# An Asian origin for a 10,000-year-old domesticated plant in the Americas

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**New genetic and archaeological approaches have substantially improved our understanding of the transition to agriculture, a major turning point in human history that began 10,000–5,000 years ago with the independent domestication of plants and animals in eight world regions. In the Americas, however, understanding the initial domestication of New World species has long been complicated by the early presence of an African enigma, the bottle gourd (*Lagenaria siceraria*). Indigenous to Africa, it reached East Asia by 9,000–8,000 before present (B.P.) and had a broad New World distribution by 8,000 B.P. Here we integrate genetic and archaeological approaches to address a set of long-standing core questions regarding the introduction of the bottle gourd into the Americas. Did it reach the New World directly from Africa or through Asia? Was it transported by humans or ocean currents? Was it wild or domesticated upon arrival? Fruit rind thickness values and accelerator mass spectrometer radiocarbon dating of archaeological specimens indicate that the bottle gourd was present in the Americas as a domesticated plant by 10,000 B.P., placing it among the earliest domesticates in the New World. Ancient DNA sequence analysis of archaeological bottle gourd specimens and comparison with modern Asian and African landraces identify Asia as the source of its introduction. We suggest that the bottle gourd and the dog, two ''utility'' species, were domesticated long before any food crops or livestock species, and that both were brought to the Americas by Paleoindian populations as they colonized the New World.**

ancient DNA | archaeology | bottle gourd | domestication

Innovative approaches in genetics and archaeology continue to provide substantial new information regarding the origins of agriculture and the independent domestication of different species of plants and animals between 10,000 and 5,000 years ago in at least eight separate regions of the world (1–4). Not surprisingly, as they come into clearer focus, the developmental histories of each of these independent centers of domestication are turning out to be far more complex and nuanced than previously thought. Here we consider one of these imbedded complexities, the consistent occurrence of the bottle gourd (*Lagenaria siceraria*), an Old World plant, in close association with the earliest indigenous New World domesticates. In the process of answering basic questions regarding the early presence of this African plant in the Americas, we reach a number of unexpected conclusions regarding the cultural, environmental, and temporal contexts of initial human domestication of plants and animals.

The bottle gourd has been grown worldwide for thousands of years, usually not as a food source, but for the value of its strong, hard-shelled, and buoyant fruits, which have long been prized as containers, musical instruments, and fishing floats (5). This lightweight ''container crop'' would have been of particular importance to human societies before the advent of pottery and settled village life. Along with the five wild perennial species that also belong to the genus *Lagenaria*, the bottle gourd has long been recognized as being indigenous to Africa (5, 6). Until the recent discovery and morphological and genetic characterization of a wild population of *L. siceraria* in Zimbabwe (7), however, the bottle gourd had only ever been adequately documented as a domesticated plant.

Morphological and genetic differences between present-day African and Asian bottle gourd cultivars are substantial enough to sustain the designation of two subspecies, *Lagenaria siceraria* ssp. *siceraria* and *L. siceraria* ssp. *asiatica*, suggesting an ancient eastward diffusion of the species out of Africa and the subsequent genetic isolation of African and Asian subgroups (5, 6, 8–10). The modes of diffusion of the bottle gourd out of Africa remain unknown, but the buoyant fruits of domesticated plants have been shown to yield still-viable seeds after floating in sea water for more than 7 months (11), and early diffusion of the species by ocean currents has frequently been suggested. Bottle gourd has been documented in archaeological contexts in China and Japan dating to 8,000–9,000 before present (B.P.) (12), providing a conservative time-certain framework for the pantropical spread of the species across Asia, while leaving unresolved its actual antiquity, routes, and mechanisms of dispersal.

