

Current Biology

Genome Sequence of a 5,310-Year-Old Maize Cob Provides Insights into the Early Stages of Maize Domestication

Highlights

- Researchers characterized genome of a 5,310-year-old maize cob
- The ancient maize genome is a basal lineage equally related to modern landraces
- There is genetic evidence of naked kernels 5,310 years ago in Tehuacan, Mexico
- Human selection on maize domestication traits occurred as a gradual process

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In Brief

Ramos-Madrigal et al. sequence the genome of 5,310-year-old maize cob, which represents a basal lineage equally related to all modern varieties. They provide an in-depth genomic characterization of maize at an early point during its evolution as a domesticate, suggesting that human selection occurred as a gradual process.

Genome Sequence of a 5,310-Year-Old Maize Cob Provides Insights into the Early Stages of Maize Domestication

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SUMMARY

The complex evolutionary history of maize (*Zea mays* L. ssp. *mays*) has been clarified with genomic-level data from modern landraces and wild teosinte grasses [1, 2], augmenting archaeological findings that suggest domestication occurred between 10,000 and 6,250 years ago in southern Mexico [3, 4]. Maize rapidly evolved under human selection, leading to conspicuous phenotypic transformations, as well as adaptations to varied environments [5]. Still, many questions about the domestication process remain unanswered because modern specimens do not represent the full range of past diversity due to abandonment of unproductive lineages, genetic drift, on-going natural selection, and recent breeding activity. To more fully understand the history and spread of maize, we characterized the draft genome of a 5,310-year-old archaeological cob excavated in the Tehuacan Valley of Mexico. We compare this ancient sample against a reference panel of modern landraces and teosinte grasses using *D* statistics, model-based clustering algorithms, and multidimensional scaling analyses, demonstrating the specimen derives from the same source population that gave rise to modern maize. We find that 5,310 years ago, maize in the Tehuacan Valley was on the whole genetically closer to modern maize than to its wild counterpart. However, many genes associated with key domestication traits existed in the ancestral state, sharply contrasting with the ubiquity of derived alleles in living landraces. These findings suggest much of the evolution during domestication may have been gradual and encourage

further paleogenomic research to address provocative questions about the world's most produced cereal.

RESULTS

DNA Sequencing of an Ancient Maize Cob

The Tehuacan Valley of Mexico is a prominent locale in the story of ancient maize cultivation, as much of the knowledge about the practices of early maize farmers comes from macrobotanical remains found in a series of cave sites with archaeological deposits [6]. The valley, located in the Mexican state of Puebla, is approximately 450 km northeast of the Balsas River Valley, maize's putative domestication center [1, 7] (Figure 1A). The maize cob fragment used for genetic analysis in this study, henceforth referred to as Tehuacan162, was excavated by an archaeological team led by Richard MacNeish in the 1960s and curated by the Robert S. Peabody Museum, where it had been exhibited as an example of "wild corn" (Figure 1B). The cob's age was determined using accelerator mass spectrometry (AMS) radiocarbon dating, yielding an age of 4,460 ± 30 radiocarbon years before present (5,310 calibrated calendar years BP) [9]. Thus, this ancient sample likely represents part of an early population of maize transported from the domestication center by humans and can be used to trace the early steps in the cultivation history of maize.

We sequenced Tehuacan162 to an average depth of coverage of 1.7×, which corresponds to 6× in the regions of the genome that are accessible with short reads (21% of the genome) based on DNA libraries prepared using a single-stranded methodology [10] (Table S1; Supplemental Information). The final dataset consists of whole-genome shotgun sequencing data (98%) and sequencing data from a library enriched for a set of 348 loci (2%) that were previously selected for analysis of ancient material [11]. Fragment length distribution and nucleotide misincorporation patterns in the sequencing data are consistent with that expected for ancient DNA (aDNA) (Figure S1; Supplemental Information).

