Starch Fossils and the Domestication and Dispersal of Chili Peppers (*Capsicum* spp. L.) in the Americas

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Chili peppers (*Capsicum* spp.) are widely cultivated food plants that arose in the Americas and are now incorporated into cuisines worldwide. Here, we report a genus-specific starch morphotype that provides a means to identify chili peppers from archaeological contexts and trace both their domestication and dispersal. These starch microfossils have been found at seven sites dating from 6000 years before present to European contact and ranging from the Bahamas to southern Peru. The starch grain assemblages demonstrate that maize and chilies occurred together as an ancient and widespread Neotropical plant food complex that predates pottery in some regions.

Chili peppers, members of the genus *Capsicum*, have been cultivated extensively, initially in the Americas and, after Columbus, around the globe (*1*, *2*). The lack of a comprehensive archaeobotanical record has hampered accurate reconstructions of the origins, domestications, and dispersals of these plants. Macroremains of the fruits are confined to rare sites in arid environments, and reports of seeds and pollen are even less common (Table 1). We found that a widespread, but previously unidentified archaeological starch morphotype is derived from chili pepper fruits and is commonly preserved on artifacts. We documented this microfossil from seven archaeological sites ranging from the Bahamas archipelago to Andean South America (Fig. 1) beginning 6000 years ago (Table 2).

The five most economically notable species of chili pepper are *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*. Although it is generally agreed that the genus *Capsicum* originated in Bolivia (*2*), the centers of domestication and dispersal patterns of these

species remain speculative. A combination of archaeological evidence, genetic analyses, and modern plant distributions have led researchers to suggest that *C. annuum* was initially domesticated in Mexico or northern Central America, *C. frutescens* in the Caribbean, *C. baccatum* in lowland Bolivia, *C. chinense* in northern lowland Amazonia, and *C. pubescens* in the mid-elevation southern Andes (*2*, *3*).

All five species of domesticated chili peppers produce large, flattened lenticular starch grains with a shallow central depression, not unlike a red blood cell in appearance (Fig. 2, A to C). When rotated into side view, a central linear figure—a clean line or split figure with sharp edges—runs parallel to the long axis of the grain. This figure can extend for the entire length of the grain or just a part of it (Fig. 2, E and F). Ranging from about 13 to 45 μm in length, the starches of domesticated peppers are easily distinguishable from smaller wild types in the microfossil record (Fig. 2D and table S1). Although the basic three-dimensional morphology is consistent among all species of *Capsicum*, micromorphological characters differ between species.

Three of the species—*C. baccatum*, *C. frutescens*, and *C. pubescens*—can be identified on the basis of diagnostic morphotypes that have unique features of the central depression. However, these features are rare even in modern starch grain assemblages. Otherwise, the morphologies

of starch grain assemblages from *C. annuum* and *C. frutescens* are so similar that, in the absence of a diagnostic, it is not possible to assign grains to a single species. The morphology of starch from *C. chinense* is similar to but not identical to that of *C. annuum* or *C. frutescens*, and the morphologies of all three starches differ significantly from those of *C. baccatum* and *C. pubescens* which, in turn, differ from one another. Because similar types occur in all congeneric species of *Capsicum*, either a diagnostic or a large archaeobotanical assemblage is required for a secure species identification.

The presence of a basic genus-diagnostic starch morphotype for *Capsicum* is predictable because of the lack of perfect barriers to intraspecific hybridization (*4*). *C. annuum*, *C. chinense*, and *C. frutescens* have been described as a species complex with a single ancestral gene pool (*4*). Therefore, it is not surprising that the starches of these three species are morphologically similar to one another. In contrast, *C. baccatum* and *C. pubescens* are distinct domesticated species in South America (*4*). Starches derived from other economically significant species in the Solanaceae including *Lycianthes*, the genus that recent phylogenetic studies indicate is the most closely related to *Capsicum* (*5*), differ from those of chili peppers (table S1 and fig. S1) (*6*). Thus, we have eliminated those plant species with the potential to confuse the source of the microfossils.

