Edward S. Buckler IV
and Natalie M. Stevens

CHAPTER 4

Maize Origins, Domestication, and Selection

> Although man does not cause variability and cannot even prevent it, he can select, preserve, and accumulate the variations given to him by the hand of nature almost in any way which he chooses; and thus he can certainly produce a great result.
>
> —Charles Darwin

Wild on a Mexican hillside grows teosinte, its meager ear containing only two entwined rows of small, well-armored kernels. This unassuming grass might easily have been overlooked, were it not for the hand of nature that beckoned with abundant variation, a gift not lost on early agriculturists. Within the last 10,000 years, early Native Americans were able to transform teosinte into a plant whose ear, brimming with row upon row of exposed kernels, feeds the world over. It was a transformation so striking and so complex that some would not believe it possible, leading to years of competing theory and intense debate. But as Darwin himself recognized, when human desires collide with the diversity of nature, the result can be great indeed.

Although controversy still lingers over the origin of maize, the molecular revolution of the last decade has provided compelling evidence in support of teosinte as the progenitor of modern maize. This chapter reviews that evidence in light of several different domestication hypotheses. We also discuss the rich genetic diversity at the source of such a remarkable morphological conversion and examine how human selection has affected this diversity, both at individual loci and for an entire metabolic pathway.

Taxonomy

Maize is a member of the grass family Poaceae (Gramineae), a classification it shares with many other important agricultural crops, including wheat, rice, oats, sorghum, barley, and sugarcane. Based on fossil evidence, it is estimated that these major grass lineages arose from a common ancestor within the last 55–70 million years, near the end of the reign of dinosaurs. Maize is further organized in the genus *Zea,* a group of annual and perennial grasses native to Mexico and Central America. The genus *Zea* includes the wild taxa, known collectively as teosinte (*Zea* ssp.), and domesticated corn, or maize (*Zea mays* L. ssp. *mays*).

For many years, the relationships within genus *Zea* were the subject of much controversy. The central difficulty in the taxonomy of maize and the identification of its closest relatives was the absence of a coblike pistillate inflorescence—or "ear"—in any other known plant. Whereas teosinte produces only 6 to 12 kernels in two interleaved rows protected by a hard outer covering (figure 4.1), modern maize boasts a cob consisting of 20 rows or more, with numerous exposed kernels. In fact, teosinte is so unlike maize in the structure of its ear that 19th-century botanists failed to recognize the close relationship between these plants, placing teosinte in the genus *Euchlaena* rather than in *Zea* with maize (Doebley, 1990b).

Despite these profound physical differences, various morphological, cytological, and genetic studies eventually delineated the relationships within genus *Zea*. H.G. Wilkes (1967) laid the foundation for the current classification scheme in 1967 with the first thorough monograph on teosinte. Wilkes did not attempt a formal hierarchy but instead presented a system of classification using different geographic populations, with separate racial designations based on distinguishing morphological features. In 1980, Hugh Iltis and John Doebley (Doebley and Iltis, 1980; Iltis and Doebley, 1980) produced a system of classification that considered the probable evolutionary relationships between taxa. With the quantitative evaluation of numerous traits and the discovery of many additional populations, Jesus Sanchez (Sanchez G. et al., 1998) provided further characterization of this genus.

Based on the morphological characteristics and geographic delineations established in these systematic treatments, five species of *Zea* are currently recognized:

- *Zea diploperennis* Iltis, Doebley & Guzman, a perennial, diploid teosinte found in very limited regions of the highlands of western Mexico

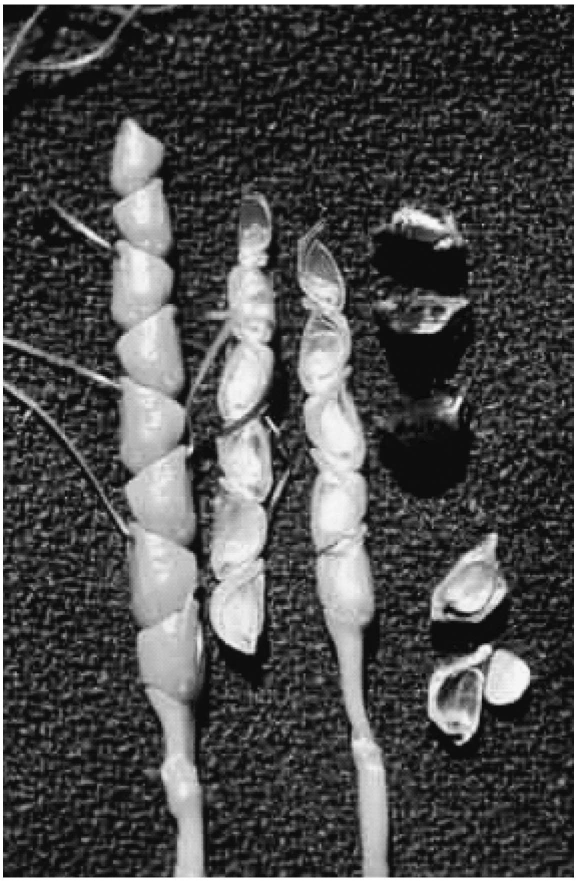

FIGURE 4.1 The seed spike, or ear, of teosinte (*Zea mays* ssp. *parviglumis*) consists of 2 interleaved rows of 6–12 kernels enclosed in a hard fruitcase (cupule). This female inflorescence, which differs so dramatically from that of maize, has led to much controversy and debate surrounding the origins of maize. (Photo by Hugh Iltis.)

- *Zea perennis* (Hitchcock) Reeves & Mangelsdorf, a perennial tetraploid teosinte, also with a very narrow distribution in the highlands of western Mexico
- *Zea luxurians* (Durieu & Ascherson) Bird, an annual teosinte found in the more equatorial regions of southeastern Guatemala and Honduras
- *Zea nicaraguensis* Iltis & Benz, closely related to *Zea luxurians* and found in mesic environments in Nicaragua (Iltis and Benz, 2000)
- *Zea mays* L., a highly polymorphic, diploid annual species, including both wild teosinte and cultivated maize

This last species, *Zea mays,* is further divided into four subspecies:

- *Z. mays* L. ssp. *huehuetenangensis* (Iltis & Doebley) Doebley, an annual teosinte found in a few highlands of northwestern Guatemala
- *Z. mays* L. ssp. *mexicana* (Schrader) Iltis, an annual teosinte from the highlands of central and northern Mexico
- *Z. mays* L. ssp. *parviglumis* Iltis & Doebley, an annual teosinte, common in the middle and low elevations of southwestern Mexico
- *Z. mays* L. ssp. *mays,* maize or "Indian corn," probably domesticated in the Balsas River Valley of southern Mexico

Origin of Maize

Historical Argument

As scientists labored throughout the mid- to late 1900s to piece together a system of classification for the genus *Zea,* a parallel puzzle surfaced regarding the origin of maize. Despite growing acceptance—reflected in the current taxonomy—of the view that teosinte (*Z. mays*) is the immediate ancestor of maize (*Z. mays* ssp. *mays*), consensus did not come easily. In the struggle to understand the derivation of the enigmatic corn ear, two leading hypotheses emerged.