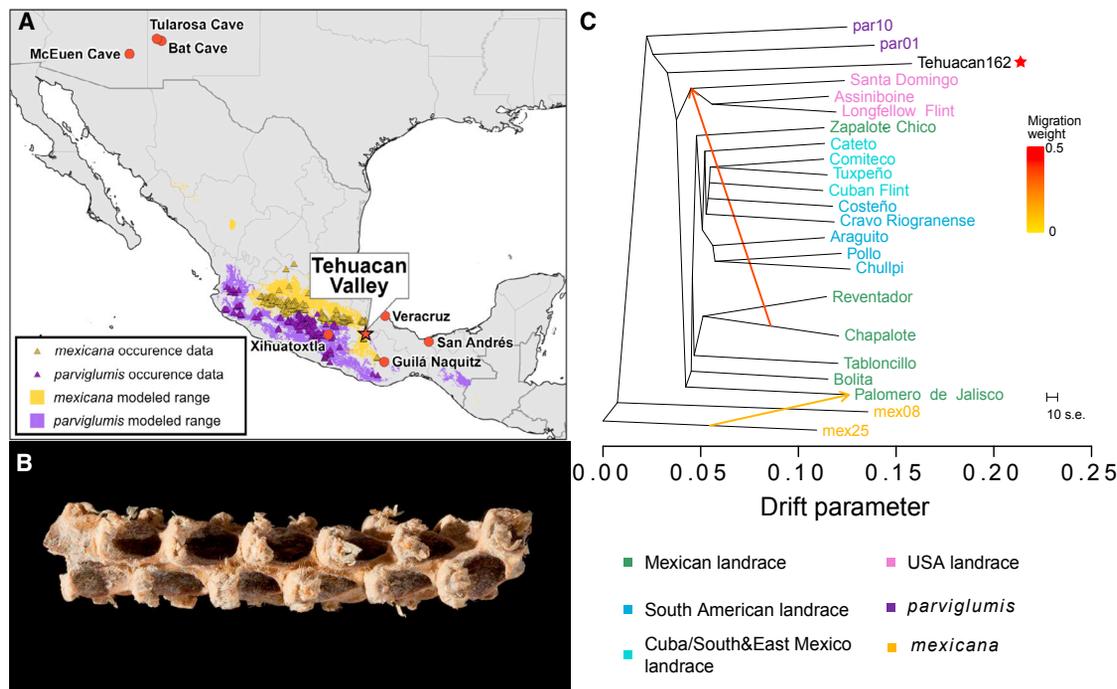


Figure 1. Tehuacan162, a 5,310-Year-Old Cob from the Tehuacan Valley

(A) Map modified from Hufford et al. [8] showing the known location of teosinte populations, modeled geographic ranges of teosinte subspecies, the location of the Tehuacan Valley (red star), and important archaeological sites in the area (red points).

(B) Photograph of the maize cob used for sequencing. Total length of the cob is 16.3 mm with an approximate diameter of 3.1 mm.

(C) Maximum-likelihood admixture graph on a dataset consisting of 18 non-admixed maize landraces (identified from the clustering analysis at $K = 5$; Figure 2B), the *Palomero de Jalisco* landrace, Tehuacan162 (red star), two *parviglumis* and two *mexicana*. A random read was chosen for each site; transitions, non-polymorphic sites, and sites with missing data or overlapping with repetitive regions were discarded ($n = 310,064$). Arrows represent admixture events between different maize populations. Colors in the figure correspond to the ancestry components obtained from Figure 2B.

See also Figure S1 and Table S1.

Phylogenetic History of Maize in the Tehuacan Valley

Two *Zea mays* subspecies have a significant role in the genetic history of maize. *Zea mays* ssp. *parviglumis* (hereafter *parviglumis*) has been established as the progenitor of modern maize [1], while introgression from *Zea mays* ssp. *mexicana* (henceforth referred to as *mexicana*) was important in the adaptation of maize to the highlands of central Mexico [12]. Both subspecies are native to Mexico and are commonly referred to as teosintes (along with *Zea mays* ssp. *huehuetenangensis* in western Guatemala) [5]. While *parviglumis* grows in the lowlands of western and southern Mexico, *mexicana* is adapted to the cooler and drier conditions of the highlands of the Mexican Central Plateau [8].

