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THE GENETICS OF MAIZE EVOLUTION

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■ **Abstract** Maize and its closest wild relatives, the teosintes, differ strikingly in the morphology of their female inflorescences or ears. Despite their divergent morphologies, several studies indicate that some varieties of teosinte are cytologically indistinguishable from maize and capable of forming fully fertile hybrids with maize. Molecular analyses identified one form of teosinte (*Zea mays* ssp. *parviglumis*) as the progenitor of maize. Analyses of the inheritance of the morphological traits that distinguish maize and teosinte indicates that they are under the control of multiple genes and exhibit quantitative inheritance. Nevertheless, these analyses have also identified a few loci of large effect that appear to represent key innovations during maize domestication. Remaining challenges are to identify additional major and minor effect genes, the polymorphisms within these genes that control the phenotypes, and how the combination of the individual and epistatic effects of these genes transformed teosinte into maize.

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INTRODUCTION

Maize and its closest wild relatives, the teosintes, present a paradox. Maize and the teosintes exhibit such extreme differences in their adult morphologies that taxonomists initially considered the teosintes more closely related to rice than to maize, whereas the maize and teosinte genomes are so similar that they share the same chromosome number, similar or identical chromosome morphologies, and they can be easily cross-hybridized. Moreover, F_1 hybrids between maize and some forms of teosinte exhibit completely normal meiosis and full fertility. Because of these conflicting observations on morphology and genetics, interpreting the relationship between maize and teosinte generated a contentious debate over much of the twentieth century.

After decades of this often bitter debate, a consensus emerged: The paradox of maize and teosinte could best be explained by regarding teosinte as the wild progenitor of maize. Since teosinte is a wild plant and maize is known only as a cultivated species, the most parsimonious interpretation was that maize is a domesticated form of teosinte and that the striking differences in their morphologies were the result of human selection during the domestication process (2). Moreover, since maize was domesticated relatively recently (6000 to 10,000 years ago), there has been insufficient time for their genomes to become differentiated at the cytogenetic level.

This conclusion has considerable appeal, but also raises a variety of questions. Can one demonstrate by data other than cross-compatibility that maize and teosinte are only recently diverged? If human selection sculpted maize from teosinte, what is the nature of the genetic changes that transformed teosinte into maize? How many genes were involved? What were these genes? How did allelic substitutions at these genes alter development to produce such different adult forms?

In this paper, I review progress in addressing these and related questions surrounding the evolution of maize. I outline some of the pioneering genetic and cytological analyses that first identified teosinte as the progenitor of maize. I review more recent population genetic analyses that demonstrated the recent divergence between maize and teosinte. Finally, I discuss progress toward identifying the genetic basis for the morphological evolution of maize from teosinte.

TEOSINTE, THE PROGENITOR OF MAIZE

What is Teosinte?

Teosinte is the common name for a group of four annual and perennial species of the genus *Zea* native to Mexico and Central America (15, 57, 67). Teosinte plants are taller and broader-leaved than most grasses (Figure 1). Their general growth form is similar to that of maize, although they have much longer lateral branches. The name, teosinte, is of Nahuátl Indian origin, and it has been interpreted to mean "grain of the gods." Some species of teosinte are distinct from maize both genetically and taxonomically, and they appear not to have played any role in the origin of maize. However, one form of teosinte, known as *Z. mays* ssp. *parviglumis*, shares a particularly close genetic relationship with maize and available evidence indicates that it is the direct ancestor of maize (15, 54). This latter teosinte grows in the valleys of southwestern Mexico. In these regions, it grows commonly as a wild plant along streams and on hillsides, although it can also invade cultivated fields as a weed. It is most common in the Balsas River drainage of southwest Mexico and hence is also known as Balsas teosinte.

Although maize and teosinte plants share a similarly robust growth form, their female inflorescences or ears are strikingly different. The teosinte ear possesses only about 5 to 12 kernels, each sealed tightly in a stony casing (Figure 2). Collectively, the kernel and its stony casing are known as a fruitcase. At maturity, the teosinte ear disarticulates such that the individual fruitcases become the dispersal units. Protected within its casing, the teosinte kernel can survive the digestive tracts of birds and grazing mammals, enabling the seed to be easily dispersed (67). By comparison, the massive maize ear can bear 500 or more kernels, each of which is attached to the central axis of the ear or cob. The kernels are naked without adequate protection from predation and are easily digested by any animal that consumes them. Since the kernels are firmly attached to the cob and the ear does not disarticulate, a maize ear left on the plant will eventually fall to ground with its full suite of kernels. When hundreds of maize kernels germinate the next season so close to one another, the emerging plants are unable to obtain adequate light and soil to grow and reproduce. Thus, maize is completely dependent on humans for its survival.

The Teosinte Hypothesis

Most crop plants differ from their wild progenitor in a simple quantitative way. For example, wild tomato is merely a small-fruited version of the large-fruited domesticated tomato. Unlike most crops, maize has no morphologically equivalent wild form, and so the identity of the wild progenitor of maize was a topic of considerable interest in the early 1900s. The stunning morphological differences between the ears of maize and teosinte seemed to exclude the possibility that teosinte could be the progenitor of maize. However, it was also known that maize and teosinte could be readily crossed and that maize and some types of teosinte

formed fully fertile hybrids (11). These conflicting observations needed to be reconciled if the origin of maize was to be solved.

In 1939, George Beadle proposed an answer to the problem of maize evolution when he published the first compelling argument that teosinte was the sole progenitor of maize (2). The idea itself was not new (58, 64); however, Beadle went further than prior authors, using experimental results to develop this idea fully. Decades later, he also followed through by leading a movement to settle the question of the origin of maize (16).

The proposal that teosinte was the sole progenitor of maize is known as *the teosinte hypothesis*. As outlined by Beadle (2–5), the teosinte hypothesis states that (*a*) teosinte provided a useful food source and ancient peoples cultivated it for this purpose, (*b*) during the cultivation of teosinte, mutations that improved teosinte's usefulness to humans arose and were selected by ancient peoples, (*c*) as few as five major mutations would be sufficient to convert teosinte into a primitive form of maize, (*d*) different mutations controlled different traits, e.g., one mutation would have converted the disarticulating ear-type of teosinte into the solid ear-type of maize, and (*e*) over the course of time, humans selected additional major mutations plus many minor ones.

Genetic Evidence and the Origin of Maize

The question of whether teosinte is the progenitor for maize has been addressed by a variety of genetic studies. The central question has been: Are maize and teosinte sufficiently similar on a genetic level to conclude that maize could have arisen from teosinte within the past 6000 to 10,000 years, the time during which virtually all crop plants were domesticated?

Some of the first evidence of consequence on this question was published in two papers by Beadle and Emerson. Beadle (1) reported that the "Florida" form of teosinte, now known as *Z. luxurians*, has chromosomes that are cytologically distinct from those of maize, and that maize-*Z. luxurians* hybrids exhibit two or more unpaired chromosomes during metaphase. These hybrids also exhibited partial sterility. In contrast, another teosinte, known to Beadle as the Mexican annual form, but now called *Z. mays* ssp. *mexicana*, has chromosomes that are cytologically similar to those of maize, and its hybrids with maize exhibit complete chromosomal pairing and full fertility. Emerson & Beadle (26) also showed that crossing-over between maize-teosinte chromosomes occurs at frequencies similar to those observed in hybrids of two varieties of maize. Later, Beadle (3) wrote that this early work convinced him that maize and Mexican annual teosinte were members of the same species as expected if maize were merely domesticated teosinte.