In the late 1930s, Paul Mangelsdorf and his colleague Robert Reeves proposed a hypothesis known as the tripartite hypothesis (Mangelsdorf, 1974; Mangelsdorf and Reeves, 1938, 1939). This theory stated that maize was domesticated from some unknown wild maize, presumably a plant with structures that resembled the modern maize ear. More specifically, as the name indicates, the hypothesis consisted of three parts: A wild maize prototype from South America, which is now either extinct or undiscovered, was the progenitor of maize; teosinte is the offspring of a cross between maize and *Tripsacum* (another genus of grasses); and sections of *Tripsacum* chromosomes had "contaminated" maize germplasm.

Thus, Mangelsdorf and Reeves invoked a missing ancestor to account for the extreme morphological differences between maize and teosinte while relying on *Tripsacum* to explain their similarities. They pointed to their own successful cross of maize and *Tripsacum* as validation for their hypothesis. Indeed, although the cross entailed significant human intervention, Mangelsdorf and Reeves were able to produce a few, largely sterile maize–*Tripsacum* hybrids. They also analyzed backcross populations of maize–teosinte hybrids and were able to identify four factors (which they interpreted as four *Tripsacum* chromosomal segments) responsible for the morphological differences between maize and teosinte.

For George Beadle, however, the morphological differences between maize and teosinte were not so large as to require an extinct ancestor. In June 1939, less than a year after the publication of the tripartite hypothesis, he responded with his own theory on the origin of maize, an idea he had convinced himself of as a Cornell graduate student under the direction of Rollins Emerson (Doebley, 2001). In his teosinte hypothesis, Beadle (1939) stated that maize is simply a domesticated form of teosinte. He believed that through artificial selection by ancient populations, several small mutations with large effects could have transformed teosinte into maize. Beadle actually used Mangelsdorf and Reeves's own data against them, claiming that their four factors might just as well correspond to four major genes, each of which controlled a single trait that differentiated teosinte from maize. He also challenged their idea that a cross between maize and *Tripsacum,* which took such Herculean efforts on their part, would have occurred in the wild.

On the surface, these dueling hypotheses focused on the origins of a humble ear of corn, but at the core of the controversy was an issue more fundamental and perhaps more far-reaching—a Darwinian debate for the ages. In one corner were evolutionary traditionalists who held that evolution proceeds slowly over time, through the accumulation of many small changes in numerous genes. Thus the dramatic transformation from teosinte to maize was simply not possible in the mere 10,000 years in which humans have been domesticating plants, and a more logical starting point was needed on which selection could act. In the other corner were minds such as Beadle's and Emerson's, where evolution could be more rapid if propelled by changes in a few significant genes. So although teosinte and maize may have looked strikingly different, this difference could be accounted for by only four or five major genes, explaining why the two plants were otherwise genetically similar (so much so that they could be easily crossed to produce fertile offspring).

From its debut in 1938 until the 1960s, the tripartite hypothesis was widely accepted. Through productive collaborations with prominent archaeologists of his day (Mangelsdorf et al., 1964, 1967) and a hemisphere-wide effort targeting maize germplasm conservation (Wellhausen et al., 1952), Mangelsdorf was able to publicize his theory among a wide audience, with his name becoming synonymous with the study of maize evolution. Meanwhile, Beadle temporarily abandoned his teosinte hypothesis for pioneering Nobel work on biochemical genetics and for the presidency of the University of Chicago. During this time his opposing ideas received little attention. Upon his retirement in 1968, however, Beadle rejoined the maize controversy, vigorously pursuing the dispute both in print and in person at several meetings specifically convened to debate the origin of maize. He came armed with additional data that supported his hypothesis (Beadle, 1972, 1977, 1980) and eventually capitalized on the lingering disbelief in the tripartite hypothesis among many maize geneticists. Before Beadle's death in 1989, a host of scientific publications had been issued in support of teosinte as the wild progenitor of maize (see review in Doebley, 1990a).

Modern Argument

The controversy continues. Although the mystery surrounding the origin of maize seemed to be solved, new pieces to the puzzle were added, given time and new technologies. Teosinte and its sister genus *Tripsacum* still take center stage in the modern argument, with one side steadfastly adhering to the teosinte hypothesis while the other revived the idea of a hybridization event. In this section we examine each contemporary hypothesis and its accompanying data in turn, demonstrating that current biological evidence in favor of Beadle's teosinte hypothesis is overwhelming.

Teosinte Hypothesis

The teosinte hypothesis has changed little since Beadle first formalized the idea more than 60 years ago, asserting that teosinte is the wild ancestor of maize. In its modern form, scientists have pinpointed one teosinte in particular, *Zea mays* ssp. *parviglumis,* as the likely progenitor (see figure 4.2 for summary of modern phylogenetics). Because ssp. *parviglumis* is the closest living relative of maize (ssp. *mays*), proponents of this theory reason that maize arose through changes—albeit large changes—to this close ancestor through human selection for specific traits. They point to a wide range of biological data from the 20th century and a wealth of new evidence ushered in with the era of molecular genetics in support of this view.

If maize were simply a domesticated form of teosinte, scientists would need to establish a close relationship between maize and its putative parent. One early indication that maize is strongly allied with *Zea mays* came from

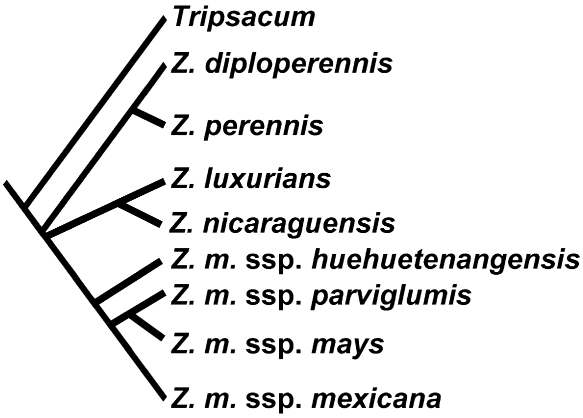

FIGURE 4.2 The summary phylogeny for the genus *Zea,* based on chromosomal number and morphology (Kato Y., 1976; Kato Y. and Lopez R., 1990), chloroplast (Doebley et al., 1987), ribosomal (Buckler and Holtsford, 1996), isozyme (Doebley et al., 1984), and simple sequence repeat (Matsuoka et al., 2002) data.

studies of chromosome morphology and number. All *Zea* species and subspecies have 10 chromosomes (Kato Y., 1976; Kato Y. and Lopez R., 1990), with the sole exception of *Z. perennis,* which has 20—clearly an example of a complete, duplicated set of chromosomes. On the other hand, most *Tripsacum* species have either 18 or 36 (Mangelsdorf and Reeves, 1938, 1939). Although polyploidy is common in the plant kingdom, either by doubling of a single genome or, more commonly, by combining two or more distinct but related genomes, neither 18 nor 36 chromosomes can easily be derived through normal meiotic associations with the *Zea* genome.

Not only do *Tripsacum* chromosomes differ in number, but they also show marked differences in constitution. Beginning in the 1930s, Barbara McClintock, Paul Mangelsdorf, and collaborators undertook a formal study of chromosome morphology among teosinte plants (Kato Y., 1976; Mangelsdorf, 1974; McClintock et al., 1981). Focusing on chromosomal knobs, or highly repetitive sections of DNA that present as enlarged, deep-staining regions on simple smears, their research revealed that certain grasses such as *Tripsacum* and several *Zea* species had terminal knobs only, whereas others, including three subspecies of *Zea mays,* displayed interstitial knobs. Thus, when coupling basic chromosome numbers with highly conserved chromosomal knob data, maize scientists found early evidence that *Tripsacum* represented a distinct group from *Zea,* with *Z. mays* ssp. *parviglumis, mays,* and *mexicana* forming a natural subgroup within this latter genus.

