

Transoceanic drift and the domestication of African bottle gourds in the Americas

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Bottle gourd (*Lagenaria siceraria*) was one of the first domesticated plants, and the only one with a global distribution during pre-Columbian times. Although native to Africa, bottle gourd was in use by humans in east Asia, possibly as early as 11,000 y ago (BP) and in the Americas by 10,000 BP. Despite its utilitarian importance to diverse human populations, it remains unresolved how the bottle gourd came to be so widely distributed, and in particular how and when it arrived in the New World. A previous study using ancient DNA concluded that Paleoindians transported already domesticated gourds to the Americas from Asia when colonizing the New World [Erickson et al. (2005) *Proc Natl Acad Sci USA* 102 (51):18315–18320]. However, this scenario requires the propagation of tropical-adapted bottle gourds across the Arctic. Here, we isolate 86,000 base pairs of plastid DNA from a geographically broad sample of archaeological and living bottle gourds. In contrast to the earlier results, we find that all pre-Columbian bottle gourds are most closely related to African gourds, not Asian gourds. Ocean-current drift modeling shows that wild African gourds could have simply floated across the Atlantic during the Late Pleistocene. Once they arrived in the New World, naturalized gourd populations likely became established in the Neotropics via dispersal by megafaunal mammals. These wild populations were domesticated in several distinct New World locales, most likely near established centers of food crop domestication.

long-distance dispersal | New World domestication | archaeogenomics

In independent centers of plant domestication worldwide, distinct suites of food crops tend to emerge from native flora under human selection. An exception to this is the bottle gourd (*Lagenaria siceraria*, Cucurbitaceae), which is native to Africa, but was used by diverse human cultures not only in Africa, but also across Eurasia, the Pacific Islands, and the New World during pre-Columbian times (1–4). Although bottle gourd fruits are edible, they are used by humans mostly for other purposes, including as lightweight, durable containers, fishnet floats, and musical instruments (5). This variety of utilitarian applications likely explains why bottle gourds are so globally pervasive.

In the New World, bottle gourds appear in archaeological contexts as early as 10,000 BP (6) (Table 1), and become increasingly ubiquitous and widespread during the latter half of the Holocene (9). Bottle gourds were long proposed to have arrived in the Americas via long-range dispersal on ocean currents (10–13). However, an analysis of DNA from living and archaeological gourds suggested that the bottle gourd may have been transported into the New World by the first colonizing humans (6). In this scenario, the bottle gourd, like the dog (14), crossed the Bering Land Bridge with colonizing humans already in its domestic form, making the bottle gourd one of the earliest domesticated species (1, 6).

Two factors suggest that bottle gourd colonization of the Americas via the Bering Land Bridge is unlikely, however. First, bottle gourds thrive in tropical and subtropical habitats. Based

on the physiological requirements of diverse modern cultivars (15), the growing season in Late Pleistocene Beringia would simply have been too cold and too short for bottle gourds to propagate and survive. Second, no archaeological or ethnographic evidence is known that supports the use of bottle gourds by humans in either Siberia or Alaska. In arctic regions, natural containers tend to be derived from animal products—hides, for example—rather than from plants (e.g., ref. 16). Given this lack of supporting evidence, the small amount of genetic data used to confirm this mode of colonization into the Americas deserves additional scrutiny. Furthermore, to explain why only pre-Columbian gourds appeared genetically Asian, authors of the previous study (6) suggested that a continent-wide replacement of New World gourd lineages by introduced varieties took place following European arrival. With no obvious explanation or mechanism for such a sweeping displacement of native varieties, however, this theory also warrants reconsideration.