Other cytological studies also indicated that maize and the Mexican annual teosintes were indistinguishable. Longley (48) showed that chromosome arm lengths, centromere positions, and the sizes and positions of knobs in Mexican annual teosintes are identical to those of maize. Longley noted that *Zea luxurians* has a primitive karyotype with knobs only in telomeric positions like that of other genera related to *Zea*, but unlike maize and Mexican annual teosintes, which

possess knobs in interstitial positions. Longley concluded that the Mexican annual teosintes might have been the ancestor of maize. Kato (41) considered frequencies of knobs of different size and at different chromosomal locations in large population samples. His data show that maize and the Mexican annual teosintes possess knobs at the same positions and at similar frequencies. Kato concluded that teosinte was ancestral to maize.

Molecular Evidence and the Origin of Maize

The development of protein and DNA-based molecular markers afforded another opportunity to assess the degree of genetic relatedness between maize and teosinte. Again, a central question was whether teosinte and maize were so similar that teosinte could be considered the progenitor of maize. Molecular data also offered the opportunity to apply a molecular clock and estimate the date of the maize-teosinte divergence.

My colleagues and I (18) examined isozyme variation in maize and teosinte populations. This work enabled us to classify teosinte populations on the basis of their isozyme allele frequencies. The results both confirmed previous cytological analyses and provided additional detail. Isozyme allele frequencies of teosintes such as *Z. luxurians*, *Z. diploperennis*, and *Z. perennis* (the latter two perennials) are strongly differentiated from those of maize. However, the allele frequencies of one Mexican annual teosinte, *Z. mays* ssp. *mexicana*, are more maize-like, although still distinct from maize. The allele frequencies of another Mexican annual teosinte, *Z. mays* ssp. *parviglumis* or Balsas teosinte, are essentially indistinguishable from those of maize. These data suggest that Balsas teosinte is the teosinte most closely related to maize, and therefore the likely progenitor of maize.

More recently, Matsuoka et al. (54) studied microsatellite diversity in maize and teosinte. First, these authors asked whether maize was the product of a single or multiple domestication(s) from teosinte. Phylogenetic analyses based on the microsatellite data strongly favor a single domestication (Figure 3). In agreement with the isozyme data, the single domestication of maize is derived from Balsas teosinte. The microsatellite data go a bit further and imply that the populations of Balsas teosinte in the central portion of its distribution (where the states of Guerrero, Michoacán, and México meet) are ancestral to maize. Second, Matsuoka et al. (54) used microsatellites to date the time of the maize-teosinte divergence. The molecular dating indicate that maize and Balsas teosinte diverged about 9000 years ago, a date that agrees well with archaeological evidence (55).

The Population Genetics of Maize Domestication

Given the model that maize is a domesticated form of Balsas teosinte and that there was a single domestication event, one can address some penetrating questions about the domestication process. How much genetic diversity did maize lose during the domestication bottleneck? How long did the bottleneck last? What were population sizes during the domestication bottleneck? For some questions, precise answers

are not yet possible; however, addressing these questions has greatly advanced our understanding of maize evolution.

During domestication, a restricted portion of the wild progenitor's gene pool is used to create a new cultivated "species." The reduction in population size during domestication should cause a reduction in genetic diversity in a crop relative to its progenitor. For maize, isozyme data indicated that maize lost ~25% of the gene diversity found in Balsas teosinte (15). This figure might seem like a relatively modest loss, but it is comparable to the degree of loss seen in other crops relative to their progenitors (14).

Nucleotide sequence data can provide more discerning answers than isozymes to questions surrounding the domestication process because the mutational process underlying nucleotide polymorphism is better understood. Estimates of the loss of gene diversity in maize relative to teosinte from nucleotide data are around 30%, roughly consistent with the isozyme estimate (27, 35, 37).

Gaut and colleagues (27, 37) have also made some estimates about the relative length versus severity of the bottleneck using coalescent simulations. These analyses suggest that if the bottleneck lasted between 500 to 2000 years [reasonable estimates given the archaeological record (59)], then maize population sizes during the bottleneck would have been between about 500 and 4000 individuals. These relatively small estimates of population size suggest that the early phases of maize domestication could have involved a relatively small human community within a restricted geographic area.

There are some important assumptions in these analyses that need to be kept in mind. For example, postdomestication gene flow from teosinte into maize would cause an underestimate of the bottleneck effect. Similarly, although there is evidence for a single maize domestication event, the actual domestication process may have been more complex, with neighboring communities separately cultivating teosinte but exchanging seed from time to time. Finally, our modern estimates of diversity in Balsas teosinte may be much larger than diversity in the specific local population with which ancient peoples were working, potentially causing an overestimate of the bottleneck effect (62).

THE TEOSINTE TO MAIZE TRANSFORMATION

Genetic Control of Differences in Ear Morphology

Early cytological evidence that maize and teosinte were closely related generated a natural interest in understanding the inheritance of the differences in their ear morphologies. Mangelsdorf & Reeves (53), among the first to investigate this question, sought to identify genetic factors that controlled the differences in general without ascribing specific traits to specific factors. They identified a minimum of four factors of large effect, each of which controlled a defined morphological syndrome. Mangelsdorf and Reeves believed that the four factors that they identified each represented a block of many linked genes. This interpretation was tied to their hypothesis that maize evolved in nature from a now extinct form of wild maize

over a long evolutionary period. They believed that the differences between maize and teosinte were too great to have arisen in the few thousand years during which virtually all crops were domesticated. Their data were not sufficient to draw this conclusion as noted by Beadle (2) and their hypothesis has not withstood the test of time (16).

Like Mangelsdorf and Reeves, Beadle (3) tried to understand the inheritance of the differences in ear morphology while ignoring individual traits. Beadle grew an F_2 population of 50,000 plants and classified the plants as having ears identical to teosinte, identical to maize, or intermediate to some degree. This approach had some real advantages. When classifying the plants, there was no need to force quantitative variation for any one trait into a Mendelian framework. Any intermediate forms were simply not identical to either parent. Also, Beadle did not know ahead of time what ratio of the two parental types to intermediate types to expect. Thus, Beadle was not likely to subconsciously bias his classification to obtain expected ratios. Finally, Beadle selected a primitive 8-rowed variety of maize as his maize parent. Thus, he minimized the complexity of the genetic differences between maize and teosinte. Beadle wanted to identify the number of genes involved in maize domestication in a population where genes involved in maize improvement were not segregating.

Beadle's experiment has become a classic in the field. He observed that about 1 in 500 of the F_2 plants was identical to the teosinte parent and about 1 in 500 to the maize parent. These were the numbers one would expect if between four and five major genes were involved. Beadle recognized that linkage and dominance complicated this interpretation, but he also believed that he had demonstrated that the genetic differences between maize and teosinte were simple enough to have arisen under the influence of human selection during domestication.

Inheritance of Key Traits

An alternative approach to that taken by Mangelsdorf, Reeves, and Beadle was to define key traits that distinguish maize and teosinte and determine the number of genes controlling the inheritance of each trait. The ears of maize and teosinte differ for four discrete traits such that a switch in the states of these four traits would convert the teosinte ear into a primitive maize ear (53). In this section, I review studies of the inheritance of the key traits distinguishing maize and teosinte. I begin with a description of the four key traits.

