1996

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Recommended Citation

Doebley, John (1995) "Genetic Dissection of the Morphological Evolution of Maize," *Aliso: A Journal of Systematic and Floristic Botany*. Vol. 14: Iss. 4, Article 8.

Available at: <https://scholarship.claremont.edu/aliso/vol14/iss4/8>

GENETIC DISSECTION OF THE MORPHOLOGICAL EVOLUTION OF MAIZE

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ABSTRACT

Maize (*Zea mays* ssp. *mays*) and its wild progenitor, teosinte (*Z. mays* ssp. *parviglumis*) differ dramatically in inflorescence and plant architecture despite the fact that their evolutionary divergence occurred within the past 10,000 years or less. To elucidate the genetic control of the morphological differences between maize and teosinte, my colleague and I employed quantitative trait locus mapping with molecular markers. Results indicated that most of the variation in plant and inflorescence morphology between maize and teosinte can be explained by five restricted regions of the genome. In this paper, characterization of three of these regions and their effects on plant and inflorescence development will be discussed. Each of these regions appears to contain a single major locus of large effect. One of these loci, *teosinte branched1*, largely controls the difference in plant architecture. Another, *teosinte glume architecture1*, controls the formation of the teosinte cupulate fruitcase that encases the kernel. A third candidate, *terminal ear1*, is hypothesized to control internode elongation within the inflorescence. In addition to their main effects, each locus appears to have pleiotropic effects on other traits. Genetic analyses also demonstrate that some of these loci exhibit epistatic interactions. The results suggest that mutations at a small number (five) of regulatory loci may have been the initial steps in the domestication of maize, supporting a model for maize evolution proposed by George Beadle in 1939.

Key words: development, gene, maize, QTL mapping, teosinte

INTRODUCTION

Maize (*Zea mays* L. ssp. *mays*) shows striking differences in both inflorescence and plant architecture from its nearest wild relative, teosinte (*Z. mays* ssp. *parviglumis* Iltis & Doebley). Nevertheless, maize and its teosinte ancestor are members of the same biological species, being fully interfertile and having no more difference between them in the structure of their genes and chromosomes than exists between two different forms of maize itself (Beadle 1932; Kato 1976; Doebley 1990). Thus, paradoxically, there exists a large morphological difference in the absence of a commensurate level of genetic divergence.

Beadle (1939) proposed a simple but controversial solution to this paradox. He hypothesized that teosinte was the ancestor of maize and that a relatively small number of mutations of large effect were responsible for the evolution of the basic set of morphological differences between maize and teosinte. Based on experimental work of Mangelsdorf and Reeves (1939), Beadle (1939) hypothesized that about five gene changes were involved. Later, he obtained independent support for his hypothesis by demonstrating that the proportion of maize-like and teosinte-like segregants in a large maize-teosinte F₂ population was approximately that

expected if there were five major gene differences between these plants (Beadle 1972). Archaeological data suggest that the teosinte to maize transition took place between 5,000 and 15,000 years ago (Smith 1995; see also Hanson et al. 1996).

QTL MAPPING

Over the past six years, my laboratory has been investigating the inheritance of the morphological differences between maize and teosinte. Like Beadle, my colleagues and I wish to determine the genetic basis of these differences and to infer from this information the genetic steps involved in the evolution of maize. We have been fortunate to be able to take advantage of a new technology called Quantitative Trait Locus mapping, or QTL mapping for short (Tanksley 1993). Our QTL mapping studies involved creating two segregating maize-teosinte populations, and then determining the genotype of the individual plants in these populations at a series of molecular marker loci throughout the genome and measuring each plant for the morphological traits that differentiate maize and teosinte. Once these data were compiled, we performed statistical tests of association between the genotypes at the individual marker loci and the mea-

Fig. 1. The ten maize chromosomes show the position of five chromosomal regions (stippled rectangles) that possess QTL with large effects on the traits that distinguish maize and teosinte. Cross-marks indicate the position of the molecular markers used in one of the QTL mapping experiments. Small black circles indicate the approximate positions of the centromeres.

surements for the morphological traits. Where a statistically significant association was observed for a particular trait with a particular marker locus, we could infer that a gene or QTL controlling the trait was located at or near that marker locus.

