The Putative Mesoamerican Domestication Center of *Phaseolus vulgaris* Is Located in the Lerma–Santiago Basin of Mexico

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**ABSTRACT**

Mesoamerican food agriculture is defined by the milpa cropping system, consisting of maize (*Zea mays* L.), common bean (*Phaseolus vulgaris* L.), and squash (*Cucurbita* spp.). In recent years, a domestication center for maize has been proposed in the Balsas River basin in west-central Mexico, raising the question whether the Balsas basin was also the center of origin for Mesoamerican food agriculture in general. We conducted a survey of genetic diversity for 26 microsatellite markers in a representative sample of 155 wild and domesticated common bean from its Mesoamerican gene pool. Microsatellite diversity was analyzed with STRUCTURE, neighbor-joining tree construction, and principal coordinate analysis. Most Mesoamerican domesticated accessions clustered in a single group, suggesting a single domestication. Furthermore, the most closely related wild beans to the domesticated clade originated from a restricted region in the Rio Lerma–Río Grande de Santiago basin in west-central Mexico, distinct from the Balsas basin. Although wild maize and *Phaseolus* beans grow together in the wild, they appear to have been domesticated in different regions to be reunited later on in a single cropping system. Crop domesticaions in Mesoamerica may therefore have a diffuse rather than a single geographic origin. Archaeological and ecological investigations into the origins of agriculture should be refocused from the arid eastern half of Mexico to the west-central part of the country.
point to Mexico as a center of domestication of the three crops (Smith, 1995; Doebley, 2004; Gepts, 1988; Gepts et al., 1986; Piperno and Flannery, 2001). (For common bean, there is an additional center of domestication in the Andes [Gepts et al., 1986; Gepts 1998]; however, this center is not discussed further in this report.)

Beans, maize, and squash are often cultivated together in traditional cropping systems in Mexico (e.g., milpa cropping system) and other countries of Latin America and in eastern Africa because they complement each other agronomically (e.g., plant nitrogen balance; Cardoso et al., 2007) and nutritionally (e.g., balance of essential amino acids; Bressani, 1983). The actual area of domestication of these crops within Mexico—and particularly, whether these domestications can be traced back to a single region—is still the subject of both archaeological and genetic research. Maize was domesticated from teosinte (Zea mays subsp. parviglumis), its closest wild relative (Doebley, 2004), in a region presumably located in west-central Mexico around the Balsas River basin, based on genetic data (Matsuoka et al., 2002). The oldest archaeological remains of incipient maize domestication have been identified in the Guilá Naquitz cave to the south of the Balsas basin (Piperno and Flannery, 2001), as were 8000- to 10,000-yr-old squash (C. pepo) seeds (Smith, 1997). Given the close association of maize and beans before and after domestication (Delgado Salinas et al., 1988), these findings raise the question whether the common bean was domesticated in the same general area as maize. Recent experimental developments prompted us to examine the question of the origin of domestication of common bean in its Mesoamerican center of domestication using a broadly distributed range of wild populations (Freytag and DeBouck, 2002) and a set of 26 putatively neutral microsatellite markers distributed over the 11 linkage groups of common bean (Blair et al., 2003).

