INVESTIGATING CROP PLANT ORIGINS IN THE AMERICAS USING ANCIENT DNA AND EXPERIMENTAL PLANT DEVELOPMENTAL RESEARCH

A Dissertation in

Anthropology

by

Logan Kistler

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The dissertation of Logan Kistler was received and approved* by the following:

Lee Newsom
Associate Professor of Anthropology
Dissertation Adviser
Chair of Committee

George Milner
Professor of Anthropology
Head of the Department of Anthropology

Dean Snow
Professor of Anthropology

Beth Shapiro
Associate Professor of Biology

*Signatures are on file in the Graduate School.
The integration of molecular biology and plant physiology into archaeology allows us to more comprehensively and directly address difficult questions regarding past human activity. The former can be used to trace plant and animal domestication and phylogeography, demographic histories, human dispersal and demographics, etc. The latter is inexorably linked with plant life history and, eventually, site formation processes and the physical composition of the archaeological record. This dissertation comprises three individual studies: Parts 1 and 2 utilize ancient and modern DNA to elucidate the domestication histories of two important North American crop plants, chenopod (*Chenopodium berlandieri* Moq., Chenopodiaceae), and bottle gourd (*Lagenaria siceraria* [Molina] Standl., Cucurbitaceae). Part 3 is an experimental study conducted to understand the effects of pathogenic stress on the formation of silica phytoliths in gourd/squash (*Cucurbita pepo* L., Cucurbitaceae), and potential implications for archaeological phytolith analyses.

In Part 1, I test whether chenopod, an important prehistoric starchy seed crop in the Eastern Woodlands of North America was locally and independently domesticated, or whether it was introduced from Mesoamerica, where morphologically identical crops are grown today. I conclude that it was native to the Eastern Woodlands, and argue that this finding strengthens the existing evidence for eastern North America as an entirely independent center of plant domestication.

In Part 2, I test competing hypotheses regarding the arrival and proliferation of bottle gourds, one of the world’s earliest and most broadly distributed crop plants, in the New World. I conclude that bottle gourds traveled on ocean currents from their native Africa to the New World during the Pleistocene, established genetically diverse naturalized populations, and underwent multiple domestication events in the Americas during the Holocene.

In Part 3, I extract and analyze silica phytoliths from wild gourd fruits grown in controlled experimental conditions to test for morphological changes driven by two common diseases. I find that pathogenic stress has the potential to significantly impact phytolith size in ways that might confound archaeological phytolith analyses. I argue that extensive experimental assessment of ecological effects on phytolith morphology is in order to strengthen archaeobotanical phytolith analyses.
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Chapter 1: Introduction and Background

1. Introduction

The integration of molecular biology and plant physiology into archaeology allows us to more comprehensively and directly address difficult questions about past human activity. The former can be used to trace plant and animal domestication and phylogeography, demographic histories, dispersal events, biodiversity through time, and more (e.g. Leonard et al. 2002; Jaenicke-Depres et al. 2003; Shapiro et al. 2004; Edwards et al. 2007, 2011; Larson et al. 2007; Schlumbaum et al. 2008; Palmer et al. 2012); and with the refinement of ancient DNA recovery and analytical procedures, the domestication histories of individual taxa are under increasing scrutiny to better understand the onset of plant and animal husbandry.

Complex physiological processes driven by the interplay between genetics and the environment determine the morphological characters of wood, seeds, fruits, starch grains, silica phytoliths, and other plant materials that frequently occur in archaeological deposits (e.g. Pearsall 2000; Ford [Ed.] 1978), as with all living tissues. Understanding the causes of normal variation in these systems is critical for developing a meaningful interpretation of any archaeological assemblage’s biotic component. Therefore, plant physiology is inexorably linked with plant life histories and, ultimately, site formation processes and the composition of the archaeological record.

Plant domestication carries considerable interest within archaeology and related fields due to the massive social, biological, and ecological shifts that tend to accompany the adoption of plant cultivation as a food production strategy. Tendencies to study domestication in terms of wholesale agricultural complexes have been steadily giving way to more nuanced and dynamic models of food production based on co-evolutionary adaptation of humans and specific taxa (e.g.
Rindos 1984; Fritz 1999; Smith 2006a). Here, I present three studies that are designed to better understand the nature of crop domestication on the level of individual plant taxa.

In Part 1, I use ancient and modern DNA analysis to confirm eastern North America as the site of independent domestication of chenopod (*Chenopodium berlandieri* Moq., Chenopodiaceae), an important starchy seed crop that played a central role in Eastern Woodlands subsistence beginning in the Late Archaic Period and persisting into the Mississippian (e.g. Cowan 1985; Gremillion 1993a, 1993b; Fritz 1995; Fritz and Lopinot 2007; Fritz and Smith 1988; Smith 1984, 2006, 2007; Smith and Yarnell 2009). In Part 2, I use a large modern and ancient DNA dataset generated by target capture and high-throughput sequencing to investigate the origins of Precolumbian bottle gourds (*Lagenaria siceraria* [Molina] Standl., Cucurbitaceae) in the New World by 10,000 B.P., and bottle gourd phylogeography on a worldwide scale. In Part 3, I conduct an experimental study to understand the effects of two common diseases on silica deposition in wild gourd/squash (*Cucurbita pepo* L., Cucurbitaceae). This study is largely a proof-of-concept that normal sources of ecological variation have the potential to affect phytolith formation in ways that might confound archaeological phytolith analyses, and that further experimental research should be directed at understanding the details of these relationships.

### 2. Methods

The lab and analytical methods used in these studies are described in detail in the individual sections. Part 1 relies on PCR and Sanger sequencing-based data generated from modern and ancient DNA from single chenopod seeds sampled throughout their geographic range. Part 2 utilizes DNA library preparation, target capture, and massively parallel sequencing to generate a large dataset comprising genetic information from 41 modern bottle gourd
accessions—including diverse cultivars and landraces, putatively wild populations from Kenya and Zimbabwe, and a single outgroup in *L. sphaerica*—and nine ancient gourd rind samples from North, Central, and South America. Part 3 uses a wet-oxidation phytolith extraction protocol and an *ad hoc* slide mounting procedure designed to facilitate rapid and accurate quantification of phytoliths using a compound microscope fitted with an eyepiece micrometer.

Part 1 is the first study to utilize ancient DNA to address the long-debated issue of eastern North America’s status and importance as an independent center of plant domestication. Part 2 is designed to re-assess the findings of a previous ancient DNA-based study (Erickson et al. 2005), which has been widely cited as evidence that Precolumbian gourds originated in Asia, and which has ultimately not withstood the more stringent analysis described here. Part 2 is also the one of the first applications of so-called next-generation sequencing (NGS) in a hypothesis-driven study dealing with archaeological plants and plant domestication, demonstrating the efficiency and usefulness of these techniques in plant domestication research (see also Ávila-Arcos 2011; Palmer et al. 2012).

Part 3 is the only study to date to test the potential for ecological variables to affect phytolith morphology with the specific goal of understanding possible confounding effects on archaeological phytolith analyses. This study stands as a proof-of-concept that phytoliths, like other botanical structures and tissues commonly recovered in archaeological deposits, are subject to significant developmental variation as a result of environmental stimuli. Similar experimental treatments of phytolith formation should play a role in the archaeobotanical literature, given the apparent sensitivity of silica allocation and phytolith morphology to external influences, and the increased reliance on archaeological phytolith assemblages as indicators of complex human behavior over the past three decades (see Piperno 2009 for a recent review).
3. Project Goals and Background

3.1. Pre-Maize Plant Cultivation in the Eastern Woodlands

Before maize-based agriculture’s rise to prominence in the Eastern Woodlands beginning around ca. A.D. 900, a food production system based at least in part on indigenous domesticated plants was in place throughout the midcontinent since the Late Archaic Period (Linton 1924; Gilmore 1931; Jones 1936; Yarnell 1974; Watson 1974; Fritz 1986, 1995; Smith 1984, 2006; Smith and Yarnell 2009; Streuver 1962). This suite of crops, commonly the “Eastern Agricultural Complex,” grew tremendously in importance during the Woodland Period (see Milner 2004:87). Ralph Linton (1924) first suggested the existence of a pre-maize crop complex, noting that maize material culture in the Eastern U.S. differed sharply from that in the Southwest and Mexico. He attributed this to “the superposition of maize upon some older food complex which was itself rather elaborate,” and suggested that “the eastern tribes had developed at least the beginnings of agriculture” (Linton 1924:349). This reflects Nels Nelson’s (1917) earlier findings in the Mammoth Cave Vestibule that evidence of maize cultivation was conspicuously absent, but remains of sunflowers, gourd, and squash were present.

Smith (2007) comprehensively outlines the development of archaeological ideas about Eastern Woodlands agriculture, beginning with Linton’s contribution and working up to recent research. Early key figures were Melvin Gilmore (1931) and Volney Jones (1936), who were the first to expand Linton’s general concept by identifying potential pre-maize cultivars in dry rockshelters of the Ozarks and the Cumberland Plateau. Stuart Streuver (1962) coined the phrase, “Eastern Agricultural Complex,” which has become a mainstay in Eastern Woodlands literature, despite having lost favor with some researchers because it may imply an unrealistic level of crop complex homogeneity during domestication and proliferation (Smith 2007).
Yarnell’s (1969, 1974) work in the Mammoth Cave System was critically important in terms of identifying and quantifying cultivated plant remains in coprolites and general deposits, allowing quantitative estimates to be made of various foodstuffs’ relative contributions to the typical diet. Watson (1974) synthesizes these data and provides a holistic overview of prehistoric food production, drawing heavily on information from her extensive work in the Mammoth Cave System. Interpretations based on deep cave paleofecal assemblages initially drew some skepticism based on the possibility that they represented a unique “cavers’ diet” (for reviews, see Kennedy and Watson 1997; Gremillion 2008). Subsequent research—including Gardner’s (1987) analysis of stratified Salts Cave Vestibule flotation samples and numerous other studies in the midcontinent (e.g. Asch and Asch 1985a; Cowan 1979, 1985; Fritz 1986; Gremillion 1993a; and see Fritz 1990)—has shown, however, that “the diet represented in the Salts Cave and Mammoth Cave paleofeces is a faithful indication of what the Early Woodland cavers were eating above ground as well as below” (Kennedy and Watson 1997:6).

The pre-maize agricultural complex was based largely on four domesticated food crops: Chenopod (Chenopodium berlandieri Moq.), sumpweed (Iva annua L.), sunflower (Helianthus annuus L.), and gourd/squash (Cucurbita pepo L.), which also served utilitarian purposes (e.g. fishnet floats or containers) (Fritz 1995; Fritz 1999; Smith 2006b). In addition to these taxa, maygrass (Phalaris caroliniana Walter), erect knotweed (Polygonum erectum L.), and little barley (Hordeum pusillum Nutt.), starchy seed-bearing taxa, were likely cultivated, stored, and consumed alongside the four cultigens, but their archaeological remnants do not carry morphological signs of domestication (Fritz 1995; Smith 2006b; and also see Asch and Asch [1985b] for a discussion of a possible domesticated morphotype of knotweed). Finally, a domesticated form of bottle gourd (Lagenaria siceraria Molina) was widely utilized in the
Eastern Woodlands—and throughout the Americas—for utilitarian purposes as mentioned above with gourd/squash (Doran et al. 1990; Erickson et al. 2005; Richardson 1972; Cutler and Whitaker 1961).

Bottle gourd, which first appeared in Florida by ca. 10,000 B.P. (Newsom and Gifford unpublished data), might represent the first cultivated plant taxon in the Southeast. The domestication status of these early gourd remains is unclear, but they most likely arrived in a wild form by oceanic drift from Africa (see Chapter 3). Florida is one likely ingress point for bottle gourds in the Americas, along with parts of Central and South America (Guppy 1917; Heiser 1985), and it is possible that bottle gourds traveled northward by human dispersal from Florida into much of the midcontinent (see below for a detailed discussion).

Gourd/squash was another crop that was first used for non-food purposes, probably as a container and/or fishnet float (e.g. Doran et al. 1990; Fritz 1999; Newsom 1986). The earliest thin-walled, wild forms of gourd/squash fruits recovered from eastern sites have been dated to roughly the beginning of the eighth millennium B.P., and consistently appear outside of their presumed native range prior to showing any morphological signs of domestication (Fritz 1999). New selection pressures eventually led to the appearance of thicker rinds and larger seeds indicating domestication—which appeared some three millennia later—and the suppression of bitter secondary chemical production, probably as gourd/squash transitioned from a strictly utilitarian item into something considered edible (King 1985; Cowan 1997; Cowan and Smith 1993; Fritz 1999).

The archaeological record in eastern North America reveals widespread wild-type gourd/squash use followed by the gradual appearance of domesticated forms, suggesting in situ domestication of *C. pepo* ssp. *ovifera*, a lineage of diverse cultivars including acorn, crookneck,
scallop, and other squashes, plus several ornamental gourds (King 1985; Fritz 1999; Decker-Walters et al. 2002). However, the modern ssp. *ovifera* lineage falls within a clade containing several wild North American taxa, including *C. pepo* var. *ozarkana*, var. *texana*, and ssp. *fraterna*, found in the midcontinent, Texas, and northern Mexico, respectively (Decker-Walters et al. 2002; Sanjur et al. 2002). These North American populations are interfertile and difficult to discriminate based on existing molecular data, and they probably represent the remnants of a large contiguous population with components ranging well into Central America (Decker-Walters et al. 2002). While archaeological evidence and limited molecular data suggest domestication in the Eastern Woodlands from wild *C. pepo* var. *ozarkana*, an alternative hypothesis argues that northeastern Mexico should also be considered as the site of domestication, with ssp. *fraterna* as the progenitor (Sanjur et al. 2002). Higher resolution molecular data will be necessary to further elucidate this issue.

Sunflower is known to have been domesticated in eastern North America, due to archaeological abundance and two recent genetic studies (Nelson 1917; Yarnell 1974; Asch and Asch 1985a; Fritz 1986, 1990, 1995; Harter et al. 2004; Wills and Burke 2006; Smith 2006b). Sunflower achenes (dry, indehiscent fruits [Simpson 2006]) first appear in the accepted size range for domestication at the Hayes Site in Tennessee, around the beginning of the fifth millennium B.P. (Crites 1993). Sunflower was a major dietary constituent during the Woodland Period, and was a component in domestic and feasting assemblages at Cahokia (Fritz and Lopinot 2007; Yarnell 1974). Sunflower was also utilized heavily by native groups, along with maize, beans, and squash, in historic times (e.g. Wilson 1917).