We assessed the broad phylogenetic context of Tehuacan162 by computing admixture graphs using *TreeMix* [13] and a set of modern maize landraces and teosinte genomes available from the maize HapMap2 panel [2] (Figure 1C). The reference panel comprises 23 modern landraces spanning the Americas, as well as 15 *parviglumis* and 2 *mexicana* genomes. We expanded the reference panel with the addition of a highland Mexican maize landrace (*Palomero de Jalisco*) previously published in Fonseca et al. [11]. The inferred maximum-likelihood tree placed Tehuacan162 as an outgroup to extant maize landraces (Figure 1C). Importantly, we were able to recapitulate the gene flow between *Palomero de Jalisco* and *mexicana*, as well as a

previously identified gene flow signal between lowland Mexican and ancient US Southwest maize [11] (Figure 1C), which were shown not to involve Tehuacan162.

Based on the admixture graphs results, we used the D statistic framework [14] to formally test the specific hypotheses on the relationship between Tehuacan162 and teosinte and modern landraces. D statistics were computed using one randomly sampled allele from each genome with the exclusion of transitions in order to avoid potential bias caused by aDNA damage in the ancient sample (see Supplemental Information). Consistent with previous genetic work [1], we found that Tehuacan162 is more closely related to *parviglumis* than to *mexicana* (Figure 2A). Furthermore, when computing D (landrace, *parviglumis*; Tehuacan162, *tripsacum*), we find Tehuacan162 is more closely related to modern landraces than *parviglumis* in all comparisons ($n = 360$; Figure 2B). Together with the *TreeMix* inference, these results support that Tehuacan162 belongs to the same phylogenetic clade as maize.

To investigate how the Tehuacan162 genome is related to modern-day maize, we compared the ancient sample to all possible pairs of landraces in the panel ($n = 264$). By computing D (Landrace1, Landrace2; Tehuacan162, *Tripsacum*), we tested whether Tehuacan162 shares more derived alleles with any particular landrace when compared to another (Figure 2C). Overall, we could not reject the hypothesis of Tehuacan162 being an

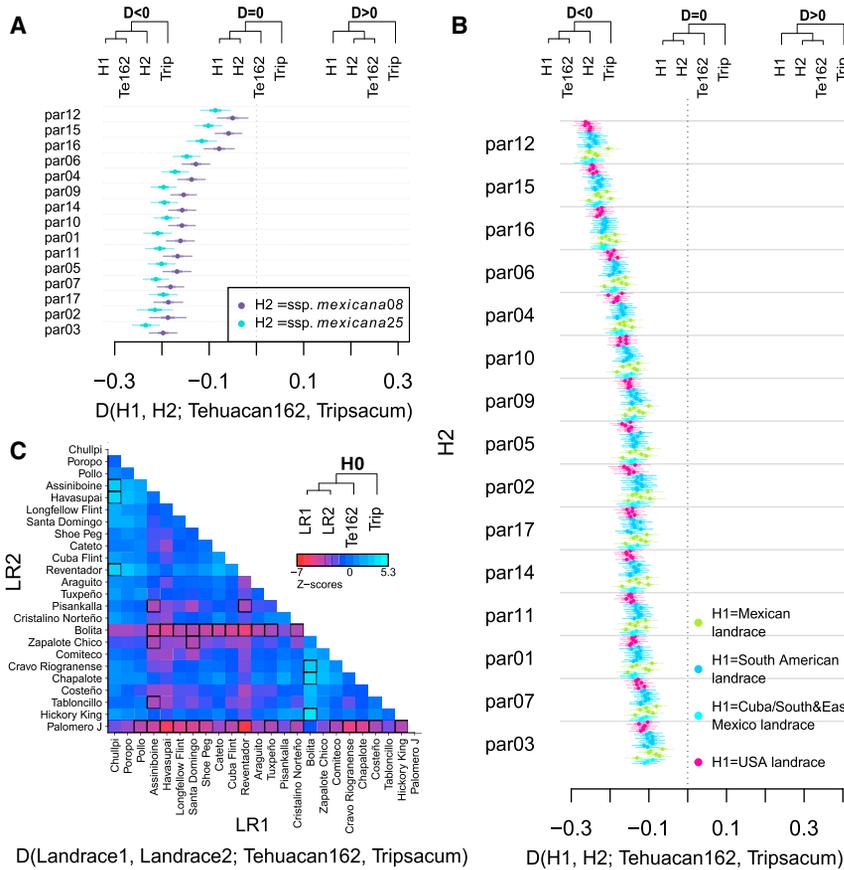


Figure 2. Genetic Relationships between Tehuacan162, Modern Maize, and Teosinte Inferred Using D Statistics