Chloroplast and ribosomal studies in the late 1980s and 1990s corroborated the story told by earlier chromosomal evidence, showing maize to be only distantly related to *Tripsacum* and more closely aligned within the genus *Zea*. Phylogenies based on the maternally inherited chloroplast clearly place *Z. mays* ssp. *mays* in a group with ssp. *parviglumis* and *mexicana,* along with the fourth subspecies *huehuetenangensis* (Doebley et al., 1987). Phylogenetic studies using nuclear ribosomal internal transcribed spacer (ITS) sequences further delineated these infraspecific *Z. mays* relationships (Buckler and Holtsford, 1996). Ribosomal ITS [illegible]uences, which evolve rapidly and are inherited from both parents, indicate that *Zea* species have evolved very recently in comparison to *Zea*'s divergence from *Tripsacum*. In addition, the phylogenetic position of *Z. mays* ssp. *huehuetenangensis* was clearly defined for the first time as being the basal (most diverged) taxon within *Z. mays* (Buckler and Holtsford, 1996).

Thus, the field was narrowing in the quest for maize's wild ancestor. The aforementioned studies had all but eliminated *Tripsacum* as a sister genus that diverged several million years ago. Instead, teosinte fielded the most likely candidates, first as a genus, then within the species *Z. mays,* and finally pared down to just two subspecies, *parviglumis* and *mexicana*. In 1984, isozyme data specifically implicated ssp. *parviglumis* in the origin of maize (Doebley et al., 1984). Simple sequence repeat (SSR) [illegible]rkers—the highest-resolution approach currently available in the arsenal of molecular genetics—later corroborated the isozyme data in naming ssp. *parviglumis* from the Balsas River Valley as the progenitor of maize (Matsuoka et al., 2002). SSR loci, or microsatellite DNA, [illegible] only are polymorphic because of the high mutation rate affecting the number of repeat units but also are abundantly distributed throughout broad expanses of eukaryotic DNA [illegible] such, they provide an easily detectable, genome-wide method for determining similarities in evolutionary history between taxa. Comprehensive phylogenetic analyses for maize and teosinte were performed using 99 microsatellite loci from plant samples that encompassed the full geographic range of pre-Columbian maize and Mexican annual teosinte. The study revealed that ssp. *mexicana* is separated from all maize (ssp. *mays*) samples, whereas samples of ssp. *parviglumis* overlap those of maize, documenting the close relationship between ssp. *parviglumis* and maize and supporting the phylogenetic result that the latter subspecies was the sole progenitor of maize (Matsuoka et al., 2002). Furthermore, all maize appears in a single monophyletic lineage that is derived from within ssp. *parviglumis,* thus supporting a single domestication for maize. Using microsatellites that follow a stepwise model and have a known mutation rate, divergence time was estimated at 9188 bp.

Having established *Z. mays* ssp. *parviglumis* as the likely parent of modern maize, and even pinpointing the Balsas River Valley as a candidate for the cradle of maize domestication, research focused on the loci involved in the dramatic transformation from wild grass to cultivated crop. Modern molecular techniques using linkage maps and quantitative trait locus (qtl) analysis have increasingly provided evidence in direct support of another fundamental tenet of the teosinte hypothesis: that a few regions of the maize genome specify the traits that distinguish maize from teosinte. Using basic Mendelian ratios from 50,000 maize and teosinte hybrids, Beadle (1972, 1977, 1980) first recognized that as few as five loci may be involved in important ear and plant morphological changes. More than 20 years later, qtl mapping would validated this idea, identifying five regions of the maize genome with large effects on basic morphology (Doebley et al., 1990; Doebley and Stec, 1991).

Although far from complete, the maize mystery is slowly unraveling through concentrated studies of these important regions. For example, a single major locus, *teosinte glume architecture1* (*tga1*), has been identified that controls the development of the glume, a protective covering on teosinte kernels that is mostly lacking in maize (Dorweiler et al., 1993). Because teosinte's hard glume makes it very difficult to eat, a mutation in this gene leading to a softer glume probably was one of the first targets of selection by Native Americans during domestication. A second locus, *teosinte branched1* (*tb1*), which dictates a difference in plant architecture (long lateral branches terminated by male tassels in teosinte vs. short lateral branches tipped by female ears in maize) has been successfully cloned (Doebley et al., 1995, 1997; Wang et al., 1999). QTLs at genes responsible for three more distinguishing traits, shattering versus solid cobs, single versus paired spikelets, and distichous versus polystichous condition, are the subject of current investigations.

Caution must be exercised in advocating a one-gene, one-trait model. Although a small number of genes, such as *tga1* and *tb1,* clearly have a striking effect on ear and plant morphology and represent major steps in maize evolution, most genes have modest effects. Even Beadle recognized that additional "modifier" genes would be necessary to complete the transition, and perhaps hundreds or even thousands of genes were involved in steps such as increasing the size of the ear, adapting growth to different agricultural environments, and modifying the nutrient content of the maize kernel. However, the essence of the argument remains intact: A small number of single-gene mutations could be sufficient to go from teosinte to a plant that possesses the key morphological features of cultivated maize.

Tripsacum–Z. diploperennis Hypothesis

A modern version of the tripartite hypothesis, formalized in 1995, is Eubanks's *Tripsacum–Z. diploperennis* hypothesis. Still challenging the idea that maize is a domesticated form of teosinte, this theory proposes that maize arose from the progeny of a cross between *Z. diploperennis* and *T. dactyloides* (Eubanks, 1995, 1997, 2001). At the heart of this proposal are two putative hybrids, dubbed Tripsacorn and Sundance, that originated from these two grasses (figure 4.3). Unlike the parents, the rudimentary ear of these hybrids has exposed kernels attached to a central rachis, or cob. If such hybrids once

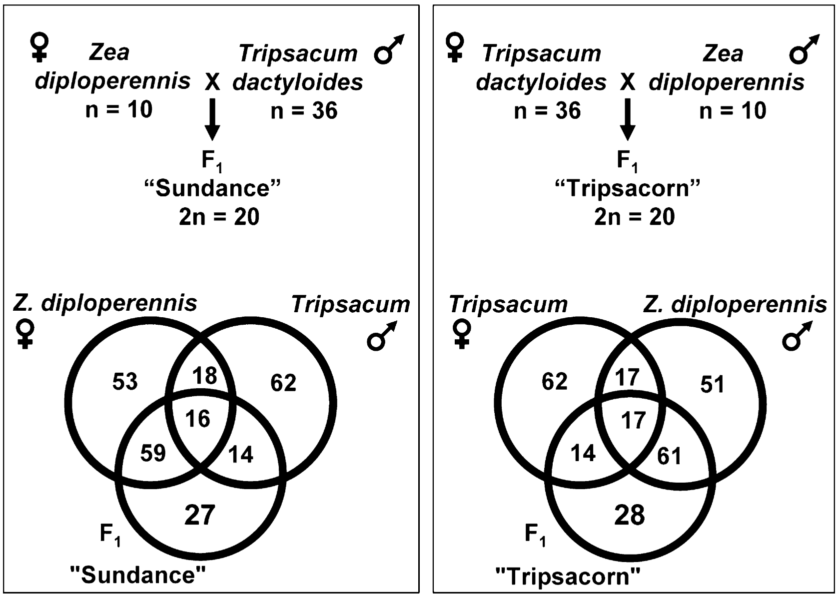

FIGURE 4.3 Sundance (*left*) and Tripsacorn (*right*) are the putative hybrids from a cross between *Z. diploperennis* and *T. dactyloides.* RFLP molecular analysis for these hybrids calls into dispute the successful hybridization of these plants because 23% of polymorphisms in the F_1 generation were not found in either parent. Overlapping regions of the Venn diagrams correspond to the number of shared bands between parent and putative offspring, whereas the numbers that appear in a single circle represent unique RFLP bands (data from Eubanks, 1997).

occurred naturally, then—at least according to proponents of the *Tripsacum–Z. diploperennis* hypothesis—the evolutionary puzzle of the origin of maize and its unparalleled architecture is solved.