First, teosinte has a cupulate fruitcase protecting each kernel. The cupule is formed from an invaginated rachis segment (internode) and a glume (modified bract) that covers over the kernel sitting in the cupule (Figure 2). The cupule and glume are present in maize, but they are reduced in size such that they do not surround the kernel. Rather, in maize, these organs form the cob. Thus, maize domestication involved a change in ear development such that the rachis segments and glumes formed a cob rather than fruitcases. Second, teosinte ears disarticulate at maturity such that the individual fruitcases become the seed dispersal units. Maize ears remain intact at maturity for easy harvest by humans. Maize domestication involved a disruption of the abscission layer of cells between

adjacent cupulate fruitcases. Third, in teosinte, each cupulate fruitcase holds a single-spikelet (kernel-bearing structure). Early in teosinte ear development, there are two spikelet primordia on each rachis segment; however, one is aborted early. In maize, there are two mature spikelets (kernels) on each cupule because the one aborted in teosinte develops to maturity in maize. Thus, maize evolution involved the derepression of the second spikelet primordium. Fourth, in teosinte, the cupulate fruitcases are borne in two ranks on opposite sides of the longitudinal axis of the ear. In maize, the cupules are borne in four (or more) ranks. Thus, maize evolution involved a switch from two to four ranks of cupules.

Collins & Kempton (12) were the first to attempt to study the inheritance of the key traits in teosinte-maize hybrid populations. Contrary to simple Mendelian expectations, these authors observed a continuous distribution of trait phenotypes and demurred from forcing this variation into discrete Mendelian categories, although they suggested that single- versus paired-spikelets approached a 3:1 ratio. Langham (45) also studied maize-teosinte crosses and reported unifactorial inheritance for two traits. He named the locus *two ranked* (*tr*) as controlling two- versus multiple-ranks of cupules, and *paired spikelets* (*pd*) as controlling single- versus paired-spikelets. Rogers (56) performed a similar analysis but came to a very different conclusion, reporting no cases of unifactorial inheritance for the key traits. Galinat (33) named two genes (*ph* and *ri*) controlling ear disarticulation, and he (32, 34) proposed that two genes (*pd1* and *pd2*), rather than one as reported by Langham, controlled single- versus paired-spikelets. Finally, Szabó & Burr (60) performed similar Mendelian analyses, reporting simple inheritance for two- versus many-ranked ears, single- versus paired-spikelets, and glume induration (i.e., cupulate fruitcase development).

The designation of gene names and tables of segregation ratios in these publications imply that the inheritance of the key traits was well understood. However, a careful reading of the papers indicates that the reported results were mostly suggestive. Where some authors report discrete Mendelian classes, others observed a continuum of phenotypes. To obtain Mendelian ratios, some authors fit quantitative variants into discrete categories. In addition, the morphology of the hybrid progeny is complex, yet only one of the authors explained in detail how they dealt with this complexity. Few of these studies attempted to confirm their initial results using advanced generation experiments. Finally, in most of these studies, the maize parent carried marker genes on multiple chromosomes, allowing the authors to map the genes controlling differences between maize and teosinte (Table 1). It is revealing that the gene(s) for any one key trait is usually mapped to different chromosomes in the different studies.

Linkage Blocks of Key Genes

As part of their theory on maize evolution, Mangelsdorf & Reeves (53) proposed that the genes differentiating maize and teosinte were grouped into four or five blocks. Subsequently, Galinat (30, 33, 34) incorporated this idea into the teosinte

TABLE 1 Number of publications reporting genes controlling key trait differences between teosinte and maize on the 10 maize chromosomes

	Chromosome										
Trait	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	**References**
Two versus multi-ranked ears	3	3	2	1	1	2	–	2	1	1	(31, 42, 50, 56, 60)
Single versus paired spikelets	1	–	4	2	–		1	2	–	–	(42, 45, 50, 51, 56, 60)
Hard versus soft glumes	–	–	1	4	–	2	2	1	1	1	(50, 51, 56, 60)
Shattering versus solid ears	–	–	1	1	1	1	–	1	–	–	(33, 50)

hypothesis. Here, I review the evidence for block inheritance of genes controlling the differences between maize and teosinte.

Mangelsdorf (52) studied backcross populations between a maize inbred line containing a portion of teosinte chromosome arm *4S* and the maize recurrent parent, and he reported that 21% of the progeny had intermediate phenotypes. He concluded that the intermediates represent cross-overs within a block of genes in arm *4S*. This evidence is not conclusive because he did not have flanking markers that would have enabled him to show that the intermediate phenotypes were associated with actual cross-overs. Galinat (30, 33, 34) also proposed block inheritance of the genes on chromosome arm *4S* citing unpublished data. Galinat refers to this block as the "chromosome *4* complex," which covers nearly all of *4S* from *ph* at position 0 to *su1* at position 66.

The concept that maize and teosinte are differentiated by many genes located in four or five blocks is an interesting one, although one for which there is at present no persuasive evidence. To evaluate the importance of block inheritance in maize evolution, it will first be necessary to map most of the genes differentiating maize and teosinte. At that point, it should be possible to discern whether the amount of linkage is greater than that expected by chance alone. These data are necessary because some linkage of the genes differentiating maize and teosinte should be expected by chance if Beadle's (2) model of five major plus many minor genes is correct.

Reinterpreting Maize Evolution

The literature on the inheritance of the morphological differences between maize and teosinte can be confusing. Where some authors report that traits exhibit quantitative variation (12, 56, 53), others report simple Mendelian ratios (30, 45, 60). Where some authors report unifactorial inheritance of a specific trait, others report bifactorial inheritance for the same trait (30, 34, 45). This confusion invited a reassessment of the genetic model for the evolution of maize. In 1983, Iltis (39)

proposed (*a*) that the key Mendelian genes (*pd1*, *pd2*, *ph*, *ri*, *tr*, and others) do not exist, (*b*) that the inheritance of the morphological differences between maize and teosinte is polygenic, (*c*) that selection during domestication acted on pre-existing variation in teosinte populations (i.e., no new mutations were involved), and (*d*) that reconfiguration of this pre-existing genetic variation elevated the incipient maize population above a genetic threshold for trait expression and thereby precipitated the transformation of a teosinte into maize.

Iltis's reassessment of the genetics arose from a morphological observation. While others focused on the differences in ear morphology, Iltis noted the differences in plant architecture. In typical annual teosinte plants, each primary lateral branch is elongate and bears a tassel at its tip (Figure 1). In maize, each lateral branch is short and has an ear at its tip. In teosinte, the leaves along the lateral branches are fully formed and composed of two parts—the sheath that clasps the stem and a well-developed blade that extends away from the stem. In maize, these leaves have the same two parts but the blade is typically small (or missing) and the sheath (husk) clasps around the ear rather than the stem per se, since the internodes of the branch are much shorter than in teosinte. In teosinte, there are secondary (and higher-order) lateral branches that are terminated by female inflorescences or ears, each ear surrounded by a single husk. In maize, secondary lateral branches terminated by ears are uncommon.

