Trends in Genetics

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Review

# Understanding Grass Domestication through Maize Mutants

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**As an economically important model crop plant, rich in genetic resources, maize has been useful for uncovering the genetic pathways responsible for domestication and plant improvement. However, several of the pathways that have been shown by recent studies to be important for domestication and/or yield in other grasses function differently in maize. In several cases, this unexpectedly wide functional divergence between genes from closely related grasses appears to be due to alternative modes of regulation rather than to simple differences in protein function. This indicates that domestication genes need to be understood within the architecture of the whole genome and the species-specific processes that they influence before they can serve as the foundation to improve plants.**

## Introduction to the Domestication Process

The wild relatives of crop plants are often poorly suited for farming and widespread agricultural production. Modern agronomic practices demand numerous changes to plant architecture and morphology, many of which comprise the 'domestication syndrome' (Box 1) [1,2]. In the case of maize, the resulting morphological changes were so dramatic that the domesticated form scarcely resembles the wild form, making the idea that maize evolved from **teosinte** (see Glossary) appear untenable [3]. George Beadle was the first maize geneticist to address this controversy directly by attempting to genetically map causative loci from maize–teosinte hybrids and determine the number of genes involved in domestication [4]. This approach has been fruitful in several species, and led to the identification of multiple domestication genes when combined with materials found in the archeological record [5]. In addition, new efforts involving genome resequencing have allowed researchers to rapidly identify putative domestication and/or yield genes in multiple grasses based on signatures of selection [6]. For some traits, such as the self-dispersal of grain or seed shattering, domestication has selected upon the same functionally conserved genes in maize, rice, and sorghum [7] as well as wheat [8]. However, there are surprising exceptions that are becoming more frequent as other domestication and/or yield loci are uncovered. The identification and analysis of these new loci in different grasses have revealed surprising aspects that could not have been predicted based on their function in maize. Such unpredictability in gene function has important implications for future crop plant design and breeding.

In this review, we highlight how the analysis of several maize mutants has helped us understand the developmental pathways that are important for either domestication or crop improvement. In several instances, there appears to be wide functional divergence between orthologous genes in maize compared with other grasses (Table 1) and yet, those genes were still selected for because of their positive effect on yield. In two cases, it has been shown that this functional diversity was caused by rare dominant mutations that resulted in novel modes of gene

## Highlights

A key domestication gene, *tb1*, encodes a transcription factor that targets other domestication genes, thus placing it at the top of a domestication hierarchy.

Despite having divergent functions in different grasses, domestication genes can still improve yield traits depending on where they are expressed.

Dominant domestication phenotypes can result from alternative modes of gene regulation due to transposon insertion or a lack of miRNA-mediated repression.

Mutations in developmental genes that normally result in sterility may improve yield when weak alleles are used in diploid crop plants. Alternatively, in polyploid crop plants, the presence of extra gene copies may partially compensate for strong loss-of-function alleles to achieve similar positive effects on yield.

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 https://doi.org/10.1016/j.tig.2018.10.007
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### Box 1. Domestication Syndrome

For many crop plants, especially cereals, there is a set of common traits and pathways that led to the formation of cultivated crops from their wild ancestors, resulting in increased yields, easier harvest, and greater seed size. They include lack of shattering, a reduction in lateral branching, a reduction of glumes and other appendages, and more determinate growth. The cloning and analysis of maize domestication genes revealed that many of these traits are under the control of single genes, such as *tb1*.

Table 1. Homologs and Orthologs of Domestication and/or Yield Genes

| Maize | Rice | Barley | Wheat | *Arabidopsis* | Gene family | Refs |
|---|---|---|---|---|---|---|
| *TB1* | *FC1* | *INT-C* | *TaTB1* | *BRC1* | *TCP* | [18,35,40] |
| *GT1* | *HOX12* | | *TaGT1* | *HB21* | *HD-ZIP I* | [25,26] |
| | | *VRS1* | | | *HD-ZIP I* | [30] |
| *TRU1* | *OsTRU1* | *CUL4* | *TaTRU1* | *BOP1/2* | *BOP* | [27–29] |
| *BD1* | *FZP* | *COM2* | *bh$^{t}$/WFZP* | *PUCHI* | *AP2* | [57–60] |
| *IDS1* | *OsIDS1* | *HvIDS1* | *Q* | *AP2* | *AP2* | [44,52] |
| *RA2* | *OsRA2* | *VRS4* | *TaRA2* | *ASL4/LOB* | *LBD* | [33,34] |
| *TGA1* | *OsSPL16/GW8* | *HvTGA1* | *TaTGA1* | *SPL13* | *SBP* | [22] |