However, there are several fundamental problems with the *Tripsacum–Z. diploperennis* theory. First, although producing a *Tripsacum–Z. diploperennis* hybrid may very well be possible, the documentation provided by Eubanks (1995, 1997) in support of these hybrids does not demonstrate that these two grasses were successfully hybridized. The chromosome number of both Tripsacorn and Sundance is 2n = 20. If *Tripsacum* (2n = 36 or 72) had indeed been one of the parents, then these hybrids would be expected to have 28 or 46 chromosomes, as evidenced by previous crosses between maize and *Tripsacum*. For example, successful experimental crosses between *T. dactyloides* and *Z. mays* ssp. *mays* by Mangelsdorf and Reeves (1939) produced hybrids with 2n = 28. Many other *Zea* and *Tripsacum* crosses were made by de Wet (de Wet and Harlan, 1974; de Wet et al., 1972), and a single generation conversion to 2n = 20 was never seen. Although the creation of a *Z. diploperennis* doubled haploid—in which all 10 *Zea* chromosomes are spontaneously doubled and all 36 *Tripsacum* chromosomes are immediately eliminated from the embryo—might be invoked to explain such a hybrid, the 2n = 20 condition is more likely to be the result of a contaminated cross. Indeed, 2n = 20 is also the chromosome number of maize and thus the number one would expect in a maize–*Z. diploperennis* hybrid.

A second concern regarding the validity of the *Tripsacum–Z. diploperennis* hypothesis centers on the analysis of rflp data for the putative hybrids (Eubanks, 1997). Because these molecular markers are inherited directly from the parents, restriction fragments present in a true hybrid must be traced back to at least one parent. Of the polymorphisms identified in Tripsacorn and Sundance, there was indeed some sharing between putative parent and offspring. It is interesting to note that the hybrids shared four times as many bands with *Z. diploperennis* as with *Tripsacum,* indicating a much closer relationship with teosinte than with *Tripsacum*. Perhaps more telling, however, is that 23% of the molecular markers surveyed were not found in either parent (figure 4.3). How does one account for these novel bands?

Proponents of the *Tripsacum–Z. diploperennis* hypothesis would argue that these restriction fragments are a consequence of the hybridization event itself: interactions between the combined genomes causing novel patterns of gene sequence. However, producing such novel gene sequences would entail either a point mutation at 2% of dna sites in one generation, or about 120 mutations per gene;[1] or a large insertion every 17,800 base pairs in one generation, or 168,000 total insertions across the genome.[2] Such genome activity is extremely unlikely and almost certainly lethal. Roughly 120 point mutations per gene in one generation is more than 3 million times the normal rate of mutation (6×10^{-9} substitutions per site per year from Gaut et al., 1996). And although the combination of two novel genomes may activate a few transposons here or there, it is doubtful that a genome could survive a rearrangement on the order of 168,000 large insertions because it would most certainly interfere with vital gene function. It seems far more plausible, as suggested earlier, that these novel bands are the product of a contaminated cross.

Even if these experimental hybrids are indeed true hybrids, they do not in themselves constitute proof that maize arose from the progeny of a cross between *Z. diploperennis* and *T. dactyloides*. Problems also exist with an argument often cited in support of the *Tripsacum–Z. diploperennis* hypothesis that attempts to tie together maize and *Tripsacum* evolution. The argument is based on shared ancestral polymorphisms between samples of teosinte (*Z. mays*), *Tripsacum,* and maize (*Z. mays* ssp. *mays*). A recent rflp study by Eubanks (2001) found that maize and *Tripsacum* share 92 unique polymorphisms (figure 4.4). From these data, it was inferred that "polymorphisms uniquely shared between *Tripsacum* and maize were likely derived from a

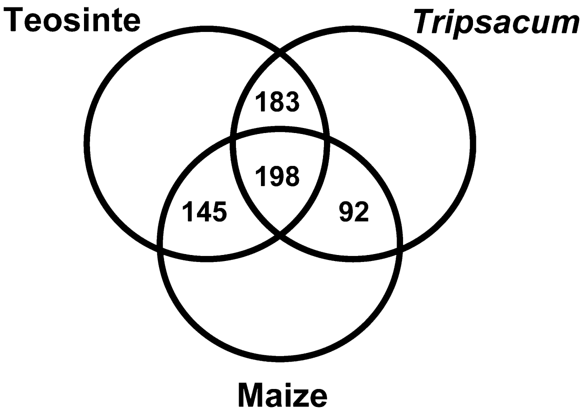

FIGURE 4.4 Shared ancestral polymorphisms between samples of teosinte (*Z. mays*), *Tripsacum,* and maize (*Z. mays* ssp. *mays*) as reported by Eubanks (2001). RFLP data revealed 92 polymorphisms unique to maize and *Tripsacum* and 198 shared by all three samples. The unique sharing of bands between maize and *Tripsacum* results from poor sampling of teosinte and the impossibility of sampling extinct alleles.

Tripsacum ancestor" (Eubanks, 2001:507). However, this can be true only if *all* alleles—both extant and extinct—are sampled from the three taxa, obviously an impossible feat. On the contrary, rather than providing proof of a *Tripsacum* origin, these shared polymorphisms are simply what one would expect to see between two grasses that share 93.5% of sites by vertical descent; indeed, 45% of rflp [illegible]ls should be shared between any *Zea* and *Tripsacum* pair because these grasses diverged from a common ancestor several million years ago.

Furthermore, there is also some question as to whether these 92 polymorphisms are uniquely shared between *Tripsacum* and maize. The teosinte sample used for the study is not reflective of the extremely high diversity inherent in the *Zea* genome (a closer look at this diversity follows later in the chapter). It included only one *Z. mays* ssp. *parviglumis* individual, thus underrepresenting a group that is not only one of the most diverse grasses but also is the one group most likely to possess alleles in common with maize (Doebley et al., 1984; Matsuoka et al., 2002). If the ssp. *parviglumis* sample had been larger and the teosinte alleles already extinct could also be considered, it is certain that many of the 92 bands would no longer be uniquely shared between *Tripsacum* and maize. Additionally, the *Tripsacum* sample can be called into question because it included *T. andersonii,* a natural, sterile *Zea–Tripsacum* hybrid with 64 chromosomes (Dewet et al., 1983). Thus, the *Tripsacum* sample already captured some *Zea* alleles, leading to inflated band sharing with both the maize and teosinte samples and calling into dispute the extent of *Tripsacum*'s unique contribution to the maize genome.