In addition, Bonzani and others (2007) report the presence of inflorescence and stalk remains from a weedy form of sunflower deep in Mammoth Cave that directly dates to the Early
Woodland Period. This suggests persistent use of weedy forms after domestication that might have resulted from cross-pollination between crops by nearby wild plants, a phenomenon common in sunflowers and responsible for persistent gene flow even in modern commercial sunflower cultivation (Linder et al. 1998). Bonzani and others (2007) also suggest that we should consider the possible utilitarian value of other sunflower parts besides the edible seeds, pointing out that the deep cave stalk and inflorescence material probably does not represent discarded parts after processing the plant for food.

All modern varieties of sunflower are apparently derived from a single domestication event in eastern North America accompanied by a substantial genetic bottleneck (Harter et al. 2004; Wills and Burke 2006). This directly contradicts the hypothesis of some researchers that domestic sunflower might have originated in Mesoamerica, based on the recovery of a small number of early specimens identified as sunflower from the San Andres and Cueva del Gallo sites in Mexico (Lentz et al. 2001, 2008; Piperno 2001; Pope et al. 2001; Tarighat et al. 2011). While we cannot presently rule out the possibility of an independent local sunflower domestication in Mexico, no remnants of it are known in crop fields today. Notably, sunflower is the only pre-maize crop in the Eastern Woodlands in which researchers have investigated the specific genetic mechanisms related to domestication, revealing “numerous wild alleles with cultivar-like effects” that made the sunflower “readily domesticated” (Burke et al. 2002:1257).

Sumpweed’s eastern origin is also well established, with a long period of low level wild-type use preceding the morphological changes associated with domestication, which had occurred by the middle of the fifth millennium B.P. (Asch and Asch 1985a; Smith 2006b). Sumpweed, an oily seed plant related to sunflower, has been characterized as an “extinct minor cultigen” in the Eastern Woodlands, implying that it contributed little to prehistoric subsistence
(Harter et al. 2004:201). It is no longer grown as a cultivar, but it was, at times, a significant food source. Yarnell (1974) estimates that sumpweed constituted around fourteen percent of dietary intake of Salts Cavers, based on paleofecal analyses.

There has been a tendency to discount the role of the pre-maize crops, including sumpweed, after the rapid growth of maize-based agriculture during the Mississippian Period (see Fritz and Lopinot 2007 for a discussion). However, Fritz (1997) reports that the largest sumpweed achenes recovered to date were found at a protohistoric site in Arkansas, suggesting that in parts of the midcontinent, cultivation and selection for larger achenes persisted directly through the rise and fall of Mississippian chiefdoms. She remarks that sumpweed appears “to have thrived in undiminished splendor in some fields at a time and place very close to the De Soto entrada” (Fritz 1997). Although its contribution was arguably not as profound as sunflower or chenopod, sumpweed was a substantial component of the prehistoric diet at times.

Chenopod is the final pre-maize cultigen, and has remained the least understood in terms of site and phylogeny of domestication. No derivatives are cultivated today, so the types of modern biomolecular studies carried out with sunflower and gourd/squash cannot be used to link wild populations with domestic counterparts. The only modern vestiges of the prehistoric crop chenopod are characteristics in some free-living eastern chenopods that suggest persistence of certain traits associated with domestication, including seed retention and synchronized flowering (Asch and Asch 1977; Smith 2007). Nevertheless, chenopod was grown and stored in huge quantities (Fritz and Smith 1988; Smith 1984), consumed year round (Gremillion and Sobolik 1996), and formed an estimated twenty-five percent of the total diet—matching sunflower as the greatest dietary constituent—among the Early Woodland Salts Cavers (Yarnell 1974). Chenopod is treated in greater detail below.
Finally, it is worth noting that no domesticated animals were raised for food in eastern North America’s prehistory. Dogs were present, probably having entered the New World with colonizing Paleoindians, but were a “utilitarian species,” not typically raised for eating (Erickson et al. 2005; but see Hudson 1976). The burial contexts of dogs at the Indian Knoll in Kentucky, for example, suggest that they shared a social relationship with humans, who probably valued them for their assistance in hunting and companionship (Morey 2006). The absence of livestock may be attributed to an absence of animals predisposed to domestication—large herbivorous mammals with a natural dominance hierarchy that can be co-opted by humans and a tendency to naturally herd into large, efficient groups (e.g. Diamond 1997; Zeder 2006). Whatever the reason, domestic animals other than dogs were not present prehistorically in the Eastern Woodlands.

3.2. *Domesticated Chenopodium in Eastern North America*

The eastern domesticated chenopod is actually a crop complex, comprising two domestic morphotypes and an occasional “companion weed” (Figure 1; Gremillion 1993b; Smith and Yarnell 2009). Throughout the Eastern Woodlands, chenopod seeds (actually simple, indehiscent, dry fruits called utricles [e.g. Simpson 2006]) recovered from archaeological deposits take three forms, distinguished by color, testa (outer seed coat) thickness, and other morphological characters:

The most common form is a dark morph cultivar in the size range of wild types, having a reduced testa—probably related to selection against germination dormancy under cultivation—and exhibiting a distinctive truncate margin, giving the seeds a blocky appearance and rectangular cross-section distinct from their lens-shaped wild counterparts (Smith 1984; Smith and Funk 1985). This morph was first described in detail and assigned to the species level (*C.*
Figure 1.1. Domesticated chenopod morphology. Top left, thin testa *C. h. ssp. jonesianum* from Russell Cave (Smith 1984). Bottom left, early pale morph cultigen from Riverton (Smith and Yarnell 2009). Top right, comparing modern wild *C. berlandieri* (left) and domestic *huazontle* seeds in cross-section displaying testa reduction and truncate margin (Wilson 1981). Bottom right, plant architecture in modern domestic *huazontle* (left), modern wild *C. berlandieri* (right), and archaeological inflorescence with attached fruits from Holman Shelter, Arkansas (Wilson 1981).

*berlandieri* from a large cache of seeds in Russell Cave, Alabama, dating to the Middle Woodland Period (Smith 1984). It has been recovered from numerous Eastern Woodlands sites, as early as the Late Archaic from Cloudsplitter and Newt Kash rockshelters in eastern Kentucky (Smith and Cowan 1987) and the Riverton site in southeastern Illinois (Smith and Yarnell 2009).
In addition to the dark morph, a pale-seeded cultivar is also recovered archaeologically, though less frequently. The Ozark Bluff Dweller rockshelter assemblages have produced the greatest quantities of the pale morph, whose seeds carry an extremely reduced testa and loss of the hard black epidermal layer, and are slightly larger overall than the dark morph (Fritz 1984, 1986; Smith and Yarnell 2009; Wilson 1981). The most complete known pale morph specimen is a preserved inflorescence retaining several seeds from the Holman Shelter in Arkansas, which Wilson (1981) assigned to the modern Mexican cultivar, *huazontle* (*C. berlandieri* ssp. *nuttalliae*), based on multiple morphological characters. Although this assignment is contentious (e.g. Smith and Yarnell 2009, and see Chapter 2), the pale morph is very similar to modern *huazontle*. Recent analysis of materials from the Riverton site in Illinois have revealed the presence of substantial numbers of pale morph seeds in a Late Archaic context contemporaneous with the earliest known dark morph specimens (Smith and Yarnell 2009).

Finally, a “companion weed” seed form occasionally occurs in archaeobotanical assemblages, having wild-type morphological characters such as a relatively thick testa, but apparently being culturally associated with one or both cultivars (Gremillion 1993b; Smith and Yarnell 2009). This condition might result from wild plant introgression, or from persistent ancestral variation in a domesticated population, leading to the expression of wild-type traits in low frequencies. The weed morph seeds recovered in eastern assemblages are often discussed in terms of wild populations that interact genetically with cultivars due to shared habitat and indiscriminate harvesting, and that, over time, might take on some domesticate-type traits (Gremillion 1993b).

The pattern of two chenopod cultivars and a companion weed appears in two other modern crop complexes in the New World. In the Andes, *quinoa* (*C. quinoa* Willd.) and *cañihua*
(C. pallidicuale Aellen) are cultivated crops, and a hybrid companion weed of unclear taxonomic position is also present (Bruno 2006). In this case, differences in ploidy between the cultivars make interfertility unlikely. In Mexico, huazontle and chia (both classified as C. berlandieri ssp. nuttalliae) are cultivated for different uses, but are interfertile (Wilson and Heiser 1979). Huazontle produces a pale seed and chia a dark one, and it has been pointed out that the two Mexican cultivars are essentially indistinguishable from their ancient eastern counterparts on the basis of seed morphology (Wilson 1990). Regardless of whether the Mexican cultivars are linked to the eastern ones, it is clear by comparison with the Andean complex that the two cultivar, one weed pattern is a typical configuration of chenopod agriculture that arose independently in at least two and possibly three New World locales (Bruno 2006; Smith and Yarnell 2009; Wilson 1990; and see Chapter 2).

3.2.1 Competing Domestication Hypotheses

As with gourd/squash and sunflower, eastern chenopod has been alternately suggested to originate in eastern North America and in Mexico (Smith 1987, 2007; Smith and Yarnell 2009; Wilson 1981, 1990). Unlike the other crops, however, neither potential site of domestication has previously been supported by satisfactory data.

The eastern origin hypothesis (e.g. Smith 1984, 2006, 2007; Smith and Yarnell 2009) suggests that all eastern cultivars of chenopod were derived from local C. berlandieri, with no contribution from introduced Mexican cultivars. Smith (2006) argues that no archaeological evidence shows domestic chenopod use in Mexico prior to historic times, and that the Mexican crops are the result of an independent and much later domestication of C. berlandieri. This model favors cultivation of local wild chenopods gradually leading to domestication in Late Archaic midcontinental riverine habitats, a scenario developed at length in Smith’s (2007)
“floodplain weed theory.” Under this model, the archaeological companion weed is essentially considered to be the mother taxon from which the two cultivars were derived, and which continued to interact with the new, domesticated forms (Gremillion 1993b; Smith and Yarnell 2009). Persistence of domesticate-type traits in some free-living modern eastern populations is explained as the taxon “retaining several attributes of its prior life as a domesticate” (Smith 2007:262).

The Mexican origin hypothesis (Wilson 1981, 1990) draws on similarities in morphology as evidence that huazontle and chia were introduced to eastern North America, where they formed part of the basis for eastern agriculture as the pale and dark morph eastern chenopods, respectively. In this model, Wilson (1981, 1990) suggests C. berlandieri ssp. bushianum—formerly C. bushianum, an eastern variety traditionally considered to be native and wild (Asch and Asch 1977)—to be the free-living weedy remnant of a crop/weed complex based on Mexican cultivars. Sympatric companion weeds are hypothesized to have occurred near crop fields of the cultivars as they do today in Mexico, and may have been generated by “direct derivation of the domesticate, or through genetic interaction with a local, wild species” (Wilson 1981:238). Under Wilson’s interpretation, the pale and dark morph archaeological seeds can be considered exotic, and the weed morph is a native taxon interacting genetically and becoming ecologically embedded with the cultivars. Modern C. berlandieri in eastern North America is known to be interfertile with the Mexican cultivars (Wilson and Heiser 1979).

An eastern origin seems more parsimonious archaeologically, with numerous chenopod remains in wild, weedy, and domesticated forms recovered from across the midcontinent (e.g. Fritz 1986; Fritz and Smith 1988; Gremillion 1993a, 1993b; Smith and Cowan 1987; Smith and Yarnell 2009; Yarnell 1974). Complexes of two cultivars and a companion weed are known to
have arisen independently at least twice in the New World (Bruno 2006; Smith and Yarnell 2009; Wilson 1990), so the expectation of a third independent emergence of this pattern is not unreasonable. The morphological similarities between ancient eastern cultivars and their modern Mexican counterparts could be explained in terms of parallel evolution of traits under similar selection pressures introduced during cultivation. The plants in both locales might have initially responded to the anthropogenic habitat by developing at the limits of their phenotypic plasticity, favoring rapid germination and abundant nutritive tissue in seeds, among other traits. Continued selection pressures would then have driven population-wide shifts in these characters, resulting in the morphological novelties of testa reduction and blocky, truncate-margin seeds. Given that the progenitor populations are closely related and human selection favors similar changes over time, consciously or unconsciously (e.g. Rindos 1984; Harlan et al. 1973), emergence of the same morphological traits can be expected. Many other changes would have occurred during chenopod domestication, including several that would not be apparent via seed morphology (Smith 2007), but this scenario illustrates the possibility of parallel evolution under human selection. For other examples, breadwheats, rices, and various millets are all known to have been domesticated multiple times, resulting in the evolution of morphologically similar and genetically compatible cultivars (Murphy 2007).

The eastern archaeological record, however, does not preclude Wilson’s model of Mexican cultivar introduction. Pre-domestication use of wild-type chenopod in eastern North America (e.g. Asch and Asch 1985a; Hollenbach and Walker 2009) followed by the appearance of domesticates could be interpreted as early cultivation of a local variety preceding widespread “displacement by ‘improved’ strains,” the Mexican cultivars (Wilson 1990:107). Upon arrival of the cultivars, the local variety would have been relegated to companion weed status, the remains
of which are visible archaeologically as weed morph seeds, and ecologically in the presence of seed retention and synchronized flowering in eastern free-living populations of *C. berlandieri* (Gremillion 1993, Smith 2007). The morphological similarities between eastern and Mexican cultivars certainly suggest the possibility of a Mexican introduction, especially in the case of the well-preserved Holman Shelter inflorescence that Wilson (1981:234) calls “identical” to those produced by *huazontle*.

In the case of the exceptional Holman Shelter specimen, Wilson (1981) was able to analyze informative characters such as fruiting body architecture and seed dispersal, offering greater insight than is possible with only seed remains. But, as previously discussed, parallel evolution is a reasonable explanation for shared morphological characters in domesticated plants. The absence of sufficiently early archaeological evidence for Mexican chenopod domestication is the greatest detraction from the Mexican origin hypothesis (e.g. Smith 2006b; Emily McClung de Tapia personal communication).

3.2.2. Study and Results Summarized

In Chapter 2, I sequence and screen chloroplast DNA from modern chenopod populations sampled throughout North America, and target informative genetic regions in archaeological chenopods from Kentucky and Arkansas. I conclude that the dark and pale domestic morphotypes both arose from wild eastern populations, evidently free of influence from Mesoamerican varieties. Along with sunflower, sumpweed, and probably gourd/squash, chenopod was locally domesticated in the Eastern Woodlands.