### Glossary

**Cupule:** hardened cup-shaped receptacle in which the grain sits.
**Ear:** female inflorescence of maize that occupies a lateral position.
**Epistasis:** genetic phenomenon where the effect of one gene is dependent on other modifiers.
**Floret:** grass flower.
**Glume:** outer floral bract leaf produced by spikelets.
**Inflorescence:** group of flowers arranged on a stem.
**Lemma:** inner floral bract leaf of spikelets.
**Silk:** female floral organ comprising fused carpels.
**Spikelet:** a compact floral branch system that holds florets in all grasses.
**Stamen:** male floral organ that produces pollen.
**Tassel:** male inflorescence of maize that occupies a terminal position.
**Teosinte:** wild ancestor of maize.
**Tiller:** vegetative branch made at ground level in grasses that recapitulates the growth of the main shoot.

regulation. Here, we highlight several of these events in three crop plants (maize, wheat, and barley), and focus on two transcription factor gene families belonging to the TCP and AP2 clades that had critical roles.

## Maize Domestication via the TCP Transcription Factor Gene *teosinte branched1*

Maize was domesticated from teosinte nearly 10 000 years ago in the Balsas River Valley in Mexico [9]. Although originally controversial, the idea that maize evolved from teosinte is now universally accepted and confirmed by molecular and archeological evidence [10,11]. Perhaps one reason why this idea was so difficult to accept initially is the fact that the divergent plant architecture between maize and teosinte made the relatedness between the two appear implausible. For example, domesticated maize produces few or no **tillers**, while teosinte produces several (Figure 1). Teosinte also produces several long aerial branches tipped by **tassels**, while maize produces short, single **ears** at the same position. While teosinte also produces ears, they are small, and several are produced at each node, a trait called 'prolificacy' [12]. Finally, teosinte grains are covered by several hardened structures, including a **cupule** as well as heavily elaborated lateral organs called **glumes**, which protect the grain from predation. In comparison, maize glumes are soft and highly reduced [13]. The paradox of the origin of maize from teosinte was addressed experimentally by George Beadle, who scored parental phenotypes from 50 000 F2 plants from a maize–teosinte cross, allowing him to deduce that as few as five genes were required to create maize from teosinte [4,14]. Building on Beadle's experiment, subsequent efforts highlighted several regions of the maize genome as being important for domestication [15]. A key outcome of this work was the identification of *teosinte branched1* as a major domestication locus acting **epistatically** on several domestication traits, including tillering, **inflorescence** sex, prolificacy, and glume hardness (Figure 1A–C) [16,17]. Cloning of the *tb1* gene showed that it encodes a *TCP* transcription factor that is overexpressed in domesticated maize [18] and is induced by high levels of far-red light [19]. Based on the loss-of-function phenotype in maize that