Finally, time itself tells a story inconsistent with the *Tripsacum–Z. diploperennis* hypothesis. Regardless of the progenitor involved, the domestication of maize cannot be older than the significant human migrations to the New World, which occurred roughly 15,000 years ago (Dillehay, 1989). By using the 18 currently sequenced genes in both maize and *Tripsacum* (Tenaillon et al., 2001; Whitt et al., 2002), we found that, on average, the genes diverged by 6.5% at noncoding and silent sites. If a mutation rate of 6×10^{-9} substitutions per site per year (Gaut et al., 1996) is assumed, this suggests that maize and *Tripsacum* alleles diverged around 5.2 million years ago, long before Native Americans could have combed the Mexican hillsides in search of food. In contrast, ssp. *parviglumis* and maize have an average divergence time of 9188 bp (Matsuoka et al., 2002). This date is consistent with the date of 6250 bp for [illegible] oldest known maize fossil (Piperno and Flannery, 2001).

Thus, from improbable hybrids to incongruous timelines, it appears that a *Tripsacum* key will not unlock the mystery of the origin of maize. However, we would be remiss not to acknowledge its potential contribution to the development of the maize genome. Because horizontal transfer of mitochondrial genes has been demonstrated between distantly related plants (Bergthorsson et al., 2003), there is a chance that some *Tripsacum* alleles could have introgressed into maize, but the contribution, if any, probably was very small. No phylogenetic, cytological, or molecular evidence exists in support of the *Tripsacum–Z. diploperennis* hypothesis, but the horizontal transfer of perhaps a handful of genes cannot formally be ruled out. If such a genome "jump" did occur, the genes involved probably conferred disease resistance rather than drove domestication because pathogens can provide intense selection pressure over billions of plants, making defense genes ideal candidates for transfer.

The Final Verdict

In short, the teosinte hypothesis best fits the evidence. For most maize geneticists and evolutionists (Bennetzen et al., 2001) familiar with the issues and data surrounding the origin of maize, there is little doubt that maize is a domesticated derivative of the wild Mexican grass teosinte (*Z. mays* ssp. *parviglumis*). However, questions persist in regard to the precise morphogenetic steps needed to complete the extreme transition from wild teosinte to cultivated maize. Just how did early Native American farmers achieve what is arguably the most remarkable breeding accomplishment of all time?

Domestication

The evolution of maize and the development of Native American societies were intimately connected; indeed, maize has been credited as the grain that civilized the New World. These early farming communities used corn not only for food but also for art and religious inspiration. Maize probably was domesticated over a period of a few thousand years in south central Mexico, the principal habitat of its immediate ancestor, *Z. mays* ssp. *parviglumis*. Archaeological remains of the earliest maize cob, found at Guila Naquitz Cave in the Oaxaca Valley of Mexico, date back roughly 6250 years (Piperno and Flannery, 2001). There is also much microfossil evidence suggesting dispersal to Central and South America by 7000–5000 BP (Piperno and Pearsall, 1998). Therefore maize probably was domesticated between 12,000 and 7500 years ago, as the first steps of domestication necessarily preceded this evidence, and its initiation cannot be older than the significant human migrations to the New World in roughly 15,000 BP (Dillehay, 1989).

Although the extraordinary morphological and genetic diversity among the maize landraces led some researchers to propose multiple, independent origins for maize (Kato Y., 1984), recent phylogenetic analyses based on comprehensive samples of maize and teosinte indicate a single domestication event. As noted earlier, a microsatellite-based phylogeny for a sample of 264 maize and teosinte plants showed all maize in a single monophyletic lineage that is derived from within ssp. *parviglumis* (Matsuoka et al., 2002). After this domestication, maize spread from Mexico over the Americas along two major paths (Matsuoka et al., 2002).

Domesticated maize was the result of repeated interaction with humans, with early farmers selecting and planting seed from plants with beneficial traits while eliminating seed from plants with less desirable features. As a result, alleles at gen[illegible]ontrolling favored traits increased in frequency within the population[illegible]ss favored alleles decreased. Thus with each succeeding generation these ancient agriculturists produced a plant more like modern maize and less like the wild grass of their ancestors.

This human selection process probably was both conscious and unconscious (Rindos, 1984). Native Americans may have combed the Mexican hillsides in search of teosinte plants with promising mutations, deliberately choosing the plants that provided more of and easier access to the sustenance they needed. For example, teosinte kernels are surrounded by a hard protective covering, or glume. Because this glume makes them very difficult to eat, plants with a softer glume were conceivably targeted during domestication. However, loss of shattering (a natural mechanism for seed dispersal) was more likely to be an inadvertent consequence of the harvesting process because early farmers could only plant the seeds that arrived home with them, still attached to the central rachis, or eventual maize cob.

Over time, these ancient agriculturists were able to select, consciously or not, the combination of major and many minor gene mutations that now distinguish maize from its wild ancestor. As it turns out, many of the same genes involved in this transformation might also be involved in that of other grasses, including wheat, rice, and sorghum (Paterson et al., 1995). Despite the independent domestication of these cereal complexes, it now appears that the earliest plant selectors desired the same sets of traits, as evidenced by selection at a common set of loci. QTLs for seed size, seed dispersal (shattering), and photoperiod have been mapped in maize, rice, and sorghum. These qtls c[illegible]espond to homologous regions between taxa more often than would be expected by chance and provide further evidence that domestication of these grasses was the result of mutations in a small number of genes with large effects (Buckler et al., 2001).

Diversity

The ability of Native Americans to transform a wild grass into the world's largest production grain crop is not only the product of skillful breeding but also a tribute to the tremendous diversity of the teosinte genome. Years before his time, these ancient farmers first practiced what Darwin later preached: that selection must be combined with natural variation in order for evolution to take place. As it turns out, teosinte is extremely diverse, with modern molecular studies measuring nucleotide diversity at silent sites in *Z. mays* ssp. *parviglumis* at roughly 2–3% (Eyre-Walker et al., 1998; Goloubinoff et al., 1993; Hilton and Gaut, 1998; White and Doebley, 1999; Whitt et al., 2002). Maize retained much of the diversity of its wild ancestor, with any two maize varieties differing from one another in 1.4% of their DNA (silent sites) (Tenaillon et al., 2001). For the sake of comparison, this level of nucleotide diversity found in maize is 2–5 times higher than that of other domesticated grass crops and is 14 times higher than that of humans; indeed, the divergence between two maize lines is roughly equivalent to the difference between humans and chimpanzees (Chen and Li, 2001).

This begs the question as to why *Z. mays* ssp. *parviglumis* has such high levels of diversity. Population genetics theory shows that levels of molecular diversity are the product of high mutation rates coupled with large effective population size. New alleles appear in a population by the natural process of mutation, and the random loss of these alleles (genetic drift) affects small populations more severely than large ones, as alleles are drawn from a smaller parental gene pool. *Z. mays* ssp. *parviglumis* conforms to both these criteria: A[illegible]h rate of mutation has been documented in grasses (Gaut et al., 1996), and population size for this wild grass historically has been quite large. Scientific literature documents such high diversity in several other species that also enjoy large population size, including *Drosophila simulans* (the fruit fly), with measures as high as 3.5% (Begun and Whitley, 2000).

In contrast, humans, whose founding populations in Africa were quite small in comparison, have only 0.1% diversity (Cargill et al., 1999).