QTL mapping also provides some basic information about each QTL. For example, because the chromosomal locations of the marker loci are known, one also learns the approximate chromosomal location of each QTL. The magnitude of the effect of each QTL is also estimated so one can distinguish between those of small versus large effect. Comparison among the different genotypic classes for each QTL enables one to estimate the degree of dominant versus additive gene action for each QTL. If one has marker loci closely spaced (every 20 centimorgans) throughout the genome and a large population size, one can obtain a reasonable estimate of the minimal number of gene changes involved in the evolution of each trait. Thus,

Fig. 2. Mexican annual teosinte and maize plant architectures.

one learns the number of genes involved, their chromosomal locations, the magnitudes of their effects and their mode of gene action. Compared to what was possible just 10 years ago, QTL mapping provides remarkable power to dissect the inheritance of complex traits that distinguish any pair of cross-compatible species.

The principal results of the QTL mapping work with maize and teosinte are that most of the key taxonomic traits that distinguish maize and teosinte are controlled by a relatively small number (5–8) of QTL with detectable effects (Doebley and Stec 1993). For most traits, we observed at least one QTL of large effect (i.e., controlling 20–50% of the phenotypic variance). Moreover, the QTL of large effect are restricted to five regions of the genome (Fig. 1); this result corresponding nicely with Beadle's estimate that five genes of large effect were involved in the early evolution of maize. In the remainder of this paper, I will summarize the present understanding of the nature of the QTL in three of these five regions and models for how these QTL may alter morphogenesis to produce the very different adult morphologies of maize and teosinte.

CHROMOSOME 1: THE TEOSINTE BRANCHED LOCUS

Teosinte plants normally have long lateral branches that are tipped by tassels or male inflorescences, while maize has short lateral branches that are tipped by ears or female inflorescences (Fig. 2). This difference between maize and teosinte is controlled by several loci; however, the locus of largest effect is on the long arm of chromosome 1 (Doebley and Stec 1993). This region of the maize genome contains a known maize mutant called *teosinte branched1* (*tb1*) that makes maize resemble teosinte; i.e., *tb1* causes short branches

Fig. 3. Plants homozygous for the teosinte (left) and maize (right) alleles at the QTL on chromosome arm 1L. These plants demonstrate how the maize allele at this QTL severely reduces lateral branch length.

with ears to be replaced by long branches with tassels). *tb1* seemed a good candidate for the QTL on chromosome 1 and my colleagues and I have recently been able to use a simple genetic complementation test to show that our QTL and *tb1* are the same locus (Doebley et al. 1995).

To understand how *tb1* might have altered teosinte morphology, we used backcross breeding to transfer the maize chromosome segment carrying *tb1* into teosinte. The resulting plants have elongation of their lateral branches suppressed and teosinte ears, not tassels, at the tips of these short branches (Fig. 3). This introgressed segment had other effects as well. First, the shorter lateral branches did not result from fewer internodes but actually from a larger number of shorter internodes. Second, the introgressed segment also alters the pattern of internode elongation in the ear such that there are a larger number of shorter internodes in the ear (Fig. 4). Thus, this segment contains a QTL that affects the pattern of internode elongation in both the lateral branch and the ear. Third, this chromosome segment increases the frequency of paired spikelets (as in maize) relative to single spikelets (as in teosinte). Fourth, this introgressed chromosome segment dis-

rupts the normal process of disarticulation of the teosinte ear so that it remains intact as found in maize. Doebley et al. (1995) argue that all of these effects are the result of a single gene, *tb1*.

A model for teosinte branched1: Plants of many species can respond to their local environment and grow into slender unbranched plants under strong competition (shading) from surrounding vegetation or into robust highly branched plants with little competition (Givnish 1988). In other words, the degree of apical dominance that plants exhibit is strongly influenced by environment. Based on my observations, teosinte also appears capable of this type of plastic response to local environment. Given its role in regulating apical dominance, it is easy to envision that *tb1* is involved in regulating this response. This effect could be produced if *tb1* functioned to repress axillary meristem development. Accordingly, Doebley et al. (1995) proposed the following model for the function of *tb1* in teosinte. Under favorable environmental conditions, *tb1*+teosinte is turned off (no repression), allowing axillary meristems to develop fully into tillers or long lateral branches tipped by tassels. Under unfavorable condi-