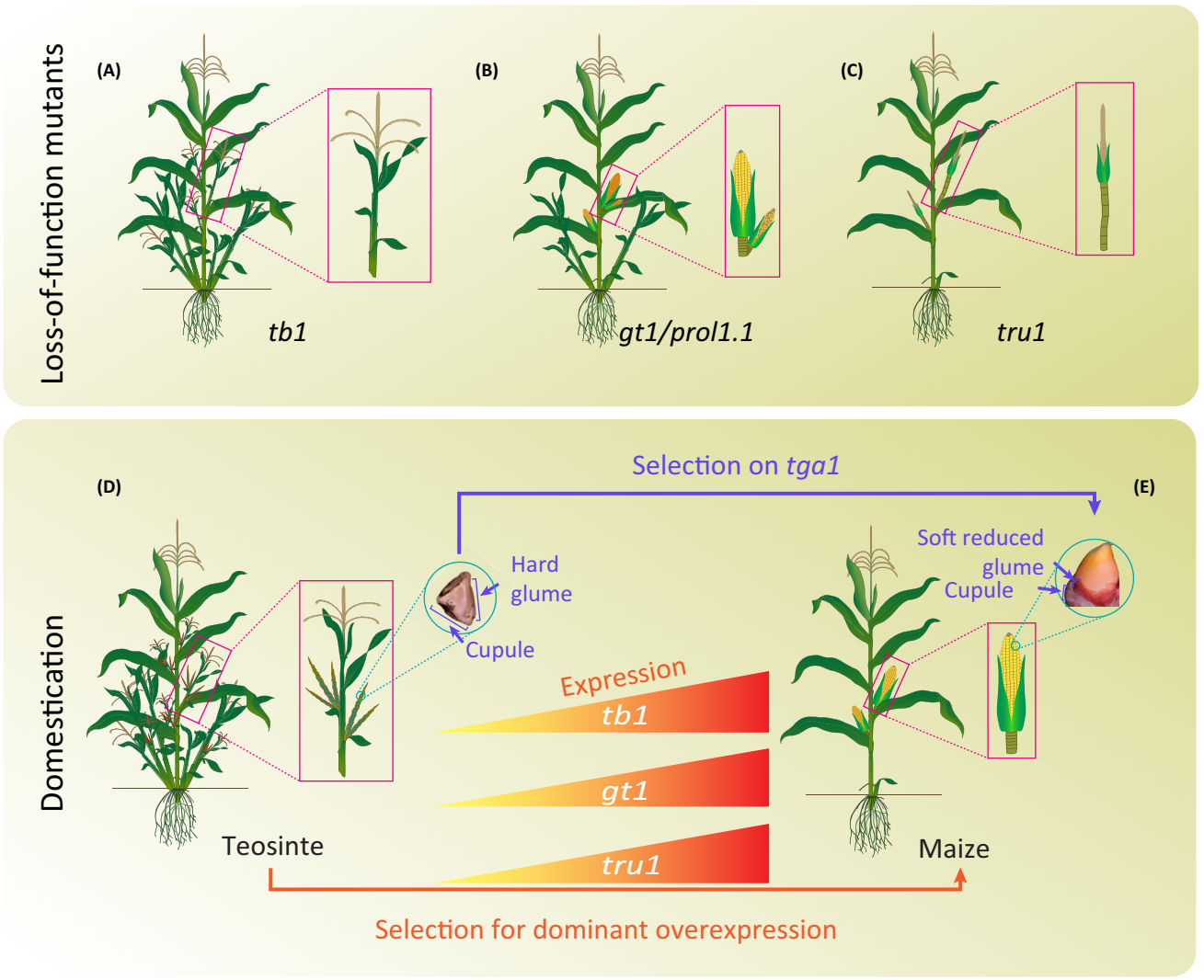


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Figure 1. Maize Domestication Genes and Phenotypes. Mutant phenotypes that have facilitated the cloning of domestication genes in maize (A–C). (A) *tb1* recapitulates the excess branching of teosinte, including excess tillering and female ears replaced by elongated aerial branches tipped by male tassels. (B) The *gt1* mutant enhances tillering at the base of the plant. Allelic variation in the *gt1* promoter (*prol1.1*) is responsible for the prolificacy trait in which multiple ears are produced at the same node (inset). (C) *tru1* mutants produce long axillary branches tipped by male tassels in place of female ears. (D,E) Four major genes are essential for the transition from teosinte to maize morphology. Selection on *tga1* is responsible for the softening of the reduced glume in maize, while selection for the overexpression of *tb1*, *gt1*, and *tru1* contributes to the suppression of axillary branches and prolificacy, and promotion of lateral branch sex identity.

produces excess axillary branches, TB1 was proposed to function as a repressor of branching. Modern maize is generally untillered because the *tb1* gene is overexpressed due to the insertion of a *hopscotch* retrotransposon ~58 kb upstream of the promoter [20].

The fact that TB1 is a transcription factor that has a quantitative effect on numerous domestication traits indicated that it may target other domestication loci [21]. This was later confirmed with respect to genes that control the glume reduction and prolificacy traits. For example, glume softening and reduction in domesticated maize is a dominant trait caused by an amino

acid change at the beginning of a SQUAMOSA PROMOTER BINDING PROTEIN transcription factor gene called *teosinte glume architecture1* (*tga1*) [22] (Figure 1D,E). TB1 directly regulates *tga1*, as confirmed by ChIP-qPCR and gel shifts that identified a TB1-binding site in the *tga1* promoter [23,24]. Similarly, the dominant ear prolificacy trait was mapped to the promoter of a known repressor of tillering, *grassy tillers1* (*gt1*) [12] (Figure 1B). *gt1* encodes a homeodomain-leucine zipper (HD-ZIP) protein that is genetically downstream and positively regulated by *tb1* [25]. This pathway is also conserved in eudicots, since the *Arabidopsis* ortholog of *gt1* is also a direct target of the *TB1* ortholog [26].