(A) D statistics testing the relationships between *parviglumis* (par01–par17), *mexicana* (mex08, mex25), and the ancient genome (Te162). Points indicate the D value obtained for each test and each of the possible pairs (*parviglumis*, *mexicana*). Horizontal bars show 1 (thick) and ~ 3.3 (thin) standard errors.

(B) D statistics testing the relationships between *parviglumis* (par01–par17), modern maize, and Tehuacan162 (Te162). Points indicate the D value obtained for each of the possible pairs (*parviglumis*, landrace). Horizontal bars show 1 (thick) and ~ 3.3 (thin) standard errors.

(C) D statistics testing the relationship between all possible pairs of maize landraces (LR1/LR2) and Tehuacan162 (Te162). Each cell represents the Z score obtained for the comparison, through a block-jackknife procedure. Significant deviations from $D = 0$ ($|Z| > 3.3$) are highlighted with darker outlines.

See also Figures S3B and S4 and Table S2.

can162 genome diverged from the source population before all other maize lineages, we expect the average sequence divergence between Tehuacan162 and any other maize genome to be similar. Consistent with this prediction, genomic divergence between Tehuacan162 and modern maize landraces ranged between 18.4% and 23.2%, with an average of 19.7% and overlapping confidence intervals for all estimates (Figure S4; Supplemental Information). In contrast, we see both lower average values (17.7%, 17.7%, and 17.9%, respectively, for *Zapalote Chico*, *Tuxpeño*, and *Chullpi* landraces) and higher variance in comparisons involving two modern landraces (Figure S4; Supplemental Information). This analysis further supports the hypothesis that Tehuacan162 diverged from the maize ancestor before extant lineages did. We note that these divergence estimates are also compatible with a strong population structure prior to the diffusion of maize from the domestication center.

Tehuacan162 Genomic Composition and Affiliations

To explore the genetic composition of the Tehuacan162 genome, we compared it to the genomes included in the HapMap2 panel and to a previously published set of low coverage ancient genomes [11] using a multidimensional scaling (MDS) [16] approach and a model-based clustering algorithm (*NGSadmix*; [17]). The MDS plot places the ancient genome in an intermediate position between modern maize and teosinte (Figure 3A). Similarly, the clustering analysis revealed that Tehuacan162 is not closely affiliated with any particular group of landraces (Figure 3B), but rather its genome displays components of the three principal maize clusters identified (46%) as well as the *parviglumis*-specific component (54%) (Figure 3B).

Hybridization between maize in the Mexican highlands and *mexicana* is a well-established phenomenon. Up to 20% of the

outgroup to any given pair of landraces (Figure 2C). We note there are some exceptions to this finding, in particular for *Palomero de Jalisco* and *Bolita* landraces, which in some cases show support for the alternative hypothesis (Landrace, Tehuacan162; *Palomero de Jalisco* or *Bolita*, *Tripsacum*) ($|Z| > 5.3$). However, we detected significant signals of introgression from *mexicana* in these two landraces (Figure S3B; Supplemental Information), leading them to contain a higher number of alleles derived from this teosinte population. Thus, our data are consistent with Tehuacan162 being an outgroup to modern-day landraces present in the panel. Collectively, these results indicate that Tehuacan162 represents a population that derives from the same lineage as modern maize but branched off before the diversification of the modern landraces represented in the reference panel.