We therefore returned to the previously studied archaeological gourds and used a capture-enrichment approach (17) to sequence and assemble the complete, 86,000 base pair large single-copy (LSC) region of the maternally inherited, nonrecombining plastid genome. In addition to two of the archaeological specimens previously analyzed (6), we included gourds from seven newly sampled New World archaeological assemblages and 36

Significance

Bottle gourd, one of the most cross-culturally ubiquitous crops, had a pan-tropical distribution by the beginning of the Holocene. Our findings overturn a major component of the current model for bottle gourd's early global dispersal, specifically regarding how it entered the Americas. Our findings also indicate that the domestication process itself took place in a diffuse pattern throughout the bottle gourd's New World range, explaining early and nearly contemporaneous use of bottle gourds in North, Central, and South America. Bottle gourd's weedy growth habit and the diffuse domestication pattern also suggest that early cultivation were probably not restricted to known centers of domestication. It is likely, however, that domesticated phenotypes emerged in these centers alongside food crops.

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. [KJ399838–KJ399882](https://doi.org/10.26434/chemrxiv-2014-01)) for assembled large single copy sequences, and the NCBI Sequence Read Archive (SRA Bioproject no. [PRJNA236372](https://doi.org/10.26434/chemrxiv-2014-01)) for short read sequence data.

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Table 1. Archaeological samples and associated AMS radiocarbon dates for gourd rind fragments used in this study

Site	Location	Accession no.	AMS Lab no.	C ¹⁴ age (y)	Cal. age (2σ)
Loreto Cave	Baja California, Mexico	3-12793	Beta-316171	80 ± 30 BP	AD 1690–1925
Putnam Shelter	Washington County, AR	32-44-396c	Beta-316173	870 ± 30 BP	AD 1045–1244
Tularosa Cave	Catron County, NM	A246294	Beta-316172	1120 ± 30 BP	AD 824–994
Spring Branch Shelter	McCreary County, KY	aLsF2	Beta-316174	1910 ± 30 BP	AD 21–210
El Gigante	La Paz, Honduras	18-13b.3	Beta-316169	2110 ± 30 BP	203–46 BC
Alred Shelter	Benton County, AR	32-4-1176	Beta-316170	3850 ± 30 BP	2459–2206 BC
Quebrada Jaguay	Arequipa, Peru	S1-U4-PA-N1f	Beta-134112	7650 ± 50 BP	6594–6431 BC
Guila Naquitz	Oaxaca, Mexico	E10-B2	Beta-97237	7940 ± 60 BP	7043–6679 BC
Little Salt Spring	Sarasota County, FL	1408551A01	Beta-261466	8890 ± 50 BP	8241–7832 BC

Sample ages were calibrated using Oxcal 4.2 (7) assuming the IntCal09 calibration curve (8).

modern landraces and wild gourds from the Americas, Europe, Asia, Africa, and the South Pacific, representing all major geographic populations (Fig. 1 and Tables S1 and S2).

Results

Phylogenetic Analysis. Phylogenetic analysis of these sequences shows that all New World archaeological gourds fall within the diversity of their African counterparts (Fig. 2). This genetic relationship indicates that pre-Columbian bottle gourds were not derived from Eurasian gourd lineages, and were therefore not brought into North America from Asia via the Bering Land Bridge. Instead, the African (*L. siceraria* ssp. *siceraria*) and Eurasian (*L. siceraria* ssp. *asiatica*) lineages (18) are distinct and strongly supported evolutionary lineages, and Africa is the clear source region of the bottle gourds that populated the Americas.

The previous conclusion that New World gourds were more closely related to Asian than to African gourds was reached by typing three variable sites within the plastid genome, two 5-bp insertion/deletions (indels) and one single nucleotide variant (SNV), that were thought to reflect fixed differences between Asian and African gourds (6). Our results highlight the risk of basing conclusions on very small genetic datasets. To resolve the discrepancy between our results and the previous findings, we used the same target-enrichment approach to capture these three variable sites from our larger dataset. Unfortunately, we were unable to capture one of the indels from any of our archaeological samples. We hypothesize that this inability was because of the large evolutionary distance separating the bottle gourd and the cucumber plastid genome, which we used to generate our probes (see *SI Methods* for discussion of additional methods). At the SNV, we captured data from four archaeological gourds, all of which we identified as carrying the African variant (Fig. S1). Although we were not able to type the SNV for either the Guila Naquitz or the Quebrada Jaguay samples, the

previous study indicated that these carried the Asian variant. Because this finding indicates both variants are present in New World gourds, we conclude that this marker is not ancestry informative.