A third domestication locus was found to affect axillary branch length as well as lateral inflorescence sex in a manner epistatic to *tb1* [16]. The map position of this mutation correlated with the position of a known mutant called *tassels replace upper ears1* (*tru1*) [27]. *tru1* mutants produce long axillary branches tipped by male tassels in place of female ears (Figure 1C). The recent cloning of *tru1* revealed that it encodes a BTB/POZ ankyrin repeat protein that was under selection during maize improvement after domestication [28]. Similar to *tga1*, *tru1* is a direct target of TB1, as confirmed by ChIP-qPCR and gel shifts, thus explaining the genetic epistasis. Immunolocalization of TRU1 and TB1 showed that both are expressed in early axillary buds and the surrounding leaves in overlapping patterns, but not specifically in the growing axillary meristem [28]. Comparing the expression of TB1 and TRU1 in maize versus teosinte showed that both genes are overexpressed in the stem tissue below the female inflorescence only in maize. Such tissue specificity is an important aspect of how TB1 is able to effect desirable plant architecture. Since TB1 is a growth repressor, strong expression throughout the ear would likely cause sterility and, thus, would be selected against.

As an intriguing example of functional divergence, the analysis of recessive loss-of-function alleles of the *tru1* ortholog in barley showed a completely different phenotype compared with maize. The *uniculme4* (*cul4*) mutant causes a shift in distal leaf identities to be more proximal, phenotypes not seen in maize. Surprisingly, *cul4* plants display a dramatic reduction in the number of axillary branches and tillers, the complete opposite of the maize *tru1* phenotype [29]. This difference may lie in the fact that, in domesticated maize, *tru1* has a unique expression pattern and is dominant compared with the loss-of-function mutant in barley [28].

The studies described earlier highlight how a single dominant mutation in *tb1* led to its overexpression, which helped generate a suite of other domestication and agronomic traits. It is interesting that this pathway is caused by a gain-of-function mutation, an increasingly common phenomenon being discovered in domestication genes in other plants. The isolation of corresponding loss-of-function phenotypes in related grasses can give a clearer picture of how this pathway normally functions in different evolutionary contexts.

## The TB1 Pathway in Barley and Wheat Controls Row Number and Yield

Five loci that increase row number have been identified in barley, but several of them converge on the same *tb1/gt1* module identified in maize. The wild ancestor of barley is two-rowed, meaning that the inflorescence contains one fertile **spikelet** (Box 2) at each node in an alternating opposite pattern, providing an appearance of two parallel rows of grain proceeding up the rachis (Figure 2A). In two-rowed cultivated barley and its wild progenitor, each of these fertile spikelets is flanked by two additional sterile spikelets, resulting in narrow spikes. In modern six-rowed barley, these sterile spikelets are fertile, producing a potentially threefold yield increase (Figure 2B). The six-rowed trait was mapped to mutations in the *Vrs1* gene, an HD-zip transcription factor gene homologous to *gt1,* although not the direct ortholog. *Vrs1* functions to repress lateral spikelet fertility, and loss-of-function mutations are sufficient to

### Box 2. Structure of the Spikelet

All grain is produced by structures called spikelets ('little spike') that produce a variable number of florets enclosed by leaf-like lateral organs. In maize, the female spikelets produce a single floret (see Figure 2D in the main text), the male spikelets produce two (see Figure 2C in the main text), while other grasses, such as wheat, can produce an indeterminate number of florets (see Figure 2G in the main text). The number of florets per spikelet is under the genetic control of genes such as *indeterminate spikelet1* in maize. After the florets are fertilized, the resulting grain in many wild species continues to be covered by several lateral organs of the spikelet, including a pair of bract leaves called lemmas as well as a pair of hard glumes that protect the grain. During domestication, mutations that render the glumes more fragile or reduced were selected for, allowing the grain to be easily accessible.

convert the rudimentary lateral spikelets to be fertile [30]. In addition, there are several modifiers of *Vrs1* that can enhance its phenotype. For example, *Vrs1* is regulated by several other *vrs* loci, including *vrs3*, a histone demethylase [31,32], and *Vrs4*, a LOB domain transcription factor gene orthologous to the *ramosa2* gene of maize [33], which functions to promote branch determinacy [34]. The functions of these *LOB*genes appear to be conserved in maize and barley since loss-of-function mutations in both grasses lead to greater inflorescence meristem indeterminacy (Box 3). *int-c,* the barley ortholog of *tb1*, was identified as a modifier of the *Vrs1* phenotype [35] that increases its effect on row number, producing an intermediate phenotype between two-row and six-row spikes [35,36]. As yet another example of functional divergence, the *intc* loss-of-function mutations significantly reduce tillering in barley, the opposite of the recessive *tb1* mutant phenotype in maize [37]. This effect was also seen with *Vrs1* mutants that display reduced tiller number [38,39], the opposite of *gt1* mutants in maize [25]. Conversely, no spikelet or row number phenotypes were noted in the *tb1* or *gt1* recessive mutants in maize.