Average DNA Divergence between Tehuacan162 and Modern Maize

To achieve another perspective of the genetic relationship between Tehuacan162 and modern-day maize, we estimated the average DNA divergence between Tehuacan162 and landraces in the HapMap2 panel, using the method described in Green et al. [15]. In brief, we counted the number of lineage-specific differences in the tree (Tehuacan162, Landrace; *Tripsacum*) and estimated the average divergence between Tehuacan162 and landraces as the percentage of “landrace-specific” differences from the differences relative to the branch leading from the common ancestor of *Tripsacum* and Tehuacan162. If the Tehua-

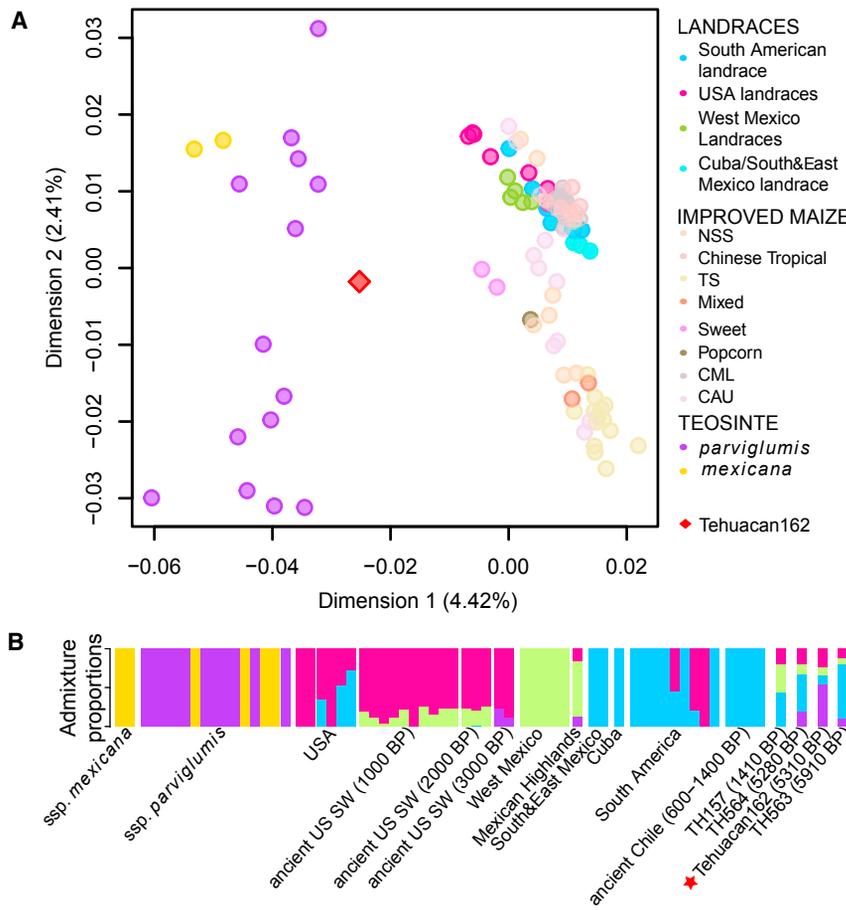


Figure 3. Admixture Components in Tehuacan162 Estimated Using Whole-Genome Data

(A) Multidimensional scaling plot computed in the HapMapV3 panel (23 landraces, 101 improved maize lines, 15 *parviglumis*, and 2 *mexicana* genomes) and called genotypes from the ancient sample using *bammis* (3,149,198 sites).

(B) *NGSadmix* analysis computed in a panel composed of the HapMap2, a set of previously published ancient maize samples and Tehuacan162 in a total of 1,821,135 sites assuming $K = 5$. Figure shows the result with the best likelihood among 300 replicates. See also Figures S2 and S3.

parviglumis ($n = 13$) and modern maize (23 landraces and 30 randomly selected improved maize lines) using the HapMapV3 panel [20]. For each site in the panel, we estimated informativeness (I_n) as described in Rosenberg et al. [21] and defined ancestry informative markers (AIMs) as those sites with an $I_n > 0.1$. This value corresponds to a difference in allele frequency of ~ 0.49 between populations. From a total of 72,800,922 SNP loci, we identified 67,830 AIMs for which Tehuacan162 has sufficient data (coverage of at least 10x) for SNP calling (see Supplemental Information). For each AIM, we calculated the probability of finding two maize alleles (A_{MM}), two *parviglumis*