Iltis (39) observed that the ear of maize was terminal on a primary branch, a position in which teosinte bears a tassel. Thus, he proposed that the maize ear was derived from the central spike of the teosinte tassel and not from the teosinte ear. Under this model, the search for genes that converted the teosinte ear into a maize ear was misguided. Rather, by assembling pre-existing genetic variants in teosinte populations, ancient Mexicans had brought a teosinte population across a genetic threshold such that the long teosinte branch was shortened and its central tassel spike transformed in a single step into the ear of maize.

This model for maize evolution has been found inconsistent with new data and it has been reassessed by its author (40). Nevertheless, it has changed how the question of maize evolution is framed by putting plant architecture into the picture. Moreover, it has exposed the inconsistencies in the literature on the "key genes" and clearly suggested for the first time that the critical genetic variants involved in maize evolution were pre-existing in teosinte populations.

QUANTITATIVE GENETIC ANALYSIS

My laboratory used quantitative trait locus (QTL) mapping to study the inheritance of the traits differentiating maize and teosinte (17, 19–21, 23). We borrowed heavily from the insights of earlier researchers. Like Beadle, we used primitive maize varieties as the maize parents of our maize-teosinte populations so that we could map the genes involved in maize domestication rather than those involved in maize improvement. Following Collins & Kempton (12), we scored the traits

quantitatively rather than forcing continuous variation into qualitative categories. Incorporating Iltis's (39) observations, we scored traits related to plant architecture and scored ear traits on ears from equivalent morphological positions on each plant.

Identifying Genomic Regions (QTL) for Plant and Ear Architecture

We performed QTL mapping in two F_2 populations, identifying QTL on all 10 chromosomes. We concluded that the genes involved in maize morphological evolution are scattered throughout the genome (20). However, we also observed five (or six) regions of the genome that have particularly strong effects on the trait phenotypes (Figure 4). Thus, our analyses confirmed the prior observations that the inheritance of the differences between maize and teosinte were governed by either a few major genes (2) or a few blocks of multiple linked genes (53) plus a larger number of small effect loci.

When we examined the distribution of QTL effects for specific traits, some patterns were apparent (20). A region on chromosome arm *1L* is most strongly associated with the differences in plant architecture. A region on chromosome arm *2S* is most strongly associated with differences in ear rank. A region on chromosome arm *4S* largely controls the formation of the cupulate fruitcase. Regions of large effect on chromosome arms *1S*, *3L* and *5S* have more general effects on

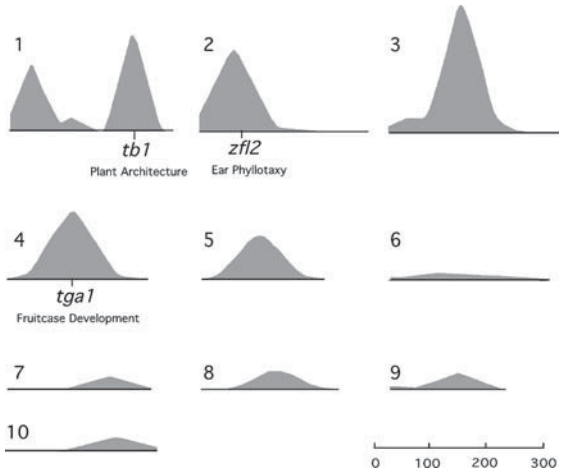

Figure 4 Plot of the ten maize-teosinte chromosomes showing the estimated effects of QTL summed over nine traits that measure plant and inflorescence architecture (20). The height of the gray shaded areas indicates the relative QTL effect at different positions along the chromosomes. Candidate genes and regions with large effects on specific traits are indicated. Six regions of particularly large effect on chromosomes 1 to 5 are visible. Scale in centimorgans.

multiple traits. We identified nine QTL for ear disarticulation with little consistency between the two populations, suggesting that the inheritance of this trait was complex. For single- versus paired-spikelets, we identified ten QTL, although the three regions of largest effect were on chromosome arms *1L*, *1S* and *3L* and were consistent between our two populations. In no case did any trait segregate in a simple Mendelian fashion.

Linkage and Pleiotropy

A fundamental question that our QTL studies did not answer is: Do the QTL regions of large effect on a trait represent single major genes or a block of multiple linked genes? For example, we mapped a large effect QTL for the length of the lateral branch to chromosome arm *1L*. Does this represent a single gene of large effect or multiple linked genes of small effect? Fine mapping of QTL to single transcription units is the only way that this question can be answered with certainty. In two cases where this question has been addressed in tomato, the QTL resolved to a single gene with no evidence for linked factors (28, 29).

A related question is: Where QTL for different traits map to the same genomic region, does this represent a single gene with pleiotropic effects or multiple linked genes, each controlling a different trait? For example, we have mapped QTL for branch length, inflorescence sex, single- versus paired-spikelets, and ear size to the same region on chromosome arm *1L*. Arguments can be made to support either pleiotropy or linkage. Since many developmental genes often have pleiotropic effects on multiple traits, it is reasonable to expect that naturally occurring polymorphisms in these genes will also act pleiotropically. However, theoretical arguments have been made that selection favors the assemblage of adaptive gene complexes in linkage blocks (36).

Gene Action and Epistasis

One model for crop evolution proposes that domestication involves the accumulation of recessive loss-of-function alleles at multiple genes (47). Disruption of the genes that form the abscission layers necessary for ear disarticulation would result in a nondisarticulating ear. Disruption of the genes necessary for the formation of the cupulate fruitcase would lead to an improperly formed fruitcase and thus a naked grain. The underlying logic of this model is that crops are crippled versions of natural species and that crop evolution was driven by new mutations that arose during the domestication process or existed as rare variants in the wild progenitor.

Maize does not appear to closely fit this model since our data indicate that most QTL involved in maize evolution behave in an additive fashion. First, a comparison of the parental and F_2 means for the traits that differentiate maize and teosinte reveals that F_2 values are usually close to the midparent value (20). Second, estimates of the modes of gene action from our QTL analyses indicate that most QTL exhibit additive gene action (17, 23). However, to the extent that the F_2 means deviate from the midparent value, they tend to be more teosinte-like

(20), suggesting some dominance of the teosinte factors. Similarly, to the extent that QTL deviate from additivity in the F_2 populations, the teosinte alleles of the QTL tend to be more dominant (17, 23).

We also examined the effect of genetic background on the magnitude of QTL effects by reciprocally transferring the teosinte allele of a QTL into a maize genetic background and the maize allele into a teosinte genetic background (21). This type of analysis was performed for two large effect QTL and the results suggested that genetic background influences QTL effect. We observed a tendency for the QTL to have their largest effects in genetic backgrounds that possessed a mixture of maize and teosinte germplasm (e.g., an F_2) and for the QTL to exhibit diminished effects in pure maize or pure teosinte genetic backgrounds. These observations are only suggestive because of the small number of traits, QTL and genetic backgrounds analyzed. However, they imply that maize evolution may have involved selection for multiple small effect QTL that enhanced the expression of the favored alleles at other QTL.

Other QTL analyses that we performed suggest that background genetic factors may act epistatically on other QTL. First, in our original QTL studies, we identified a QTL of large effect on chromosome arm *3L*. When the maize allele of this QTL was transferred into a teosinte background, its effects were severely diminished to the point that our teosinte line possessing the maize allele of this QTL was morphologically well within the range of normal teosinte. However, when the maize alleles of both the QTL on *3L* and a second QTL on *1L* were jointly transferred to teosinte background, the large effect of the *3L* QTL was restored, and we observed a novel phenotype not found in teosinte. In another experiment, we used a maize-teosinte hybrid genetic background to uncover genetic variation that is not normally expressed at the phenotypic level in a pure teosinte background (46). This approach enabled us to detect genetic variation in teosinte for domestication traits that are phenotypically invariant in teosinte. Our results suggest that teosinte populations contain a pool of cryptic genetic variation upon which selection could have acted during maize domestication as proposed by Iltis (39).