Interestingly, all of the eight archaeological gourds that we were able to type for the third marker carried the African variant (indel absent). These included the Guila Naquitz and Quebrada Jaguay specimens, both of which were reported previously to carry the Asian variant (indel present). However, further analysis of the previously published data revealed that, in the region surrounding the indel, the published archaeological gourd sequences were highly divergent both from each other and from the modern gourd sequences (6) (Fig. S1). As horizontal gene transfer from the chloroplast to the mitochondrial genome is a common phenomenon in the Cucurbitaceae (20), these data may be explained if a homologous region of the mitochondrial genome that closely matched the target in the plastid genome had been inadvertently amplified from the archaeological gourds. To test this theory, we compared this region from the bottle gourd plastid with several Cucurbitaceae plastid sequences and with the *Cucurbita pepo* mitochondrial genome. We identified a homologous mtDNA region in *C. pepo* that forms an outgroup to all plastid sequences, providing evidence for an ancient transfer event (*SI Methods* and Fig. S1). We conclude, therefore, that this marker is also not ancestry informative.

In contrast, our complete LSC dataset robustly supports an African ancestry for ancient New World gourd lineages. Assuming an evolutionary rate of 1×10^{-9} substitutions per site per year (21, 22), the African and Eurasian lineages share a most recent common ancestor (MRCA) 105–181 kya (Fig. 2, node A), and all New World gourds share a MRCA with African gourds at 60–103 kya (Fig. 2, node B), significantly predating the earliest possible domestication dates. Interestingly, archaeological gourds do not cluster together within the African clade. That is, there is no

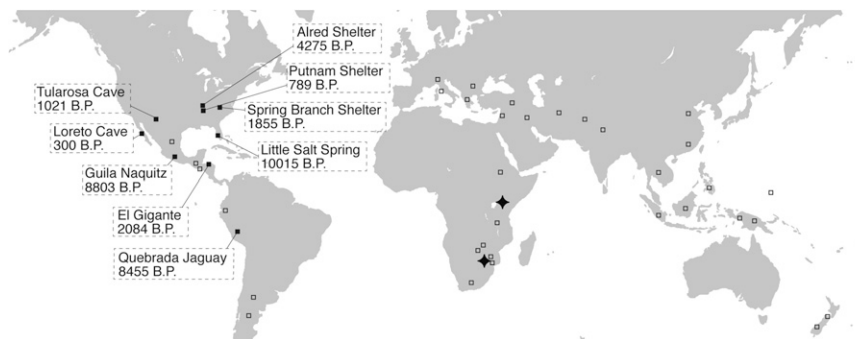


Fig. 1. Sample map showing modern domestic gourds (□), modern wild gourds (★), and archaeological gourd rind samples (■) used here. Dates reported with archaeological specimens give the weighted mean of the calibrated age invoking the IntCal.09 calibration curve (8) in Oxcal 4.2 (7). See Table 1 for complete details of archaeological samples, and Table S1 for modern sample information.