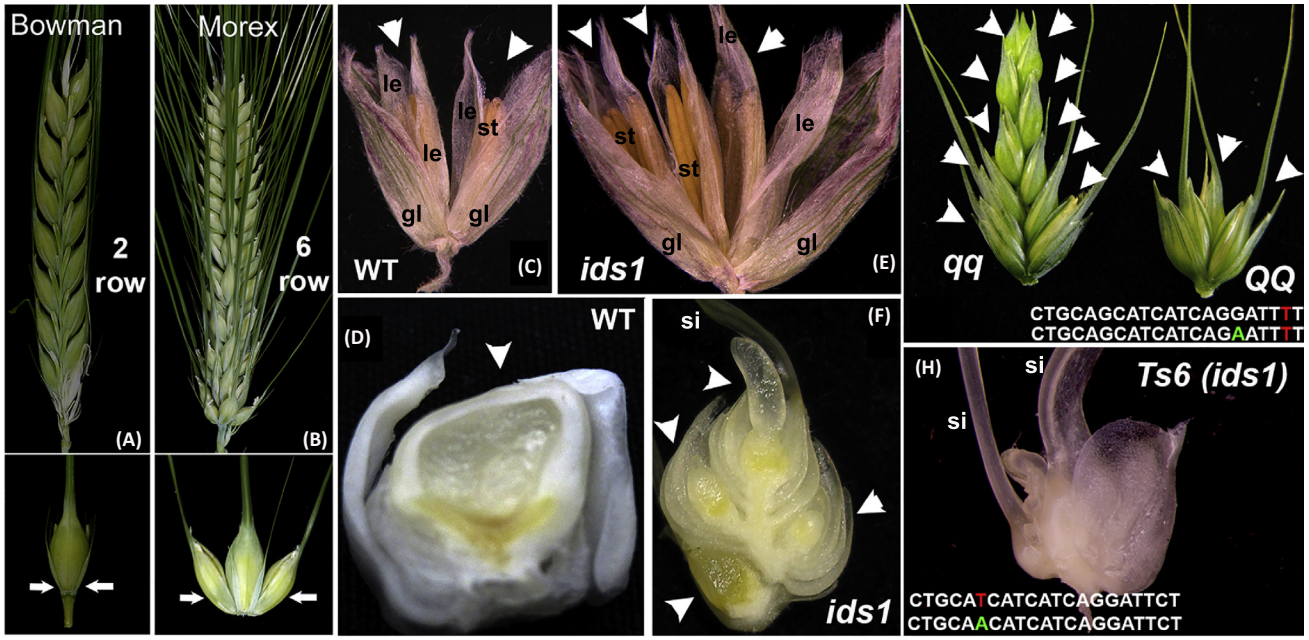


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Figure 2. Barley, Maize and Wheat Spikelets and Mutant Phenotypes. (A) Two-row barley inflorescence and spikelet below. Arrows point to sterile spikelets. (B) Six-row barley and spikelet below. Arrows point to fertile spikelets. (C) Normal male maize tassel spikelet with two florets (arrowheads). (D) Normal maize female ear spikelet with one floret. (E) *ids1* tassel spikelet with three florets. (F) *ids1* ear spikelet with four florets. (G) *QQ* (right) versus *qq* highly indeterminate wheat spikelet (left). The *miR172*-binding site sequence of *q* is shown below marking the positions of mutations (green and red) responsible for two dominant *Q* alleles. (H) T*s6* male tassel spikelet showing conversion to female identity. *miR172*-binding site sequence of *ids1* is shown below, marking the positions of mutations (green and red) responsible for two *Ts6* alleles. Abbreviation: gl, glume; le, lemma; si, **silk**; st, **stamen**; WT, wild type.

Box 3. Determinate versus Indeterminate Meristems

Meristems are groups of plant stem cells necessary for the growth of all the tissues and organs of the plant. After every organ initiation event, meristem cells are lost and, thus, cells must be renewed for growth to continue. Meristems that make a limited number of cells and organs and then terminate are 'determinate', while those that make an unlimited number and constantly renew themselves are 'indeterminate'. The spikelet meristem of maize is determinate, producing two florets and then terminating. By contrast, root meristems are indeterminate and never terminate. In grasses, several genes are necessary to specify whether a meristem is determinate or indeterminate, some of which have had a role in domestication.

Elucidating the entire *tb1/intc* pathway in both species will be required to fully comprehend these opposing effects. However, despite this divergence in *tb1* and *int-c* function, both genes were still selected for during domestication and breeding for crop improvement.