Charles Pickering's discovery of bottle gourds in Peru in the 1840s (13, 14) extended these questions of the timing, routes, and mechanisms of the plant's global diffusion to include the Americas, and a complete spectrum alternative explanations, Asia vs. Africa, wild vs. domesticated, and ocean current vs. human-mediated have subsequently been proposed (15): i.e., the bottle gourd was carried from Africa to the New World by ocean currents (6, 14–16), by a boatload of African fishermen (17), by ocean currents from Asia (16), or perhaps by boat (14, 16, 18). Because of observed morphological similarities between present-day bottle gourds grown in Africa and the Americas, a substantial majority of researchers writing on the topic over the past 150 years have formed a consensus that *L. siceraria* reached the Americas by ocean currents directly from Africa.

Because of its Old World origins and equivocal status as a domesticate, *L. siceraria* has long been a difficult and unresolved aspect of initial plant domestication and the origins of agriculture in the Americas. Here we address the complex and long-standing set of questions that surround the initial arrival and early history of this plant in the New World via an interdisciplinary approach involving direct accelerator mass spectrometer (AMS) radiocarbon dating, morphological analysis, and ancient DNA (aDNA) sequencing of bottle gourd rind fragments recovered from early archaeological contexts in South America, Mexico, and the eastern United States. This combined analysis provides independent, complementary lines of evidence that

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Abbreviations: aDNA, ancient DNA; AMS, accelerator mass spectrometer; B.P., before present; InDel, insertion deletion; SNP, single nucleotide polymorphism.

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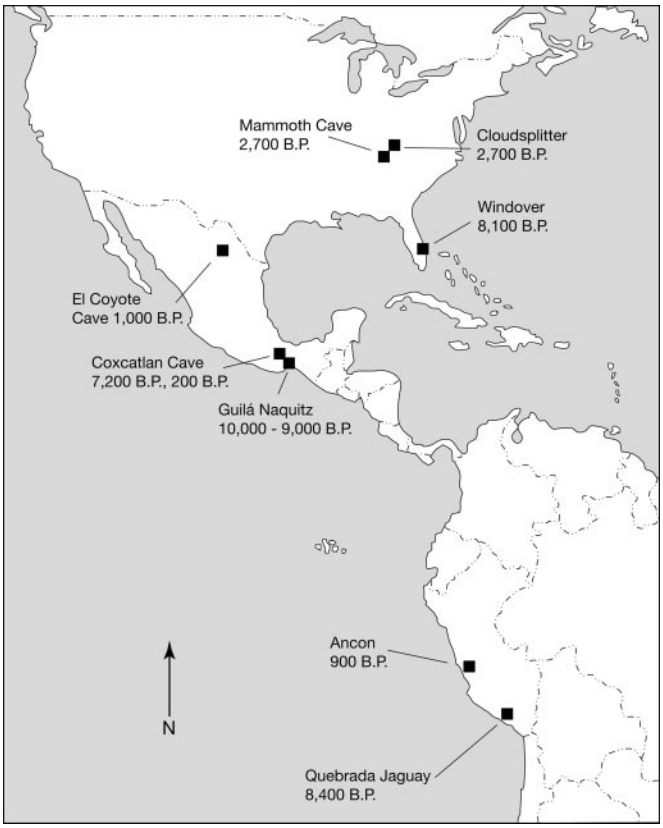


**Fig. 1.** The archaeological sites that yielded bottle gourd rind samples included in the present study, and direct AMS radiocarbon age determinations (calibrated calendar years) obtained on the samples.

together offer new and unexpected insights regarding the cultural, environmental, and temporal contexts of the initial human domestication of this species.

## The Temporal Context of Arrival and Early Dispersal *L. siceraria* in the Americas

To establish a more accurate time frame for the initial arrival and subsequent early dispersal of the bottle gourd in the New World, we obtained direct AMS radiocarbon dates on bottle gourd rind fragments recovered from archaeological sites in Peru, Mexico, and eastern North America (Fig. 1 and Table 1). All of the specimens included in the study exhibit the distinctive cross-section cellular structure and phytolith morphology diagnostic for bottle gourd (19, 20). Detailed documentation and provenience information for archaeological rind fragments and the criteria used for their taxonomic identification is provided in *Supporting Text*, which is published as supporting information on the PNAS web site.