We recovered securely identified genus-diagnostic *Capsicum* starch microfossils from seven sites throughout the Americas. The oldest positively identified starches were found at the contemporaneous sites of Loma Alta and Real Alto in southwestern Ecuador. Interpreted as a village-sized, permanent settlement, Loma Alta was occupied for more than a millennium beginning about 6100 years before present (yr B.P.) (*7*). We recovered chili pepper starches from sediment samples, milling stones, and food residues from ceramic sherds of cooking vessels, all of which were excavated from the lower levels of the site.

Similar to Loma Alta, Real Alto was a village site at about 6100 yr B.P.; however, by about 4750 yr B.P., it had expanded into a regional ritual-ceremonial center (*8*, *9*). The chili starches were extracted from milling stones from two house floors dating to the period of expansion. Microfossil evidence of maize, *Canna edulis* (achira), *Maranta arundinacea* (arrowroot), *Calathea* sp.

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Table 1. Published reports of archaeological *Capsicum* from well-dated sites with clearly defined stratigraphy.

Species	Plant part	Region	Site(s)	Date(s) (yr B.P.)	Source
C. annuum	Fruits	Mexico	Tehuacan Valley	500–6000	(*24*)
C. annuum	Seeds and peduncles	El Salvador	Ceren	1400	(*25*)
C. baccatum	Fruits	Peru	Huaca Prieta, Punta Grande	4000	(*26*)
C. chinense	Fruits	Peru	Huaca Prieta, Punta Grande	4000	(*26*)
C. chinense	Fruits	Peru	Casma Valley	Ca. 2500–3500	(*3*)
Capsicum sp.	Seeds	Haiti	En Bas Saline	600	(*27*)
Capsicum sp.	Pollen	Venezuela	La Tigra	450–1000	(*28*)

(leren), manioc, cucurbits (squash), *Canavalia* sp. (jack bean), and the Arecaceae family (palms) has also been recovered from Real Alto (*10–12*). A combination of evidence, including plant remains and site proximity to seasonally flooded bottomland, indicates that agriculture was important in the economies of both Ecuadorian sites. Ecuador is not considered to be the center of domestication for any of the five major economic species of chili peppers. Therefore, the presence of domesticated chilies within this early, complex, agricultural system indicates that these plants must have been domesticated elsewhere earlier than 6000 yr B.P. and brought into the region from either the north or the south.

In central Panama, the Aguadulce Rock Shelter was occupied from about 13,000 to 3200 yr B.P. during both the Preceramic and Early Ceramic periods (*13*). The site has yielded evidence for the cultivation of other plants not native to southern Central America, including maize, manioc, and squashes dating from about 9000 to 5800 yr B.P. We identified chili pepper starch on a groundstone tool recovered from the top of the preceramic deposits; the tool and thus its associated starch residues have a stratigraphic date of about 5600 yr B.P. This artifact also yielded starch grains from maize and domesticated yam (*13*).

The occupation of the coastal shell-midden site of Zapotal coincides with the Early Ceramic period of this region of Panama, beginning about 4800 yr B.P. (*14*). We recovered starches of chilies from groundstone tools, indicating that the peppers were processed and consumed alongside a number of other domesticates at the site, including maize, manioc, and yams (*15*). By this time, swidden cultivation of several domesticated species, including maize and manioc, was well established in the region, and farmers had significantly deforested the foothills near both Panamanian sites (*16*). Thus, the Panamanian record documents the use of domesticated chilies as components of the diet of swidden agriculturists in both Preceramic and Ceramic era groups.

Farther south at 3600 m in the Peruvian Andes lies the site of Waynuna, a Late Preceramic house occupied beginning about 4000 yr B.P. At Waynuna, we found chili starches on processing tools in association with maize, arrowroot, and the remains of what is likely *Solanum* sp. (potato) (*17*). These data indicate that the residents of Waynuna were cultivating maize, tubers, and peppers and were processing them into food on site. Waynuna yielded the only starch assemblage that contained a species-diagnostic morphotype. These chili pepper starches appeared to be derived from *C. pubescens*, the species that includes varieties such as the rocoto pepper, a chili that is cultivated at mid-altitude in the Andes (*2*). When combined with macrofossil evidence (Table 1), the starch data indicate that the cultivation of three domesticated species of chili pepper was contemporaneous on the coast and in the highlands of Peru as early as 4000 yr B.P. in the Late Preceramic period. The presence of numerous other cultivars within the assemblages of each region indicates that sophisticated agriculture was practiced in both regions before the introduction of pottery.