Fig. 4. Immature ears from plants homozygous for the teosinte (left) and maize (right) alleles at the QTL on chromosome arm 1L. These ears demonstrate how the maize allele at this QTL alters ear morphology by producing some yoking of the cupulate fruitcases and by producing a larger number of fruitcases. Bar = 1 cm

tions, *tb1+teosinte* is turned on (repression) so that the plant produces few or no tillers and only short lateral branches tipped by ears. Thus, *tb1* is hypothesized to be a locus involved in the plastic response of the teosinte plant to its local environment by adjusting the degree of apical dominance.

This model can be extended to explain the evolution of maize plant architecture by hypothesizing that in maize the expression of *tb1* is no longer tied to an environmental signal (degree of shading) but rather that *Tb1+Maize* is constitutively expressed during the development of the branches, keeping both tillering and full elongation of the upper lateral branches repressed. Under this model, both the *tb1+teosinte* and *Tb1+Maize* alleles would encode functional products, although ones that are differentially regulated. Also,

Fig. 5. Immature ears from plants homozygous for the teosinte (left) and maize (right) alleles at the QTL on chromosome arms 1L and 3L, and one ear (center) from a plant heterozygous for the QTL on 1L and homozygous for teosinte allele for the QTL on 3L (center). These ears demonstrate the dramatic effect that the combination of the maize alleles at these two QTL have on ear morphology by producing a nondisarticulating ear with fully yoked cupulate fruitcases and twice the number of fruitcases. Bar = 1 cm.

under this model, the maize mutant (*tb1-ref*) can be explained as a recessive loss-of-function allele. With complete loss of the repressor function, the axillary meristems of homozygous *tb1-ref* plants elongate to produce either basal tillers or elongated upper lateral branches tipped by tassels.

CHROMOSOME 3: THE *TERMINAL EAR* LOCUS?

The teosinte ear is composed of roughly 5–12 small segments called cupulate fruitcases which are arranged one on top of the other (Fig. 5, left ear). Among the F_2 plants in our QTL mapping populations, my colleagues and I observed numerous plants in which the cupulate fruitcases in the ear were side-by-side in addition to being one on top of the other (Fig. 5, right ear). This side-by-side arrangement has been termed

Fig. 6. Mature ears from plants homozygous for the teosinte (left) and maize (right) alleles at the marker loci in the target region on chromosome arm 3L. These ears demonstrate how this QTL alters ear morphology by producing somewhat shorter (or plumper) cupulate fruitcases and a larger number of fruitcases in the ear. Bar = 1 cm.

“yoked” cupules by those who have studied teosinte morphology. QTL-mapping placed a major QTL controlling this phenotype on the long arm of chromosome 3 (Doebley et al. 1995).

Using backcross breeding, we transferred the region on chromosome arm 3L from maize into teosinte (Doebley et al. 1995). Surprisingly, the teosinte line containing this maize chromosomal segment failed to show the yoked cupule phenotype. There were some significant effects on ear morphology in that the maize segment caused a larger number of shorter (or plumper) fruitcases in the ear (Fig. 6); however, we could not initially explain the loss of the yoked cupule phenotype. This segment had other effects similar to *tb1* including partially changing the sex of the lateral inflorescence from male to female and causing the lateral

branch to produce a larger number of shorter internodes (Doebley et al. 1995).

One possible explanation for the apparent loss of the yoked cupule QTL was that this phenotype resulted from an interaction between two or more QTL. In fact, tests of epistasis among QTL provided a tantalizing hint that this might be true. In the original F_2 population for QTL mapping, there was a suggestion of an epistatic interaction between this QTL on chromosome arm 3L and the one on chromosome arm 1L (*tb1*). The test statistic fell just below the normal threshold for statistical significance; however, when there are multiple QTL segregating at once as in our maize-teosinte F_2 populations, this can obscure epistatic interactions among some of them. One way around this difficulty would be to generate a population in which only these two QTL were segregating in a uniform genetic background. This was possible by crossing our teosinte line containing the maize segment on chromosome arm 3L with the one containing the maize segment on chromosome arm 1L and selfing to producing a segregating population.