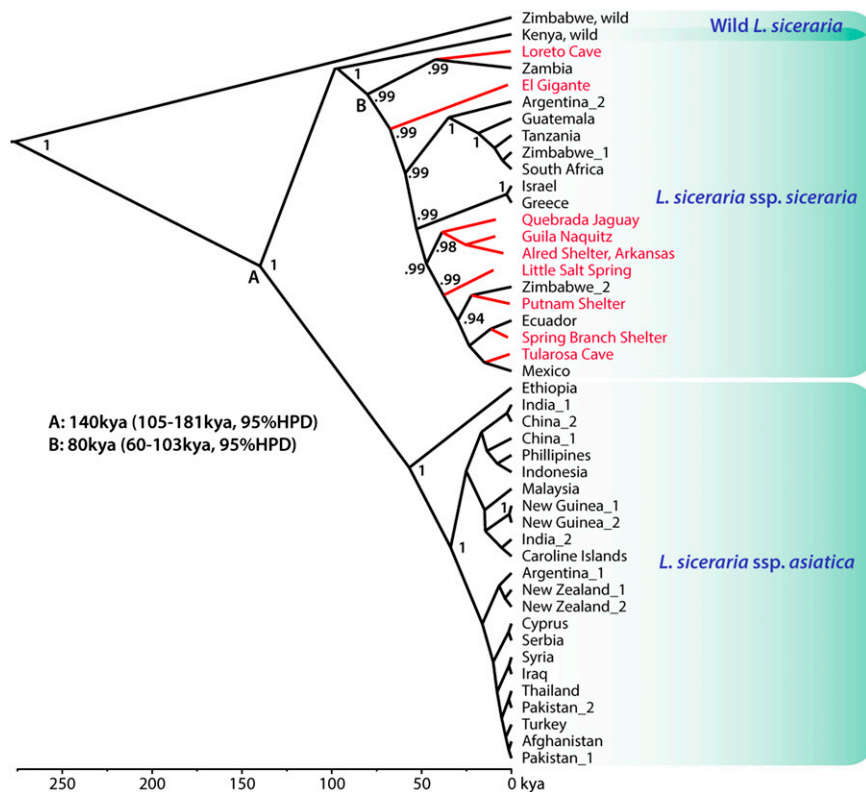


Fig. 2. Maximum clade credibility tree of modern and ancient bottle gourd LSC plastid genome data, showing Bayesian posterior probability at nodes where Bayesian posterior probability ≥ 0.9 . Ancient branches and samples are shown in red, and the scale bar assumes an evolutionary rate of 1.0×10^{-9} substitutions site⁻¹ per year⁻¹. The two subspecies, *spp. siceraria* and *spp. asiatica* [after Heiser (18)], are outlined, and a wild gourd from Zimbabwe [described by Decker-Walters et al. (25)] and one of only two known wild populations) forms an outgroup to all others, indicating some of the intraspecific diversity lost in *L. siceraria* during recent population declines. The Argentinian specimen within the *asiatica* lineage, from Heiser's (18) collections (Table S1), may represent a historic introduction to South America. Alternatively, it may be of particular interest regarding possible prehistoric contact, material culture exchange, and domesticated germplasm transmission between Polynesia and South America (15, 19). The *asiatica* lineage is subtended by a domestic Ethiopian landrace, whereas a wild gourd from Kenya falls at the base of the *siceraria* group, suggesting that the Horn of Africa might have been an important ancestral center of *Lagenaria* diversity, and a source region for *asiatica* gourds dispersing from Africa north and east into Eurasia, as discussed elsewhere (15).

clear founder effect within New World gourd plastid genomes. Thus, the mechanism that dispersed gourds from Africa to the Americas likely brought multiple, genetically diverse wild gourds across the Atlantic Ocean.

Oceanic Drift Modeling. To test the plausibility of oceanic dispersal on a scale sufficient to explain this level of diversity, we conducted a series of oceanic drift simulations based on wind values from the National Center for Environmental Protection/National Center for Atmospheric Research, and surface current data from the Estimating the Circulation and Climate of the Ocean consortium (23, 24). Both datasets constitute the best fit of numerical model output to empirical observations, and are used to calibrate a model that simulates drifting object displacement over time based on currents and winds. We varied model parameters—drifter sensitivity to wind speed, deflection angle between drifter and wind direction, and the intensity of wind-induced currents—to encompass the range of plausible variation in Late Pleistocene circulation patterns (*SI Methods* and *Table S3*). Wild bottle gourds are extremely rare, and little is known about their natural variation in either rind thickness or water-tight durability (25). However, seeds contained within the fruits of domesticated bottle gourds are known to remain completely viable after nearly a year floating in seawater (11). We therefore constrained the duration of individual drift events to 1 y, so that all transoceanic crossings would be completed within a time-frame that is compatible with seed viability.