3.3. Phylogeography of the American Bottle Gourd

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.), native to Africa, was one of the earliest domesticated taxa in the New World, first appearing in Florida by 10,000 B.P. (Newsom
and Gifford, unpublished data), and throughout Central and South America shortly thereafter (Erickson et al. 2005). Its large, hollow fruits were widely used throughout the Americas as natural containers (Richardson 1972; Cutler and Whitaker 1961; King 1985), and in other utilitarian applications such as fishnet floats (Cutler 1975; Hudson 2004). Bottle gourds have a complex biogeographic history, including early distribution and use throughout the tropics and multiple independent domestication events (Whitaker and Cutler 1965; Heiser 1973, 1979, 1985; Decker-Walters et al. 2001; Richardson 1972). Demographic events and a shortage of known wild populations have confounded our understanding of this taxon. Its intercontinental movement and worldwide usage likely make the bottle gourd the most cosmopolitan crop plant of worldwide prehistory (e.g. Richardson 1972), and its archaeological abundance highlights its importance in North America (see Doran 2002, Table 1.2). The study described in Chapter 3 clarifies elements of the bottle gourd’s complex biogeographic history, including its colonization of the New World and independent domestication in the Americas.

3.3.1. A Diverse Range of Applications

The durable, lightweight, and easy-to-grow fruits of the bottle gourd are employed in an astonishing range of uses (Heiser 1979). Most commonly, the dried and hollow gourds are used as containers for storage and easy transport of food, liquids, and other items. Among specialized container applications, Maori gourds in New Zealand are used for long-term storage of fatty preserved meat (Burtenshaw 2003; Leach 2003), and gourds grown in special molds are used for traditional cricket keeping and cricket fighting in China (Walters 1989). Some traditional African agricultural practices utilize gourds as seed containers during planting (Carney and Rosomoff 2009), and Garwood (1956:225) refers to gourds being used “to store everything from clothes to vegetables or milk” in Texas boarding houses of the 19th Century. Gourd milk
strainers were favored in the U.S. until the Civil War, and gourds were sometimes used in the same era with a piece of mesh fabric to bolt flour (McCulloch-Williams 1908).

Many musical instruments from a variety of cultures employ bottle gourds as natural sound chambers (Heiser 1979; Figure 1.2). Most notably in modern western musical culture, the banjo was originally constructed from a gourd cut in half and covered tightly with an animal skin to create resonance (Bluestein 1964), but hundreds of other examples exist around the world (see Heiser 1979 for an overview). In Egypt, several gourds are lashed together to provide buoyancy, and are covered in a deck of wood or reeds to construct river rafts (Cooper 2010; Figure 1.2). Gourds are also sometimes used as fishnet floats, an application which is known archaeologically from Peru and Florida (Cutler 1975; Hudson 2004; Newsom et al. 2012; Figure 1.2). Bottle gourds also have a prominent place in many shamanistic and magical traditions. They are invoked in creation myths, used as amulets and fetishes, instruments of divination, containers for sorcerers’ goods, and similar applications (Wilson 1954). Various parts of the plant are used medicinally (Yang and Walters 1992; Moerman 1998), and mature gourd fragments have even been used to repair skull fractures and trepanation wounds (Bandelier 1904; Durham 1923; Heiser 1979).

The young bottle gourd fruit is sometimes eaten as a vegetable, especially in the Old World, and bottle gourd is known from the recipes of the high-society Roman chef Apicius (Bakels and Jacomet 2003). In addition, seeds were sometimes eaten prehistorically in the Americas (Yarnell 1974). However, the bottle gourd’s global importance derives mainly from its usefulness in a wide variety of utilitarian applications (Heiser 1979).
3.3.2. Distribution and Systematics

The broad range of variation in modern bottle gourd cultivars and landraces is encompassed in two recognized subspecies (after Heiser 1973). The first, *L. siceraria* ssp. *siceraria*, likely derives from an African domestication event within approximately the last five thousand years (Richardson 1972; Decker-Walters et al. 2001). It contains nearly all modern varieties currently found in the New World, and the majority of African types. The second subspecies, *L. siceraria* ssp. *asiatica*, encompasses most known Eurasian varieties and a small proportion of extant New World gourds (Heiser 1973; Decker-Walters et al. 2001; Clarke et al.}

*Figure 1.2.* Top, Egyptian gourd rafts used on the Nile (Cooper 2010). Bottom left, archaeological fishnet and gourds from Peru (Hudson 2004). Bottom center, snake charmer playing a gourd *pungi* (public domain image). Bottom right, second generation fruit from seed of wild Zimbabwean *L. siceraria* (Decker-Walters et al. 2004).
2006; and see Chapter 3). This subspecies stems from one or more domestication events independent of ssp. *siceraria* in Eurasia or northern Africa as early as *ca.* 10,000 B.P. (Richardson 1972; Decker-Walters et al. 2001; Fuller et al. 2010a).

Only two apparently wild populations have been discovered, one near Lake Baringo in Kenya (Decker-Walters personal communication), and the other in southeastern Zimbabwe (Decker-Walters et al. 2004; Figure 1.2). The Zimbabwe population is almost certainly wild, carrying a primitive genetic profile distinct from known cultivars. The Kenyan example cannot be entirely ruled out as a cultivar escape, but its basal phylogenetic position to the African cultivar lineage suggests that it might be legitimately wild (see Chapter 3 for supporting data). In addition, the vast diversity and *in situ* evolution of landraces in Kenya suggest it as a likely source region for wild germplasm, and a possible site of domestication within Africa (Morimoto et al. 2005; Morimoto et al. 2006). These two wild populations could potentially represent relict members of a broader ancient distribution that has been heavily depleted over time. Because these are the only two known wild populations, and only the Zimbabwe gourds have been described in detail (Decker-Walters et al. 2004), comparative materials by which to study the biology of wild bottle gourds are very scarce. Accordingly, it has been difficult to establish morphological measurement thresholds to delineate domestic vs. wild specimens, which often creates uncertainty as to the domestication status of archaeological bottle gourds.

Molecular analyses have partially elucidated bottle gourd’s phylogenetic distribution and paths of dissemination. At least two independent domestication events corresponding to the two subspecies are widely accepted, and phylogenetic analyses tend to tightly group modern cultivars into the two distinct lineages described above (Decker-Walters et al. 2001; Erickson et al. 2005; Clarke et al. 2006; and see Chapter 3). Previous ancient DNA analysis has drawn a link between
ssp. *asiatica* and prehistoric American gourds, whereas modern American gourds almost invariably fall within ssp. *siceraria* (Erickson et al. 2005). This was interpreted as native gourd population displacement by newly introduced varieties following European contact (Erickson et al. 2005), but see Chapter 3 for further discussion.

Bottle gourds evolved in Africa, but enjoyed much broader pre-Columbian distribution and human use (Richardson 1972). In addition to their early presence in the New World, bottle gourds were used in Asia by ca. 11,000 B.P., Africa by ca. 5,000 B.P., and Polynesia by ca. A.D. 1,000 (Richardson 1972; Decker-Walters et al. 2001; Burtenshaw 2003; Matsui and Kanehara 2006; Fuller et al. 2010a). Like other Cucurbitaceae taxa, bottle gourd fruits are well adapted for long-distance water dispersal, and are known to maintain viable seeds for nearly a year while floating in saltwater (Whitaker and Carter 1954; Schaefer et al. 2009). A combination of oceanic drift and human-mediated dispersal may be responsible for bottle gourd’s broad ancient distribution (Heiser 1985; Decker-Walters et al. 2001; Erickson et al. 2005; Clarke et al. 2005), but many details of these dispersal events remain unclear.

### 3.3.3. Bottle Gourds in the Americas

The earliest archaeological bottle gourds in the New World are from Little Salt Spring in Florida, and Guila Naquitz in the Valley of Oaxaca, both dating directly to ca. 10,000 Cal. B.P. (Newsom and Gifford unpublished data; Whitaker and Cutler 1971; Erickson et al. 2005). These two examples are followed by scattered finds from Florida and Latin America during subsequent millennia (see Doran 2002, Table 1 for an extensive listing). In the Southeast, bottle gourd remains are sometimes recovered in Archaic deposits, most commonly in Florida but occasionally from Middle and Late Archaic sites in Missouri, Illinois, Kentucky, and Tennessee (Doran 2002). Toward the Early Woodland Period, bottle gourd shifts from being a sporadic
component of Eastern Woodlands archaeobotanical assemblages to a broadly distributed and widely utilized member of the region’s suite of pre-maize domestic plants (King 1985).

3.3.4. Competing Theories of Origin

It has long remained unclear whether bottle gourds in the New World originated in Africa or Asia, and whether the earliest New World gourds represent a domesticated or wild lineage. Initial molecular research showed that modern American gourd varieties cluster with modern African ones, lending support to the theory of an African origin (Decker-Walters et al. 2001). However, we now know that modern American varieties do not reflect the range of variation in their prehistoric counterparts (Erickson et al. 2005, and see Chapter 3), so comparisons with modern New World varieties are uninformative as to the complete history of prehistoric gourds.

If African gourds originally populated the Americas, they would have travelled on ocean currents and arrived in wild form, given that archaeological evidence in Africa suggests domestication only around 5,000 B.P. (Richardson 1972; Decker-Walters et al. 2001). Mature fruits could have made a trans-Atlantic journey on ocean currents via the North and South Equatorial currents, and then moving with the Gulf Stream, washed up with viable seeds virtually anywhere in the Caribbean islands, along the coastal Gulf of Mexico, or on the Atlantic coast of much of North and South America (Heiser 1985; and see Montenegro et al. 2006 for a detailed drift model involving intercontinental movement of boats). As inherently weedy “supertramp” taxa, bottle gourds could have proliferated in naturalized populations upon arrival for a substantial period prior to domestication, as suggested by Heiser (1985) and Pickersgill (personal communication cited in Flannery 1973). Alternatively, they might have been collected immediately upon arrival by humans realizing their potential. They could have been noted as useful due to their similarity with native *Cucurbita* sp. gourds, which are known from early sites
in both Mexico and Florida (Cutler and Whitaker 1961; Newsom et al. 1993; Smith 1997).

After discovery and use by humans, bottle gourds are likely to have persisted as useful weeds that were occasionally utilized and casually tended, and were eventually brought under domestication. Whitaker (personal communication, cited in Ferg 1977) describes the bottle gourd as a “camp follower,” an opportunistic weed that thrives in disturbed habitats and benefits symbiotically with humans, exchanging useful fruits for seed dispersal and favorable management. This description is consistent with the model for bottle gourds washing ashore, establishing naturalized populations with or without human assistance, and eventually being brought under domestication.

Even so, a trans-Pacific float via the Pacific Basin Equatorial Counter Current is plausible, and could be responsible for the early gourd remains from Central America and coastal South America (Erickson et al. 2005). Dispersal from Central America to Florida would then have been possible through the Gulf of Mexico or the Southeast Coastal Plain via natural or human-assisted dissemination. Early domesticated landraces from Asia, or free-living feral or wild individuals—reportedly present in Japan at ca. 11,000 Cal. B.P. (Fuller et al. 2010a)—could have seeded the ocean current with mature fruits that ferried their germplasm to the New World. It has also been suggested that Paleoindians traveling overland or along the southern Beringian coast might have introduced bottle gourds to the New World along with dogs, another domesticate kept mainly for reasons other than eating (Erickson et al. 2005). However, this seems unlikely in light of the growth requirements of bottle gourds and the inhospitable landscape traversed by Paleoindians colonizing the New World near the end of the Pleistocene, and the likely protracted duration of migration through Beringia (e.g. Fagundes et al. 2008).

It is possible that bottle gourds entered the New World via multiple pathways, human or
natural, and could have been domesticated more than once independently in the Americas. Bottle gourds’ propensity for oceanic drift and their early pantropical distribution mark them as a classic “supertramp” taxon (Diamond 1974), highly resilient and capable of long-distance dispersal and colonization. Their weedy growth habit and attractiveness to humans as natural containers increase their adaptive potential in anthropogenic ecosystems, and we already know of two independent domestication events worldwide. It is conceivable that some combination of the models described above is responsible for the gourds’ distribution and usage in the Americas.

3.3.5. Study and Results Summarized

In Part 2, I use targeted high-throughput sequencing to generate whole long single copy (LSC) region sequences from the plastid genome (86kb) for 38 broadly-sampled modern bottle gourd landraces and cultivars, two putative wild accessions of $L. sicera$ria from Kenya and Zimbabwe, and one accession of $L. sphaerica$. I also extract DNA and sequence target regions in nine archaeological bottle gourd rinds from the New World. Coverage in ancient accessions ranges from 10% to 96% of the total LSC, but is sufficient to characterize their positions relative to modern populations, and to draw conclusions regarding their origins in the New World.

I conclude that prehistoric New World bottle gourds were not derived from either extant cultivar lineage. Instead, they appear to reflect ancestral variation that is unknown in modern populations, and that evolved in Africa before direct dissemination to the New World, most likely via trans-atlantic drift. I conclude that domestic forms arose during one or more independent domestication events in the Americas. I conclude further, based on modern evidence from Argentina and several Pacific islands, that the Eurasian lineage of gourds might have also reached the New World in prehistoric times via contact between Polynesia (or the greater South Pacific) and South America, but was probably not widely cultivated in the
3.4. **Archaeological Phytolith Analysis**

In numerous plant taxa, monosilicic acid in the soil and groundwater is brought into the plant’s tissues via the xylem (primary water-conductive tissue) (Simpson and Volcani 1981), a process which we are coming to understand often involves specialized transporter proteins to control the movement and allocation (Ma et al. 2006, 2007; Chiba et al. 2009; Mitani et al. 2009, 2011; Mitani-Ueno et al. 2011). In its soluble form, silica acts as a broad-spectrum signaling molecule involved in mobilizing adaptive responses to a wide range of biotic and abiotic stressors (Fauteux et al. 2005, 2006). Furthermore, silica is polymerized in and between plant cells in a wide range of tissues to form tough silica accretions (Cseke and Kaufman 1999; Simpson and Volcani 1981). These are thought to confer a mechanical defense or physical barrier against herbivory, as the glassy structures strengthen organic tissues and abrade the mouthparts and digestive anatomy of herbivores (Cseke and Kaufman 1999; Epstein 2009; Evert 2006), and also have been shown to form effective barriers against fungal hyphae (Samuels et al. 1991; see Chapter 4 for more on silica deposition and bioactivity).