**MATERIAL AND METHODS**

**Microsatellite Marker Analysis**

Twenty-six microsatellite markers from all 11 linkage groups were selected on the basis of their map location (Supplemental Table S1; Blair et al., 2003). Forward primers were designed with a 5′-TGTAACGACGGCCAGTATTGC M-13 reverse sequence tail added to the 5′ end of the forward primer. Genomic DNA was extracted from young leaves using the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). Except for simple sequence repeat markers BM146 and BM157, two independent polymerase chain reactions (PCRs) were performed. For the primary PCR, the pairs of forward and reverse primer were used to amplify microsatellite fragments. Thus, the fragments amplified in the primary PCR included the M13 sequence extension at forward primer site. The secondary PCR reactions were performed with the reverse primer and the M-13 primer labeled with the 6-FAM, NED, PET, or VIC fluorescence dyes (Applied Biosystems, Foster City, CA). For the primary PCR reaction, PCR reaction mixtures contained approximately 30 ng of total genomic DNA, 200 μM of dNTP, 0.2 μM of forward primer and reverse primer, the standard Taq buffer with 1.5 mM MgCl₂, and 1 unit of Taq polymerase (New England Biolabs, Ipswich, MA) in a 20 μL total reaction volume. The primary PCR cycle consisted of 2 min at 94°C and 35 cycles of 30 s at 94°C, 1 min at 47°C (BMd45, BMd10, BMd1, Pv-cct001, BMd53, BMd37, BMd12, BMd25, BMd42, and BMd41), 49°C (Pv-ag003, BM143, BM172, Pv-ag004, BM151, Pv-at007, and BM212), 52°C (GATS91, BM160, and BM210), 55°C (BM188), 57°C (Pv-ag001), or 60°C (BM53 and BMd20), and then 40 s at 72°C followed by a 3-min extension at 72°C. For the secondary PCR reaction, the PCR reaction mixtures contained 1 μL of primary PCR product, 0.2 μM of fluorescence-labeled M13 universal primer and reverse primer, 0.34 μM of forward primer and standard Taq buffer with 1.5 mM MgCl₂, and 1 unit of Taq polymerase in a total volume 20 μL reaction. For M13 primer labeling, the 6-FAM, NED, PET, or VIC dye was attached to the 5′ end of the 5′-TGTAACGACGGCCAGTATTGC M-13 universal primer sequence. The secondary PCR cycle consisted of 2 min at 94°C and 30 cycles of 30 s at 94°C, 45 s at 56°C, and 45 s at 72°C followed by 8 cycles of 30 s at 94°C, 45 s at 53°C, and 45 s at 72°C, and then 3 min at 72°C for the final extension. For the BM146 and BM157 amplification, PCR reaction mixtures contained approximately 30 ng of total genomic DNA, 200 μM of dNTP, 0.16 μM of labeled M-13 universal primer and reverse primer, 0.04 μM of reverse primer, standard Taq buffer with 1.5 mM MgCl₂, and 1 unit of Taq polymerase (New England Biolabs) in a 20 μL total reaction volume. Polymerase chain reaction cycles consisted of 5 min at 94°C and 30 cycles of 30 s at 94°C, 45 s at 56°C, and 45 s at 72°C followed by 8 cycles of 30 s at 94°C, 45 s at 53°C, and 45 s at 72°C, and a final extension of 3 min at 72°C. The amplified fragments were multiplexed depending on their size variation and analyzed in an ABI 3730 (Applied Biosystems). Marker genotypes were determined using the GeneMarker program (version1.51; SoftGenetics LLC, State College, PA) and are included in Supplemental Table S2.

**Defining Mesoamerican Accessions Using STRUCTURE**

Three hundred forty-nine wild, landrace, and commercial cultivars and advanced germplasm accessions of common bean were analyzed in this study (Supplemental Table S3). These accessions were obtained from the Phaseolus World Collection at CIAT, Cali, Colombia, or from the Bean Collection of the USDA National Plant Germplasm System. Because crop varieties can be disseminated across different regions or continents, the geographic origin of individual accessions is not a reliable indicator of the origin of domestication (Andean vs. Mesoamerican; Gepts et al., 1986; Gepts 1998). Thus, the program STRUCTURE (Pritchard et al., 2000) was run with a clustering number (K) = 2 setting. Five independent simulations were performed using the admixture model and 5000 replicates for burn-in and 50000 replicates during analysis. A between-simulation similarity coefficient (SSC) was calculated using the STRUCTURE sum program (Ehrlich 2006), which showed that the clustering in different runs was almost identical (SSC = 0.9969). Among the five simulations, the run with the highest likelihood value was selected, and the accessions...
with more than 50% of membership coefficient for the Mesoamerican cluster were assigned to the Mesoamerican gene pool. Through this process, 155 accessions out of 349 were defined as belonging to the Mesoamerican gene pool. Among these 155 accessions, 88% had a membership coefficient above 90% and 95% above 80%. The remaining 5% had membership coefficients between 50 and 80%.

**Population Structure in Mesoamerican Gene Pool**

To define the population structure in the Mesoamerican gene pool, the 155 accessions were further analyzed with the STRUCTURE program (Pritchard et al., 2000). For each of the $K = 2$ to $K = 13$ settings, 20 independent simulations were performed using the admixture model. To compare likelihoods of each assumption, a Wilcoxon two-sample test was performed (Rosenberg et al., 2001). A between-simulation similarity coefficient was calculated using the STRUCTURE-sum program (Ehrich, 2006). To detect the optimal clustering number ($K$), the ad hoc statistic $\Delta K$, which is based on the rate of change in the log probability of data between successive $K$ values, was calculated using the STRUCTURE-sum program (Evanno et al., 2005). $\Delta K$ identified the optimal $K$ number as 8 based on the lowest $\Delta K$ value and no further significant decrease beyond $K = 8$. The STRUCTURE bar graph was established with the software Distuct (Rosenberg, 2002).