THE *TEOSINTE BRANCHED1* GENE

Although QTL studies provide some evidence concerning the genetic control of the differences between teosinte and maize, a full understanding requires that QTL be resolved into individual genes and the nature of the allelic differences between maize and teosinte be defined. In this section, I summarize our work on the *teosinte branched1* (*tb1*) gene, which is a candidate for the large effect QTL on chromosome arm *1L*.

tb1 is a plant architecture mutant of maize that, as its name suggests, makes the maize plant resemble a teosinte plant (22). Like teosinte, plants homozygous for the reference allele (*tb1-ref*) have long lateral branches tipped by tassels at some upper nodes of the main culm (Figure 5). *tb1-ref* plants also have

many tillers at the basal nodes. *tb1-ref* plants differ from teosinte in that they do not form normal ears, their secondary branches typically bearing only sterile, tassel-like inflorescences where teosinte bears its ears (Figure 5). The inability of *tb1-ref* plants to form ears indicates that *tb1* function is necessary for normal ear development. *tb1* can be viewed as an apical dominance mutant, in that homozygous mutant plants exhibit unrestrained outgrowth of the axillary meristems (22).

tb1 was identified as a candidate for a QTL on chromosome arm *1L* because of their coincident chromosomal locations and similar phenotypic effects. This candidacy was confirmed by complementation tests, which indicated that our QTL and *tb1* were allelic. As discussed below, the candidacy of *tb1* for this QTL was further supported by evidence that *tb1* was under selection during maize domestication and that the maize and teosinte alleles are differently regulated.

Developmental and Molecular Functions of *tb1*

The tissue-specific expression pattern of *tb1* in maize is complex. *tb1* mRNA is found in axillary buds, axillary branches, husk leaf primordia, ear primordia, the lower floret primordium of each female spikelet, and stamen primordium of developing flowers of the maize ear (22, 38). A common theme is that all of these organs are either reduced in size or aborted in wild-type maize, but more fully developed in *tb1* mutant plants. For example, wild-type modern maize has husks (leaf sheaths with little or no blade) along its axillary branches, whereas *tb1* mutant plants have fully formed leaves along their axillary branches. The contrast between fully formed organs in *tb1* mutants versus reduced (or aborted) organs in wild type suggests that *tb1* acts as a negative regulator of organ growth (21).

An interesting feature of the *tb1* mutant is that it converts the inflorescences terminating the lateral branches from ears to tassels. Hubbard et al. (38) have shown that this is not an indirect effect of having short versus long branches. One possible mechanism is that *tb1* expression in the stamen primordia of the developing flowers of the maize ear arrests the growth of these stamens. In the absence of stamens in the flower, pistil development and ear formation can proceed. With loss of *tb1* expression in stamen primordia as occurs with *tb1* mutant alleles, stamen growth is derepressed and the growing stamens produce a signal that blocks pistil development (9). Thus, *tb1* mutants have tassels rather than ears on the tips of their lateral branches, and, in absence of *tb1* function, maize cannot form a normal ear.

tb1 was molecularly cloned and found to be a class II member of the TCP family of transcriptional regulators (13, 22). Class I TCP genes bind a conserved motif found in the promoters of several genes involved in the cell cycle or cell growth, and they appear to positively regulate the transcription of these genes (43, 44). TB1 and other class II TCP proteins can bind the same sequence motif as class I TCP proteins, although their effects on transcription have not been assayed. An obvious hypothesis is that *tb1* functions as a negative regulator of cell-cycle or

cell-growth genes and thereby represses organ growth. *tb1* may exert this negative regulation by competitively binding to the same sites as class I TCP genes.

The Role of *tb1* in Maize Evolution

Assuming *tb1* functions as a repressor of organ growth, most of the effects associated with the QTL on chromosome arm *1L* can be explained by changes in the spatial pattern and/or level of *tb1* expression. In teosinte, *tb1* should be off or expressed at low levels in the primordia that form the primary branches. This would enable the growth of these primordia into fully elongated branches. *tb1* should also be off (or at low levels) in the inflorescence primordium terminating the primary branch and its stamen primordia, so that these stamens would not be repressed and a tassel rather than an ear would be formed. In teosinte, *tb1* would be expressed in secondary branch primordia, governing their development into short ear shoots with a surrounding bladeless husk leaf. Thus, the evolution of maize required an increase in *tb1* expression in the primary axillary branch primordium and its terminal inflorescence so that they form short ear shoots rather than elongated, tassel-tipped branches.

There are some data to support the above model. First, comparison of maize and teosinte alleles for the level of *tb1* message accumulation indicated that the maize allele is expressed at about twice the level of the teosinte allele in immature axillary branches and the inflorescence primordia (22, 49). Second, in situ hybridization in teosinte showed no sign of *tb1* expression in axillary buds, where maize shows strong expression (38). Third, there are no fixed amino acid differences between maize and teosinte, suggesting that a change in TB1 protein function has not occurred. If the function of the protein has not changed, then it is more likely that changes in expression are involved. Finally, our QTL on chromosome arm *3L* has a strong epistatic effect on *tb1* expression such that plants carrying the teosinte allele at this second QTL have greatly reduced *tb1* message levels (49). It appears that modification of *tb1* expression was an important target of selection during maize domestication.

Molecular Evolution

If *tb1* represents our QTL and was the target of human selection, then it may have the signature of this past selection reflected in its pattern of nucleotide diversity. Specifically, one would expect a greater reduction in nucleotide diversity in maize relative to teosinte for *tb1* than for genes that had not been the targets of human selection. As mentioned above, maize retains about 70% of the nucleotide diversity found in teosinte at a typical neutral gene. For *tb1*, we found that maize has only about 2% of the diversity found in teosinte for the 5′ upstream region, but about 30% of the diversity found in teosinte for the protein-coding region (65). Statistical tests indicate that the 5′ upstream region, but not the protein-coding region, was the target of selection during domestication. This result is consistent with the model outlined above that changes in *tb1* regulation underlie the evolution of maize plant

architecture. Recently, Clark et al. (10) extended this result to survey the entire genomic region between *tb1* and the next gene 5′ of *tb1*. The effect of selection extends between 58 and 93 kb upstream, but does not extend to the next gene, which is 163 kb upstream. This was a remarkable result, since it indicated that while selection strongly reduced diversity in the *tb1* 5′ upstream region, diversity at the neighboring gene was unaffected.

THE *TEOSINTE GLUME ARCHITECTURE1* LOCUS

One of the fundamental differences between maize and teosinte is the presence versus absence of the cupulate fruitcase. In maize, the organs (rachis internode and glume) that form the teosinte cupulate fruitcase are present and form the cob to which the naked maize kernels are attached. We mapped a QTL of large effect on the formation of the cupulate fruitcase near the centromere on chromosome arm *4S* (25). Subsequently, we reciprocally transferred the maize allele of this QTL into a teosinte background and the teosinte allele into a maize background. Observing the effect of the maize allele in teosinte background, it was demonstrated that this single QTL was sufficient to free the teosinte kernel from its casing, making teosinte a more valuable grain crop (Figure 2). Interestingly, the teosinte allele in maize background segregated like a single Mendelian locus, consistent with the hypothesis that it may represent a single transcription unit.