These polymerized silica structures are often collectively called phytoliths, especially in paleobotanical and archaeobotanical usage. They are very durable under the normal heat and moisture fluctuations of deposits in surface sites—although, high pH values typical of shell deposits, for example, may compromise phytolith preservation (Piperno 2006; Rovner 1983)—and so can often be recovered from archaeological bulk sediments or artifact surfaces. Phytoliths can also sometimes be recovered from human and animal dental calculus (Armitage 1975; Henry et al. 2011), and from ceramic pastes (Bishop et al. 1982). Phytoliths form in such a way that their surface morphology reflects the anatomy of adjacent cellular features, and so they often
carry some diagnostic potential (Pearsall 1982; Rovner 1983; Piperno 2006; Figure 1.3). Their durability and taxonomic value have drawn considerable attention from archaeobotanists over roughly the past three decades, during which time phytolith analysis has become a frequent component of archaeobotanical investigations (see Piperno 2009 for a review). Phytoliths are used to infer paleoecology, site use dynamics and formation processes, plant domestication, and more, especially at sites where conditions are not conducive to macrobotanical preservation (e.g. Ball et al. 1999; Piperno and Stothert 2003; Piperno 2006, 2009; Piperno et al. 2009; Strömberg 2004; Tsartsidou et al. 2008; Whang et al. 1998; Zhang et al. 2010).

3.4.1. Critiques of Phytolith Analysis

The taxonomic resolution of phytolith morphology has been a point of debate (e.g. Rovner 2004; and see Shillito 2012 for a review of current debates). Some researchers prefer a very conservative usage, relying upon phytolith assemblages for gross biome or ecosystem reconstruction only (e.g. Strömberg 2004; Bremond et al. 2008), while others routinely assign phytoliths to the species level and below, and draw distinctions between the phytoliths of domesticated plants and their wild progenitors (e.g. Pearsall et al. 2003; Piperno et al. 2009).

Figure 1.3. A sample of phytolith types indicative of cellular origin, from http://geo.arizona.edu
Two central arguments are used to question high-resolution phytolith assignments: First, it is relatively recently that phytoliths have been used as taxonomic identifiers, and as such, our knowledge of the range of phytolith forms and variations across plant taxa is incomplete. Therefore, it may be problematic to assign archaeological phytoliths to a given taxon without knowledge of the full range of plausible phytolith forms produced across the plant community at a given site (see Rovner 2004). This is especially concerning when phytoliths of important domesticated plants are reported at sites where closely related taxa are present (common for the grass [Poaceae] and legume [Fabaceae] families, for example). Without knowledge of an area’s background phytolith composition in the past and present, there may be a tendency to shoehorn phytolith classifications into a limited range of taxa at the erroneous exclusion of plausible alternatives.

Second, it can also be argued that intra-specific phytolith variation is not adequately understood in most taxa to make determinations based on subtle differences in phytolith forms. That is, it may be premature in many cases to use variations in the forms of a specific phytolith type in a single species to assess, for example, the domestication status of the represented plants. In several taxa, including maize (Zea mays L.), rice (Oryza sativa L.), and gourd/squash (Cucurbita s L.), phytoliths have been used to infer domestication.

In maize, a typology based on so-called “rondel” phytoliths is sometimes used to discriminate between domesticated maize, wild teosinte, and non-Zea wild grasses (Pearsall et al. 2003). However, Rovner (2004) suggests that this typology is fatally flawed due to poor experimental design and execution. He offers a credible argument that the typology represents the “reduction of a range of complex morphological variation to superficial, artificial, arbitrary and subjective ‘types’ based on a few qualitative, visually prominent attributes,” rendering it
unreliable. Nonetheless, rondel phytolith morphotypes, as delineated by Pearsall and others (2003, 2004), are still used as a signal for maize domestication in contexts lacking macrobotanical preservation (e.g. Piperno et al. 2009).

A similar methodology is used to discriminate wild from domesticated rice, as well as paddy vs. dry field cultivation, and the japonica vs. indica subspecies, based on morphological analysis of two key phytolith types. Fujiwara (1993) argues that phytoliths derived from bulliform cells—specialized storage cells involved with water storage, and previously believed to control leaf movement in response to water stress—are informative in this regard, especially as indicators of dry vs. wet field cultivation. However, it could be argued that insufficient data have been gathered on variation in bulliform phytolith morphology driven by normal intra- and inter-seasonal climatic trends, for example, and that without additional background data, researchers might infer specific cultural phenomena due to an inadequate understanding of natural variation.

Zhao and others (1998) believe that the “double-peaked glume” phytolith—derived from one of the subtending bracts at the base of the spikelet in grasses—is useful in separating wild from domestic Oryza s. However, their analysis relies heavily on statistical manipulations that would not be possible given a phytolith assemblage of unknown composition, and arguably, fail to meet a reasonable standard of reliability. In fact, Fuller and others (2010b) argue that there is sufficient “variation and overlap amongst modern rices to make this [phytolith type] an unreliable separator in many cases.”

Finally, many members of the gourd family, Cucurbitaceae, produce large, characteristic “scalloped” phytoliths in the outer mesocarp—the hard outer layer of the rind (e.g. Bozarth 1987; Piperno et al. 2002; Piperno and Stothert 2003; Figure 1.4). These are reportedly diagnostic to
the genus level in many cases, and some researchers attempt species-level assignments among
*Cucurbita* s (although these have been based on geographic and ecological inference rather than
morphological characters themselves [Piperno et al. 2009]). Furthermore, scalloped phytolith
size and surface characteristics have recently been used as indicators of prehistoric gourds’

domestication status (Piperno and Stothert 2003; Piperno et al. 2009). Piperno and Stothert
(2003) demonstrate that domestic *Cucurbita* gourds produce larger phytoliths, on average, than
their wild counterparts. This seems to be largely the effect of the increase in fruit size that tends
to accompany domestication, a trait that is closely correlated with scalloped phytolith size
(Piperno and Stothert 2003). They define overall and sample-average maximum phytolith
dimensions among wild plants, specimens above which are considered to represent domesticated
plants.

**Figure 1.4.** Wild-type *C. pepo* var. *texana*. Left, mature fruit. Top right, extracted scalloped phytoliths.
Bottom right, pistillate flower with pollinator.
Inferring domestication from *Cucurbita* phytoliths is currently problematic for several reasons. As with any plant structure, it is difficult to establish reliable size thresholds that signal domestication, and care must be taken to avoid misidentifying wild plants as domestic in the archaeological record. Phytolith size, like other continuous variables, is subject to numerous influences of genetics and the growth environment, and varies dramatically even within a single fruit (Kistler unpublished data). Growth conditions and potential confounding ecological factors have not been considered in the studies relying on scalloped phytolith size as an indicator of domestication—or in the vast majority of archaeological phytolith studies—leaving considerable room for doubt that the normal range of variation has been accounted for. In the study described in Chapter 4, in fact, a small proportion of the phytoliths that I measured, all from wild-type plants, exceeded the suggested 100 um threshold for domestication.

In addition, an inherent problem is posed by the fact that the modern reference materials used to establish domestication size thresholds are often many millennia removed from their archaeological counterparts. While a size threshold may capture a realistic baseline for discriminating modern domesticates from wild plants, it is unwise to assume biological constancy through archaeological time.

In *Cucurbita* spp., for example, the wild antecedents of two important domestic taxa, *C. pepo* ssp. *pepo* and *C. moschata*, are unknown and possibly extinct (Nee 1990; Ferriol and Picó 2004; Sanjur et al. 2002). In *C. pepo*, a large, contiguous population likely spanned from the Eastern Woodlands into Central America, a wild southern member of which gave rise to the ssp. *pepo* cultivar lineage some 10,000 years ago (Decker-Walters et al. 2001; Smith 1997). This population is now represented by only a few relict groups, and no extant progenitor of *C. pepo* ssp. *pepo* has been discovered. In the case of *C. moschata*, it seems likely that a circum-
Caribbean distribution gave rise to domestic lineages in more than one locale, and subsequent habitat changes led to the extinction of wild populations not buffeted against new selective pressures by the adaptive advantage of cultivation. In addition, *Cucurbita* s were apparently evolved for dispersal by extinct megafauna, as inferred by zoologists (Janzen and Martin 1982; Barlow 2000) and evidenced by *Cucurbita* seeds in Pleistocene Mastodon dung from Florida (Newsom and Mihlbachler 2006). This goes to illustrate that in *Cucurbita* s, there are a number of profound events in their recent natural history that could have had meaningful consequences for their phytolith morphology. Therefore, it is misguided to equate a modern set of reference materials with an archaeological assemblage for the sake of establishing size thresholds for domestication.

Furthermore, a recent study uses qualitative morphological characters, such as “surface cavities and marks” and “faint scalloped impressions,” to infer *Cucurbita* domestication from archaeological scalloped phytoliths (Piperno et al. 2009). Piperno and others (2002, 2009) argue that these features result from selection on a genetic locus linked to rind lignification and silicification (see below), and are indicative of breeding for a softer rind in *Cucurbita* under domestication for food. These criteria remain unquantified, unscrutinized, and highly subjective, and are not suitable for use with archaeological materials without rigorous controlled evaluation of their diagnostic potential. In addition, use of these characters assumes selection for an edible fruit, whereas early *Cucurbita* in many areas was used for utilitarian applications, not food (Fritz 1999; King 1985; Newsom et al. 1993).

### 3.4.2. The Genetic Basis for Phytolith Development

Recently, Ma and others (2006, 2007) described a series of genes in rice (*Low silicon rice, Lsi* genes) responsible for active transport and allocation of soluble silica, constituting the
first direct insight into the molecular genetic basis for silica movement. Subsequently, homologous genes have been described in maize (ZmLsi) and Cucurbita (CmLsi), shedding light on the specific mechanisms responsible for silica transport in a number of economic plants (Mitani et al. 2009, 2011; Mitani-Ueno et al. 2011). Furthermore, a microarray-based transcriptome analysis in Arabidopsis identified a very large number of genes whose regulation was affected by the presence or absence of soluble silica under pathogenic stress (Fauteux et al. 2006). That is, silica had no appreciable effect under ideal growth conditions, but became very active when plants were infected with powdery mildew disease and mounted a systemic defense response. This supports other research indicating soluble silica’s role as a multipurpose signaling molecule that is integral in mediating biotic and abiotic stressors (Epstein 2009; Cooke and Leishman 2011).

Although we are beginning to understand the molecular genetic basis for soluble silica movement and bioactivity, there are no studies to date that have addressed similar mechanisms for silica polymerization and, specifically, phytolith formation. However, Piperno and others (2002; 2006) have argued for specific genetic bases for phytolith development in maize and Cucurbita spp.

In Cucurbita spp., scalloped phytolith production and rind sclerification—programmed cell death and heavy lignification in the outer mesocarp to strengthen the rind—have been shown to closely co-vary, and appear to be regulated by an upstream element that acts as single Mendelian locus described by breeders are the Hard rind (Hr) gene (Mains 1950; Piperno et al. 2002). Piperno and others (2002) refer to dominant (tough, lignified rind with abundant phytoliths) and recessive (softer, pliable rind lacking phytoliths) alleles, but the intermediate heterozygous phenotype that they describe indicates a level of codominance.
Piperno and others (2002) use the activity of \( Hr \) to argue for close genetic control over many aspects of phytolith production, ultimately invoking its activity as validation that subtle morphological variables are reliable, informative characteristics. This argument is faulty, in that we know this locus to be linked to the presence or absence of scalloped phytoliths, not their morphology. If, upon further genetic research, \( Hr \) proves to be a single gene, it is probably an upstream regulatory element in a genetic hierarchy controlling lignification, silicification, and other functions—a sort of “on-off switch” for a range of cellular functions—and is likely several steps removed from the actual deposition of silica and development of the surrounding cells. \( Hr \) could also represent one or more genomic regions exhibiting linkage disequilibrium due to shared selection for rind strength (i.e. separate loci related to sclerification and silica deposition at which alleles for specific phenotypic traits covary more than they would if recombined at random).

In any case, phytolith morphology is the product of a complex range of physiological processes and the anatomical characters of nearby cells, and it is profoundly unlikely that a single gene controls both the presence/absence of phytoliths and their morphological characters on a diagnostic level. In short, the argument that \( Hr \) represents a meaningful genetic basis for scalloped phytolith minute morphology is untenable.

Similarly, Piperno (2006) argues that maize cob phytolith development is under the control of a gene called \textit{Teosinte glume architecture 1} (\textit{Tga1}), a variant of which played a critical role in maize domestication by eliminating the tough outer tissue surrounding each seed of wild teosinte—a glume and internode modified by heavy lignification and impregnated with silica—making the grain accessible to humans (Dorweiler et al. 1993). This gene has been identified as an upstream transcriptional regulator in a developmental cascade of functions related to teosinte...
fruit maturation, including silicification of the cupulate fruitcase (Wang et al. 2005). Piperno and others (2009) argue that “tga1 underwrites the phytolith traits in maize and teosinte fruits that distinguish them from one another,” again validating the diagnostic value of subtle variation in phytolith morphological characters by invoking genetic control. However, Tga1 can only be shown to regulate a suite of developmental functions that are ultimately responsible for cell morphology and silica deposition, not to directly control the minute morphological characters of phytoliths. As with Hr, the action of Tga1 is simply too far removed from phytolith formation to make the argument that it controls phytolith morphology in a meaningful way.

Finally, if genes that directly control phytolith morphology are identified, this in no way means that morphology is not highly variable within a species (or a population, individual, or single organ). Normal variation and phenotypic plasticity have received very little attention in the development of phytolith typologies, indices, and measurement thresholds, in spite of the bioactivity of silica and the effects of external variables at all stages of plant growth and development. Arguments that point to the genetic basis for phytolith development have tended to lose sight of the fact that the environment, along with genetics, is a critical component of the developmental equation. Phytoliths in ancient deposits have outstanding informative potential, but considerable research is in order to explicate the effects of external variables on phytolith formation across a broad range of taxa.

3.4.3. Study and Results Summarized

In Chapter 4, I describe a study conducted to investigate the effects of mosaic virus and bacterial wilt disease on diagnostic scalloped phytoliths in the rind of a wild-type gourd. We observed a minimal shift in phytolith size distribution between control plants and individuals with mosaic virus. However, we observed a notable difference between plants with bacterial wilt
disease and control plants, with diseased individuals carrying a greater proportion of large-diameter scalloped phytoliths. This and similar phenomena could potentially confound archaeological interpretations from phytolith assemblages, especially in cases where a continuous variable such as size is used to infer a process like plant domestication. I suggest that the effects of this and other ecological variables should be studied in a diverse range of taxa. In this capacity, it may be possible to strengthen archaeological phytolith analyses by quantitatively integrating background information on the normal variables affecting phytolith morphology in order to mitigate the effects of confounding ecological variables.