Bottle gourd rind fragments and seeds are rarely recovered from pre-4,000 B.P. archaeological contexts in the Americas; the samples included in the present study from Windover, Florida (21), Guilá Naquitz Cave, Mexico (19), Quebrada Jaguay, Peru (22), and Coxcatlan Cave, Mexico (23) represent the six oldest specimens recovered to date from the Western Hemisphere and confirm early Holocene temporal placement of this species in the New World. Interestingly, in both eastern North America and Mexico, this Old World plant was recovered in close association with the earliest occurrence of the first New World domesticate documented to date for those regions, *Cucurbita pepo* squash. In Mexico, at Guilá Naquitz, the bottle gourd appears in the same occupational deposits and essentially contemporaneously with the earliest documented domesticated food plant (*C. pepo*) (19). In eastern North America, bottle gourd rind fragments were similarly recovered in close archaeological and temporal association with the earliest occurrence of a local domesticate (*C. pepo*) (24). Bottle gourd has also been recovered, however, from 8,000 B.P. contexts at the Windover site in Florida, a full 3,000 years earlier than the first evidence of a locally domesticated food crop (21). In South America, in contrast, the bottle gourd rind fragments from Quebrada Jaguay are not as early as currently available evidence for domesticated species of *Cucurbita* squash (25). Although patterns and rates of subsequent dispersal remain to be mapped in detail, the early occurrence of bottle gourd in the central highlands of Mexico, the Pacific coast of South America, and the Atlantic coast of Florida indicates widespread dispersal of the plant across 40° of latitude by 8,000 B.P.

## Morphological Evidence for Domestication in the Bottle Gourd

When humans begin to harvest, store, and plant seeds over a sustained period, they unconsciously, inadvertently, create a new and distinctive selective environment to which the target plant populations under their management adapt through genetic and morphological change. The morphological changes associated with this adaptive syndrome of domestication are well understood and well described, and they provide a standard set of criteria for distinguishing wild from domesticated seed plants in the archaeological record (26). Morphological changes that distinguish domesticated plants from their wild relatives can also, of course, be the result of deliberate and sustained human selection for desired traits.

For these morphological markers, both deliberate and automatic, to be used in the identification of domesticates in archaeological plant assemblages, a standard baseline of comparison is needed, and it usually involves modern specimens of the wild ancestor taxon that gave rise to the domesticated plant in question. Until very recently, any effort to establish whether a bottle gourd rind fragment recovered from an archaeological context represented a wild or a domesticated plant was seriously hampered by the absence of any well documented modern wild bottle gourd populations that could be used as a basis of comparison (5, 14, 23). The recent discovery and description of a wild population of *L. siceraria* in southern Africa (7), however, resolve this long-standing problem, and for the first time provides a comparative morphological baseline for distinguishing wild vs. domesticated bottle gourd in archaeological contexts.

When compared with the modern wild *L. siceraria* gourds recently documented in Zimbabwe, all of the archaeological specimens included in the present study exhibit a distinctive morphological difference, a substantial increase in fruit rind thickness and associated fruit durability (Table 1). Such a marked increase in rind thickness reflects a loss of natural seed dispersal mechanisms, which is one of the most important adaptive responses by target populations of seed plants to a new human-created environment and is also one of the markers of domestication most likely to be visible in the archaeological record. Loss of natural seed dispersal mechanisms is also considered perhaps the strongest single criterion for recognizing domestication in seed plants, because it reflects an inability on the part of the domesticated plant to successfully reproduce without the active and sustained intervention of humans in the planting of stored seed stock.