My colleagues and I generated this population and scored both the yoked cupule phenotype and molecular markers in each chromosomal segment (Doebley et al. 1995). We detected a significant interaction between the two QTL, and plants homozygous for the maize allele at both QTL exhibited the yoked cupule phenotype (Fig. 5, right ear). Thus, we could now explain the failure to recover yoked cupules in the teosinte line carrying the maize segment of chromosome arm 3L, i.e., this phenotype is the product of the nonadditive combined effects of two QTL.

Each fruitcase in the teosinte ear represents a single internode. The maize allele of the QTL on 3L causes a larger number of shorter (plumper) internodes or fruitcases in the ear. A developmental model for this QTL would be that it controls the rate of initiation of new internodes in the ear such that the maize allele causes them to be initiated more rapidly. If new internodes are initiated too rapidly, then they might contain a relatively small population of founder cells and be incapable of fully elongating. As discussed, *tb1* has a similar phenotypic effect and can also be seen as regulating the rate of internode initiation. In this context, it seems reasonable that these two QTL interact epistatically to produce the yoked cupule phenotype by altering the normal teosinte pattern (timing) of internode initiation.

Whether the QTL on 3L corresponds to any known maize locus is not known. Doebley et al. (1995) discussed two candidates. The locus *terminal ear1* (*te1*) controls the pattern or timing of internode initiation in the main stalk of the plant (Veit et al. 1993). If it also controls this process in lateral branches, then it would be an attractive candidate locus for this QTL. A second

candidate is *tassel replaces upper-ear1* (*tru1*). The mutant allele at this locus caused the upper ears of the main stalk to be replaced by long lateral branches tipped by tassels. The locus *tru1* seems an attractive candidate locus in that it affects the fate of axillary meristems in a way similar to the QTL on 3L.

CHROMOSOME 4S: TEOSINTE GLUME ARCHITECTURE1

In teosinte, each kernel is tightly encased in a hardened, cupulate fruitcase. As such, the kernels are not readily accessible for harvest and consumption by humans. Mutants that disrupted the formation of the fruitcase, exposing the kernel, would have been of great utility to early agriculturalists. Each fruitcase is composed of an internode that is invaginated to form the cupule in which the kernel sits. The opening of the cupule is sealed by a modified leaflike structure called the glume.

In the QTL mapping populations, we scored the degree of formation of the cupulate fruitcase. In both populations, we detected a QTL of large effect on chromosome arm 4S (Doebley and Stec 1993). We transferred this chromosomal region into both maize and teosinte genetic background and determined that in maize background, this QTL behaved like a single Mendelian locus (Dorweiler et al. 1993). We named this new locus, *teosinte glume architecture1* (*tgal*). The maize allele behaves in a more-or-less dominant fashion to the teosinte allele in maize background, and heterozygotes are more maize-like in appearance; however, the heterozygotes have some intermediacy, suggesting that both alleles may encode functional products. The demonstration that *tgal* behaved like a single genetic locus was an exciting result for us since it suggested that the evolution of a new adaptation (exposed kernels) resulted largely from changes in a single gene.

The *tgal* locus has multiple effects on ear development (Dorweiler et al. 1993). The teosinte allele renders the internodes in the ear longer and more deeply invaginated (a deeper cup for the kernel to sit in). The teosinte allele also causes the glume to grow upward (parallel to the axis of the ear) and thus cover over the opening of the cupule (Fig. 7). By covering over the opening, the glume completely obscures the kernel from view. Correspondingly, the maize allele causes the glume to grow outward, perpendicular to the axis of the ear, leaving the kernel exposed. The *tgal* locus also affects the pattern of lignification in the glume with the teosinte allele causing a larger number of cells to become lignified (Dorweiler and Doebley, unpublished). Finally, *tgal* affects the deposition of silica in the cells of the epidermis of the glume. The teosinte allele causes silica to be deposited in both the long and short cells that compose the glume epidermis, while the maize allele conditions silica to

Fig. 7. Mature ears (without kernels) of maize line W22 homozygous for the maize (A, C) and teosinte alleles (B, D) at *tgal*. With the maize allele (A), the relatively small outer glumes are not visible, being obscured by the red pigmented bracts (paleas and lemmas). With the teosinte allele (B), the paleas and lemmas are obscured by the enlarged, unpigmented outer glumes. Longitudinal cross-sections show that W22 with the maize allele at *tgal* has outer glumes (G) that are thin and perpendicular to the axis of the ear (C), while those of W22 with the teosinte allele at *tgal* are thicker and curved upward (D). The black bar in B represents 1 cm and applies to both A and B; the black bar in D represents 5 mm and applies to both C and D.

be deposited only or largely in the short cells (Dorweiler and Doebley 1994). These latter two differences probably contribute to the relatively soft glumes of maize versus the hard glumes of teosinte.