We also found starches of chili peppers at the Three Dog site located on San Salvador Island in the Bahamas. This site was occupied by a group of fisher-horticulturists about 1000 yr B.P. Representing the material remains of at least one household, the site consists of a midden, two activity areas, and a low-density (well-swept) area. Fifty-eight chert microliths, all typical of the morphology commonly described as manioc grater flakes (*18–20*), have been recovered, as were ceramic griddle sherds. The microliths yielded the starchy remains of both maize and unidentified roots or tubers. We recovered chili starches from two flakes that also contained starches of maize.

Lastly, we recovered microfossil evidence for chili pepper at Los Mangos del Parguaza in Venezuela, a large habitation site occupied about

Table 2. Summary of Neotropical archaeological starches of *Capsicum*. F, flaked tool; G, groundstone tool; C, ceramic sherd; S, sediment sample.

Sample	#	Size (µm)	Source	Date in yr B.P. (Ref.)
Los Mangos, Venezuela (Arauquinoid, Valloid)				
Lev 1, 1	1	15	G	~500–1000 (*21*)
Lev 2, 1	1	17	F	~500–1000 (*21*)
Lev 3, 1	1	15	F	~500–1000 (*21*)
Lev 3, 2	1	22	F	~500–1000 (*21*)
Lev 5, 2	2	19, 20	F	~500–1000 (*21*)
Lev 7, 1	2	20, 20	F	~500–1000 (*21*)
Three Dog, Bahamas (Lucayan)				
Z87-89	1	19	F	969–1265 Cal* (*29*)
Z1032-1035	1	21	F	969–1265 Cal* (*29*)
Waynuna, Peru (Preceramic)				
Tool 10	2	18,24	F	3564–3837 Cal† (*17*)
Tool 11	6	14–34	G	3564–3837 Cal† (*17*)
Tool 29	2	24,25	G	3564–3837 Cal† (*17*)
Tool 30	6	19–28	G	3564–3837 Cal† (*17*)
Cat 36	1	18	S	3689–3969 Cal† (*17*)
Zapotal, Panama (Early Ceramic)				
C2N8F4	4	20-28	G	3560–4850 Cal‡ (*14*)
C7N2	1	25	G	3560–4850 Cal‡ (*14*)
C32N7	1	32.5	G	3560–4850 Cal‡ (*14*)
Real Alto, Ecuador (Valdivia 3)				
Structure 1	1	20	G	4400–4800 Cal† (*9*)
Structure 1	3	24–26	G	4400–4800 Cal† (*9*)
Structure 10	3	18–24	G	4400–4800 Cal† (*9*)
Structure 10	1	24	G	4400–4800 Cal† (*9*)
Aguadulce, Panama (Late Preceramic)				
350	3	24–28	G	5600 Cal (*30*)
Loma Alta, Ecuador (Early Formative)				
SS275	5	22–26	G	5050–6250 Cal† (*9*)
SS275-2	6	16–24	G	5050–6250 Cal† (*9*)
SS292	2	19, 20	G	4550–6050 Cal† (*9*)
Sample 13	2	24, 28	C	4830–5280 Cal† (*9*)
Sample 7	1	27	C	4080–4410 Cal† (*9*)
Level 12	2	24, 28	S	4990–5310 Cal† (*9*)
Sample 11	1	18	C	4250–4860 Cal† (*9*)
Sample 10	1	22	C	4990–5310 Cal† (*9*)
Level 14	2	24, 24	S	4990–5310 Cal† (*9*)
Sample 9	1	28	C	4990–5310 Cal† (*9*)

*Standard and AMS radiocarbon dates from associated charcoal, 2σ calibrated result. †Standard radiocarbon date from associated charcoal, 2σ calibrated result. ‡Standard radiocarbon dates from associated shell, adjusted for 12C/13C ratio, 2σ calibrated results.

Fig. 1. Archaeological sites mentioned in the text. Red sites yielded starch grains of chili pepper. Blue sites yielded all other classes of remains of chili pepper.