Although no detailed rind thickness measurements of the modern wild *L. siceraria* population are yet available, a clear description of a brittle and rapidly disintegrating exocarp is provided for the Zimbabwe population: "Once dry, the rind was not durable as is typical of *L. siceraria*. Instead, the exocarp became very thin, was easily cracked, and ultimately disintegrated after several years" (7). Rind thickness values from other

**Table 1. Age, rind thickness, and genotype assignment of ancient bottle gourd samples**

| Archaeological site and excavation provenience or museum catalog nos. | Age in AMS calibrated calendar years B.P. | | Laboratory sample no. | Rind thickness, mm | Marker genotype | | |
|---|---|---|---|---|---|---|---|
| | Intercept | 1$\sigma$ age range | | | LS_InDel1 | LS_SNP | LS_InDel2 |
| Coxcatlan Cave, Mexico | 210 ± 40 | 290–0 | OS36646 | 3.8 | African | African | African |
| Square 9, Level 1, Zone 2 | 220 ± 20 | 300–0 | OS36791 | | | | |
| Ancon, Peru | 790 ± 40 | 910–760 | B203521 | 4.8 | Asian | Asian | Asian |
| NMNH A132611 | | | | | | | |
| Ancon, Peru | 900 ± 40 | 850–810 | B203522 | 4.3 | Asian | Asian | Asian |
| NMNH A132616 | | | | | | | |
| El Coyote Cave, Mexico | 1,000 ± 30 | 960–910 | OS36797 | 4.9 | Asian | Asian | NA |
| NMNH 45581 | 1,090 ± 35 | 1,060–940 | OS36789 | | | | |
| Cloudsplitter Cave, Kentucky | 2,735 ± 35 | 2,630–2,500 | OS36796 | 4.2 | Asian | NA | Asian |
| UMMA EL 7853 | | | | | | | |
| Mammoth Cave, Kentucky | 2,750 ± 40 | 2,770–2,720 | OS36649 | | | | |
| NMNH A87276, Fruit 5 | 2,760 ± 30 | 2,780–2,740 | OS36795 | 7.0 | Asian | Asian | Asian |
| Coxcatlan Cave, Mexico | 7,200 ± 50 | 7,220–7,175 | B123043 | | | | |
| Square 72, Level 12, Zone 13 | 7,230 ± 50 | 7,280–7,020 | OS36647 | 3.2 | Asian | Asian | Asian |
| Windover, Florida | 8,105 ± 120 | 8,265–7,945 | B20450 | 3.0 | No PCR amplification | | |
| B150, N148E69, Level 25 | | | | | | | |
| Quebrada Jaguay, Peru | 8,410 ± 50 | 8,440–8,390 | B134112 | 3.4 | Asian | Asian | Asian |
| S1, U4, Pozo A, Nivel 1f | | | | | | | |
| Quebrada Jaguay, Peru | 8,415 ± 50 | 8,445–8,395 | B134111 | 3.6 | Asian | Asian | Asian |
| S1, U3, Pozo B, Nivel 1d | | | | | | | |
| Guilá Naquitz, Mexico | 8,685 ± 60 | 8,970–8,545 | B97237 | 4.6 | Asian | Asian | Asian |
| Square E10, Zone B2 | 9,030 ± 35 | 9,130–9,005 | OS36794 | | | | |
| Guilá Naquitz, Mexico | 9,920 ± 50 | 9,980–9,865 | B100762 | 2.2 | No PCR amplification | | |
| Square F9, Zone C | | | | | | | |

Multiple dates, when listed, are both from the rind sample analyzed for aDNA. Windover AMS date and average rind thickness value are from ref. 21. NMNH, National Museum of Natural History (Smithsonian Institution); UMMA, University of Michigan Museum of Anthropology; NA, not available. Calibration of dates was provided by Beta Analytic (Miami).

related wild species of cucurbit gourds can also be used to provide a more general reference class for comparison. Three fruits of a related wild perennial species of *L. abyssinica* (National Herbarium catalog no. 7978), for example, yielded exocarp thickness values of 1.3, 1.4, and 1.4 mm. Similarly, maximum rind thickness measurements for more than a dozen modern wild *Cucurbita* gourds are all <2.0 mm (19, 23), providing a solid basis for the general characterization of a thin exocarp morphotype for wild cucurbit gourds, including wild *L. siceraria*.