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Chapter 2: Ancient DNA Confirms a Local Origin of Domesticated Chenopod in Eastern North America

Chapter Abstract

Domesticated chenopod was an important starchy seed crop in eastern North America before the rise of maize agriculture. Domesticated chenopod first appeared in North America during the fourth millennium B.P., but its wild progenitor and site of domestication remain unresolved. Archaeological evidence suggests a local domestication in the Eastern Woodlands, while morphological similarities with modern Mexican cultivars indicate a possible introduction from Mesoamerica. To distinguish between these two scenarios, we isolate chloroplast DNA (cpDNA) from modern and archaeological North American chenopods sampled from across their geographic range. Our results demonstrate that the chenopod grown in the Eastern Woodlands was locally derived, indicating that independent domestication events gave rise to the ancient eastern North American and modern Mexican cultivars. These results strengthen the argument for an entirely native pre-maize crop complex with chenopod as a major component.

Keywords: Chenopodium, Plant Domestication, Ancient DNA, Eastern North America
1. Introduction

Chenopod (*Chenopodium berlandieri* Moq.), a weedy relative of quinoa and spinach, was an extremely important starchy seed crop in North America from ca. 1,800 B.C. until ca. A.D. 900 or later, when maize-based agricultural intensification began throughout much of the Southeast (Smith, 1989, Smith and Yarnell, 2009). During this period, chenopod was grown from modern-day Arkansas to eastern Kentucky, at latitudes from northern Alabama to central Ohio (Cowan, 1979, Fritz, 1984, Fritz and Smith, 1988, Smith, 1984). In some regions it persisted along with other pre-maize seed crops well after maize was established as the primary staple in the midcontinent (Fritz and Lopinot, 2007, Simon and Parker, 2006). Its cultivation helped facilitate population growth and human colonization of new parts of the landscape, which affected shifts in population pressure, political organization, and other important features of prehistoric society. Its modern and archaeological distributions, morphological characters, ecosystem dynamics, and prehistoric usage in the Eastern Woodlands have been extensively studied during the past several decades (Asch and Asch 1977; Asch and Asch 1985; Cowan 1979, 1985; Fritz, 1984, Fritz, 1990, Fritz and Smith, 1988, Gremillion, 1993a, Gremillion, 1993b, Gremillion, 1998, Smith, 1984, Smith, 1987, Smith and Cowan, 1987, Smith and Yarnell, 2009, Yarnell, 1974). However, it has remained unclear whether chenopod was domesticated locally in the Eastern Woodlands or introduced from Mesoamerica, where morphologically identical chenopods are cultivated today (Wilson, 1981).

Chenopod seeds of two different domesticated variants are found archaeologically in the Eastern Woodlands. One is darker in color and has a reduced testa, or outer seed-coat, and has been formally classified as an extinct cultivar, *C. berlandieri* ssp. *jonesianum* (Smith and Funk, 1985). The other is pale or horn colored because it lacks the typical hard, black, outer epidermal...
layer (Wilson, 1981), and has not been formally described as a novel taxon. The earliest known domesticated chenopods were recovered from the Riverton Site in southeastern Illinois, where pale and dark variants were both found dating to ca. 1,800 B.C. Only very low level wild-type chenopod use is known from sites pre-dating Riverton (Asch and Asch, 1985).

The eastern origin hypothesis suggests that both eastern variants were derived from local *C. berlandieri* (Smith, 1987, Smith, 2006, Smith and Yarnell, 2009). Proponents of the eastern origin hypothesis argue that there is no archaeological evidence for domestic chenopod use in Mexico prior to historic times, and suggest that the Mexican crops are the result of an independent and much later domestication event (Smith, 2006, Smith and Yarnell, 2009). Furthermore, there is no evidence for contact between Mesoamerica and eastern North America until well after the appearance of chenopod in the latter.

Alternatively, chenopod may have been introduced into North America following domestication in Mesoamerica (Wilson, 1981, Wilson, 1990). According to this hypothesis, the two modern Mexican cultivars, *huazontle* (or *huauzontle*) and *chia* (both *C. berlandieri* ssp. *nuttalliae* [Saff.] H.D. Wilson & Heiser), were introduced to eastern North America by the early fourth millennium B.P., when they first appear archaeologically (Smith and Cowan, 1987, Smith and Yarnell, 2009, Wilson, 1990). The Mexican origin hypothesis is rooted in the morphological similarity between the ancient eastern cultivars and the two modern Mexican cultivars: *Chia* seeds are essentially indistinguishable from ancient dark morph seeds (Wilson, 1990), and an articulated pale morph inflorescence with seeds from the Holman rockshelter in Arkansas was morphologically assigned as *huazontle* (Wilson, 1981). Once established in North America, *chia* and *huazontle* may have formed part of the basis for eastern agriculture as the dark and pale morph eastern chenopods, respectively.
An eastern origin seems more parsimonious than a Mexican origin considering the archaeological record, which reveals chenopod in wild, weedy, and domesticated forms in the midcontinent. The eastern archaeological record, however, does not preclude a Mesoamerican introduction. Early use of wild-type chenopod in eastern North America (Asch and Asch, 1985, Hollenbach and Walker, 2010) followed by the appearance of domesticates could reflect early low-level exploitation of a local variety preceding widespread displacement by Mexican cultivars.

To test between the two domestication hypotheses, we generate non-coding chloroplast DNA (cpDNA) sequences from modern and ancient samples that represent both wild and domestic chenopods from throughout the U.S. and Mexico. Using these data, we characterize genetic variation among several subordinate taxa of *C. berlandieri*, and determine which potential parent population most likely gave rise to the early domesticated chenopod in eastern North America.

### 2. Materials and Methods

#### 2.1. Sample Collection

We sampled modern chenopod across as broad a geographic range as possible, so as to include representatives of all available wild and domestic North American subtaxa within *C. berlandieri* and to capture any regional variation (Table 2.1; Figure 2.1). Wild *C. berlandieri* from eastern North America and domesticated *C. berlandieri ssp. nuttalliae* from Mexico represent the two potential progenitor populations of the prehistoric crop. In addition to these, we included other wild *C. berlandieri* subtaxa. This enabled a more thorough reconstruction of the evolutionary history of chenopod, and assessment of relationship between the ancient
cultivars and the wild samples. We tested ancient chenopod seeds from three archaeological rockshelter sites in the Eastern Woodlands, spanning a temporal period from the earliest known usage in the fourth millennium B.P. to late prehistoric times. We included archaeological representatives of both pale and dark morph specimens.

We processed 35 modern chenopod samples from throughout the U.S. and Mexico (Figure 2.1, Table 2.1).

We analyzed specimens from the following taxa (sensu the USDA PLANTS database [http://plants.usda.gov]):

*Chenopodium berlandieri* is the potential wild progenitor in eastern North America, and includes, in this sample, free-living eastern populations of *C. berlandieri* var. *berlandieri* and *C. berlandieri* ssp. *bushianum*. These are two readily interfertile eastern varieties that often occur sympatrically in the Eastern Woodlands. We processed nineteen *C. berlandieri* seed samples from throughout the eastern U.S.

### Table 2.1.

<table>
<thead>
<tr>
<th>Modern taxon</th>
<th>Sampling location</th>
<th>Haplotype</th>
<th>Acc. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. berlandieri</em>, ENA</td>
<td>Calhoun Co., IL</td>
<td>A</td>
<td>PI 608030$^a$</td>
</tr>
<tr>
<td><em>C. berlandieri</em>, ENA</td>
<td>Cherokee Co., SC</td>
<td>B</td>
<td>82A$^b$</td>
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<td><em>C. berlandieri</em>, ENA</td>
<td>Cullman Co., AL</td>
<td>B</td>
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</tr>
<tr>
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<td>Fulton Co., PA</td>
<td>B</td>
<td>40$^e$</td>
</tr>
<tr>
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<td>Mississippi Co., AR</td>
<td>B</td>
<td>57$^f$</td>
</tr>
<tr>
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<td>Mississippi Co., MO</td>
<td>B</td>
<td>96$^g$</td>
</tr>
<tr>
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<td>B</td>
<td>94A$^h$</td>
</tr>
<tr>
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<td>B</td>
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</tr>
<tr>
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<td>Mississippi Co., MO</td>
<td>B</td>
<td>95A$^j$</td>
</tr>
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<td>95C$^k$</td>
</tr>
<tr>
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<td>Pike Co., OH</td>
<td>B</td>
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<tr>
<td><em>C. berlandieri</em>, ENA</td>
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<td>B</td>
<td>92E$^m$</td>
</tr>
<tr>
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<td>B</td>
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</tr>
<tr>
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<td>Washington Co., MD</td>
<td>B</td>
<td>89B$^o$</td>
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<td>B</td>
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<tr>
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<td>B</td>
<td>90C-3$^q$</td>
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<tr>
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<td>Colima, Mexico</td>
<td>C2</td>
<td>668$^r$</td>
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<tr>
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<td>C2</td>
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<td>C3</td>
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<td>C3</td>
<td>PL 433230$^w$</td>
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<td>C1</td>
<td>878$^x$</td>
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<tr>
<td><em>C. b. ssp. zschachei</em></td>
<td>Fremont Co., ID</td>
<td>C1</td>
<td>622$^y$</td>
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<td>Fremont Co., WY</td>
<td>C1</td>
<td>862$^z$</td>
</tr>
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<td>Jackson Co., CO</td>
<td>C1</td>
<td>637$^{aa}$</td>
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<tr>
<td><em>C. b. ssp. zschachei</em></td>
<td>Utah Co., UT</td>
<td>C1</td>
<td>882$^{ab}$</td>
</tr>
<tr>
<td><em>C. b. var. sinuatum</em></td>
<td>Wasatch Co., UT</td>
<td>A</td>
<td>AMES 27372$^{ac}$</td>
</tr>
<tr>
<td><em>C. b. var. sinuatum</em></td>
<td>Grant Co., NM</td>
<td>B</td>
<td>845$^{ad}$</td>
</tr>
<tr>
<td><em>C. b. var. sinuatum</em></td>
<td>Apache Co., AZ</td>
<td>C1</td>
<td>874$^{ae}$</td>
</tr>
<tr>
<td><em>C. b. var. sinuatum</em></td>
<td>Coconino Co., AZ</td>
<td>C1</td>
<td>865$^{af}$</td>
</tr>
<tr>
<td><em>C. album</em></td>
<td>Centre Co., PA</td>
<td>A</td>
<td>C.a.2$^{ag}$</td>
</tr>
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</table>

### Ancient sample

<table>
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<tr>
<th>Site</th>
<th>Haplotype</th>
<th>Estimated age</th>
</tr>
</thead>
<tbody>
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<td>Cloudsplitter</td>
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<td>B</td>
</tr>
<tr>
<td>Haystack</td>
<td>Powell Co., KY</td>
<td>B</td>
</tr>
<tr>
<td>Holman</td>
<td>Madison Co., AR</td>
<td>B</td>
</tr>
</tbody>
</table>

$^a$-USDA NPGS.
$^b$-Bruce Smith, Smithsonian Institution.
$^c$-Eric Jellen, Brigham Young University.
$^d$-collected by Logan Kistler.
C. berlandieri. ssp. nuttalliae is the potential Mexican progenitor to the prehistoric eastern cultivars, containing both huazontle and chia (Wilson, 1981). We analyzed six seeds, all huazontle collected in Mexico. C. berlandieri ssp. zschackei and C. berlandieri var. sinuatum are plains/western and southwestern U.S. wild types, respectively, with C. berlandieri var. sinuatum ranging into Mexico (Wilson and Heiser, 1979).

We tested five seeds of ssp. zschackei and four of var. sinuatum from various western and southwestern locations. Finally, to provide an outgroup, we collected a single fresh C. album leaf sample in central Pennsylvania.

To be suitable for DNA analysis, ancient samples must be preserved by processes that retain the organic material and protect the fragile DNA molecules. Desiccation in dry caves and rockshelters is the primary method by which this occurs in the southeastern U.S. Three previously excavated rockshelter assemblages containing desiccated chenopod remains were selected for analysis (Table 1; Figure 2.1):

The Cloudsplitter shelter in Menifee County, Kentucky, along with the nearby Newt Kash Hollow shelter, contains the earliest directly dated thin-testa chenopods in the Eastern Woodlands. Seeds from these sites were directly dated to the early fourth millennium B.P. (Smith and Cowan, 1987), making them contemporaneous with the Riverton assemblage (Smith...
and Yarnell, 2009). We sampled well-preserved seeds from the Cloudsplitter assemblage housed at the William S. Webb Museum of Anthropology, University of Kentucky.

The Late Woodland (ca. A.D. 200-A.D. 900) Haystack shelter in Powell County, Kentucky, yielded several seeds of both the thin-testa and pale variants. Occupation at Haystack ranged from ca. A.D. 400-700, when pre-maize agriculture was well-established (Cowan, 1979). Dr. Kris Gremillion of The Ohio State University provided seeds from this site for analysis.

Finally, the Holman shelter is an Ozark Bluff Dweller site in Madison County, Arkansas, with occupation dating to ca. A.D. 1,100 (Fritz, 1984, Fritz and Smith, 1988). This site yielded one of only a small number of articulated chenopod inflorescences, complete with pale seeds, from the Eastern Woodlands (Wilson, 1981). This specimen is remarkably preserved, and beautifully shows the compaction and terminalization of the fruiting structure associated with seed crop domestication. As indicated above, this specimen has been assigned previously as huazontle based on morphology (Wilson, 1981), but other researchers have suggested that it instead represents a distinct lineage native to the Eastern Woodlands (Smith and Yarnell, 2009). The University Museum at the University of Arkansas provided seeds from this specimen for analysis.

2.2. DNA amplification and sequencing

We selected chloroplast DNA intergenic spacers for analysis. Certain chloroplast regions have a rapid rate of evolution in comparison to other genetic loci, and are informative in studies of closely related plant taxa (Shaw, et al., 2005, Shaw, et al., 2007). Chloroplast DNA is also present in greater copy-number than nuclear loci, and therefore has a significantly improved chance of survival in ancient materials. Finally, like mitochondrial DNA, cpDNA is maternally inherited in most plant taxa (Schlumbaum, et al., 2008), which simplifies the phylogenetic
Using a small number of modern samples, we screened eight cpDNA intergenic spacer regions (Shaw, et al., 2005, Shaw, et al., 2007) for polymorphisms fixed between the two potential parent populations. Of these, trnQ-5’rps16 proved the most informative, and offered the best clustering of informative sites to target using new primers in ancient accessions. This spacer is located on the long single copy region of the chloroplast genome, and was shown to provide high phylogenetic resolution within several divergent angiosperm lineages (Shaw, et al., 2007). The trnQ-5’rps16 spacer of 697 bp (aligned length) was sequenced in all modern samples (Genbank Accession no. JN646817-JN646851).