highland maize genome derives its ancestry from *mexicana* [12], conferring advantages to high altitude environments (e.g., $>2,000$ m) [18, 19]. The Tehuacan Valley (up to $\sim 1,700$ m) is surrounded by high elevation regions that overlap with the geographic range of *mexicana* [8] and where hybridization between maize and this subspecies can occur [19]. Therefore, we compared the genotypes called from Tehuacan162 to a panel of highland and lowland maize ($n = 243$), *parviglumis* ($n = 98$), and *mexicana* ($n = 96$), genotyped over 936 SNP loci [12] in order to improve our understanding on the timing of gene flow from *mexicana*. We ran *ADMIXTURE* assuming three ancestral populations to assess whether Tehuacan162 carried admixture from *mexicana* (Figure S3D; Supplemental Information) and found no evidence for *mexicana*-specific admixture in Tehuacan162, consistent with the low levels of introgression found in previous studies of maize from geographic locations with similar elevation as the Tehuacan Valley and growing in sympatry with *mexicana* [19]. While we cannot entirely exclude admixture from *mexicana* with this limited dataset, we note that we were able to detect admixture proportions as low as $\sim 5.5\%$ admixture in the *Palomero de Jalisco* landrace (Figure S2; Supplemental Information).

Domestication-Related Loci in the Tehuacan162 Genome

In order to investigate the effect of selection on the Tehuacan162 genome, we identified a set of SNP markers that differentiate

alleles (A_{PP}), or a combination of one *parviglumis* and one maize allele (A_{PM}) given the allele frequencies in both populations (see Supplemental Information). We focused on the A_{MM} estimate (Figure 4A) to identify sites with high similarity to maize in the ancient sample.

To identify genes that could be associated with selection in maize during the early stages of domestication, we assessed A_{MM} in two groups of genes previously identified by Hufford et al. [22] as important during initial maize domestication as well as subsequent improvement during recent breeding and local adaptation. Additionally, we included a list of genes that have been identified as responsible for the morphological differences between maize and *parviglumis* or important during the domestication process (Table S4). For each of these genes, we estimated the average probability of deriving from maize (A_{MM}) in Tehuacan162, 23 landraces, 15 teosintes, and 10 randomly selected improved maize lines. As expected, for genes related to domestication or improvement, we observed a clear distinction between modern maize and teosinte genomes (Figure 4B). A distance-based dendrogram using this set of genes placed Tehuacan162 as intermediate between the maize and *parviglumis* clusters, suggesting that the ancient sample is a step that links modern maize with its wild ancestor (Figure 4B; Table S4). We hypothesize that genes with high A_{MM} in Tehuacan162 are either targets of selection or located close to regions that were important during the early stages of the domestication process.