In comparison, all but one of the archaeological rind fragments are 3.0–7.0 mm in thickness (the specimen with a rind thickness of 2.2 mm is missing part of the inner rind), which is substantially thicker and more durable than modern wild cucurbit gourds and comparable in thickness to modern domesticated *L. siceraria*, providing strong evidence that the archaeological rind fragments represent domesticated plants. It is also possible, and perhaps even likely, that in addition to automatic inadvertent selection for thicker exocarp fruits, humans deliberately selected for this highly desirable trait. Bottle gourds with a thick and durable exocarp represented a uniquely valuable source of raw material for a wide variety of strong and lightweight containers, which would have been highly prized, particularly before the development of ceramic containers.

## Establishing the Source of Introduction of *L. siceraria* into the New World

**Identifying Chloroplast DNA Markers.** To identify DNA sequence polymorphisms that would enable us to accurately and consistently distinguish between African and Asian landraces of bottle gourd, it was important to address the problem presented by the increasing availability of bottle gourds and seed stock worldwide, and the associated possibility of African genotype gourds currently being grown in Asia, and Asian ones in Africa. We addressed this problem in the construction of our modern reference collections by acquiring a majority of our plant material either directly from rural farming contexts or from well provenienced ethnographic specimens (Table 3, which is published as supporting information on the PNAS web site).

Screening of modern reference classes focused on markers that exhibited fixed differences between the landraces. In doing so, we sought to identify polymorphisms that represent the broadest geographic patterns of migration, as opposed to local, random differences among populations. Overall levels of genetic variation were extraordinarily low, and very few fixed differences between the landraces were identified. The entire internal transcribed spacer regions 1 and 2 from ribosomal DNA as well as >10 kb of mitochondrial sequence revealed no sequence differences between the landraces. We did identify one nuclear microsatellite marker that exhibits fixed differences between the modern African and Asian landraces; however, it could not be amplified from the ancient bottle gourd material.

Sequencing within the chloroplast genome was more successful, and we observed three diagnostic polymorphisms (Table 2). Two of the polymorphisms [a 5-bp insertion deletion (InDel) and a G/A transition single nucleotide polymorphism (SNP)] are located in the *trn*C–*trn*D intergenic regions and were discovered by using published PCR primers (27). The third polymorphism (a 5-bp InDel) is located in the *trn*S–*trn*G intergenic region and was discovered by using novel combinations of published primers (28). The markers were mapped onto the cucumber (*Cucumis sativus*) chloroplast genome (Fig. 7, which is published as supporting information on the PNAS web site). The InDels serve as particularly robust indicators of landrace origin and are immune to many of the postdepositional mutations that plague interpretations of ancient material based on SNP variation (29, 30).

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**Table 2. Details of the three PCR-based markers used to distinguish Asian from African landraces of bottle gourds**

| Primer name | Primer position in *Cucumis sativus* (5′ → 3′) | Primer sequence (5′ → 3′) | Annealing temperature, °C | Product length, bp | Marker type |
|---|---|---|---|---|---|
| LS_InDel1L | 30,449–30,468 | TGCTCAATCAATTACTTCTT | 50 | 97 | InDel: GAAAT |
| LS_InDel1R | 30,545–30,526 | GCTTCATAATTCATGTTGAT | | | |
| LS_SNPL | 31,389–31,408 | AACTCAAGCAAAGAATAGCA | 50 | 95 | SNP: G ↔ A transition |
| LS_SNPR | 31,483–31,460 | AAGAAGATTTGATAAGTACAAAAA | | | |
| LS_InDel2L | 9,071–9,046 | TGGTATTATTTATATATTAGGATTGG | 47 | 125 | InDel: AATCA |
| LS_InDel2R | 8,943–8,967 | GATGGATATCTATAAAATCGATAAA | | | |

Primer positions were determined from the cucumber (*Cucumis sativus*) complete chloroplast genome (GenBank accession no. NC_007144).