We find strong support in our simulations for trans-Atlantic crossings in under a year, the majority of which occurred between 20° S and the equator and lasted an average of approximately 9 mo, but as little as 100 d (Fig. 3 and *Table S4*). For gourds leaving Africa within these latitudes, every combination of model parameters led to some successful crossings. In addition, regardless of the latitude of their departure from Africa, more than 80% of drifting gourds that arrived in the New World landed within this latitude zone. High crossing success was also observed for drifters originating between 10° N and 20° N, and this zone had the second highest proportion of successful landings (16%). Drifters originating between the equator and 10° N, however, only rarely arrived in the New World, and drifters that departed from latitudes poleward of $\pm 20^\circ$ almost never successfully crossed the Atlantic in our model. Any African gourd that reached the ocean within the tropical zone—with the help of inland river systems, for example—had a reasonable chance of making a successful trans-Atlantic crossing in sufficient time to maintain the capacity for germination upon landfall.

Discussion

It is most likely that the first bottle gourd populations in the Americas were established in the Neotropics. Like similar large, robust fruits, bottle gourds are adapted for dispersal by both water and large mammals, the latter of which may have provided important dispersal vectors within the New World (11, 21, 26–28). Wild *Cucurbita* seeds have been found in Late Pleistocene

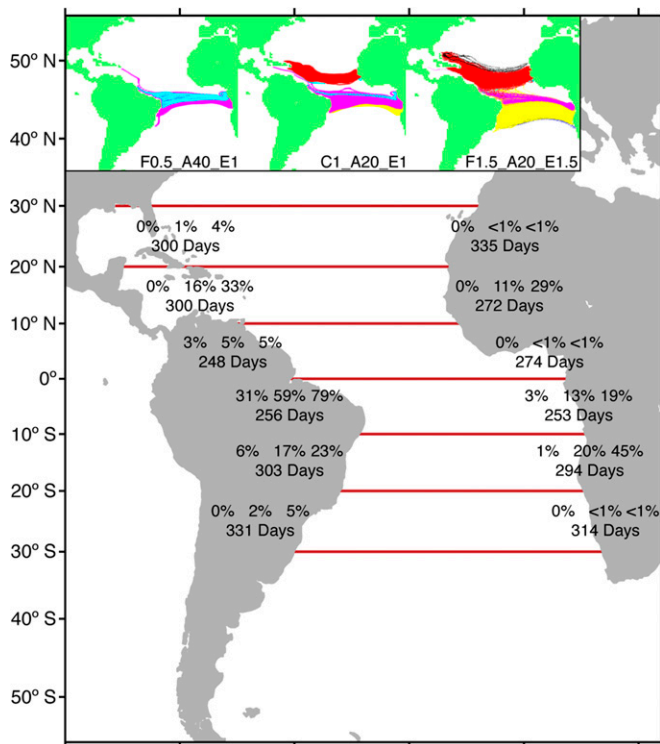


Fig. 3. Results of oceanic drift simulation experiments summarized in 10° latitude bins (Tables S3 and S4). African coastal bins give the minimum, average, and maximum drifter crossing success under all configurations of model parameters, and the average crossing times of successful drifters. New World figures indicate the minimum, average, and maximum percentage of all successful drifters arriving in a given bin, and the average crossing times of local arrivals. The *Inset* shows successful drifter paths over the course of three selected experiments (Table S3), with colors reflecting different departure latitude bands: yellow, 20°–10° S; magenta, 10°–0° S; cyan, 0°–10° N; red, 10°–20° N; black, 20°–30° N.