Wheat yields can also be affected by variations to the *tb1* regulatory module, primarily through modifications of the inflorescence. The wheat spike is alternate distichous with only one spikelet at each node. A variant called *highly branched* (*hb*) produces paired spikelets at each node [40] and is due to the presence of extra copies of the *TaTB1* gene in hexaploid wheat. The presence of paired spikelets has a direct effect on yield, and is a defining characteristic of all members of the Andropogoneaetribe of grasses, including economically important crops, such as maize, sugar cane, and sorghum. Wheat is not a member of the Andropogoneae and, thus, the paired spikelet phenotype of *hb* is morphologically and phylogenetically distinct. As yet another example of functional divergence, the effect of increased TB1 in wheat appears to enhance branching, which is the opposite effect seen in maize. However, this divergence is consistent with its conserved role as a growth repressor. In wheat, TaTB1 protein interacts with the floral regulatory protein FLOWERING LOCUS T1 (FT1), which is necessary for promoting the floral transition. This interaction is proposed to suppress FT1 function, thereby reducing expression of the spikelet meristem identity genes and prolonging branching activity of the spikelet [40]. Thus, depending on the organism, both increases and decreases in TB1 activity can produce highly ramified branching depending on whether it expresses during the floral phase versus the vegetative phase.

## Wheat Domestication and the Role of AP2 Transcription Factors

Dominant mutations were also important for the domestication of grain characteristics in wheat. Modern bread wheat *(Triticum aestivum)* was domesticated nearly 10 000 years ago near the Fertile Crescent [41] from tetraploid wheat hybridized with a wild diploid goatgrass, *Aegilops tauschii*, resulting in a hexaploid genome [42]. In wild wheats (or 'hulled wheat'), the glumes of the spikelet tenaciously enclose the grains, requiring a strong force to liberate them. In addition, the rachis is fragile and shatters easily into individual spikelets, complicating its harvest. However, in domesticated bread wheat, the rachis is not fragile, the glumes are soft, and the grains release easily from the spikelets, a property called 'free threshing'. Several domestication loci were mapped that affect the free threshing character [43], and one of them, a dominant trait called *Q*, was localized to a region containing an AP2 transcription factor gene orthologous to a maize gene called *indeterminate spikelet1* (*ids1*) [44]. *Q* not only controls the free threshing character, but also positively affects several other agronomic traits, including earlier flowering time, reduced height [45,46], increased grain density, and spike compactness [47]. The molecular lesion responsible for the *Q* domestication phenotype was initially ambiguous, but was narrowed down to an amino acid change that reportedly affects protein dimerization [44]. However, another amino acid change was observed in a *miR172*-binding site. All wheat lines that possess the dominant *Q* trait have an identical polymorphism in the second to last nucleotide of the microRNA binding site compared with undomesticated lines [48]. This

observation indicated that the dominant *Q* phenotype maybe due to this polymorphism, although any direct experimentation supporting this hypothesis was lacking. However, recent analyses by a pair of research groups have shown that this microRNA binding site mutation is in fact responsible for the *Q* domestication phenotype. Degradome analysis showing reduced microRNA mediated degradation in a *Q* background, phenocopy of the *Q* phenotype by suppressing *miR172* activity [49], as well as the isolation of new dominant *Q* mutants with unique SNPs within the *miR172* binding site [50] (Figure 2G) all support the hypothesis that the *Q* phenotype is due to a lack of *miR172*-mediated suppression. The mechanism behind the dominant glume-softening traits of *Q* was hypothesized to be due to a homeotic conversion of glumes to **lemma** organ fates [49], consistent with the role of *AP2* transcription factor genes in specifying floral organ fate [51].

## Conservation of Loss-of-Function Phenotypes, but Divergence of Gain-of-Function Phenotypes of a Domestication Gene

Compared with the various *tb1* orthologs in different grasses, the function of the maize *ids1* gene appears to be conserved in wheat. *ids1* in maize was originally identified as a meristem determinacy mutant that makes several extra **florets** in both the female and male inflorescences [52] (Figure 2E,F). The function of *ids1* is conserved in wheat since *qq* loss-of-function mutants show a similar extra floret phenotype [49] (Figure 2G). Interestingly, a domestication quantitative trait locus (QTL) for kernel row number in maize also identified *ids1* as a strong potential candidate gene [53]. This appears plausible since *ids1* mutants strongly reduce kernel row number in combination with mutations in its duplicate locus, *sister of ids1* (*sid1*) [54], although further confirmation is needed to determine whether *ids1* is responsible for the increase in row number in modern maize.