What is *tgal* in a developmental genetic sense? The fact that *tgal* affects several distinct aspects of fruitcase development suggests that it acts as a regulatory locus that sits on top of a developmental cascade. At what point in ear/fruitcase development does *tgal* act? Inflorescences in *Zea* are bisexual in their early development, having both male (stamens) and female (ovary) organ primordia. During their development, the adult sex is determined by an internal signal and then either the male organs are aborted to make an ear or the female organs aborted to make a tassel. In teosinte, if an inflorescence is determined to become female, then each internode will form a cupulate fruit-

TEOSINTE INFLORESCENCE DEVELOPMENT

Fig. 8. Model for the position and function of *teosinte glume architecture1* (*tga1*) in maize inflorescence development. Boxes contain specific developmental processes and arrows indicate their hierarchical relationships.

case (hardened, invaginated internodes). If it is determined to become male, the internodes remain soft and uninvaginated. The locus *tga1* can be seen as a locus that is activated after the decision to become female is made and one that has the role of regulating the development of the cupulate fruitcase. In this latter capacity, *tga1* activates the programs for invagination of the internode, internode elongation, three dimensional growth of the glume, silica deposition and the pattern of lignification (Fig. 8).

IMPLICATIONS FOR THE EVOLUTION OF NATURAL SPECIES

The processes involved in crop evolution are not fundamentally different from those operating during the evolution of natural species. For this reason, studies of crop evolution can reveal processes operative in plant evolution in general. Several results of our work on maize evolution may apply more broadly. First, the results indicate that genes of large effect can be an important force in morphological evolution (see Gottlieb 1984; Orr and Coyne 1992). This is especially true for *tb1* and *tga1* which as shown above (Figs. 3–4) have striking effects on ear and plant architecture. Similarly, the combined effects of alleles at only two

QTL transform the ear extensively (Fig. 5). The differences in ear structure among the wild teosintes (see Wilkes 1967) are minute in comparison to the change conferred by the maize alleles of these two QTL. Had such a difference occurred in nature, it would be judged sufficient by taxonomists to name a new genus. This provides further evidence that a few genes can induce a major morphological shift. Other recent studies of natural species provide similar evidence that genes of large effect can be involved in species differentiation (e.g., Bradshaw et al. 1995).

Second, the demonstration that the epistatic interaction between two QTL is required to produce the yoked fruitcase trait raises the question of how important epistasis is in the evolution of natural species. The QTL on chromosome arm 3L has rather modest effects in teosinte background even when homozygous. As such, the maize allele of this QTL could probably exist as a natural variant in a teosinte population. If this is true, then hybridization among teosinte populations would produce new combinations of such cryptic alleles and rapidly generate novel phenotypes, even where one sees little phenotypic differentiation among populations. In *Arabidopsis*, such a cryptic lo-

cus (*cauliflower*) has recently been discovered which in combination with a standard major mutant (*apetalal*) radically transforms the inflorescence into a cauliflower-like mass of undifferentiated flowers, despite the fact that the *cauliflower* locus has no discernible effects of its own (Bowman et al. 1993).

Lastly, our model for *tb1* suggests that, during the evolution of maize, the key change was in its regulation rather than in the function of the protein it encodes. Specifically, we hypothesize a shift from environmental regulation to constitutive expression. If this model is confirmed once *tb1* is cloned, it will provide support for the view that regulatory changes underlie most morphological evolution (Wilson 1976).

ACKNOWLEDGMENTS

This research was supported by the National Science Foundation grant BSR-9107175, USDA-NRI Competitive grant 93-37300-8773, and the Minnesota Agricultural Experiment Station.

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