mastodon dung in Florida (29), revealing megafaunal dispersal of very similar fruits. Today, no wild bottle gourd populations survive in the Neotropics, and wild gourds are near extinction in Africa (5, 25). This result may reflect ecological changes since the Late Pleistocene, including the widespread disappearance of large mammal dispersers. In the absence of a natural vector for dispersal, gourds under cultivation would have had a significant reproductive advantage over their wild relatives, so that the only New World gourds likely to survive into the Holocene were the domesticates. By 10,000 y ago, humans in the Americas had begun to use bottle gourds both for food and in a variety of utilitarian capacities (Table 1) (13, 30, 31). As humans encountered free-living gourds, a level of cultivation or wild population management likely ensued, followed by selection for fruits better suited to the specific needs of local populations, and ultimately, the emergence of morphologically distinct, domesticated lineages. Although our data do not address where in the Americas these domesticated phenotypes arose, established centers of food crop domestication are strong candidates. South, Central, and

eastern North America each independently gave rise to diverse suites of food crops, and early bottle gourds appear more-or-less concurrently with domestic food crops in all three of these localities.

Rather than a single domestication event followed by extensive cultural diffusion and varietal diversification, our results indicate that bottle gourd domestication was most likely a diffuse process of human intervention, taking place at multiple times across a geographically and culturally diverse landscape. Although we explore this process only in the New World, the phylogenetic intermingling of deeply divergent domestic African and New World lineages suggests a pattern of diffuse domestication in Africa similar to what we propose for the Americas. That is, the model of gourd domestication that we infer is probably not restricted to the New World, and higher-resolution analyses could help parse the details of bottle gourds' complex global movements and human interaction.

Methods

We extracted DNA from modern seed and leaf samples using Qiagen's Plant DNeasy Mini Kit (Qiagen). For ancient gourd rinds, we ground tissue using a ball mill or pellet pestle, and extracted DNA using a PTB-based extraction protocol (32) (*SI Methods*). We confirmed DNA presence using LS_INDEL1 and LS_SNP primers (6), and prepared barcoded Illumina sequencing libraries after Meyer and Kircher (33). We used the MycroArray MySelect system for in-solution RNA hybridization capture (17) of the LSC based on the cucumber (*Cucumis sativus*) plastid genome (34). We pooled and sequenced libraries in parallel on an Illumina HiSeq. 2000 at the University of California at Berkeley using 150-nt paired-end reads, and we demultiplexed reads, merged forward and reverse reads, and performed read quality control as described by Kircher (35). We used MIA (github.com/udo-stenzel/mapping-iterative-assembler) to construct a 99.8% complete LSC reference sequence from sample Ls6A based on the cucumber plastid genome, and used Burrows-Wheeler Aligner and Samtools to assemble the remaining reads for all other samples. We reconstructed a dated phylogeny (Fig. 2) using BEAST 1.7.4 (36), combining three independent Markov-chain Monte Carlo simulations, and assuming the Bayesian skyride coalescent model (37), the HKY + G nucleotide substitution model, and 1.0×10^{-9} substitutions per site per year (21, 22). We conducted oceanic-drift simulations as developed by Montenegro et al. (38, 39), integrating simulated general circulation model estimates of ocean current movement with empirical data on drifter behavior. Although observational and model-based studies indicate that large-scale wind and surface current systems in the Atlantic have remained relatively unchanged from the Late Pleistocene to the present (40–44), we varied all model inputs through parameter space to conservatively encompass reasonable behavior of drifting objects under Late Pleistocene conditions.

Short read sequence data are curated in the National Center for Biotechnology Information Sequence Read Archive (SRA Bioproject no. PRJNA236372) and assembled LSC sequences used in analysis are annotated and available in GenBank (accession nos. KJ399838–KJ399882).

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