**Neighbor-Joining Tree Construction and Principal Coordinate Analysis**

The genetic distance among 155 Mesoamerican accessions was calculated using the C.S. Chord distance (Cavalli-Sforza and Edwards, 1967). The C.S. Chord distance does not require any mutation model for microsatellite evolution and is free from bottleneck effect (Takezaki and Nei, 1996). Based on this genetic distance, an unrooted neighbor-joining (NJ) tree was constructed in PowerMarker (Liu and Muse, 2005). Principal coordinate analysis was performed using the GenAlEx 6 Program (Peakall and Smouse, 2006).

**RESULTS**

**Identification of Mesoamerican Accessions**

Extensive research has shown that common bean consists of two major ecogeographic gene pools. The existence of Andean and Mesoamerican gene pools was initially suggested on the basis of the geographic distribution of hybrid lethality genes based on electrophoretic patterns of phaseolin seed protein (Gepts and Bliss, 1985; Gepts et al., 1986). This reproductive isolation preexisted domestication (Koinange and Gepts, 1992). Additional evidence for these two gene pools was provided by morphological (Gepts et al., 1986; Singh et al., 1991a,b), molecular marker (Singh et al., 1991c; Khairallah et al., 1990, 1992; Becerra-Velásquez and Gepts 1994; Tohme et al., 1996; Chacón et al., 2007), and phytopathological (Guzmán et al., 1995; Kelly and Vallejo, 2004) data. Thus, domesticated common bean resulted from at least two domestinations from an already diverged ancestor, wild common bean. A common origin for these two gene pools may reside in the wild common bean of Ecuador and northern Peru (Kami et al., 1995). Because of exchanges after domestication between the Andean and the Mesoamerican gene pools (Gepts et al., 1988; Paredes and Gepts, 1995), geographic origin is an ambiguous indicator of gene pool membership. Neutral molecular markers such as microsatellites provide the opportunity to assess the gene pool origin of common bean accessions, independently of geographic information.

To identify a more specific region for the Mesoamerican domestication of common bean, we followed a two-step strategy. First, we identified the presumptive Mesoamerican accessions in a large representative sample following a model-based approach of microsatellite diversity, implemented in the STRUCTURE software. Next, we analyzed genetic distances among these Mesoamerican accessions by a STRUCTURE again, combined with NJ and principal coordinate analyses of microsatellite diversity.

A representative sample of 349 wild and domesticated accessions of common bean was developed, which comprised 100 wild and 249 domesticated types. Twenty-six microsatellite markers, distributed on the 11 linkage groups of the common bean genome, were chosen for this analysis (Blair et al., 2003; Yu et al., 2000; Gaitán-Solis et al., 2002). Genetic diversity was analyzed with a model-based approach implemented in STRUCTURE (Pritchard et al., 2000) assuming two populations ($K = 2$) (see “Materials and Methods”). This procedure assigned 155 accessions to the Mesoamerican gene pool, including 76 wild and 79 domesticated types, and 199 Andean gene pool materials. Further discussion of results in this article addresses only the 155 accessions assigned to the Mesoamerican gene pool. The wild-types in this Mesoamerican sample included 8 accessions from the ancestral populations in Ecuador and Peru, 8 from Colombia, 3 from Guatemala, and 57 from Mexico. The 79 domesticated entries included 2 from Bolivia, 6 from Brazil, 1 from Chile, 5 from Colombia, 1 each from Costa Rica and Ecuador, 5 from Guatemala, 3 from Honduras, 35 from Mexico, 2 from Nicaragua, 1 each from Panama and Peru, 14 from the United States, and 2 from Venezuela.