As expected, the target fragment length of this primer pair was too large for successful amplification from the ancient specimens. We therefore designed new primers using PrimerSelect (DNASTAR Inc., Madison, WI) to target the most informative short regions in trnQ-5’rps16. Two new primer sets were used to amplify adjacent regions, resulting in a total amplified product of 284 bp, excluding primers (Genbank Accession no. JN646814-JN646816, total aligned length is 291 bp due to length polymorphisms). This fragment contained four SNPs that were fixed between Mexican cultivars and eastern North American wild-types.

We extracted DNA from modern single seeds following a modified CTAB protocol (Doyle and Doyle, 1987). We incubated seeds in 500 µL of CTAB extraction buffer with 0.02 g polyvinylpyrrolidone and 2.5 µL β-mercaptoethanol at 55°C for one hour, agitating them periodically. We then ground seeds in the buffer using pellet pestles, adding small amounts of sterile sand as necessary to assist with grinding. We added 700 µL chloroform, centrifuged, and isolated the aqueous phase. We incubated this on ice with 0.08 volumes 7.5 M ammonium acetate and 0.54 volumes of isopropanol for 45 minutes. The mixture was centrifuged and
supernatant was discarded, and DNA was washed once with 70% ethanol and once with 95% ethanol, then allowed to dry completely. DNA was resuspended overnight in Low TE buffer. We extracted DNA from archaeological seeds using QiagenDNEasy Plant Mini kits [Qiagen, Valencia, CA] according to the manufacturer’s protocol, except that seeds were ground in the kit’s buffer AP1 using pellet pestles before the initial incubation period. DNA was eluted in 100 µL Qiagen buffer AE. We observed severe PCR inhibition in dark morph seeds, probably caused by lignin and/or other compounds in the epidermal tissue. To counteract this, the testa was removed from dark morph specimens and DNA was extracted directly from the seeds’ internal tissues.

For modern seeds, we PCR-amplified the trnQ-5’rps16 spacer using primers trnQ (5’-GCG TGG CCA AGY GGT AAG GC-3’) and rps16x1 (5’-GTT GCT TTY TAC CAC ATC GTT T-3’) (Shaw, et al., 2007). Amplification took place in 25 µL reactions comprising: 2.5mM MgCl2, 75 µM each dNTP, 0.8 µM each primer, 0.1 ng BSA, 1.25 units taq DNA polymerase, and 2 µL template DNA. We used the rpl16 PCR program designed by Shaw and others (2005) with an optimized annealing temperature of 63 °C.

We used the two newly designed primer pairs to target informative regions in trnQ-5’rps16: trnQA(5’-TCA TCC CGG CAA AGA AMG T-3’) and rps16A (5’-ATC CCT AAG AAA TAC AAA TCC AT-3’); and trnQB (5’-TCA AAT GAA AGG AAG ATA AGT GTT-3’) and rps16B (5’-CCG GGA TGA ATA AAA AAA KAA CTA-3’). Amplification took place in 25 µL reactions comprising, for trnQA-rps16A: 2 mM MgSO4, 250 μM each dNTP, 1 µM each primer, 50 µg RSA, 1.25 units PLATINUM taq DNA polymerase, and 11.5 µL template DNA. For trnQB-rps16B, MgSO4 was decreased to 1.5 mM and template DNA was increased to 12 µL. We found that using a large amount of template DNA from ancient seeds improved PCR product
yield and decreased non-specific amplification. We used the following PCR program for both primer pairs: A two-minute denaturation step at 94 °C was followed by 45 cycles of 45 second denaturation at 94°C, 45 second annealing at 59 °C for the A primers and 48 °C for the B primers, 90 seconds extension at 68 °C, and a five-minute final extension step at 68°C. PCR using the A primers more frequently produced a product suitable for sequencing, and both fragments could be amplified in one accession from each site.

Amplified PCR products were cleaned using Exo-Sap IT, and underwent chain-termination sequencing at The Pennsylvania State University Genomics Core Facility. Sequences were assembled and chromatograms checked by eye using CodonCode Aligner [CodonCode Corp., Dedham, MA].

During each experimental phase, we implemented standard protocols designed to avoid and detect contamination by modern DNA sources (Gilbert, et al., 2005). DNA isolation and PCR preparation using ancient samples was carried out in a dedicated, sterile ancient DNA facility at The Pennsylvania State University in a building containing no PCR facilities that is geographically isolated from all modern molecular biology research. All work surfaces were cleaned with bleach and ethanol before and after each use. Protective suits, masks, gloves, and footwear were worn at all times, and no reagents, samples, or other materials were carried into the ancient DNA lab from a building containing PCR facilities. Following PCR preparation, we performed all downstream work in the Anthropological Genetics Lab at The Pennsylvania State University. Control negatives lacking tissue or template DNA were included during isolation and PCR to detect any contamination, and multiple PCR products were sequenced from each archaeological context. Finally, we cloned one PCR product representing each of the two amplicons in each of the three archaeological sites using an Invitrogen TOPO TA Cloning Kit.
[Invitrogen Corporation, Carlsbad, CA], following Millipore cleanup [Millipore, Billerica, MA]. From each product, between two and eight clones were sequenced to assess damage and contamination in ancient samples.

2.3. Phylogenetic Analysis

We aligned the sequences using ClustalW (Larkin, et al., 2007), and checked the alignment by eye. We trimmed the resulting data set to include only the two regions amplified from the ancient specimens. To assess the evolutionary relationships between the isolated sequences, we first produced a median-joining network using Network v. 4.516 (Bandelt, et al., 1999) with default settings. We then constructed maximum likelihood (ML) and maximum parsimony (MP) trees using PhyML v. 2.0.2 (Guindon and Gascuel, 2003) and PAUP* v. 4.0b10 (Swofford 2003), both with and without assuming C. album as outgroup. C. album proved to be an unsuitable outgroup due to incomplete lineage sorting, so Beta vulgaris (common beet, GenBank Accession no. EF534108) was used instead. For the ML analysis, we used the F81 nucleotide substitution model, which was selected by ModelTest (Posada and Crandall, 1998) as the best-fitting evolutionary model. Starting trees were generated by NJ, followed by an heuristic search with NNI branch swapping. Clade support was assessed via 1000 bootstrap replicates generated using parameters as for the full analysis.

For the MP analysis, we used PAUP* to perform an heuristic search and construct a tree with minimum total branch length. 1000 bootstrap replicates were again analyzed to assess clade support.

3. Results

All modern samples were successfully processed with minimal experimental optimization. For the ancient specimens, we isolated DNA suitable for PCR amplification and
sequencing from 12 of 44 single seed samples. In all cases, replicate PCRs, which necessarily begin from different template molecules, produced consistent results. We detected low levels of DNA damage and environmental contamination during PCR product cloning, but clone sequences at informative sites were always consistent with directly sequenced PCR products.

ML and MP analyses result in similar, well-supported phylogenies with identical branching order (Figure 2.2). Modern samples cluster into three distinct clades with little variation within each clade: The domesticated Mexican samples and most wild eastern North American samples fall into two distinct clades separated by four single nucleotide polymorphisms (SNPs). One eastern specimen carries a third, distinct haplotype. All ancient samples carry the eastern haplotype. Median-joining network analysis supports the close evolutionary relationship between the ancient samples and the modern eastern populations (Figure 2.2).

4. Discussion

Our results demonstrate that chenopod was locally domesticated in eastern North America from native wild populations independent of the cultivated Mexican lineage. Ancient seeds of both pale and dark variants carry the same cpDNA haplotype as modern wild chenopods from throughout the eastern U.S. Because cpDNA is inherited only via maternal germplasm, these results cannot be attributed to native wild chenopod pollination of introduced cultivars.

However, given that a progenitor population is expected to carry greater overall genetic diversity than its domesticated subset, the modern wild plants might represent feral derivatives of the ancient crop. This is not likely to be true, since we sampled modern chenopods from outside the known range of prehistoric use, including materials from Pennsylvania, Maryland, and South
Figure 2.2. a): Median-joining network from a 291 bp alignment of the trnQ-rps16x1 spacer in modern and ancient samples. Circles represent unique haplotypes, and circle size is proportional to the number of individuals carrying each haplotype. Colors indicate the samples carrying each haplotype, and slices are proportional to the number of specimens represented. The length of lines connecting the haplotypes is not proportional to genetic distance, and all lines except where noted otherwise represent a single mutation. Internal connections between lines represent putative ancestral states. 
b): Tree showing branching order from ML and MP analyses. Trees were estimated using Beta vulgaris as an outgroup; this branch has been removed here. Bootstrap support values are given for ML (above line) and MP (italics, below line) analyses. Ancient specimens from all three sites carry the characteristic haplotype of wild chenopods in eastern North America. Two specimens, one each of C. berlandieri and C. b. var. sinuatum, cluster with C. album.
Carolina. This sampling helps control for the possibility of feral populations, assuming that domesticated chenopods would not have spread prolifically and displaced local wild varieties outside their range of cultivation. Further, the use of wild chenopod at ca. 6,000 B.C. in Illinois and even earlier in Alabama (Asch and Asch, 1985, Hollenbach and Walker, 2010) suggests that native chenopod populations significantly pre-date cultivation. The broadly-sampled modern materials from eastern North America therefore most likely represent native, wild populations, rather than naturalized escaped cultivars.

Interestingly, the chenopod varieties analyzed here do not cluster into haplotype- or population-specific taxonomic clades. One specimen each of eastern North American *C. berlandieri* and southwestern *C. b. var. sinuatum* clustered with *C. album*, the intended outgroup, and one other specimen of *C. b. var. sinuatum* carried the eastern haplotype, demonstrating incomplete sorting of haplotypes among recognized taxa. This suggests that, while the cpDNA locus selected for this analysis is evolving quickly, the divergence between the sampled varieties has occurred too recently to be resolved using these data alone. Sharing of chloroplast haplotype lineages between recognized taxa is known to occur in other angiosperms (Serrano-Serrano, et al., 2010, Vrancken, et al., 2009). Given the recent divergence between North American chenopods (Wilson and Heiser, 1979), our observation of such partitioning is not particularly surprising. However, the two hypothesized parent populations of the prehistoric eastern crop carry distinct cpDNA haplotypes and are reliably distinguishable in our phylogeny. The observed incomplete lineage sorting makes it impossible to rule out a low level of haplotype sharing between these two populations. However, our results demonstrate dominance of the respective haplotypes in the two groups.

The pre-maize eastern North American agricultural complex consisted of at least four
domesticated plants, as well as a handful of others that were cultivated but show no morphological signs of domestication. Those that were domesticated include chenopod, sunflower, sumpweed, and gourd/squash (Cucurbita pepo L.) (Fritz, 1990, Fritz, 1995, Smith, 2006). Reportedly cultivated plants whose archaeological remains show no recognized signs of domestication include maygrass (Phalaris caroliniana Walter), knotweed (Polygonum erectum L.), and little barley (Hordeum pusillum Nutt.) (Fritz, 1995, Smith, 2006). Chenopod and these presumably non-domesticated plants were used for their starchy seeds, sunflower and sumpweed for their oily seeds, and squash for its seeds and flesh. Along with recent genetic and archaeological literature on sunflower, sumpweed, and gourd/squash (Cucurbita pepo L.) (Asch and Asch, 1985, Cowan, 1997, Crites, 1993, Decker-Walters, et al., 1993, Harter, et al., 2004, Wills and Burke, 2006), our findings here provide compelling support for the development of an entirely indigenous agricultural complex in eastern North America. The crop complex developed from a diverse group of weedy taxa within the context of a mainly hunting and gathering lifestyle, and came to dominate Eastern Woodland archaeobotanical assemblages during the Woodland Period (Milner, 2004, Smith and Yarnell, 2009, Yarnell, 1974).

We have also demonstrated a third independent domestication within the genus Chenopodium in the New World, including two within the species C. berlandieri, along with the C. b. ssp. nuttalliae complex in Mexico and C. quinoa in South America. In the future, it might be informative to identify genetic loci associated with the derived morphological characters of domesticated chenopods in Mexico, and conduct additional analysis of ancient eastern North American samples using more extensive DNA capture and sequencing techniques to compare the molecular evolutionary mechanisms of domestication.

Our results agree with previous work on other taxa suggesting that the food crops
cultivated in the Eastern Woodlands prior to the rise of maize agriculture were locally-derived products of long-term interactions between the area’s plants and people. Probing the ecological and anthropological histories of individual economic taxa via molecular, archaeological, and ethnohistoric investigation is central to our understanding of the complex dynamics of ancient food production systems.

References


Chapter 3: Bottle Gourds in the Americas: New Molecular Data Reveal African Origin and New World Domestication

Chapter Abstract

Bottle gourd (Lagenaria siceraria) was one of the world’s earliest and most broadly distributed crop plants. It evolved in Africa, but its robust fruits are known archaeologically in eastern Asia by ca. 11,000 B.P., and in the Neotropics by ca. 10,000 B.P. Following bottle gourd’s early archaeological appearance in the Americas, it enjoyed broad New World distribution and use throughout the Holocene. Bottle gourd fruits serve as perfect natural containers, fishnet floats, and other utilitarian items, and bottle gourd was brought under domestication more than once in diverse world regions because of its usefulness and versatility. Modern cultivars form two accepted subspecies widely thought to originate from two independent domestication events. However, the origins of prehistoric bottle gourds in the Americas are poorly understood. Competing theories include trans-oceanic drift from Africa or Asia, or introduction by Paleoindians entering the New World during the Late Pleistocene. We use targeted high-throughput DNA sequencing and analysis of diverse modern cultivars and wild specimens, as well as archaeological gourds from North, Central, and South America, to assess the most likely model of origin for bottle gourds in the New World. We conclude that wild gourds probably traveled on ocean currents from Africa, established naturalized populations, and were brought under cultivation and independent domestication on one or more occasions in the Americas.