Like most other grasses, maize maintained a substantial proportion of the variation of its wild progenitor, with only a 30% drop in diversity at the average locus (Buckler et al., 2001). This is probably because humans—both ancient and modern—depend on domesticated grains as a basis for subsistence, so large quantities of plants are needed before they are useful. If 10 people derive 10% of their calories from maize, it is estimated that roughly 250,000–350,000 plants would have to be grown annually (Buckler et al., 2001; Hillman and Davies, 1990).

Such abundant variation in the maize genome presents an intriguing paradox in light of the dramatic morphological differences between it and its closest living relative. On one hand, the extreme phenotypic and molecular variation found in maize is consistent with a large historical population size, as discussed in the preceding paragraph. On the other hand, maize is so unlike teosinte in ear morphology and plant architecture as to suggest strong selection during domestication, a decidedly diversity-limiting process. In other words, the initial steps of most domestication events probably included a population bottleneck.

Coalescent theory has been used to study the likelihood of such a domestication bottleneck in maize. Based on sequence diversity at the neutral *Adh1* locus in maize (*Z. mays* ssp. *mays*), its progenitor (*Z. mays* ssp. *parviglumis*), and a more distant relative (*Zea luxurians*), current diversity in maize can indeed be explained by a founding population with a modest number of diverse teosinte individuals (Eyre-Walker et al., 1998). However, the exact size of this founding population depends on the duration of the domestication event (the more founding individuals, the longer the bottleneck), something archaeological evidence has yet to elucidate with any certainty. Despite the virtual necessity of a population bottleneck to initiate maize domestication, its effects probably were limited by high rates of outcrossing and the impressive diversity among the founding teosinte population.

Targets of Selection

Individual Loci

Although the maize genome as a whole is extremely diverse, individual targets of selection can be identified because domestication should strongly reduce sequence diversity at genes controlling traits of human interest. As previously discussed, *tb1* is responsible for some of the major distinguishing morphological differences between teosinte and modern maize. Because this locus represents a key step in maize domestication, its nucleotide polymorphism should be lower than that of neutral sites. Indeed, within the promoter region of *tb1,* maize possesses only 3% of the diversity found in teosinte, or 61-fold lower diversity in the domesticated crop than in the closest wild relative (Wang et al., 1999). The timing and sequence of such character selection by early farmers is now being revealed by the fusion of molecular biology and archaeological research. Surveys of *tb1* in ancient DNA suggest that selection at this locus occurred before 4400 BP (Jaenicke et al., 2003).

A recent large survey of 1772 maize loci suggests that roughly 3–5% of these genes have undergone selection since domestication (Vigouroux et al., 2002). Coalescent simulations were used to compare the genetic diversity (or divergence) at a locus with what one would expect under a neutral model that incorporates the domestication bottleneck. This approach to screening large numbers of loci for the signature of selection appears to offer a powerful method for identifying new candidate genes of agronomic importance.

Starch Pathway

Whereas changes in plant shape and ear morphology were the initial focus of Beadle and his successors, many additional traits have been the target of human selection over the last few thousand years. Some of these traits of particular significance were yield, ear size (which increased from 2 cm to 30 cm), and grain quality. Starch is the key product of maize, accounting for 73% of the kernel's total weight. Therefore the genes involved in starch synthesis are among the most important for grain production, critical to both the yield and the quality of the grain.

A simplified pathway of starch production in maize is outlined in figure 4.5. Amylopectin makes up roughly three-quarters of the total product, with amylose the remainder. Amylopectin is primarily responsible for granule swelling and eventual thickening of pastes upon addition of heat, and amylose typically is thought to affect the gelling of starch, all chemical and structural properties important in food processing. For example, starch pasting modifies the ability of foods to hold fat and protein molecules that enhance flavor and texture, certainly an aspect of maize that

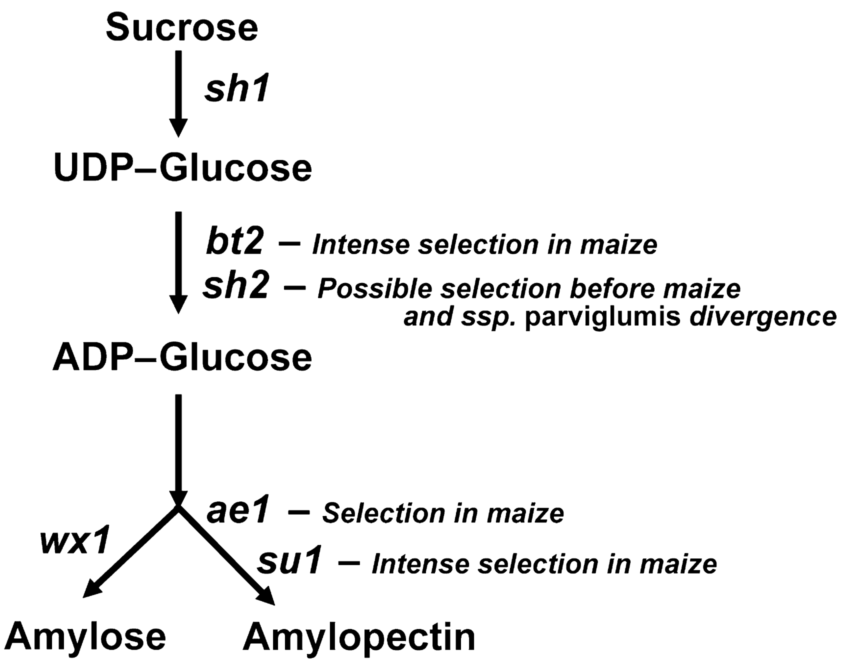

FIGURE 4.5 A simplified pathway of starch production in maize, indicating the relative position of the 6 sampled genes in the pathway: *amylose extender1* (*ae1*), *brittle2* (*bt2*), *shrunken1* (*sh1*), *shrunken2* (*sh2*), *sugary1* (*su1*), and *waxy1* (*wx1*). The genes *bt2, sh1,* and *sh2,* located upstream in the pathway, aid in the formation of glucose, whereas the enzymes coded by *ae1, su1,* and *wx1* produce the final products of starch metabolism: amylose and amylopectin. The signature of selection at each locus is also noted, as revealed by low nucleotide diversity. ADP = adenosine diphosphate; UDP = uridine diphosphate.

Native American breeders might have included in the domestication and improvement process.

Although plant genetics and biochemistry have thus far identified more than 20 genes involved in starch production, Whitt et al. (2002) focused on six key genes known to play major roles in starch production: *amylose extender1* (*ae1*), *brittle2* (*bt2*), *shrunken1* (*sh1*), *shrunken2* (*sh2*), *sugary1* (*su1*), and *waxy1* (*wx1*). For each locus, diversity estimates (π) were performed by sequencing 6–13 kb from 30 diverse maize lines with 1–2 kb from *Z. mays* ssp. *parviglumis* and 2–4 kb from *Tripsacum dactyloides* for comparison. The Hudson–Kreitman–Aquade (HKA) test (Hudson et al., 1987), a test that compares rates of divergence between species to levels of polymorphism within species, was then used to formally test for selection.

The results were striking: Four of these six starch loci exhibited evidence of selection (Whitt et al., 2002), whereas random loci in maize showed almost no proof of selection. Three maize loci in particular, *su1, bt2,* and *ae1,* revealed a dramatic three- to sevenfold reduction in diversity over *Z. mays* ssp. *parviglumis,* which is consistent with artificial selection in the starch pathway during maize domestication and improvement (figure 4.5). The significant HKA [illegible]lts for both *bt2* and *su1* indicate that this selection probably occurred b[illegible]e the dispersal of maize germplasm throughout the world, whereas with *ae1* the HKA t[illegible]in conjunction with a second test of selection, Tajima's test) suggests that selection is ongoing.