Despite the conservation in function between *ids1* and *q*, the gain-of-function phenotypes diverge. Dominant alleles of *ids1* caused by *miR172*-binding site mutations similar to *Q* were identified in maize based on a completely different suite of phenotypes. The dominant maize mutant *Tasselseed6* (*Ts6*) (*ts6*) switches tassel floret sexuality from male to female and alters spikelet meristem identity [55]. Chromosome walking led to the cloning of *Ts6* and its identification as a novel allele of *ids1*. Two independent *Ts6* alleles revealed two different mutations in the *miR172*-binding site of *ids1*, both of which lead to ectopic expression of IDS1 protein [48] (Figure 2H). No other *Ts6* phenotypes overlap with *Q*. A possible reason for this divergence in gain-of-function phenotypes may lie in the fact that the *ids1* gene in maize functions in the context of the sex-determination pathway dependent on the plant hormone jasmonic acid (JA) [56]. Maize is monecious, containing male and female flowers on the same plant due to differential expression of JA-specific sex identity genes, differing from wheat, where all the flowers are bisexual. In maize, the dominant activity of AP2 proteins may function differently in these tissues, indicating that the tissue- and organ-specific context in which domestication genes function can have a major impact on phenotype.

## Weak Alleles of A Conserved Regulator of Floral Branching Affect Grain Yield

In contrast to the divergence found in the *tb1* network, the maize *branched silkless1* (*bd1*) gene has been shown to have complete conservation in function among the major cereal crops. BD1 is an AP2 transcription factor that is required for spikelet meristem identity [57] and, when mutated, leads to either branched spikelets or conversion of spikelets into branches in several species, including wheat [58], barley [59], and rice [60]. While excess branching often comes at the expense of seed set, especially in maize and rice,where the recessive mutants are sterile,

wheat is an exception. The phenomenon of 'Miracle Wheat' is the result of the identification of strong *bd1* alleles that cause excess spikelet branching and increased spikelet number, thus increasing grain yield [59]. Similarly, the rice *control of secondary branch 1* (*cos1*) mutant, which produces many more panicle branches and higher yields, is another weak allele of the rice *bd1* ortholog [61].

The above examples are confounding in light of the trend during domestication to favor reduced branching, especially during the vegetative phase. However, this trend does not always apply to the floral phase, where it is clear that increased branching can have a positive effect on yield. For example, mutations in a TCP transcription factor gene in sorghum called *multiseeded1* result in nearly double the number of grains per panicle by increasing the number of fertile spikelets [62]. Branching incorporates a fertility trade-off that is best visualized along an arc between two extremes [63]. In both branching extremes, fertility suffers: zero branching, in theory, would produce low yielding single spikelets, but continuous indeterminate branching often fails to produce fertile florets, as shown by the *bd1* phenotypes in maize and rice. However, in polyploids, such as wheat, the presence of multiple redundant genomes may compensate for loss-of-function mutations, and produce an intermediate phenotype that increases yield. The introduction of partial loss-of-function alleles may achieve the same effect in diploids, such as rice, making the discovery of such alleles important for crop improvement.

## Concluding Remarks

### Phenotype Is Dependent on Biological Context

Dominant phenotypes are important, but the functions of the dominant genes can diverge depending on biological context. In the case of the *Q* and *Ts6/ids1* genes, it may not be surprising that gain-of-function mutant phenotypes differ even though the loss-of-function phenotypes are conserved, since the maize gene participates in a sex determination pathway that does not exist in wheat florets that are bisexual. Furthermore, some domestication genes normally function in specific expression domains, and their resulting phenotypic effects may differ outside of them. For example, although *tb1* is a conserved repressor in several grasses, the effects of *tb1* differ depending on the tissue, repressing axillary branching during the vegetative phase in maize, repressing floral identity in wheat, and repressing spikelet fertility in barley. When the latter two functions are lost, floral branching increases in these grasses compared with maize. While phenotypic divergence is not unexpected in related grasses, it is surprising when selection favors the same gene networks for opposing architectural traits. However, such divergent modes of gene function are not unique and have been observed with conserved rice floral regulators that promote flowering under some light regimes, but also repress it under others [64].