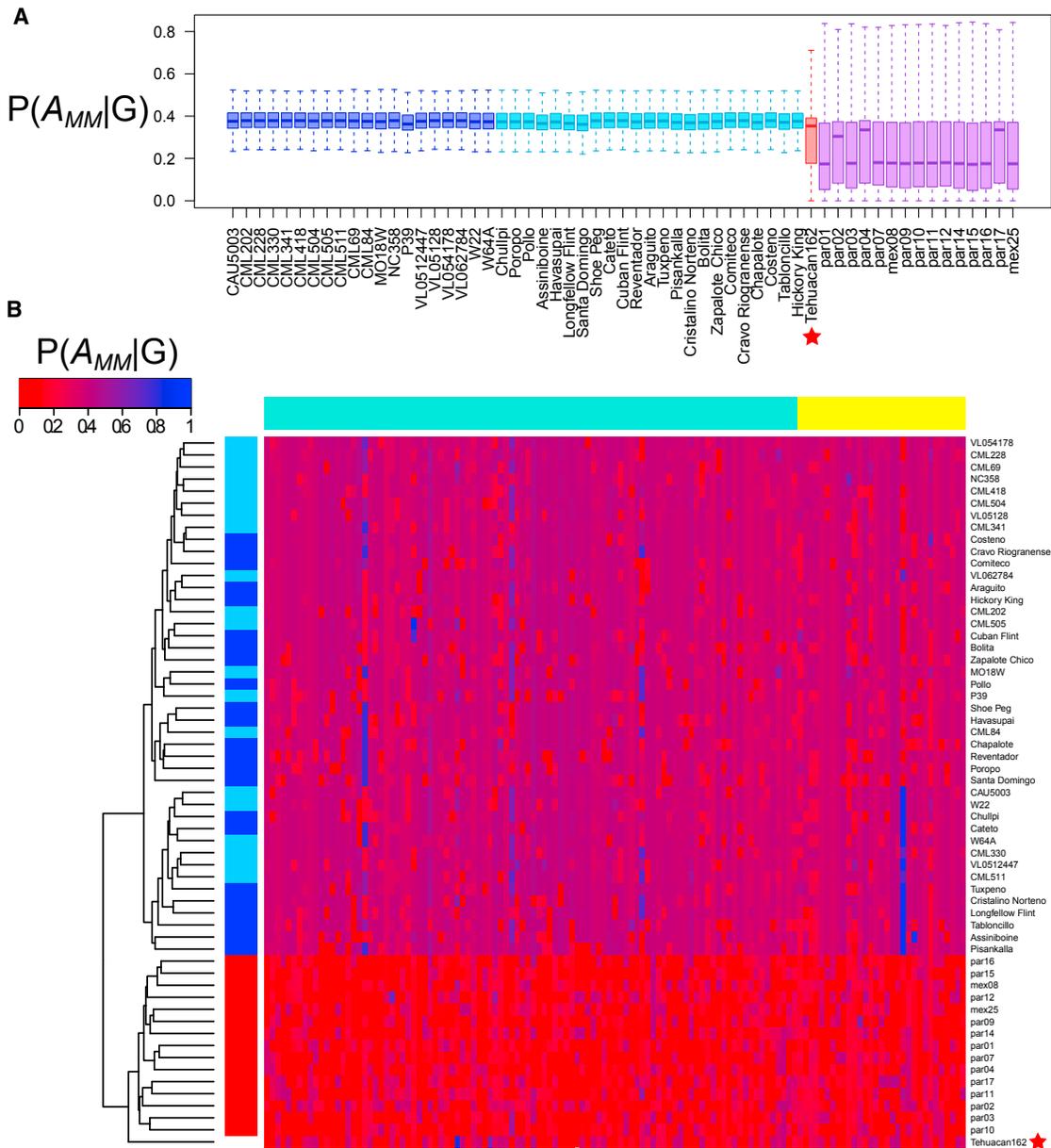


Figure 4. Ancestry Assignment to a Set of Genes Related to Domestication and Improvement in Maize

(A) Boxplot showing the distribution of A_{MM} in improved maize lines (blue), landraces (light blue), Tehuacan162 (red), and teosinte (purple) samples.

(B) Dendrogram of A_{MM} per gene. Each row represents a teosinte (red), landrace (blue), improved maize lines (light blue), or Tehuacan162. Each column (sorted by category) represents a gene from a list of domestication-related (green) or improvement-related (yellow) genes. Colors correspond to the average of the probability of finding two maize alleles (A_{MM}) in that gene.

See also [Tables S3](#) and [S4](#).

The genes for which Tehuacan162 is more maize-like (higher A_{MM}) have been associated with inflorescence architecture (*td1*) [23], circadian clock and flowering time (*zmg1*) [24], glycogen biosynthesis (*bt2*) [25], and lateral meristem development (*ba1*) [26]. Tehuacan162 also demonstrates high A_{MM} for some genes known to be important during maize domestication but whose functions are yet to be determined (Table S4). Of particular interest is *tga1*, which controls the change from encased to exposed kernels and for which a single nucleotide variant has been asso-

ciated to the difference in phenotype between maize and *parviglumis* [27]. We found Tehuacan162 to carry the variant that leads to exposed kernels production, consistent with phylogenetic analysis suggesting that selection on this gene dates to the beginning of domestication. In contrast to genes with high A_{MM} , genes with high A_{PP} in Tehuacan162 represent genes that were potentially selected after 5,310 years ago. Among those, we found genes related to ear shattering in *parviglumis* (*zag1*) [28] and starch biosynthesis (*su1* and *wx1*) [29].