Developmental analyses of *teosinte glume architecture1* (*tga1*) revealed that it has pleiotropic effects on a small suite of traits (24, 25). The teosinte allele lengthens and deepens inflorescence internodes, creating the invagination or cup within which the kernel resides. The teosinte allele also makes the glumes longer, thicker, and angled upward to act as a closure over the cupule. The increased hardening of the glumes is correlated with a thicker abaxial mesoderm of lignified cells. Finally, the teosinte allele directs silica deposition in all abaxial epidermal cells of the fruitcase, giving this structure a stony appearance.

A Developmental Model for *tga1*

What is *tga1* in a developmental sense? The fact that *tga1* affects several distinct aspects of fruitcase development suggests that it acts as a regulatory locus. At what point in ear/fruitcase development does *tga1* act? Inflorescences in *Zea* are bisexual in their early development, having both male (stamens) and female (ovary) organ primordia. During their development, 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 fruitcase. If it is determined to become male, the internodes remain soft and uninvaginated. *tga1* may be a locus that is activated after the decision to become female is made and one that regulates 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 (24, 25).

OTHER CANDIDATE GENES AND TRAITS

Zea Floricaula/Leafy2 (*zfl2*) and Inflorescence Structure

We detected a major QTL for the number of ranks of cupules around the circumference of the ear on chromosome arm *2S* (20). A gene(s) affecting ear rank had previously been mapped to this same chromosomal region by Galinat (31). We identified a single candidate gene in this region, which is the maize homolog (*zfl2*) of the *FLORICAULA* gene of snapdragon and *LEAFY* gene of *Arabidopsis* (8). The candidacy of *zfl2* for this QTL is based on the following logic. In maize as in other flowering plants, *FLO/LFY* genes control the transition from vegetative to reproductive identity. In teosinte, the vegetative (leaves) and the reproductive (spikelets) organs are both borne in two ranks. In maize, the vegetative leaves are borne in two ranks as in teosinte, but the spikelets are borne in multiple ranks. Thus, the switch in phyllotaxy is coincident with the reproductive transition, and genes involved in the reproductive transition are candidates for phyllotaxy genes. Specifically, we proposed that *zfl2* patterns inflorescence meristems so that more than two ranks of reproductive organs are formed (8).

We are in the early phases of testing the candidacy of *zfl2* for our QTL. If our hypothesis is correct, then one might expect loss-of-function alleles of *zfl2* to show an alteration in ear rank. Consistent with this hypothesis, Bomblies et al. (8) demonstrated an association between the number of wild-type copies of *zfl2* and ear rank. Plants with two wild-type alleles had about one more row of cupules than did plants with two mutant alleles. This result suggests that the developmental pathway controlling the reproductive transition was a target of selection. Confirmation of this hypothesis will require mapping of the causative site for the QTL on chromosome arm *2S* and demonstrating that it lies within *zfl2*.

Genes Under Selection During Domestication

Population genetic analyses offer another means of identifying genes for traits that were under selection during maize domestication and improvement. As described above for *tb1*, selection during domestication will cause a reduction in diversity in maize relative to teosinte. Selection can also be inferred when a specific maize gene has reduced diversity relative to an average (neutral) maize gene. Finally, as new mutations arise in a population after a selective sweep, they will occur at very low frequencies. Thus, an excess of rare variants in a population is expected in the wake of a selective sweep.

Over the past few years, several groups have applied population genetic analyses to maize with considerable success. Whitt et al. (66) presented evidence that the starch biosynthesis pathway was the target of selection during maize

domestication and improvement. Vigouroux et al. (63) examined patterns of diversity in microsatellites located in 500 maize ESTs, identifying 15 loci with evidence of past selection including one MADS box gene. Finally, Tenaillon et al. (61, 62) have identified *tassel*, *seed2*, and *dwarf8* as targets of selection, based on a screen of genes on chromosome *1*. This approach promises to become much more powerful in the near future with technological improvements and availability of the complete sequence of the maize genome. The next step will be to determine which traits these selected genes actually affect.

PERSPECTIVE

Development and Maize Evolution

The dichotomies of single- versus paired-spikelets, shattering versus nonshattering ears, soft versus hard glumes, and two- versus multi-ranked ears are striking when one compares maize and teosinte. However, in F_2 families, these discrete classes blur into a continuum of phenotypes, and novel phenotypes and interactions appear. Consider two traits: spikelet pairing and ear rank. Ears of F_2 plants can vary from 0% to 100% paired-spikelets, and one spikelet of each pair can range from rudimentary to fully formed. Some F_2 ears even show three spikelets in a single cupule. On some F_2 plants, a terminal ear may exhibit single spikelets and four ranks of cupules, while lateral ears show two ranks of cupules and paired-spikelets. In F_2 populations, ear rank and spikelet pairing are correlated and the QTL controlling them are partially coincident (20), suggesting that they are controlled by the same underlying set of genes.

The complexity of F_2 phenotypes suggests to me that the underlying genes control aspects of development that can be translated into different adult phenotypes (traits) depending on the entire genotype of the individual F_2 plant. Thus, rather than genes for paired- versus single-spikelets or two- versus multi-ranked ears, the underlying genes control aspects of development such as meristem size or patterning. The effects of these genes are specific to a given genetic background such that effects observed in the F_2 can be quite different from those seen when a QTL is transferred to a foreign background (21). It is for this reason that no one has transferred the key genes required for single spikelets and two-ranked ears from teosinte to maize and shown them to be simply inherited.

As additional QTL are cloned and their developmental functions revealed, better inferences about the underlying developmental processes will be possible. In the end, there can be a meaningful description of the underlying developmental programs and how they are executed differentially to produce the striking differences between the teosinte and maize ears. The discovery of *tb1* and its role in the repression of organ growth is a step in this direction.

One trait that does fit neatly into a key trait paradigm is the formation of the cupulate fruitcase as controlled by *tga1*. In this case, the teosinte allele could be transferred to maize and this single locus confers the teosinte phenotype in a

maize genetic background. Nevertheless, the inheritance in the F_2 is complex and multiple QTL affect this trait. While *tga1* provides an affirmation of the teosinte hypothesis, much remains to be learned about the functional differences between the maize and teosinte alleles of *tga1*.

Genetics and Maize Evolution

KEY GENES AND QTL The genes involved in maize evolution might be placed in three overlapping classes. First, major loci that exhibit simple inheritance and whose phenotypic effects are only moderately dependent on genetic background and environment. *tga1* is the only locus of this type to have been identified. Second, QTL that exhibit complex inheritance, conditioned on both genetic background and environment, but which have measurable effects across diverse genetic backgrounds and environments. These QTL have a range of magnitudes, from those that approach simple inheritance to those of diminishingly small effects. Third, cryptic QTL whose effects are highly dependent on epistatic interactions with other genes to the point that they may have no measurable effect in many contexts. This class of QTL could exist in teosinte with little or no contribution to the standing phenotypic variation (46).

The available facts favor a view that QTL of measurable additive effects likely account for most of the morphological changes brought about during maize domestication. A few QTL, like *tga1* and *tb1*, may have played a disproportionately large role for the phenotypes they affect. With the exception of *tga1*, genes that behave like simple Mendelian factors do not appear to have been involved.