**Genetic Diversity in the Mesoamerican Gene Pool**

The organization of this Mesoamerican sample was further analyzed with STRUCTURE in a stepwise fashion from 2 to 1 populations ($K = 2$ to $K = 11$). The final number of populations of $K = 8$ was chosen based on the $\Delta K$ statistical test (Evanno et al., 2005) showing no further significant subdivisions for larger $K$ values. At $K = 8$, two domesticated groups were identified, one corresponding
to the ecogeographic race Mesoamerica (K7) and the other including ecogeographic races Jalisco and Durango (K6) (Singh et al., 1991a). A close relationship between races Jalisco and Durango had already been suggested with earlier data (Pallottini et al., 2004). Wild populations were subdivided by STRUCTURE into five groups, in part along geographic lines. In the Andes, the ancestral wild group from northern Peru and Ecuador (K2) (Kami et al., 1995) was distinct from the Colombian wild beans (K1) (Fig. 1). Wild beans from Mesoamerica were divided into three groups although no geographic, topographic or ecological pattern could be identified as yet for this subdivision (K3, K4, and K8). The eighth group included wild beans from two populations near the municipality of Arandas in eastern Jalisco (K5). Segregation for seed size and color among these populations suggests they resulted from recent outcrossing events between wild and domesticated types in these locations.

To better understand the genetic relatedness among and within these different groups, genetic distances were calculated using the C.S. Chord distance, which does not assume a specific mutation model for microsatellite evolution and is free from bottleneck effect (Cavalli-Sforza and Edwards, 1967; Takezaki and Nei, 1996). A cluster analysis using these genetic distances was conducted based on the NJ method as implemented in Powermarker version 3.25 (Liu and Muse, 2005). The resulting tree (Fig. 2) identified a predominantly single-cluster origin for the Mesoamerican domestication of common bean, with 95% of Mesoamerican domesticates grouped in a single clade, confirming earlier evidence based on phaseolin data (Gepts et al., 1986) and amplified fragment length polymorphism (Papa and Gepts, 2003). The tree further confirms the subdivisions identified by the STRUCTURE analysis. These include a geographic distribution of genetic diversity in wild beans belonging to the Mesoamerican gene pool. Specifically, wild common bean from Ecuador and northern Peru (a group that is the presumed ancestor of P. vulgaris; Kami et al., 1995), Colombia, and Mexico and Guatemala were included in different branches (Fig. 2).

The tree also identified two major domesticated subgroups in the domesticated cluster, one coinciding with race Mesoamerica with small seeds (generally <25–30 g/100 seeds) and the other including races Jalisco and Durango, with medium- to large-size seeds (>30 g/100 seeds) (Singh et al., 1991a). There were two exceptions to this single-cluster pattern of Mesoamerican domestication. First, a smaller (n = 5) group of domesticated accessions was separate from the main group of domesticated accessions and which included three cultivars from the STRUCTURE K7 group (G01344; G03807; and G05059), representing the Mesoamerica ecogeographic race, and two cultivars from the STRUCTURE K6 group (G01796 and G05024), representing races Durango and Jalisco. One possibility is that this small group resulted from hybridization Mesoamerican and Andean domesticated types. Membership coefficients in the Mesoamerican gene pool, as calculated with STRUCTURE for K = 2, were G01344 (0.991), G03807 (0.989), G05059 (0.997), G01796 (0.994), and G05024 (0.995). These high values for membership coefficients suggest either that these accessions are not Andean × Mesoamerican hybrids or that introgression has affected the genome of these accessions in a minimal way. The latter is the case for accession G03807 (Brasil2), which resulted from the introgression of an Andean determinacy allele into a Mesoamerican background (Singh et al., 1991a; M. Kwak and P. Gepts, unpublished results) yet shows a high Mesoamerican membership coefficient. Another possibility is that this small group consists of intra-Mesoamerican gene pool hybrids. This appears to be the case for accessions G01796 (K6: races Jalisco & Durango: 0.437; K4: Mexican wild: 0.28; K7: race Mesoamerica: 0.185) and G05024 (K6: races Jalisco & Durango: 0.449; K7: race Mesoamerica: 0.448). This leaves accessions G01344 (unnamed from Nicaragua) and G05059 (H6 Mulatinho from Brazil), which are apparently nonhybrid and belong to the K7 (race Mesoamerica) group (membership coefficients: 0.921 and 0.964, respectively). Both accessions are small-seeded (~20–23 g/100 seeds according to the CIAT database, http://isa.ciat.cgiar.org/urg/main.do?language=en). Gepts and Bliss (1986) and Beebe et al. (2000) speculated on the existence of an additional, minor center of domestication or ecogeographic race in Central America and lowland South America. Additional materials are needed from these regions to better understand their origin.