Fig. 2. Modern and archaeological starch granules from *Capsicum*. (**A**) Starch granule from the fruit of modern *Capsicum baccatum* var. *pendulum* (aji mirasol) showing typical morphology. Note the rounded lenticular form and large, flat, central depression. (**B**) Archaeological *Capsicum* starch granule from Loma Alta. (**C**) Archaeological starch granule of *Capsicum* from Real Alto. (**D**) Starch granule from a modern specimen of *Cap-*

sicum annuum var. *minimum*. This starch granule is typical of those from wild peppers. (**E**) Side view of a modern starch granule from *Capsicum baccatum*. Note the linear figure. (**F**) Side view of an archaeological starch granule of *Capsicum* from Zapotal.
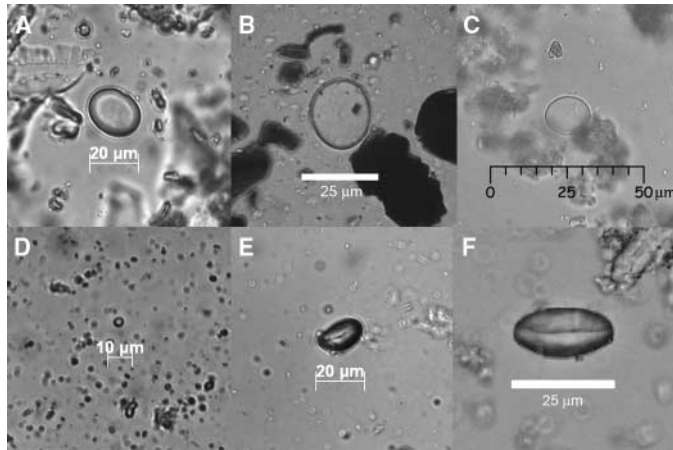
500 to 1000 yr B.P. (*21*). Several large, deep stone metates were scattered over the surface of the site. Excavation of a midden deposit yielded ceramic griddle sherds and microlithic flakes that are often associated with manioc processing (*22*). As at the Three Dog site, the remains of manioc are conspicuously absent from an excavation that yielded artifacts usually associated with manioc processing (*22*). The same processing tools that contained starches of chili pepper also contained remains of maize. Root crops, including arrowroot, *Myrosma* sp. (guapo), and a member of the Zingiberaceae family (ginger) also left their starchy remains. When combined with the data from the Three Dog site, the chili pepper microfossils from Los Mangos del Parguaza support the notion that a sophisticated mixed subsistence economy of both root and seed crops occurred at these sites that were initially categorized as being occupied by manioc horticulturists (*23*).

Neither microfossils typical of wild species nor transitional forms of *Capsicum* were recovered from any site. The presence of domesticated plants used as condiments rather than as staple foods during the Preceramic period indicates that sophisticated agriculture and complex cuisines arose early throughout the Americas and that the exploitation of maize, root crops, and chili peppers spread before the introduction of pottery. Evidence from both macrobotanical and microbotanical remains indicates that once chili peppers became incorporated into the diet, they persisted. Apart from the chili peppers, maize is present at every site we sampled. Maize and chilies occur together from the onset of this record until European contact and, thus, represent an ancient Neotropical plant food complex.

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Multipotent *Drosophila* Intestinal Stem Cells Specify Daughter Cell Fates by Differential Notch Signaling

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The adult *Drosophila* midgut contains multipotent intestinal stem cells (ISCs) scattered along its basement membrane that have been shown by lineage analysis to generate both enterocytes and enteroendocrine cells. ISCs containing high levels of cytoplasmic Delta-rich vesicles activate the canonical Notch pathway and down-regulate Delta within their daughters, a process that programs these daughters to become enterocytes. ISCs that express little vesiculate Delta, or are genetically impaired in Notch signaling, specify their daughters to become enteroendocrine cells. Thus, ISCs control daughter cell fate by modulating Notch signaling over time. Our studies suggest that ISCs actively coordinate cell production with local tissue requirements by this mechanism.

Stem cells in adult tissues frequently reside in specific anatomical positions known as niches, whose microenvironment represses premature differentiation and controls prolifera-

tion (*1*, *2*). Several different signal transduction pathways—including BMP (bone morphogenetic protein), JAK/STAT (Janus kinase/signal transducer and activator of transcription), Wnt, and