The earliest archaeological maize (*Zea mays* L.) from highland Mexico: New accelerator mass spectrometry dates and their implications

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Accelerator mass spectrometry age determinations of maize cobs (*Zea mays* L.) from Guilá Naquitz Cave in Oaxaca, Mexico, produced dates of 5,400 carbon-14 years before the present (about 6,250 calendar years ago), making those cobs the oldest in the Americas. Macrofossils and phytoliths characteristic of wild and domesticated *Zea* fruits are absent from older strata from the site, although *Zea* pollen has previously been identified from those levels. These results, together with the modern geographical distribution of wild *Zea mays,* suggest that the cultural practices that led to *Zea* domestication probably occurred elsewhere in Mexico. Guilá Naquitz Cave has now yielded the earliest macrofossil evidence for the domestication of two major American crop plants, squash (*Cucurbita pepo*) and maize.

Maize (*Zea mays* L.) is the New World's preeminent grain crop, was widely grown at the time of the European contact in both hemispheres, and was a staple food of many prehistoric societies. Despite decades of research by botanists, molecular biologists, and archaeologists, the origin and early history of maize remain controversial (1–5). Many investigators are convinced by the considerable amount of molecular, cytological, and isozyme data accumulated on the ancestry of maize, which indicates that maize is probably descended from an annual species of teosinte (*Zea mays* ssp. *parviglumis*) native to the Balsas River Valley on the Pacific slopes of the states of Michoacán and Guerrero, Mexico, at elevations between 400 and 1,200 m (6–10) (Fig. 1). A competing model, however, attributes early maize to the region of Tehuacán in the state of Puebla, Mexico, at altitudes of 1,000 to 1,500 m (5).

Much of the Balsas region receives an annual precipitation of between 1,200 and 1,600 mm and has mean annual temperatures between 20°C and 28°C, so if that region is key, it would make the potential vegetation and ecological context of maize's origins a tropical broadleaf deciduous forest (11, 12). However, whereas Tehuacán has seen intensive archaeological research, the Balsas region has seen very little. It is still unknown when the first efforts to cultivate a wild *Zea* leading to the domestication of maize occurred. Various investigators have thus proposed both "early" (*ca.* 10,000–7,000 ^{14}C years B.P.) and "late" (*ca.* 6,000–5,000 ^{14}C years B.P.) scenarios for maize emergence (1–3).

Reanalysis and direct accelerator mass spectrometry dating of *Cucurbita pepo* squash from Guilá Naquitz Cave, a previously published site in the Mexican highlands (Fig. 1), has established dates of 8,990–6,980 ^{14}C years B.P. for the onset of plant domestication in Mexico (13). No maize cobs were found in deposits this ancient, however. The earliest maize cobs previously reported from Mexico are from San Marcos Cave in the Tehuacán Valley, Puebla, and were directly dated by accelerator mass spectrometry to 4,700 ^{14}C years B.P (about 5,500 calendar years ago) (14). Reanalyses of these cobs indicated that substantial effort had already been made by prehistoric cultivators to effect genetic changes that increased grain accessibility and productivity (15). Here we report the results of a reanalysis of
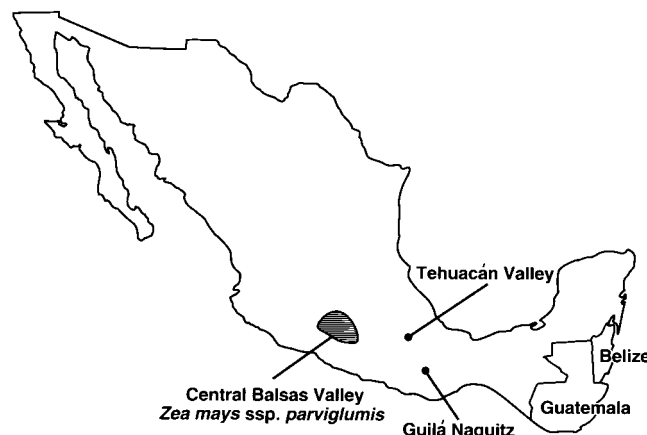

Fig. 1. Map of Mexico showing the location of Guilá Naquitz Cave and the Tehuacán Valley, together with the modern distribution of the populations of *Zea mays* ssp. *parviglumis* from the Central Balsas River Valley, the molecular profiles of which suggest that they are ancestral to maize.

maize cobs and sediments from Guilá Naquitz Cave by two techniques that were unavailable when the site was originally excavated—accelerator mass spectrometry dating and phytolith analysis.