The current descriptions of the genes controlling the morphological differences between maize and teosinte are all based on crosses of teosinte and modern maize, even if primitive varieties of modern maize. Inheritance in these crosses will necessarily exhibit more complex patterns of inheritance than one would see if we could use the maize of 6000 years ago, which had an ear of only 6 cm in length with as few as 28 kernels (6, 7). For this reason, I believe that Beadle's teosinte hypothesis (2) that a few gene changes along with some modifiers could have made teosinte into a useful food crop remains consistent with the evidence. What the QTL studies have added to this hypothesis is that the genes involved had quantitative effects rather than discrete Mendelian effects, that epistatic interactions among QTL may have been important, and that the QTL affected underlying developmental processes directly and the key traits only indirectly.

NEW MUTATION AND STANDING VARIATION There is an underlying assumption in much of the literature on maize evolution that new mutations were central to the morphological evolution of maize. The word "mutation" is used repeatedly to describe the gene changes involved, and Beadle (16) led an expedition ("mutation hunt") to find these rare alleles. The opposing view, that naturally occurring standing variation in teosinte populations could provide sufficient raw material for maize evolution, was stated clearly for the first time by Iltis in 1983 (39).

Although new mutation is likely to have made a contribution, anyone who has worked with teosinte would agree that teosinte populations possess abundant genetic variation. Even for *tb1*, a QTL of large effect, maize-like alleles exist in teosinte populations without obvious deleterious effects (10). Allowing for cryptic variants and novel phenotypes from new epistatic combinations to arise during domestication, it is easy to imagine that maize was domesticated from teosinte. Indeed, sufficient variation likely resides within many or most teosinte populations to entice some misguided individual to attempt to redomesticate maize (www.wisc.edu/teosinte/redomestication.htm).

The evolution of maize was enormously complex and involved selection on a broad array of traits far beyond those for plant and inflorescence architecture discussed in this paper. Other targets of selection included kernel size, kernel composition, palatability, rapid germination, stalk strength, leaf size, and more. Much remains to be learned.

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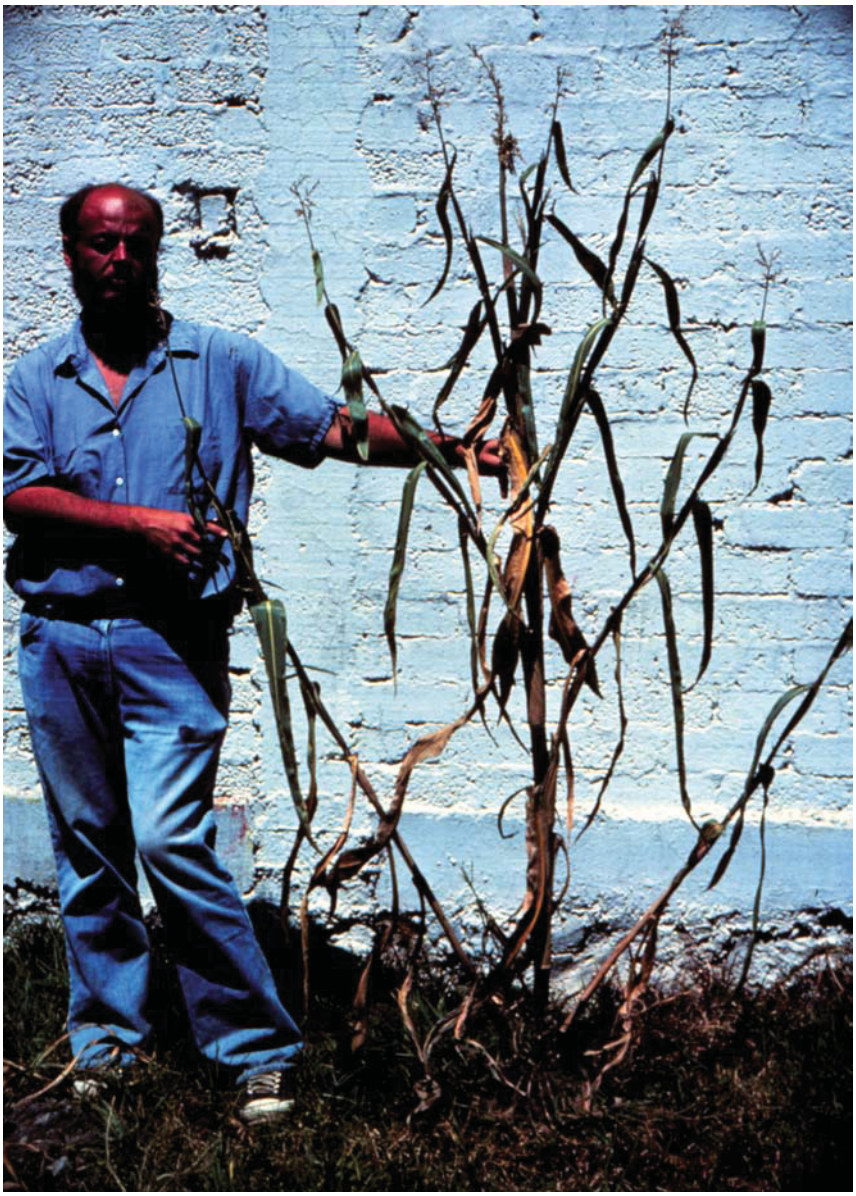

Figure 1 Annual teosinte (*Zea mays* ssp. *mexicana*) held by Stephen Solheim. The photograph shows the robust vegetative habit and long branches with tassels at their tips. Photo by Hugh Iltis.

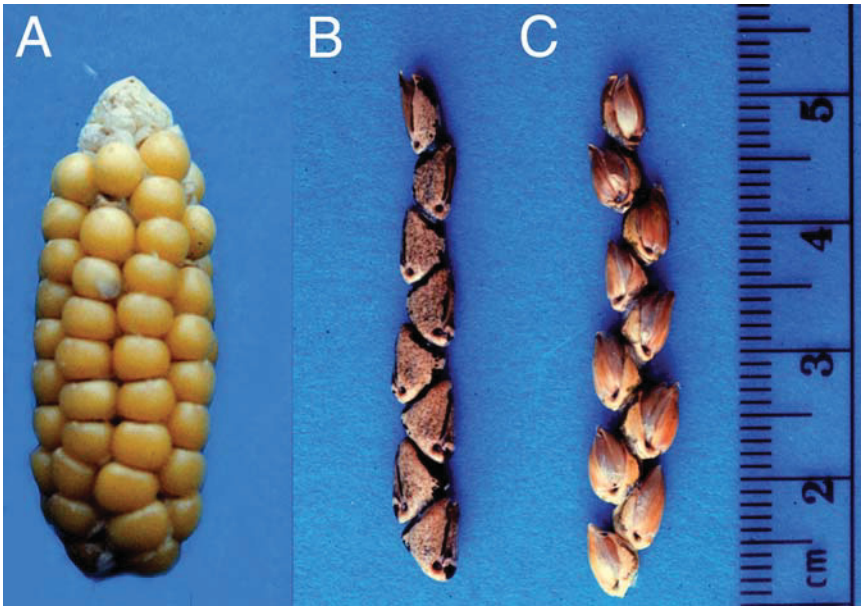

Figure 2 (*A*) A 'Reconstructed' ear of primitive maize (*left*). This small-eared form of maize was bred by George Beadle by crossing teosinte with Argentine pop corn and then selecting the smallest segregants (16). (*B*) Ear of pure teosinte (*Zea mays* ssp. *parviglumis*) composed of eight cupulate fruitcases. (*C*) Ear of teosinte carrying a segment of maize chromosome arm *4S* including the maize allele of *tga1*. The effects of the maize allele of *tga1* include cupules that are less well-developed and shallower so that the kernels are visible. Photo by John Doebley.