Keywords: Lagenaria, Bottle Gourd, Plant Domestication, Ancient DNA, Archaeology
1. Introduction

Bottle gourd (*Lagenaria siceraria*, Cucurbitaceae) was probably the most broadly distributed plant species under cultivation and use by humans around the globe in pre-Columbian times (Richardson 1972; Heiser 1989). Unique suites of food crops emerged from local flora under human selection in several regions of independent plant domestication worldwide (e.g. Bellwood 2005), but bottle gourd crosscut many of these separate food production systems in diverse world-regions. Gourds were also widely used by groups practicing no recognizable forms of agriculture, a fact that remains true today in parts of the world (e.g. Cambie and Ferguson 2003; Doran et al. 1990; Heiser 1979). Bottle gourd’s cosmopolitan nature among prehistoric cultures can be credited to two specific traits: its inherent usefulness to humans (e.g. Heiser 1979)—especially those lacking ceramic technology—and its weedy propensity for long-distance dispersal and colonization of new habitats, including those disturbed by human activity (Ferg 1977). However, many specific details regarding its dispersal out of its native Africa to other parts of the world are unclear. Its movement into the Americas, in particular, has been a point of debate (see Figure 1). Oceanic drift from Africa to the Neotropics is one plausible model (Camp 1954; Whitaker and Carter 1954; Cutler and Whitaker 1961; Heiser 1985; Doran et al. 2000; Decker-Walters et al. 2001), while an Asian origin via trans-Pacific drift or human introduction has also been suggested (Erickson et al. 2005).

Bottle gourd—which refers both to the taxon and its characteristic fruits—is an herbaceous annual plant that produces long, climbing vines bearing large globular, elongated, or variously lobed fruits (Heiser 1973). Very few wild gourd populations have ever been encountered (Decker-Walters et al. 2004), so our understanding of their morphology and growth habits outside of human symbiosis is limited. However, wild gourds recently discovered in
Zimbabwe are described as producing roughly spherical fruits of 8.5 – 9 cm in diameter (just smaller than a softball) and relatively thin rinds compared with their cultivated counterparts (Decker-Walters et al. 2004). However, Deena Decker-Walters (personal communication) suspects that the thin, brittle rind characteristics in this wild population could be related to poor growth conditions or a short growing season. As the fruits mature, the outer tissues of the rind become heavily lignified, creating a lightweight and durable housing for their seeds. The wild fruits and seeds closely resemble those of *L. sphaerica*, a congeneric and interfertile taxon (Decker-Walters et al. 2004). Bottle gourd fruits, like many other members of the Cucurbitaceae, are well adapted for both dispersal by large mammals and long-distance water movement (Barlow 2000; Janzen and Martin 1982; Whitaker and Carter 1954; Gunn and Dennis 1976; Schaefer et al. 2009). Numerous speciation events within the family have been attributed.

**Figure 3.1.** Hypothesized migration routes of bottle gourds into the Americas: trans-Atlantic drift, trans-Pacific drift, and Beringian human introduction. Yellow points: modern accessions used in this study. Blue points: ancient samples used in this study.
to intercontinental dispersal via oceanic drift (Schaefer et al. 2009), and bottle gourds have been experimentally shown to maintain viable seeds while floating in seawater for nearly a year (Whitaker and Carter 1954).

Modern domesticated bottle gourds are divided into two subspecies: *L. siceraria* ssp. *siceraria*, which consists mainly of African and New World varieties, and *L. siceraria* ssp. *asiatica*, characterized by Eurasian cultivars (Heiser 1973). Domesticated populations contain a tremendous array of fruit morphological diversity borne out of selection for a wide variety of uses (e.g. Heiser 1973; 1979). Fruits range from quite small gourds used as flasks and rattles to enormous cultivars exceeding a meter in length for elongated examples and 70cm in diameter for spheroid fruits. A thickened, durable rind is often a desirable trait for humans, and domestic gourd rinds range from a few millimeters to an impressive 27mm reported in one Hawaiian cultivar called *ipu nui* (Eames and St. John 1943).

True to their common name, bottle gourds make exceptional natural containers, being durable, lightweight, and watertight. They are also commonly used as naturally resonant sound chambers for a wide variety of wind, string, and percussion instruments (Heiser 1979), and virtually all parts of the plant have supposed medicinal value (Yang and Walters 1992; Moerman 1998). The buoyancy that confers the gourds’ capacity for long-distance water dispersal has long been recognized in Egypt, where bottle gourds are used with bundled reeds to construct traditional rafts for use on the Nile (Cooper 2010). Comparably, gourds are sometimes used as fishnet floats, a practice that is known archaeologically from Florida and coastal Peru, and continues today in the latter (Cutler 1975; Hudson 2004). The remarkably tough gourd rind has even been used to repair skull fractures and trepanation wounds in at least two independent parts of the world (Bandelier 1904; Durham 1923; Heiser 1979).
The fruits can be eaten if harvested when they are soft and immature, and bottle gourd is an extraordinarily productive crop, yielding up to thirty-six tons of fruit per hectare when harvested for food (TNAU 2008). Growing bottle gourds specifically for food is historically most common in parts of Europe and Asia, but eating gourds is a widespread, if low frequency phenomenon (Heiser 1979). Erickson and others (2005) draw an interesting comparison between bottle gourds and dogs as two “utilitarian species,” unusual taxa whose primary roles under domestication are for purposes other than food. Dogs provide companionship, hunting assistance, and protection, and gourds fulfill a wide array of utilitarian functions. Both are eaten, but neither is primarily a food crop in most of its range. Interestingly, they are likely the most widely distributed domestic animal and plant taxa, respectively, of the pre-Columbian world.

In addition to fruit morphological diversity, bottle gourd seeds exhibit a level of variation rivaled by very few cultigen species—namely maize (Zea mays), and possibly common beans (Phaseolus vulgaris) or sunflowers (Helianthus annuus) (Figure 2.2). The smallest seeds among numerous varieties currently in our possession are approximately four by eight millimeters, exemplified by a cultivar from Papua New Guinea, while one Zambian variety bears huge seeds of up to 15x27mm (accessions Ls6d and Ls4G in this study, respectively, see Appendix Table A.1). In addition to the size differential, the seeds of domesticated gourds are often ornamented with features described as “wings” and “ears,” plus changes in coloration, surface texture, and pubescence (Heiser 1973; Figure 2.2). Since the fruit as a whole, and not the seed, is usually the target of selection by humans, this wide range of seed diversity likely stems from a substantial amount of genetic drift and neutral evolution under the relaxed selective constraints of cultivation.

In the New World, bottle gourds appear archaeologically in Florida at ca. 10,000 cal. B.P.
Figure 3.2. Seed morphological diversity in modern bottle gourds. 1 – New World varieties; 2 – Europe, Asia, South Pacific; 3 – Africa; 4 – variation within a single landrace from Zambia. Black box indicates wild specimens: a – *L. sphaerica*, South Africa; b – *L. siceraria*, Kenya; c – *L. siceraria*, Zimbabwe.
(Newsom and Gifford unpublished data; Table 1) and in Mexico slightly later (Erickson et al. 2005). They were used in South America by the ninth millennium B.P. at Quebrada Jaguay, and they occur regularly in subsequent Central and South American contexts (Erickson et al. 2005; Doran 2002). In North America, several of the earliest bottle gourd remains are from wet sites in Florida; at Little Salt Spring, Windover, and Salt Springs (Doran et al. 1990; Newsom lab unpublished data). The earliest directly dated specimen north of Florida is from the fifth millennium B.P. at Alred Shelter, an Ozark Bluff-Dweller site in Arkansas (Table 1; Fritz 1986), and a roughly contemporaneous deposit at the Phillips Springs site in Missouri produced several gourd seeds and rind fragments (Kay et al. 1980). During the latter half of the Holocene, bottle gourds occur with increasing ubiquity throughout the Americas (see Table 1 in Doran 2002).

The seeds were sometimes eaten (Yarnell 1974), but this seems secondary to their use as containers and in other utilitarian applications.

In spite of their archaeological frequency, it is still uncertain how bottle gourds came to populate the Americas, having evolved in Africa (Whitaker 1971; Richardson 1972; Heiser 1979; Doran et al. 1990; Decker-Walters et al. 2001). Initial molecular research suggested a direct African origin, drawing links between modern African and New World cultivars (Decker-Walters et al. 2001). This would have occurred by oceanic drift of gourds from coastal Africa to the Neotropics, where they may have been collected immediately and cultivated by humans, or formed stable naturalized populations that eventually came under use and cultivation.

However, we now know that modern genetic diversity in the New World does not reflect the full range of pre-Columbian variation (Erickson et al. 2005; and see below). A previous ancient DNA-based study found that three genetic markers in the plastid genome could be used to discriminate between modern Asian and African cultivars, and after sequencing these regions
in a broad sample of archaeological specimens, concluded that the Asian lineage was the predecessor of all pre-Columbian New World gourds (Erickson et al. 2005). However, the possibility remains that prehistoric American gourds align with the Eurasian lineage due to shared ancestry out of Africa, not direct derivation of the former from the latter, and higher-resolution analysis is needed.

In this study, we revisit the question of whether prehistoric American gourds originated in Africa or Asia, and we attempt to discern whether gourds were independently domesticated on one or more occasions in the New World. We use targeted high-throughput sequencing to generate a dataset comprising a large section of the plastid genome from a diverse sample of modern gourds and a selection of archaeological specimens from throughout the Americas, and we use this dataset to test competing hypotheses regarding the origins of bottle gourds in the New World.

Table 3.1. Archaeological gourd fragments used in this study with AMS dates obtained from the same specimens. Calibration was in Calib 6.1.0 using IntCal09. Superscript letters after Acc. No. indicate the source of the material.

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>Acc. No.</th>
<th>AMS Lab #</th>
<th>Age RCYBP</th>
<th>Cal. Age (2σ)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loreto Cave</td>
<td>Baja California, Mex</td>
<td>3-12793a</td>
<td>Beta-316171</td>
<td>80 +/- 30 BP</td>
<td>AD 1690-1925</td>
</tr>
<tr>
<td>Putnam Shelter</td>
<td>Washington County, AR</td>
<td>32-44-396c</td>
<td>Beta-316173</td>
<td>870 +/- 30 BP</td>
<td>AD 1045-1244</td>
</tr>
<tr>
<td>Tularosa Cave</td>
<td>Catron County, NM</td>
<td>A246294c</td>
<td>Beta-316172</td>
<td>1120 +/- 30 BP</td>
<td>AD 824-994</td>
</tr>
<tr>
<td>Spring Branch Shelter</td>
<td>McCreary County, KY</td>
<td>aLsF2d</td>
<td>Beta-316174</td>
<td>1910 +/- 30 BP</td>
<td>AD 21-210</td>
</tr>
<tr>
<td>El Gigante</td>
<td>La Paz, Honduras</td>
<td>18-13b.3e</td>
<td>Beta-316169</td>
<td>2110 +/- 30 BP</td>
<td>203-46 BC</td>
</tr>
<tr>
<td>Alfred Shelter</td>
<td>Benton County, AR</td>
<td>32-4-1176b</td>
<td>Beta-316170</td>
<td>3850 +/- 30 BP</td>
<td>2459-2206 BC</td>
</tr>
<tr>
<td>Quebrada Jauuyay</td>
<td>Arequipa, Peru</td>
<td>S1-U4-PA-N1f</td>
<td>Beta-134112*</td>
<td>7650 +/- 50 BP</td>
<td>6594-6431 BC</td>
</tr>
<tr>
<td>Guila Naquitz</td>
<td>Oaxaca, Mexico</td>
<td>E10-B2e</td>
<td>Beta-97237*</td>
<td>7940 +/- 60 BP</td>
<td>7043-6679 BC</td>
</tr>
<tr>
<td>Little Salt Spring</td>
<td>Sarasota County, FL</td>
<td>1408551A01e</td>
<td>Beta-261466**</td>
<td>8890 +/- 50 BP</td>
<td>8241-7832 BC</td>
</tr>
</tbody>
</table>

- Phoebe A. Hearst Museum of Anthropology, University of California Berkeley
- University Museum at the University of Arkansas
- National Museum of Natural History, Smithsonian Institution
- Kentucky Archaeological Survey/US Forest Service
- The Pennsylvania State University, Department of Anthropology
- Dates acquired by Erickson and others (2005) and provided by BD Smith (personal communication)
- Date acquired by LA Newsom and JA Gifford (unpublished data)
- Calibrated ages exclude any outlier ranges containing less than 1% of the 2σ distribution.
2. Results

In all modern gourds, we targeted and successfully sequenced the long single copy (LSC) region of the plastid genome, totaling approximately 86kb, at ample depth (see methods below; see Figure 3.1 for sample distribution and Appendix Table 1 modern sample details). In addition, we successfully recovered and sequenced plastid DNA ranging from 10 to 96 percent of the complete LSC in nine archaeological gourd rind samples from the New World (Table 1; Figure 3). We have excluded ancient samples with less than 50 percent coverage of the LSC (n=4) until we are able to conduct additional sequencing in these accessions.

Phlogenetic analysis revealed two well-supported clades corresponding to the *asiatica* and *siceraria* subspecies (Figure 4). In addition to most of the Eurasian cultivars, the *asiatica* clade encompasses samples from Polynesia and the greater South Pacific, and a single sample from Argentina. The *asiatica* clade forms a sister group to one cultivar from Ethiopia. The *siceraria* clade contains modern African and New World cultivars, as expected, and also a sample each from Israel and Greece, extending the range of this group into the Mediterranean.

![Figure 3.3. Sites yielding archaeological bottle gourds used in this study. Map outline after Erickson et al. (2005).](image-url)
region, where it overlaps the distribution of ssp. *asiatica*. The wild Zimbabwe population discussed above falls outside the two subspecific clades, indicating that it represents ancestral variation that was excluded from extant cultivar populations.

**Figure 3.4.** Bayesian MCC tree showing phylogeny estimated using whole plastid LSC sequences. See methods, below, for details of the analysis. Archaeological specimens appear in blue. Posterior clade support greater than 0.70 is given, and the two recognized subspecies are indicated. Full sample details corresponding with lab numbers can be found in the Appendix, Table A.1.

The archaeological New World specimens carry considerable variation relative to their modern counterparts, and they do not cluster within a single lineage. The Alred and El Gigante specimens, along with the wild Zimbabwe gourd, fall outside of the large clade containing all other modern and ancient samples. The Little Salt Spring gourd subtends the *asiatica* clade, along with a modern cultivar from Ethiopia. The Loreto and Tularosa Cave samples fall within the *siceraria* clade. None of the ancient samples fall within the *asiatica* clade, indicating that
they did not derive from the lineage carrying extant Eurasian cultivars. We artificially fixed ancient and Eurasian gourds together in assorted combinations as monophyletic clades during analysis, and found no statistical support for any such relationship based on our data. These findings strongly suggest that we should reject an Asian origin via human introduction or trans-Pacific float (but see below on a possible South Pacific connection).

