *tb1*. One implication of this interaction is that the maize allele of QTL-3L could reside in natural teosinte populations without affecting the morphology or fitness of the plants. However, during the domestication process, once *tb1-maize* was present in the population, QTL-3L would have visible effects and come under human selection. Thus, the domestication of maize may have depended on selection for a gene complex rather than strictly on the additive effects of individual genes. Selection on gene complexes may be a general phenomenon and there are a variety of other recent examples of mutants at one locus uncovering otherwise silent variation at others (Bowman *et al*., 1993; Rutherford & Lindquist, 1998; Gibson *et al*., 1999).

In quantitative genetics, epistasis is a component of the non-additive genetic variance, while, in classical or biochemical genetics, epistasis refers to genes whose products function in a common pathway (Phillips, 1998). Epistasis in the biochemical sense and epistasis in the quantitative genetic sense need not be the same. In the present study, however, statistical epistasis appears to correspond to biochemical epistasis. QTL-3L and *tb1* interact non-additively to influence both morphological traits and gene transcript level. Thus, the synergistic interaction between *tb1* and QTL-3L at the morphological level may be explained in part by changes in the regulation of *tb1*. The most likely mechanism is that QTL-3L acts as an upstream regulator of *tb1*, since the maize and teosinte alleles of *tb1* have similar mRNAs and encode similar proteins (Wang *et al*., 1999).

The importance and frequency of epistasis for quantitative traits remain uncertain. While some authors have been enthusiastic proponents of epistasis, the evidence for epistasis has not been commensurate with this enthusiasm (Crow, 1987; Tanksley, 1993). Why is this? Clark & Wang (1997) have noted that little epistasis is usually observed for morphological traits, although epistasis is commonly observed for traits closer to the expression of individual genes. The reason for this may be that multiple independent pathways contribute to morphological traits, confounding attempts to detect epistasis in one pathway when genes in all pathways are segregating. Perhaps, then, we were able to detect epistasis between *tb1* and QTL-3L because we have isolated their effects in the NILs.

(iii) *Genotype by environment interaction*

The evolution of phenotypic plasticity, or predictable changes in the values of phenotypic characters in response to environmental cues, has been a subject of both theoretical and experimental interest (Schichtling, 1986; Via, 1993). Many plant species respond to shade by elongating the main stem and repressing lateral branch outgrowth so as to outgrow competitors and capture more sunlight. This 'plastic response' has been shown to be adaptive in *Nicotiana* (Schmitt *et al*., 1995). When grown at high density, mutant *Nicotiana* lacking the elongation response, have less biomass and are shorter than plants which can elongate in response to shade. Correspondingly, when grown at low density, plants that have constitutive apical dominance have lower biomass than plants that can produce lateral branches.

In its natural habitat, teosinte responds to crowding in a similar way to other plant species: it branches less (J. D., personal observation). However, maize, like many crops, is unbranched in all environments and is less responsive to changes in planting density. Doebley *et al*. (1995) hypothesized that changes in *tb1* during maize domestication underlie this loss of the ability of maize to respond to differential crowding. Our morphometric results support this hypothesis. NILs possessing *tb1-teosinte* differ widely for TILL and STAM between the high- and low-density treatments, while NILs with *tb1-maize* are less variable (Table 1). Furthermore, some traits showed a significant interaction between *tb1* (or chromosome segment T1L) and treatment (Table 5). Thus, there is genetic variation in environmental response that is associated with *tb1-teosinte*, and selection for an allele with reduced environmental plasticity may have been one of the steps in maize domestication. Maize evolution appears to fit the model that rapid morphological evolution can be achieved when selection favours one morph of a phenotypically plastic species (West-Eberhard, 1986).

(iv) *Integrating evolutionary genetics and developmental biology*

The past decade has witnessed an explosion of interest in the integration of developmental and evolutionary biology (Raff, 1996). Most of this interest is focused on comparing the patterns of expression of developmentally important genes among higher taxonomic categories with distinct body plans, and drawing inferences about the path of phenotypic evolution based upon the degree of conservation or change in gene expression (e.g. Caroll *et al*., 1995). A parallel opportunity exists to integrate evolutionary and developmental genetics and investigate how genetic variation within populations (and between cross-compatible species) is translated by development into the phenotypic variation upon which selection operates. Several recent studies have taken advantage of this opportunity (e.g. Gibson & Hogness, 1996; Stern, 1998).

The present study touches upon two areas in which a role for developmental processes in generating phenotypic variation is suggested. First, we asked whether the effects of *tb1* on morphology are articulated through repression of the cell cycle by *tb1*. Our result that *cyclinB* message levels are roughly equivalent between *tb1-teosinte* and *tb1-maize* require that we reject this hypothesis. However, we regard this result as provisional since the inflorescence primordia that we assayed are complex structures with multiple organs developing at different rates. Thus, the question of whether the effects of *tb1* are articulated via the cell cycle needs to be revisited. Secondly, it is of interest that *tb1* and QTL-3L interact epistatically to control several aspects of inflorescence morphology (sex, presence of the pedicellate spikelet, and fruitcase arrangement) (Doebley *et al*., 1995; this paper), yet,

for tillering and branch internode length, only *tb1* has an effect. One possible explanation is that *tb1* is expressed over much of the life of the plant, regulating tiller initiation, branch elongation and inflorescence development, but that QTL-3L is expressed only during the reproductive phase during which it interacts with *tb1*.

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