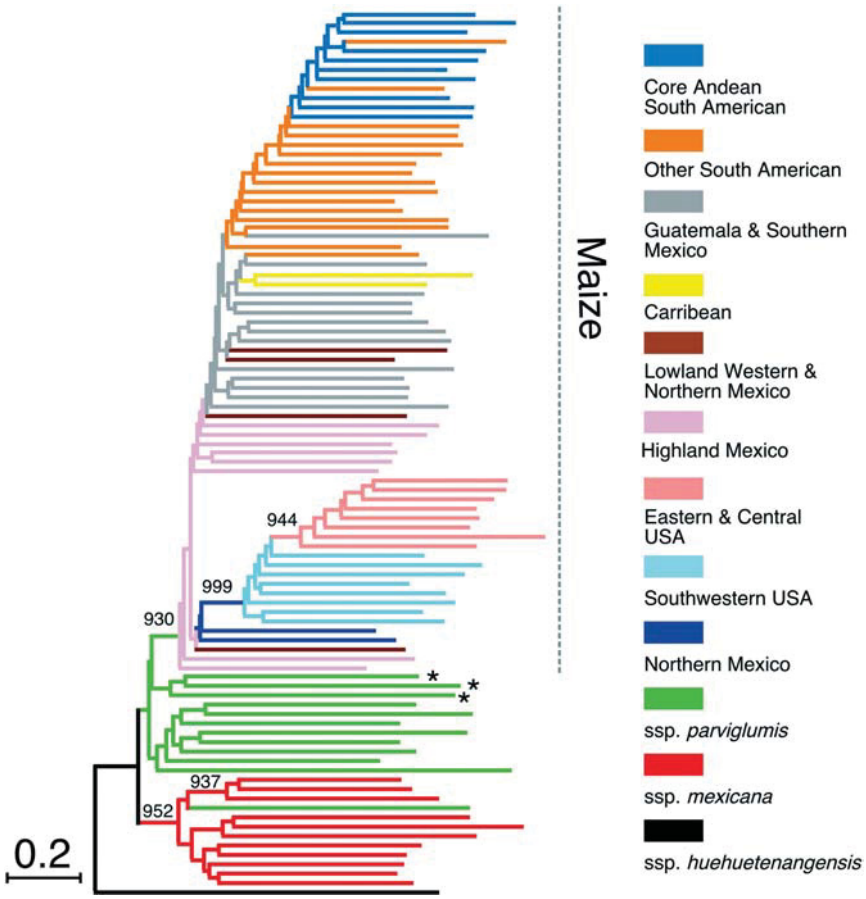

Figure 3 Phylogeny of maize and Mexican annual teosinte rooted with *Zea mays* ssp. *huehuetenangensis* based on microsatellites (54). Dashed gray line circumscribes the monophyletic maize lineage. Asterisks identify those populations of ssp. *parviglumis* basal to maize, all of which are from the central Balsas river drainage. The numbers on the branches indicate the number of times a clade appeared among 1000 bootstrap samples. Only bootstrap values greater than 900 are shown.

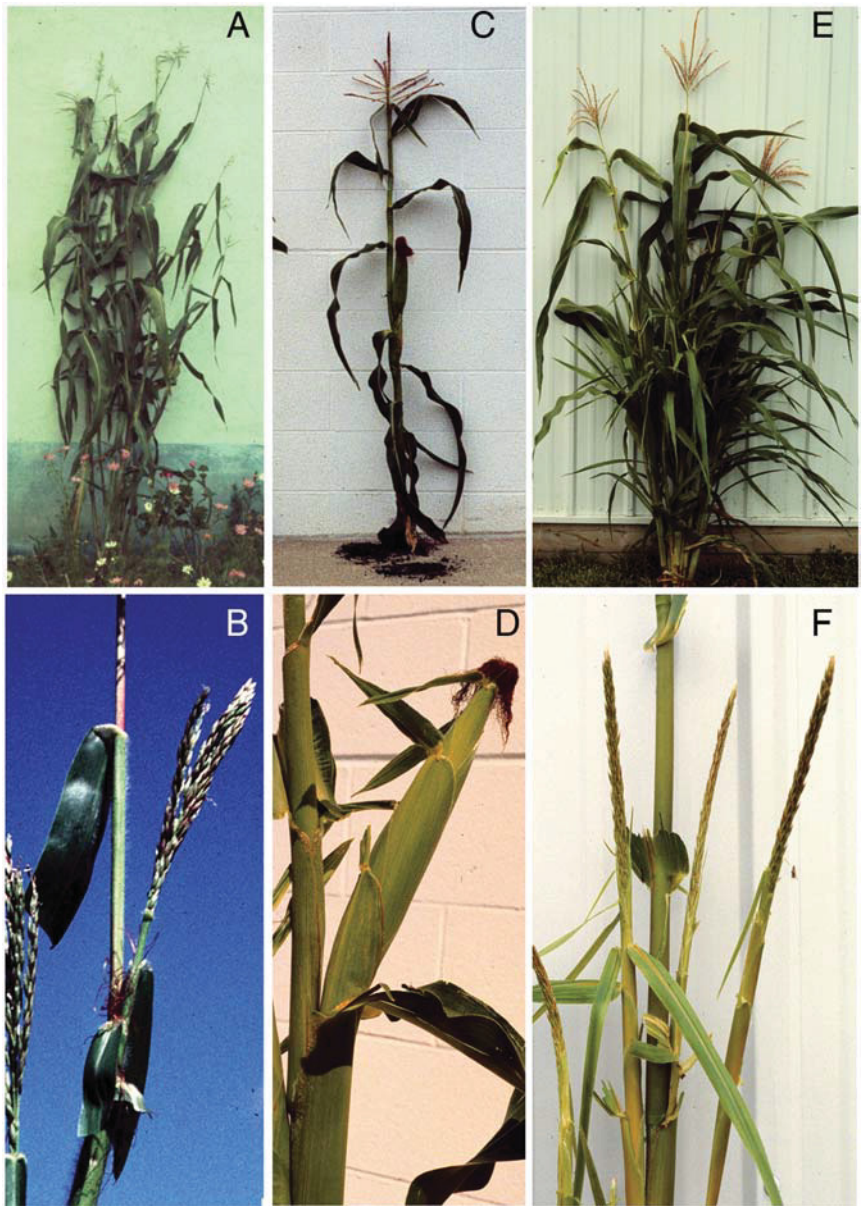

Figure 5 (*A*) Teosinte (*Zea mays* ssp. *mexicana*) plant and (*B*) its axillary branch with terminal tassel and silks emerging from teosinte ears hidden within the leaf sheaths. (*C*) Maize plant and (*D*) ear shoot. (*E*) *tb1-ref* mutant maize plant and (*F*) axillary branches that have terminal male inflorescences and lack ears. Photo by John Doebley.