Guilá Naquitz lies 5 km from the town of Mitla in the mountainous eastern Valley of Oaxaca, 1926 m above sea level in a semiarid thorn scrub forest (Fig. 1). In most years, annual precipitation does not exceed 600 mm. When excavated in 1966, the cave was found to have seven substantial "living floors" or human occupations (16). Zone A, the uppermost level, was dated to A.D. 620–740 and contained a diverse array of domesticated plants. Zones B1, B2, B3, C, D, and E were dated to the much earlier Naquitz phase (*ca.* 10,650–6,980 ^{14}C years B.P.). The Naquitz phase appears to have been a long period of preceramic hunting and gathering, and it spans the interval of incipient plant cultivation and domestication in Mexico. A seed of domesticated *Cucurbita pepo* squash recovered from zone B1 was directly dated to 8,990 B.P (about 9,975 calendar years ago) (13). Between *ca.* 8,990 and 7,000 ^{14}C years B.P., changes in fruit shape and color of the *C. pepo* remains indicated deliberate human selection for fruit characteristics (13).

In addition to the extensive living floors at Guilá Naquitz, shorter visits appear to have been made to the cave during the long hiatus between *ca.* 6,980 ^{14}C years B.P. and A.D. 620. These

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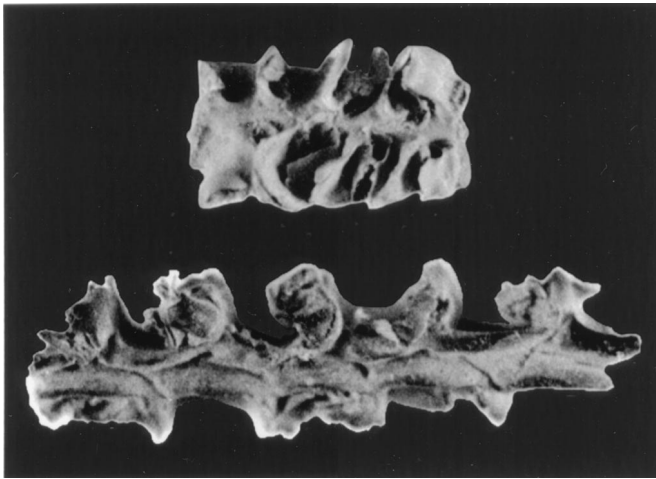
Fig. 2. The two oldest maize cobs in the New World from Guilá Naquitz Cave. The cob at the bottom is from excavation square D10 and is about 2.5 cm long. The cob at the top is from square C9.

Table 2. Phytolith percentages from Guilá Naquitz

Provenience zone (square)	Phytolith percentages			
	Zea mays cob-type	Other Poaceae	Cucurbitaceae	Other
Zone A (E5)	6	94	*	*
Zone A (C12)	16	63	17	4
Zone A (E8)	6	67	2	25
Zone B1 (E6)	0	81	11	8
Zone B1 (E9)	0	77	20	3
Zone B3 (D8)	0	75	25	*
Zone B3 (E7)	0	94	6	*
Zone C (E6)	0	83	6	13
Zone C (D8)	0	*	*	*

In the sample from zone C, square D8, phytoliths occurred infrequently and were not counted. No cob-type phytoliths were observed in this sample, and Cucurbitaceae phytoliths were the most common types noted.
*Observed on scans of the slide.

"ephemeral" occupations (17) were too brief to produce actual living floors, features, or well-defined activity areas, but the visitors left behind four small, primitive-looking maize cobs (18). They occurred in small lenses of ash that lay stratigraphically above zone B1, the youngest preceramic living floor, and below zone A. Two of these cobs, from squares C9 and D10, have been curated in the Laboratorio de Paleobotánica of Mexico's National Institute of Anthropology and History since 1984 (Fig. 2).

In July of 1999, we sampled these specimens for accelerator mass spectrometry dating (Table 1). Dates of $5,420 \pm 60$ and $5,410 \pm 40$ ^{14}C years B.P. (about 6,250 calendar years ago) were obtained on the cobs. These dates are approximately 700 years older than the most ancient specimens of maize reported from the Tehuacán Valley (14). The close agreement in the ages of the cobs and their presence in adjacent excavation squares (19) suggests that the cobs could be from a single harvest. When botanists George Beadle and Richard I. Ford inspected the cobs in the 1970s—a time when the antiquity of the specimens was still unknown—both agreed that the cobs either represented maize–teosinte hybrids or a primitive maize that demonstrated strong teosinte influence in its ancestry (18). In light of the appreciable age now documented for the specimens, together with the molecular data bearing on maize's ancestry, the latter interpretation appears to be more likely (32).