The second exception to the single-cluster pattern of Mesoamerican domestication was a cluster of accessions separating the two main domesticated subgroups (race Mesoamerica; races Durango and Jalisco). This cluster of accessions (Fig. 2: Arandas hybrids: accessions G12922–G12935) represents a cross between wild and domesticated types, all originating from El Tule, municipality of Arandas, in Jalisco.

A principal coordinate analysis (Fig. 3) provides a similar picture as the NJ tree. The first axis, which accounts for 34% of the variation, separates wild from domesticated types. The second axis separates the two domesticated groups: race Mesoamerica and races Jalisco and Durango. Among wild types, the separations among different STRUCTURE groups are not as clear. However, the wild groups from Ecuador and northern Peru and Colombia tend to separate from the three wild Mexican groups, consistent with their geographic origins. In turn, wild types from Ecuador and northern Peru are separated from the wild Colombian types along the third axis (not shown).
Wild Beans Most Closely Related to the Mesoamerican Domesticated Clade

A group of seven wild bean populations most closely related to the domesticated clade (G11051, G11056, G12910, G12949, G12952, G12953, G12957; Fig. 2 and 3) is distributed in a narrow region oriented east–west across the state of Jalisco and into the western part of the neighboring state of Guanajuato. This region is centered in the Río Lerma–Río Grande de Santiago River basin (hereafter called the Lerma–Santiago basin; Fig. 4A). It was also identified earlier using electrophoretic patterns of phaseolin seed protein coded by the Phs locus (Gepts, 1988). Three wild bean populations
DISCUSSION

The close genetic relationship between wild and domesticated common bean in the Lerma–Santiago basin observed in this study confirms earlier results obtained with phaseolin seed protein electrophoretic data. Phaseolin, however, is a trait under selection during domestication because of its role in seed weight (Johnson et al., 1996; Koinange et al., 1996). Thus, the similarity suggested by phaseolin could have been the result not only of a potential ancestor–descendant relationship but of alternative causes as well, such as selection for seed weight. In contrast, microsatellite markers are most likely neutral and therefore provide a better reflection of actual genetic relationships. In our case, the corroboration of earlier phaseolin results by microsatellite data markedly strengthens the case for a domestication center in the Lerma–Santiago basin.

The significance with regard to bean domestication of the close relationship between the group of seven wild beans and the domesticated gene pool can be interpreted in three ways. It may reflect the location of the first cultivation and domestication of common bean in the Mesoamerican area. Predomestication cultivation of wild common bean, a necessary condition for domestication, would have taken place in this region, as would have selection for the different traits of the domestication syndrome (Koinange et al., 1996) before wild bean populations gave way to bean domesticates. Alternatively, it reflects the geographic origin of the most successful domesticate that came to predominate the Mesoamerican domesticated gene pool of common bean. This second hypothesis takes into account the possibility that predomestication cultivation of common bean may have been initiated elsewhere, in lieu of or in addition to the Lerma–Santiago basin. This discussion assumes that the overall distribution of wild beans has not changed in a major way since domestication. Because of uncertainties regarding this question (Buckler et al., 1998), the domestication area identified here can only be considered as putative. Nevertheless, the clear single origin in the Mesoamerican domestication process of common bean (Gepts et al., 1986; Papa and Gepts, 2003; current results) suggests that the divergence among domesticated ecogeographic races (Durango, Jalisco, and Mesoamerica; Singh et al., 1991a) must have appeared following the domestication process instead of resulting from separate domestinations. Divergence within the domesticated Mesoamerican gene pool cannot be interpreted in this case as an indication of multiple domestinations.

Alternatively, the close genetic relationship could be due to gene flow between domesticated and wild types. Although common bean is a predominantly self-pollinated species, high levels of outcrossing can occasionally be observed (Ibarra-Pérez et al., 1997). Overall, gene flow is asymmetric in common bean as it occurs predominantly from domesticated to wild types (Papa and Gepts, 2003). It affects the distribution of genetic diversity both among populations (Payró de la Cruz et al., 2005) and within the bean genome (Papa et al., 2005). However, these outcrossing events are rare, as suggested by very high levels of observed homozygosity (>97%; M. Kwak and P. Gepts, unpublished results). Furthermore, it would be very unlikely that separate outcrossing events in the putative domestication region, which stretches over a distance of some 300 km (Fig. 4A), would yield progeny with similar microsatellite profiles. The majority of microsatellites used in this study are unlinked; in the event of an outcross, one would therefore expect free recombination among these microsatellite loci, unless higher-order epistatic interactions among the genome regions marked by these loci would lead to similar microsatellite profiles. There is no evidence for such interactions to our knowledge. Thus, the close genetic relationship...
between wild populations from the Lerma–Santiago basin and Mesoamerican domesticates suggests a wild progenitor–domesticated descendant relationship.