Although the exact nature of this selection cannot be fully understood until a wide range of teosinte starch alleles are examined in maize genetic backgrounds, our results provide an intriguing glimpse into the preferences of early Native American breeders. Given the particular roles of *ae1, bt2,* and *su1* in the starch pathway, it appears that selection favored increased yield and different amylopectin qualities. Because starch (unlike protein) is often lacking in hunter–gatherer diets of tropical and subtropical societies, it is reasonable to presume that early cultivators of maize focused on improving starch yield. Starch pasting properties are also logical targets of selection in maize because the ratio of amylose to amylopectin and the chemical structure of amylopectin (specifically the length of branched glucose chains) affect everything from porridge to tortilla texture.

A timeline indicating when these early breeders selected for starch production and other advantageous traits is being constructed with help from archaeology. Ancient DNA a[illegible]ysis from maize samples unearthed in Mexico and the southwestern United States has revealed that *su1* alleles known to occur in modern maize probably were under selection between 1800 and 900 years ago (Jaenicke et al., 2003). Future studies that integrate important archeological questions, such as when and how ancient peoples used maize, with molecular evidence of selection will make it possible to trace the genetic consequences of domestication over time.

The enduring legacy of ancient maize agriculturalists is far more than the germplasm for a softer tortilla, however. As evidenced by our research, the reduction of diversity in starch loci is dramatic and should motivate a paradigm shift in maize breeding. Although tremendous variation at most loci has allowed maize to respond to centuries of artificial selection and industrial farming practices, limited diversity in the starch pathway and perhaps other pathways of critical importance may prevent current breeding practices from reaching their full potential. The ability of plant breeders and scientists to improve current maize lines and develop new products to meet future needs depends on useful variation within the maize germplasm. Perhaps the most efficient way to introduce this potentially useful diversity into maize is to introgress or transform the abundant allelic variation present in teosinte for selected genomic regions or specific genes. By using this raw genetic material from maize's wild relatives, the next generation can continue what the early Mexican natives so deftly began: the most impressive feat of genetic modification and morphological evolution ever accomplished in any plant or animal domesticate.

Acknowledgments

We would like to thank Sherry Whitt and Larissa Wilson for their research contributions to this chapter; both played important roles in establishing the signature of selection in the maize starch pathway. This work was supported by the U.S. Department of Agriculture Agricultural Research Service and the National Science Foundation (DBI-0321467).

References

Beadle, G.W. 1939. Teosinte and the origin of maize. *Journal of Heredity* 30: 245–247.

Beadle, G.W. 1972. The mystery of maize. *Field Museum of Natural History Bulletin* 43: 2–11.

Beadle, G.W. 1977. The origin of *Zea mays*. In C.A. Reed (ed.), *Origins of Agriculture,* 615–635. Mouton Press, The Hague, The Netherlands.

Beadle, G.W. 1980. The ancestry of corn. *Scientific American* 242: 112–119.

Begun, D.J. and P. Whitley. 2000. Reduced X-linked nucleotide polymorphism in *Drosophila simulans*. *Proceedings of the National Academy of Sciences (USA)* 97: 5960–5965.

Bennetzen, J., E. Buckler, V. Chandler, J. Doebley, J. Dorweiler, B. Gaut, M. Freeling, S. Hake, E. Kellogg, R.S. Poethig, V. Walbot, and S. Wessler. 2001. Genetic evidence and the origin of maize. *Latin American Antiquity* 12: 84–86.

Bergthorsson, U., K. Adams, B. Thomason, and J. Palmer. 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. *Nature* 424: 197–201.

Buckler, E.S. IV and T.P. Holtsford. 1996. *Zea* systematics: Ribosomal ITS evidence. *Molecular Biology and Evolution* 13: 612–622.

Buckler, E.S. IV, J.M. Thornsberry, and S. Kresovich. 2001. Molecular diversity, structure and domestication of grasses. *Genetical Research* 77: 213–218.

Cargill, M., D. Altshuler, J. Ireland, P. Sklar, K. Ardlie, N. Patil, N. Shaw, C.R. Lane, E.P. Lim, N. Kalyanaraman, J. Nemesh, L. Ziaugra, L. Friedland, A. Rolfe, J. Warrington, R. Lipshutz, G.Q. Daley, and E.S. Lander. 1999. Characterization of single-nucleotide polymorphisms in coding regions of human genes. *Nature Genetics* 22: 231–238.

Chen, F. C. and W. H. Li. 2001. Genomic divergences between humans and other hominoids and the effective population size of the common ancestor of humans and chimpanzees. *American Journal of Human Genetics* 68: 444–456.

de Wet, J. M. J., L. M. Engle, C. A. Grant, and S. T. Tanaka. 1972. Cytology of maize–*Tripsacum* introgression. *American Journal of Botany* 59: 1026–1029.

de Wet, J. M. J., G. B. Fletcher, K. W. Hilu, and J. R. Harlan. 1983. Origin of *Tripsacum*–Andersonii (Gramineae). *American Journal of Botany* 70: 706–711.

de Wet, J. M. J. and J. R. Harlan. 1974. *Tripsacum*–maize interaction: A novel cytogenetic system. *Genetics* 78: 493–502.

Dillehay, T. D. 1989. *Monte Verde, a Late Pleistocene Settlement in Chile*. Smithsonian Institution Press, Washington, DC, USA.

Doebley, J. 1990a. Molecular evidence and the evolution of maize. *Economic Botany* 44: 6–27.

Doebley, J. 1990b. Molecular systematics of *Zea* (Gramineae). *Maydica* 35: 143–150.

Doebley, J. 2001. George Beadle's other hypothesis: One-gene, one-trait. *Genetics* 158: 487–493.

Doebley, J. F., M. M. Goodman, and C. W. Stuber. 1984. Isoenzymatic variation in *Zea* (Gramineae). *Systematic Botany* 9: 203–218.

Doebley, J. F. and H. H. Iltis. 1980. Taxonomy of *Zea* (Gramineae). I. A subgeneric classification with key to taxa. *American Journal of Botany* 67: 982–993.

Doebley, J., W. Renfroe, and A. Blanton. 1987. Restriction site variation in the *Zea* chloroplast genome. *Genetics* 117: 139–147.

Doebley, J. F. and A. Stec. 1991. Genetic analysis of the morphological differences between maize and teosinte. *Genetics* 129: 285–295.

Doebley, J., A. Stec, and C. Gustus. 1995. *Teosinte branched1* and the origin of maize: Evidence for epistasis and the evolution of dominance. *Genetics* 141: 333–346.

Doebley, J., A. Stec, and L. Hubbard. 1997. The evolution of apical dominance in maize. *Nature* 386: 485–488.

Doebley, J., A. Stec, J. Wendel, and M. Edwards. 1990. Genetic and morphological analysis of a maize–teosinte F_2 population: Implications for the origin of maize. *Proceedings of the National Academy of Sciences (USA)* 87: 9888–9892.

Dorweiler, J., A. Stec, J. Kermicle, and J. Doebley. 1993. *Teosinte glume architecture 1:* A genetic locus controlling a key step in maize evolution. *Science* 262: 233–235.