DISCUSSION

The genomes of modern landrace and teosinte samples can reveal only part of the complex story of maize domestication and cultivation, yet a complete history of maize has far-reaching implications. From the biological standpoint, domesticated maize represents a quintessential example of the extreme morphological changes that can occur during domestication. For archaeology, maize domestication and its prehistoric cultivation is intrinsically linked to cultural development of large-scale societies and empires across the Americas, made possible through dependable, calorie-dense maize products. And from an economic angle, understanding how maize adapted to diverse environments and how nutritional content changed over millennia may aid plant breeders in developing new lines by utilizing untapped sources of genetic diversity to resist diseases, adapt to extreme and changing environmental conditions, and improve nutritional content.

Our data complement other recent studies of domestic animals [30, 31] and plants [11, 32] to show how paleogenomic-scale datasets from archaeological materials can help clarify our understanding of the process of domestication and early selection pressures. Using genomic data of a 5,310-year-old maize cob, we have obtained a genomic snapshot of the past, finding Tehuacan162 represents an ancient form of maize that is closely related to the ancestor of all modern maize, yet distinct from *parviglumis*, the closest living relative to maize.

While the Tehuacan162 genome is more closely related to modern maize than teosinte, it differs from modern landraces and improved maize lines in genes related to the domestication syndrome [33]. For these genes, we observe a mosaic pattern where the ancient sample is an intermediate step between maize and teosinte. Genes with a high A_{MM} in the ancient sample would indicate that selection already acted upon these loci. Genes with a high A_{PP} provide evidence that these genomic regions were associated with selection only after 5,310 years ago. Some genes matched the expected pattern, such as *tga1*, a gene that determines whether kernels are encapsulated by a hard seed coat, and for which Tehuacan162 has the exposed, edible type of kernel. Based on previous findings [11, 34], we expected the specimen to predate selection on genes in the starch metabolic pathway and observed genes like *su1* and *wx1* were of the ancestral type. Intriguingly, we observed the gene that controls for ear shattering (*zgl1*) [28] remains in the ancestral state, more similar to *parviglumis*. This is somewhat unexpected because analogous genes have been implicated in the initial steps of domestication of other cereals [35, 36]. Previous studies have identified two stages of selection during maize domestication [11, 22]: a strong selection bottleneck during domestication that greatly restricted allelic diversity of some genes in modern maize and a much more recent period of adaptation and improvement of landraces to local conditions [22]. Our data suggest that the history of selection is a more complex and gradual process, far richer than a simple two-stage process.

As a whole, the genomic-level data from Tehuacan162 highlight the gradual nature of the domestication process in maize, wherein different genetic loci were selected at different points in time. The sample provides a new snapshot of the genetic background on which modern maize is based, in a manner that

cannot be achieved using only modern samples or other archaeological methods. In addition, these results underscore the utility of a single well-preserved archaeological sample for aDNA research. Other samples from the same collection yielded much lower endogenous content and could not provide the same amount of useful data. Therefore, we encourage the prudent screening of other ancient maize macroremains, sourced from both museum collections and ongoing excavations. In this manner, researchers can access a vast archive of paleogenomic data, permitting detailed investigations of prehistoric and recent selective pressures, and ultimately achieving new understandings of the maize landraces and improved maize lines that now serve as dietary staples for hundreds of millions of people.

EXPERIMENTAL PROCEDURES

See [Supplemental Information](#) for complete materials and methods.

ACCESSION NUMBERS

The accession number for the raw sequence reads reported in this paper is NCBI SRA: PRJNA352392.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2016.09.036>.

AUTHOR CONTRIBUTIONS

Conceptualization, J.R.-M., B.D.S., J.R.-I., M.T.P.G., and N.W.; Methodology, J.R.-M., J.R.-I., and N.W.; Software, J.R.-M. and S.G.; Formal Analysis, J.R.-M., J.V.M.-M., and S.G.; Investigation, N.W.; Resources, M.T.P.G. and B.D.S.; Data Curation, J.R.-M.; Writing – Original Draft, J.R.-M. and N.W.; Writing – Review & Editing, J.R.-M., B.D.S., J.V.M.-M., S.G., J.R.-I., M.T.P.G., and N.W.; Visualization, J.R.-M.; Supervision, N.W.; Funding Acquisition, M.T.P.G.

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