The dates on the cobs raise the question of *Zea* exploitation during earlier periods at Guilá Naquitz, when human occupations were of longer duration and *Cucurbita pepo* was domesticated and developed into a productive crop plant (13). Previous pollen work (20, 21) resulted in the identification of 10 *Zea* pollen grains in zones C-B1 (*ca.* 9,500–6,980 ^{14}C years B.P). These grains were too small to be classified as maize and were considered more likely to have originated from teosinte. Although teosinte is not found near the site today, it may once have had a more widespread distribution in the southern Mexican highlands (22). To provide more information on how, to what degree, and what kind of *Zea* was exploited at this early period,

nine sediment samples from zones C through A were analyzed for phytoliths. (Sediments were processed by standard techniques. D.R.P.'s modern reference collection of phytoliths comprises 2000 neotropical taxa and includes vegetative and reproductive structures from 25 races of maize, all known races of teosinte, all known species of *Tripsacum*, and more than 300 different species of wild neotropical grasses.) Our attention focused on the phytoliths produced in teosinte fruitcases and maize cupules and glumes. Phytolith analysts are well agreed that these structures form recognizable phytolith assemblages that can be distinguished from assemblages produced by the vegetative organs of *Zea* and other Poaceae (23–26). Unlike pollen grains, the presence of these phytoliths in archaeological sites can be used to infer harvesting and processing of *Zea* grains (23, 24).

Moreover, recent molecular and developmental studies have demonstrated that phytolith formation in *Zea* fruits is under the control of the important *teosinte glume architecture 1* (*tga1*) locus, which also controls the development of the cupulate fruitcase in teosinte and the degree of glume induration (lignification) in wild and domesticated *Zea* (27). Differential expression of *tga1* (presence of the teosinte or maize alleles) results in the production of different types of phytoliths in different loci of maize and teosinte fruits and largely accounts for the considerable morphological differences in their phytolith assemblages [e.g., having many elongated and irregularly shaped epidermal phytoliths in teosinte, or almost exclusively possessing spherical, short cell phytoliths (called "rondels" by phytolith analysts) in maize] (25, 27). The fact that these phytoliths are largely under genetic control means that any past environmental variability should not have influenced their production and visibility in phytolith assemblages.

Analysis of nine sediment samples from zones C through A, recovered during the excavation in 1966, showed that phytoliths from vegetative parts of grasses and other plants were abundant in samples from zones C through B1. However, no phytoliths characteristic of either teosinte fruitcases or maize cobs were present (Table 2). Types of phytoliths consistent with those found in modern maize cobs (23–25) (e.g., undecorated rondel

Table 1. Radiocarbon dates of the Guilá Naquitz maize cobs

Provenience	^{14}C years B.P.	Dendrocalibrated 2σ age ranges, years B.C.	Dendrocalibrated 2σ age ranges, years B.P.	Beta analytic lab number
Square D10	5410 ± 40	4340–4220	6290–6170	β132510
Square C9	5420 ± 60	4355–4065	6305–6015	β132511

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phytoliths with indented upper or lower faces), were, however, common in zone A, where numerous maize cobs were recovered. These results show that the absence of *Zea* macrofossils in levels dating between *ca.* 10,000 and 6,980 ^{14}C years B.P. is probably not due to inadequate preservation or sampling error. Although the consumption of immature *Zea* ear branches as a vegetable food supplement cannot be ruled out, the combined macro- and microfossil data indicate that neither wild nor domesticated *Zea* fruits were harvested for food and manipulated at Guilá Naquitz during the occupation of zones C through B1.

This finding, in addition to the unequivocally domesticated nature of the dated cobs, suggests that Guilá Naquitz cannot resolve the question of where the cultural practices began that resulted in maize domestication. The dates of *ca.* 4700 ^{14}C years B.P. currently available for the earliest maize cobs from the Tehuacán Valley, combined with an absence of macrofossils from teosinte there in levels dated 10,000 B.P. to 5,000 ^{14}C years B.P. (28), suggest that the Tehuácan area also has yet to produce the earliest stages in maize domestication. It is possible, given the ecological preferences of the populations of *Zea mays* ssp. *parviglumis* that have been genetically fingerprinted as the likely wild ancestors of maize, that early cultivated maize was adapted to longer, moister, and more predictable growing seasons than are typical of the arid Tehuacán Valley or the semiarid Oaxaca Valley today. Obviously, until intensive work is done in the region where *Zea mays* ssp. *parviglumis* is native, the question of where maize was first domesticated will remain unresolved. It should also be remembered that people who began maize cultivation were mobile hunters and gatherers, and the handful of caves and rock shelters so far excavated were not necessarily occupied at the key moments when domestication began.

The dates of the Guilá Naquitz cobs do indicate that the age of initial maize domestication falls before 5,400 ^{14}C years B.P. This is a conclusion supported by the presence of maize pollen in various archaeological and paleoecological sites from southern Central America and northern South America that date to between *ca.* 6,600 and 4,700 ^{14}C years B.P (2, 29–31).

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