Eubanks, M. 1995. A cross between two maize relatives: *Tripsacum dactyloides* and *Zea diploperennis*. *Economic Botany* 49: 172–182.

Eubanks, M. W. 1997. Molecular analysis of crosses between *Tripsacum dactyloides* and *Zea diploperennis* (Poaceae). *Theoretical and Applied Genetics* 94: 707–712.

Eubanks, M. W. 2001. The mysterious origin of maize. *Economic Botany* 55: 492–514.

Eyre-Walker, A., R. L. Gaut, H. Hilton, D. L. Feldman, and B. S. Gaut. 1998. Investigation of the bottleneck leading to the domestication of maize. *Proceedings of the National Academy of Sciences (USA)* 95: 4441–4446.

Gaut, B. S., B. R. Morton, B. C. McCaig, and M. T. Clegg. 1996. Substitution rate comparisons between grasses and palms: Synonymous rate differences at the nuclear gene *Adh* parallel rate differences at the plastid gene *rbcL*. *Proceedings of the National Academy of Sciences (USA)* 93: 10274–10279.

Goloubinoff, P., S. Pääbo, and A. C. Wilson. 1993. Evolution of maize inferred from sequence diversity of an *Adh2* gene segment from archaeological specimens. *Proceedings of the National Academy of Sciences (USA)* 90: 1997–2001.

Hillman, G. and M.S. Davies. 1990. Domestication rates in wild-type wheats and barley under primitive cultivation. *Biological Journal of the Linnean Society* 39: 39–78.

Hilton, H. and B.S. Gaut. 1998. Speciation and domestication in maize and its wild relatives: Evidence from the *globulin-1* gene. *Genetics* 150: 863–872.

Hudson, R.R., M. Kreitman, and M. Aquade. 1987. A test of neutral molecular evolution based on nucleotide data. *Genetics* 116: 153–159.

Iltis, H. and B. Benz. 2000. *Zea nicaraguensis* (Poaceae), a new teosinte from Pacific coastal Nicaragua. *Novon* 10: 382–390.

Iltis, H.H. and J.F. Doebley. 1980. Taxonomy of *Zea* (Gramineae). II. Subspecific categories in the *Zea mays* complex and generic synopsis. *American Journal of Botany* 67: 994–1004.

Jaenicke, V., E.S. Buckler, B.D. Smith, M.T.P. Gilbert, A. Cooper, J. Doebley, and S. Pääbo. 2003. Early allelic selection in maize as revealed by ancient DNA. *Science* 302: 1206–1208.

Kato Y., T.A. 1976. Cytological studies of maize (*Zea mays* L.) and teosinte (*Zea mexicana* Schrader Kuntze) in relation to their origin and evolution. *Massachusetts Agricultural Experiment Station Bulletin* 635: 1–185.

Kato Y., T.A. 1984. Chromosome morphology and the origin of maize and its races. *Evolutionary Biology* 17: 219–253.

Kato Y., T.A. and A. Lopez R. 1990. Chromosome knobs of the perennial teosintes. *Maydica* 35: 125–141.

Mangelsdorf, P.C. 1974. *Corn: Its Origin, Evolution, and Improvement*. Harvard University Press, Cambridge, MA, USA.

Mangelsdorf, P.C., R.S. MacNeish, and W.C. Galinat. 1964. Domestication of corn. *Science* 143: 538–545.

Mangelsdorf, P.C., R.S. MacNeish, and W.C. Galinat. 1967. Prehistoric wild and cultivated maize. In D.S. Byers (ed.), *The Prehistory of the Tehuacan Valley: Environment and Subsistence,* Vol. 1, 178–200. University of Texas Press, Austin, TX, USA.

Mangelsdorf, P.C. and R.G. Reeves. 1938. The origin of maize. *Proceedings of the National Academy of Sciences (USA)* 24: 303–312.

Mangelsdorf, P.C. and R.G. Reeves. 1939. The origin of Indian corn and its relatives. *Texas Agricultural Experiment Station Bulletin* 574: 1–315.

Matsuoka, Y., Y. Vigouroux, M.M. Goodman, J. Sanchez G., E.S. Buckler, and J.F. Doebley. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. *Proceedings of the National Academy of Sciences (USA)* 99: 6080–6084.

McClintock, B., T.A. Kato Y., and A. Blumenschein. 1981. *Chromosome Constitution of Races of Maize*. Colegio de Postgraduados, Chapingo, Mexico.

Paterson, A.H., Y.R. Lin, Z. Li, K.F. Schertz, J.F. Doebley, S.R.M. Pinson, S.C. Liu, J.W. Stansel, and J.E. Irvine. 1995. Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. *Science* 269: 1714–1718.

Piperno, D.R. and K.V. Flannery. 2001. The earliest archaeological maize (*Zea mays* L.) from highland Mexico: New accelerator mass spectrometry dates and their implications. *Proceedings of the National Academy of Sciences (USA)* 98: 2101–2103.

Piperno, D.R. and D.M. Pearsall. 1998. *The Origins of Agriculture in the Lowland Neotropics*. Academic Press, San Diego, CA, USA.

Rindos, D. 1984. *The Origins of Agriculture: An Evolutionary Perspective*. Academic Press, San Diego, CA, USA.

Sanchez, G. J., T.A. Kato Y., M. Aguilar S., J.M. Hernandez C., A. Lopez R., and J.A. Ruiz C. 1998. *Distribucion y Caracterizacion del Teocintle*. Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias, Guadalajara, Mexico.

Tenaillon, M.I., M.C. Sawkins, A.D. Long, R.L. Gaut, J.F. Doebley, and B.S. Gaut. 2001. Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. *mays* L.). *Proceedings of the National Academy of Sciences (USA)* 98: 9161–9166.

Vigouroux, Y., M. McMullen, C.T. Hittinger, K. Houchins, L. Schulz, S. Kresovich, Y. Matsuoka, and J. Doebley. 2002. Identifying genes of agronomic importance in maize by screening microsatellites for evidence of selection during domestication. *Proceedings of the National Academy of Sciences (USA)* 99: 9650–9655.

Wang, R.L., A. Stec, J. Hey, L. Lukens, and J. Doebley. 1999. The limits of selection during maize domestication. *Nature* 398: 236–239.

Wellhausen, E.J., L.M. Roberts, E. Hernandez X., and P.C. Mangelsdorf. 1952. *Races of Maize in Mexico: Their Origin, Characteristics and Distribution*. Bussey Institute of Harvard University, Cambridge, MA, USA.

White, S.E. and J.F. Doebley. 1999. The molecular evolution of *terminal ear1,* a regulatory gene in the genus *Zea*. *Genetics* 153: 1455–1462.

Whitt, S.R., L.M. Wilson, M.I. Tenaillon, B.S. Gaut, and E.S. Buckler. 2002. Genetic diversity and selection in the maize starch pathway. *Proceedings of the National Academy of Sciences (USA)* 99: 12959–12962.

Wilkes, H.G. 1967. *Teosinte: The Closest Relative of Maize*. Bussey Institute of Harvard University, Cambridge, MA, USA.

Notes

1. Necessary point mutation frequency was obtained by dividing the frequency of novel bands in the putative hybrids (0.23) by the length of nucleotides in RFLP cut sites (6 + 6).

2. Insertion number was obtained by dividing the average band size (4096 for restriction enzymes with 6 bp recognition sites) by the frequency of novel bands in the putative hybrids (0.23).