**Ancient DNA Extraction.** DNA extractions were conducted on the ancient bottle gourd fruit rind (exocarp) fragments (Table 1), by following procedures outlined for specimens considered as representing a "medium risk" for contamination (domesticated animals and plants) (31). Extraction of DNA from archaeological samples was conducted by using a modification of the procedure of Goloubinoff, Pääbo, and Wilson (32). All DNA extractions were performed in a Smithsonian Institution laboratory that is physically separated from the Laboratories of Analytical Biology (LAB), where PCR, cloning and sequencing were conducted. The extraction laboratory was a nonmolecular laboratory where no PCR of any kind is conducted. The ancient bottle gourd samples were the only plant material stored in the laboratory, and each sample was individually sealed in a separate plastic bag. PCRs were set up in the extraction lab, and tubes were not opened in the PCR laboratory until thermocycling was complete. Further details of the precautions taken with the aDNA samples are provided in *Supporting Text*.

Tissue samples were initially prepared for extraction by shaving off the surface of the gourd rind fragment to remove fungal and microbiological contaminants. Then ≈0.2 g of tissue was removed from the fresh surface and cut into small pieces with a sterile single-use razor blade. Each tissue sample was then placed in a sterile 2-ml plastic O-ring tube with a single 6-mm ceramic bead combined with 0.01 g of 0.5-mm zircon beads, and pulverized in a bead-mill (Bio 101 FastPrep FP120) until reduced to a fine powder. The pulverized tissue was mixed with 1.2 ml of extraction buffer (1% SDS/10 mM Tris, pH 8.0/5 mM NaCl/50 mM DTT/20 mg/ml proteinase K/10 mM EDTA/2.5 mM *N*-phenacylthiazolium bromide). The extraction was incubated for 18–24 h at 37°C while being gently mixed on a flat-bed shaker. Only one sample and one parallel blank (containing no plant tissue) were extracted at any one time. After incubation, the sample was centrifuged at 9,000 × *g* for 5 min to pellet undigested cellular debris. DNA was isolated from the supernatant, which had been transferred to a fresh tube, by using the Qiagen Plant Mini Kit (catalogue no. 69181). DNA was eluted in 50 μl of 10 mM Tris (pH 8.0). Use of the kit substantially reduced coextraction of chemical byproducts that would otherwise have inhibited Taq DNA polymerase activity.

**PCR Amplification of aDNA Templates.** After extraction, 1 μl of aDNA template was used in PCRs to amplify the three diagnostic chloroplast DNA sequences. The PCR primer sequences, annealing temperatures, and product lengths for each locus are given in Table 2. PCR was performed in 50-μl reactions, consisting of 1 μl of aDNA, 1× *Ex Taq* buffer (Takara), 2.5 mM $MgCl_2$, 0.2 M betaine (Sigma), 0.1 mg/ml nonacetylated ultrapure BSA (BSA from Ambion), 125 μM of each dNTP, 0.2 μM concentrations of each primer, and 2 units of Takara *Ex Taq* DNA polymerase. PCR cycling was conducted on a MJ Research PTC-225 thermal cycler. Reaction conditions consisted of an initial denaturation of 94°C for 3 min, followed by 50 cycles of 30 s at 93°C, 30 s at 48–50°C (Table 2), and 30 s at 72°C, followed by 10 min at 72°C and then a hold at 10°C. PCR products ranged from 95 to 125 bp in size (Table 2). Each aDNA sample was amplified separately from other aDNA samples and was prepared in parallel with two negative PCR controls: one containing "template" from the DNA extraction blank and a PCR blank (water in place of template).