### Why Was *tb1* a Target for Selection in Multiple Species?

Until the entire *tb1* pathway is elucidated and its targets functionally characterized in additional crop plants besides maize, it will remain unclear why both gain-of-function and loss-of-function variants of *tb1* were recurrent targets of selection in different grasses (see Outstanding Questions). Given the current data for *tb1* function, we propose three possible explanations for why it was a common target for crop domestication and improvement (Figure 3, Key Figure). First, there is a large body of evidence showing that TB1 can modify branching according to environmental signals [19], even in eudicots,such as potato, where the ortholog has a role in tuber formation in response to light quality [65]. Thus, a rapid way to decouple plant development from its dependence on environmental inputs to fit the needs of early farmers would be to select for *tb1* mutations. Second, since spikelets are produced in lateral positions where *tb1* normally functions and is expressed, selection on *tb1* would have a direct impact on yield in

### Outstanding Questions

Are there other factors responsible for the functional divergence in the *tb1* pathway in maize, barley, and wheat? Do the downstream targets differ and are there other protein cofactors responsible for the functional diversity?

Since TB1 targets two other domestication and/or improvement loci, did the selection of the domesticated *tb1* allele occur simultaneously with these other genes, or were they selected for after the initial *tb1* domestication event?

Could the positions of the microRNA binding site mutations in the *miR172* sites of *ids1* versus *Q* contribute to the differences seen in the dominant phenotypes?

Why was *tb1* selected for in both barley and maize for domestication and/or plant improvement in light of their functional differences? Are there genetic constraints, such that only a few genes can serve as domestication and/or improvement loci?

Since *tb1* regulates other domestication loci, is it possible to domesticate a new crop plant simply by altering *tb1* expression?

## Key Figure

### TB1 Functions as a Repressor in Maize, Wheat, and Barley with Different Phenotypic Outcomes

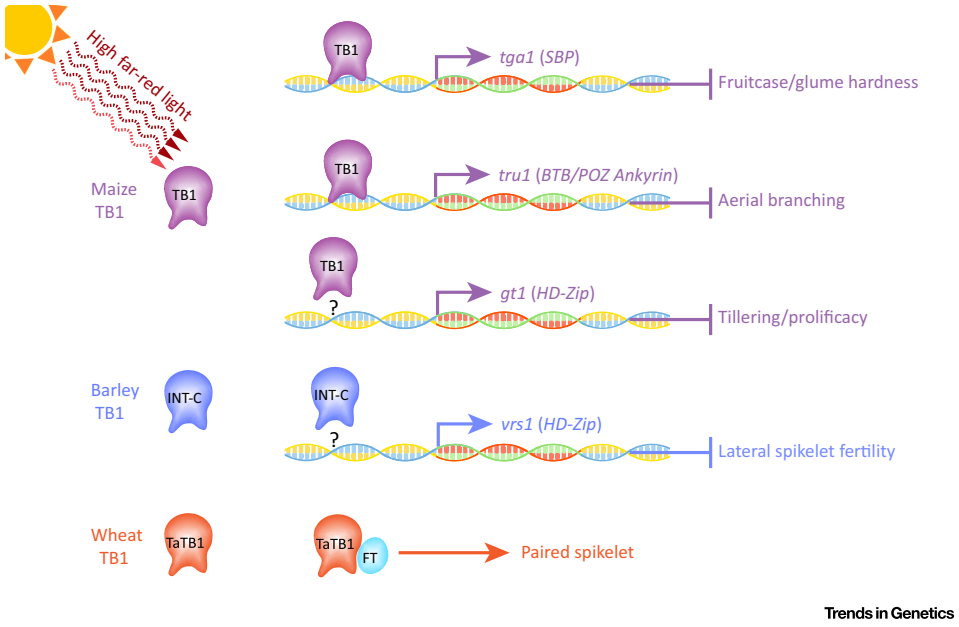


Figure 3. A high ratio of far-red light activates *tb1* expression in maize (purple), which binds to the promoters of the *tga1* and *tru1* genes to control glume softness and aerial branch fates. *tb1* also activates *gt1* to repress vegetative tillering and prolificacy. In barley (blue), the *tb1* ortholog *int-c* either directly or indirectly activates expression of the *gt1* homolog *vrs1* to repress lateral spikelet fertility. In wheat (red), the TB1 ortholog controls flowering by interacting with the FT protein to restrict floral determinacy and promote paired spikelet formation.

grasses. Third, TB1 appears to be a master regulator operating in a large regulatory hierarchy that targets other domestication loci. Therefore, mutations in this gene may quickly cause numerous, potentially beneficial phenotypic changes. While there are many genes that could bring about any one of these effects, *tb1* may be the only one to influence all three. Moving forward, a major advance in crop improvement will be engineering the ability to separate the full array of phenotypes that accompany variations in the *tb1* network to improve specific organs without affecting others.

### Acknowledgments

The authors thank China Lunde and Sam Leiboff for reviewing the manuscript. Z.D., M.A., and G.C. are supported by National Science Foundation grant PGRP IOS-1339332 (to G.C.).

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