The Lerma–Santiago river system drains part of central Mexico before flowing into the Pacific Ocean. The westernmost population close to the domesticated clade (G11056) is actually located in the Río Ameca basin, which also flows into the Pacific Ocean. The domestication area is located between the Transverse Neovolcanic Axis to the south and the southern edges of the Sierra Madre Occidental and Altiplano Mexicano to the north (Fig. 4A). Altitudes of the different wild populations that are part of the domestication group range from 1400 to 2100 m. The climate of this region is characterized as...
subtropical (temperature of the coldest month between 5 and 18°C), subhumid (between 4 and 6 mo of humidity), and semiwarm (average annual temperature between 18 and 22°C) (López Soto et al., 2005). The original vegetation changes gradually from a conifer–oak forest at the western end through a dry deciduous forest in the central part to a drier thorn forest at the eastern end of this area (Rzedowski, 1981).

The proposed domestication area for common bean does not overlap with the area proposed for maize domestication, based on genetic data (Matsuoka et al., 2002), nor does it overlap with any of the three major archaeological locations pertaining to the origin of agriculture in the Mesoamerican area: the Oaxaca Valley, the Tehuacán Caves in Puebla, and the Ocampo Caves in Tamaulipas (Fig. 4B). Buckler et al. (1998) already observed that these archaeological sites were situated in an arid ecological zone that was unimportant in the domestication of most Mesoamerican crops. Thus, the question whether the major food crops in the Mesoamerican center of agricultural origins resulted from domestication in a single or multiple locations in Mexico remains to be answered. The oldest archaeological records for common bean are from the southwestern United States (2470 and 2140 BP), the Tehuacán Caves (2285 BP), and the Oaxaca Valley (2098 BP) (Kaplan and Lynch, 1999) (all Accelerator Mass Spectrometry data, uncalibrated years; Fig. 4B). That these domesticated remains of approximately the same age are distributed over a distance of some 2300 km suggests that they reflect an established, agriculture-based economy. Older remnants, representing the transition from wild to domesticated beans, remain to be discovered. Further support for the utilization of beans at an earlier stage than suggested by archaeological dating is provided by linguistic analyses of ancient indigenous languages such as proto–Mayan, which suggest an origin of at least 3400 BP (Brown, 2006). Our data also suggest that a portion of the Lerma–Santiago basin should be the focus of archaeological efforts to identify new sites pertaining to the origin of agricultural origins in Mesoamerica. Furthermore, Jalisco has also been suggested as one of two possible domestication centers for tepary bean (P. acutifolius A. Gray) (Muñoz et al., 2006).

DNA marker analyses of cereals domestiated in Southwest Asia (the Fertile Crescent), combined with archaeological data, had identified a “core area” for agricultural origins within the Fertile Crescent (Abbo et al., 2006). It is from this core area that agriculture rippled out to other areas of the Fertile Crescent and, eventually, to other regions in Asia, Africa, and Europe. (Recently, this centric model has been questioned; Kilian et al., 2007). Our data, showing that the two main components of the milpa cropping system were domesticated in different areas of Mesoamerica, question whether a similar core area could be identified for this center of agricultural origins. The assembly of the traditional milpa cropping system, consisting of beans, maize, and squash, may have resulted from a more diffuse and extensive pre- or postdomestication cultivation and domestication process.

Finally, our data should provide additional impetuses for a refocused archaeological effort into the river basins of west-central Mexico. The moister areas may provide a more appropriate target to identify sites relevant to the origins of agriculture than the existing sites in arid regions. Recent progress in archaeobotanical approaches has demonstrated the possibility of identifying crop remains such as phytoliths or starch grains in moister environments (Holst et al., 2007; Perry et al., 2007). Our results should also encourage additional research on wild relatives and landraces of crops in west-central Mexico, including additional explorations and ecological studies on adaptation to climatic and edaphic factors and interactions with pathogens and pests.

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References