Samples that did not amplify, or which produced a product that was too weak to clone, were used as template in a second PCR amplification. For reamplification of PCR products, 1 μl of a 10:1 dilution of the first PCR was used as template, but otherwise followed the same PCR amplification protocol as before, except that the annealing temperature was raised 1°C from the initial thermocycling profile.

All PCR products were TA cloned by using the TA Cloning Kit (Invitrogen), and subsequent positive clones were sequenced with M13 forward and reverse primers by using BigDye sequencing chemistry (Applied Biosystems). Sequences were separated on an ABI 3100 capillary sequencer. When PCR products contained multiple bands, samples were screened on the Bioanalyzer (Agilent) for presence or absence of the correct band size product. If the correct product was present, the sample was run on a low melting point agarose gel, the correct band excised from the gel and then TA cloned.

A minimum of eight clones were sequenced for each PCR product. Point mutations in nondiagnostic sites were observed among the samples and different clones from the same sample. These mutations may be due to amplification of random mutations that are attributable to accumulated physical and chemical damage over time. For all of the reported loci, we were able to replicate the diagnostic nucleotide sequences, either through independent extractions and amplifications within the LAB, or through independent verification in a different laboratory.

**Results of aDNA Analysis.** The three markers we used proved to be robust in diagnosing genotypes in the ancient samples, in that all ancient samples that could be PCR-amplified contained alleles that were consistent with either the African or Asian genotype. Thus, whereas there were point mutations at other locations along the molecule for some of the ancient samples, at the diagnostic bases the ancient samples contained alleles that were represented in either the modern African or Asian landrace samples. No novel alleles were observed in the ancient samples, and the markers proved to be informative for samples as old as 9,000 calendar years B.P. (The 10,000-year-old rind fragment from Guilá Naquitz did not yield any DNA that could be amplified.)

We observed that all of the archaeological rind fragments predating the arrival of Europeans from which DNA could be

**Fig. 2.** A 1,000-year-old bottle gourd seed from Cold Oak Rock Shelter in Kentucky (age in calibrated calendar years: A.D. 760 ± 40, Beta-195535). The seed exhibits morphology typical of Asian landraces (*L. siceraria* ssp. *asiatica*) (8, 20): light grayish brown color, length/width ratio >2, ''ears'' present (ear fragments partially broken off in photo, see Fig. 8), corky ''wings'' absent, and prominent and finely pubescent lines (seed length, 14.3 mm).

amplified were identical to the modern Asian reference group (Tables 3 and 4, which are published as supporting information on the PNAS web site). The single archaeological specimen included in the study that postdates European contact, however, corresponds to the modern African reference genotype for the three markers used. This postcontact rind fragment, recovered from Coxcatlan Cave and dated to *ca. anno Domini* (A.D.) 1660, more than a century after the establishment of Spanish settlements in the Tehuacan Valley (33), likely represents only one of many early European introductions of domesticated African bottle gourd into the Americas. After multiple early postcontact arrivals, African landraces apparently spread rapidly, and on the basis of DNA analysis of modern New World cultivars, today have almost completely replaced the Asian subspecies in the western hemisphere (34).

The multiple diagnostic loci were concordant for all 10 archaeological samples that yielded DNA (Table 1), suggesting that reproductive isolation between Asian and African landraces was early, or that genetic drift through domestication strongly differentiated them during the period of expansion from Africa into Asia, and before introduction into the Americas. Further support for the Asian origin of early bottle gourd in the Americas is provided by the distinctive shape of bottle gourd seeds occasionally recovered from pre-European archaeological contexts in both North and South America, which have long been recognized as generally conforming to the Asian seed morphology profile (20) (Fig. 2 and Fig. 8, which is published as supporting information on the PNAS web site).

## Discussion

In addition to showing that *L. siceraria* initially reached the Americas from Asia rather than Africa, and was being grown as a domesticated plant in the New World as early as 10,000 B.P., this study also provides a new perspective on a number of more general questions regarding the initial domestication of this ''container crop'' and its central role in the first efforts by humans to bring plants and animals under domestication. If the exocarp of wild bottle gourds is as thin and fragile as that documented for a range of wild cucurbit taxa, including *Lagenaria* (7, 19, 24, 26), for example, the probability that wild bottle gourd fruits drifted intact on surface ocean currents from Asia to the Americas is considerably reduced. Nor would the thin-walled fruits of wild bottle gourds have been as valued as containers, thus reducing any role that humans might have played as vectors of introduction of wild *L. siceraria* into the Americas. The fragile nature of wild bottle gourd fruits, and their resultant reduced utility to humans, along with the lack of evidence of wild bottle gourd ever having being present in the Americas thus substantially weakens the case for bottle gourd having diffused from Asia as a wild plant. In contrast to the thin-walled fruits of wild plants, however, thicker walled, domesticated bottle gourd fruits could potentially have been carried eastward from Asia to the Americas along the north Pacific current rapidly enough to reach landfall with still-viable seeds, based on recent drift and diffusion analyses of container ship spills of buoyant cargo (e.g., rubber bath toys, Nike shoes) in the North Pacific (35).

In contrast, any scenarios involving straight line, long-distance trans-Pacific transport of domesticated bottle gourds from Asia to the Americas by open-ocean seafaring vessels can be considered as having a close-to-zero probability, given the absence of evidence for watercraft capable of making such a voyage in the Late Pleistocene time frame required for bottle gourd to have reached the interior southern highlands of Mexico by 10,000 B.P. A human vector is still possible, however, as Paleoindian groups could have carried bottle gourds and still-viable seeds through the northern noncultivation zone along the south coast of Beringia, either on foot or in near-shore water craft, rapidly enough to have introduced domesticated *L. siceraria* to the New World along with the dog (*Canis familiaris*), another early utilitarian domesticate they brought to the Americas (36, 37). Although we favor a Paleoindian near-coast (land and/or water) introduction as representing the most plausible alternative, establishing the relative merits of these different possibilities will be a challenging process, with no easy or rapid resolution. Bottle gourd has yet to be recovered from any Paleoindian cultural contexts, for example, or from any early Holocene contexts along the western coast of North America.

Our documentation of the Late Pleistocene arrival of domesticated bottle gourd in the Americas, when combined with other available evidence, also strongly suggests that *L. siceraria* was independently domesticated at least twice. The significant degree of genetic separation between modern Asian and African subspecies of domesticated bottle gourd is paralleled by a very different archaeobotanical record for the plant in the two regions. The bottle gourd has been recovered from archaeological contexts in China and Japan dating to *ca.* 8,000–9,000 B.P. (12), whereas in Africa, despite decades of high-quality archaeobotanical research, the earliest record of its occurrence remains the 1884 report of a bottle gourd being recovered from a 12th

Dynasty tomb at Thebes dating to *ca.* 4,000 B.P. (14, 38). When considered together, the genetic and archaeological information points toward *L. siceraria* being independently brought under domestication first in Asia, and more than 4,000 years later, in Africa.

In addition, the early arrival of *L. siceraria* in the New World also provides strong evidence for it being one of the first species brought under domestication worldwide. Because domesticated bottle gourd had reached the interior highlands of Mexico by 10,000 B.P., it is reasonable to look for evidence of its initial domestication in Asia perhaps as much as 2,000–3,000 years earlier. Such an estimated date of domestication for the bottle gourd in Asia (by 12,000–13,000 B.P.) would place it in the same general time frame as currently available archaeological evidence for the initial domestication of the dog somewhere in Eurasia (36). If this projected time frame of domestication for the bottle gourd proves to be correct, it would join the dog as a second "utilitarian species" brought under domestication by humans long before any plants or animals worldwide were targeted for domestication as food sources (1, 2), and that these two "first domesticates" subsequently crossed Beringia into the New World with Paleoindian populations (36, 37).

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