Initial phylogeographic analysis has indicated that three of the ancient samples represent populations that are likely to have dispersed to the Americas directly from Africa. The other two, the Loreto and Tularosa Cave specimens, fall within the well-supported siceraria clade that contains a mix of extant African and New World cultivars, again strongly indicating African origin. Interestingly, Erickson and others (2005) had similar findings with a post-contact specimen from Mexico resembling modern African and New World varieties, which they attributed to introduction following European arrival in the Americas. However, the Tularosa Cave specimen predates contact by several hundred years (Table 1), indicating that members of the siceraria subspecies were present prehistorically, and cannot be entirely attributed to historic introduction.

Post-contact introduction of additional gourds to the New World is likely, especially given that African slaves in the New World were known to cultivate gourds in house gardens and use them in agricultural tasks and other purposes (e.g. Carney and Rosomoff 2009). No direct reference could be found to gourds crossing the Atlantic with slaves, but other plants made the journey and were propagated extensively in the Americas (see Carney and Rosomoff 2009). However, the Tularosa Cave specimen demonstrates that historic introduction and population displacement may not be solely responsible for modern ssp. siceraria cultivars in the Americas.
3. Discussion

Our results indicate that bottle gourds entered the New World from Africa, most likely by way of a persistent pattern of oceanic drift, and that they were independently domesticated in the Americas. We were unable to replicate previous findings of an Asian origin, which we can attribute to shared ancestry in Africa between the *asiatica* lineage and New World gourds.

3.1. A Model of Oceanic Dispersal

The level of genetic diversity in the New World does not suggest an isolated occurrence of trans-oceanic dispersal resulting in a severe bottleneck among colonizing gourds. Under such circumstances, we would expect gourds in the Americas to represent a small subset of the diversity in Africa, and to carry relatively invariant plastid haplotypes forming a monophyletic lineage. We find, however, that ancient gourds in the Americas represent a range of ancestral diversity, much of which has not survived to the present in domestic populations, and in fact may now be lost altogether due to the scarcity of wild gourds in Africa.

If prehistoric American gourds formed a distinct lineage at the exclusion of modern haplotypes, the best interpretation might be early colonization of the New World by a small population of gourds, followed by extensive diversification over a long timescale within the Americas. However, because the prehistoric haplotypes fall in several locations throughout the phylogeny—including within the *siceraria* clade in the case of the Tularosa specimen—the diversity in the New World seems to mirror an extensive subset of diversity originating in Africa rather than a monophyletic American lineage derived from a small founder group. That is, many gourds representing an array of diversity seem to have made the Atlantic crossing, and to have become established successfully in the Americas.

The specific timescale of these dispersals is unknowable based on our data, as a
molecular clock estimate can only be used to infer the timing of diversification, not migration events. Migration events could theoretically have occurred at any time subsequent to estimated divergence from modern African gourds. For example, an American haplotype sharing a common ancestor at 10kya with a modern African cultivar must have migrated to the New World at a point later than the split at 10,000 B.P.

However, the long branch lengths of several of the ancient samples indicate deep divergence from extant haplotypes. Enforcing a plastid substitution rate for the Cucurbitaceae developed by Schaefer and others (2009) of $1 \times 10^{-9}$ substitutions per site per year, all ancient haplotypes in the Americas likely diverged from African counterparts during the Pleistocene. This holds true even when doubling the substitution rate, which may not capture the quickly evolving intergenic spacer regions that carry many of the informative sites in our alignment. Interestingly, the New World subclade within the *siceraria* group containing the Tularosa and Loreto Cave gourds shares a common ancestor with a Zimbabwe cultivar at ca. 19-48kya (using the conservatively slow $1 \times 10^{-9}$ rate), indicating that oceanic drift was occurring at least as recently as the Late Pleistocene. Unfortunately, a more accurate rate estimate is needed to refine these dates, and we do not have enough information to calibrate a molecular clock rate for our dataset.

Our data suggest a pattern of trans-Atlantic drift persisting until at least the Late Pleistocene that that led to a cumulative abundance of diversity in the Americas, ultimately mirroring a sizeable subset of the variation evolved in Africa. This may have occurred over a short timescale or a fairly protracted one, depending on climatic and ecological conditions in the Americas and Africa, the physiological and ecological behavior of *Lagenaria* when it occupied a more sizeable niche and carried greater diversity within Africa, and the nature of ocean currents.
A previous study dealing with hypothetical trans-oceanic movement of boats revealed that when set adrift in parts of Africa without power or navigation, vessels can have a startlingly high probability of reaching the Neotropics (up to 13%, [Montenegro et al. 2006]). Additional simulation-based modeling of oceanic drift may help elucidate likely ingress and egress points, and provide some estimates as to, for example, the quantities of gourds that could potentially make the trip, assuming certain population and ecological parameters.

Having reached the Americas, gourds likely established naturalized wild populations in the Neotropics. The alternative model is that they were opportunistically collected and immediately cultivated by humans upon arrival, implying that they only ever proliferated in the New World under human care. However, the former hypothesis is more consistent with gourds’ early broad archaeological distribution and genetic diversity in the Americas. That is, initial propagation by humans would likely introduce a much more profound genetic bottleneck and limited geographic range than would the proliferation of naturalized populations over some period of time preceding human contact. Wild gourds are unknown in the modern Neotropics, but Heiser (1979) points out that by comparison, they seem close to extinction in the wild in their native Africa as well. It seems likely that Holocene climatic and ecological changes—for example, warming and drying coupled with extinction of several large mammal dispersers—may have severely impacted wild gourd populations in both hemispheres, leading to local extinction events and extensive loss of diversity. Ultimately, however, previous discussions of bottle gourds having an ancient pantropical distribution seem fairly accurate (Harris 1967; Richardson 1972; Flannery 1973; Heiser 1989; Decker-Walters et al. 2001, 2004).

### 3.2. Gourd Domestication Within the Americas

Sometime following their colonization of the New World, bottle gourds’ inherent
usefulness became apparent to humans, who began to utilize them as containers and other utilitarian items, and also possibly as food (Cutler and Whitaker 1961; Yarnell 1974; Doran et al. 1990). The first evidence for bottle gourd use occurs at ca. 10,000 B.P. in Florida, and shortly thereafter in Central and South America (Table 1). We might suggest that bottle gourds were domesticated early in one region and subsequently dispersed. However, our data do not support this model, under which we would expect restricted genetic diversity indicating a single domestication bottleneck. In addition, the fundamental assumptions regarding selection pressures on a food crop might be inappropriately applied with a utilitarian taxon such as bottle gourd, especially in light of its weedy “camp follower” niche and inherent usefulness to humans (Ferg 1977; Heiser 1979).

It seems plausible that virtually anywhere in the New World where gourds and humans co-existed, people could have been using gourds and subtly influencing their biology on small regional scales. The recent work of Allaby and others (2010) and Fuller and others (2011) suggests that a more protracted and geographically diffuse model of domestication is often more realistic than discrete and rapid domestication events, even when molecular data may be ambiguous. This seems likely in the case of gourd domestication in the Americas. The most plausible scenario may involve a level of cultivation or other population management in multiple regions throughout the gourd’s ancient Neotropical distribution, followed by selection for fruits better suited to the needs of humans, and ultimately, the emergence of one or more lineages carrying morphological hallmarks of domestication.

Our data do not indicate where and when morphologically domesticated gourds might have arisen. Central America is one likely candidate, with a tropical climate and plenty of potential for genetic diversity. Also, several domesticated plant taxa originated in Mesoamerica,
including, notably, multiple species of *Cucurbita* gourds and squashes that emerged as cultigens beginning at *ca.* 10,000 B.P. (e.g. Flannery 1973; Bellwood 2005; Smith 1997a). It is reasonable that bottle gourd domestication may have been most likely to occur against a cultural backdrop of crop domestication at large, and Cucurbitaceous plant cultivation and domestication in particular. South America represents a very similar situation, with plant domestication beginning as early as 10,000 B.P., and at least one species of *Cucurbita*—and possibly up to two more—domesticated in the tropical lowlands (Sanjur et al. 2002).

A third plausible region of gourd domestication is North America outside of Mexico, namely Florida and the Eastern Woodlands. The earliest and most abundant gourd remains in the New World are from Archaic deposits in Florida, and gourds were used across the peninsula from 10,000 B.P. to the historic period (see Table 1; Doran et al. 1990; Newsom and Scarry 2012). Recent work at the Archaic Period Salt Springs site has yielded at least one provisionally identified bottle gourd seed that is appreciably larger than numerous other examples from the same and other Florida sites (Newsom lab unpublished data). This could be an isolated anomaly, but it could also indicate the early release of selective constraints on seed size under human cultivation.

Gourds might have been disseminated by humans from Florida to the midcontinent, as suggested by Doran and others (1990), carrying either a wild-type or domesticated morphology. The earliest midcontinental gourds at Phillips Spring and Alred Shelter, in fact, do not show obvious rind thickening or seed evolution exemplifying domestication (Fritz 1986; Kay et al. 1980; but see below on domestication criteria). It is conceivable that if gourds populating the midcontinent out of Florida appeared more-or-less morphologically wild, intensive selection and cultivar improvement for the large, thick-rinded examples seen by the Early Woodland Period at
Mammoth Cave, for example (Kistler, unpublished data), was temporally and culturally linked with the emergence of other domesticated taxa in the region (Fritz 1990, 1995; Smith 1989, 2006). It is also worth investigating any possible link between gourds in the midcontinent and northern Mexico, where bottle gourds were used in the Ocampo Caves by the seventh millennium B.P. (Whitaker et al. 1957; Smith 1997b).

Any or all of these broad regions are valid candidates for the emergence of morphologically domesticated gourds, and ongoing quantitative morphometric research will hopefully provide additional insight. It seems likely that morphologically domesticated gourds emerged in multiple locales throughout the Americas.

3.3. Morphological Criteria for Domestication

With only a single wild population of *L. siceraria* fully described, it is very difficult to develop criteria by which to assign archaeological materials as wild or domestic. Wild seeds are in the size range of domestic ones (4x10 mm – 5x13 mm, [Kistler unpublished data, Decker-Walters et al. 2004]), so that while it may be possible to securely identify a very large seed as domestic, we cannot assume a relatively small one is wild. In addition, seeds are not typically the targets of selection in bottle gourd domestication, and so we have no predictive model for the effects of domestication on the evolution of seed morphology in gourds.

A two-millimeter rind thickness threshold has been proposed to indicate domestication (Erickson et al. 2005), but this may also be problematic. First, no measurements of wild *L. siceraria* rind thickness have been published, meaning that we do not have any direct data on normal variation in wild populations. The threshold is based on three fruits of wild *L. abyssinica*, plus several *Cucurbita* spp. wild gourds (Erickson et al. 2005), but a better model for the extent of variation might be an extensive set of measurements from *L. abyssinica* and *L.*
sphaerica from across a range of habitat and growth conditions. Second, range contraction, climate change, and the extinction of a number of potential large mammal dispersal agents leading up to and during the Holocene make modern Cucurbitaceous populations a questionable morphological analog for their archaeological counterparts. That is, the brittle rind described in the Zimbabwe wild gourds could be a vestige of the ancestral variation, representing the thin end of the spectrum that was best adapted for dispersal without megafaunal mammals, for example.

Finally, Loreto Cave, a historic period site in Mexico, contains numerous gourd fragments with rind thickness ranging from 1.5 to 8mm (Whitaker 1957). Thus, the assemblage represents an almost certainly domestic population spanning the 2mm threshold. Likewise, the Archaic Period Salt Spring assemblage from Florida contains abundant rind fragments ranging from 1.2 to 3.65 mm (Newsom lab, unpublished data), and the large bottle gourd assemblage from Romero’s Cave in Tamaulipas, Mexico, contains a wide distribution of thickness measures, including a range of 1.76 to 4.78 mm in a single occupation layer (Smith 1997; Whitaker et al. 1957). It is impractical to arbitrarily assign those below 2 mm as wild and those above as domestic in a single population. In addition, the trans-oceanic float by which gourds apparently colonized the New World might have biased founder populations with selection toward thick-rinded fruits that would have been more likely to survive the trip intact. All of these issues deserve attention, and the 2 mm threshold should probably be reconsidered.

In total, discriminating wild from domestic examples of a given taxon is fundamentally problematic with such limited comparative wild material available. It is impossible to understand the full range of variation, and even more difficult given that modern wild gourds contain a fraction of their ancestral genetic and, presumably, morphological variation. One worthwhile approach may be extensive morphological and histological study of L. sphaerica,
possibly the best taxonomic analog with an appreciable extant distribution.

Another possibility is to assess whether we can treat certain rich archaeobotanical gourd assemblages—from Florida or East Asia, for example—as wild populations for the sake of studying wild-type morphological variation. It may be possible, for example, to use modern cultivars to determine an expected minimum variance in quantitative seed morphological characters in domestic populations (after e.g. Newsom et al. 1993; Newsom and Scarry 2012), given that relaxed selective constraints under domestication can be expected to lead to morphological diversification. It may then be possible to determine with some level of confidence if archaeological gourd assemblages might represent wild populations. This and similar techniques may ultimately prove useful in tracing the specific origins of domesticated gourd phenotypes in the Americas and elsewhere.

3.4. **A Polynesian Connection?**

Finally, it deserves attention that a single Argentinian specimen in our sample—a landrace collected by Charles Heiser—falls squarely within the ssp. *asiatica* lineage characterized by modern gourds from throughout much of Europe, Asia, and the South Pacific. Only one other New World specimen tested to date appears to carry plastid haplotype consistent with ssp. *asiatica*, a Brazilian sample also collected by Heiser (Clarke et al. 2006).

These landraces could represent an independent and early colonization of the New World prehistorically from Asia, as previously suggested to account for bottle gourds’ American origins (Erickson et al, 2005). While a Beringian origin seems unlikely on the basis of plant physiological restrictions and the Pleistocene climate (Clarke 2009), trans-pacific float is a viable model for an Asian origin (Ebbesmeyer and Ingraham 1994; Montenegro et al. 2006). Our molecular data from ancient New World gourds give no indication that this took place, but
additional sampling could reveal an important missing link. It is also possible that these South American *asiatica* samples represent historic introduction of Eurasian germplasm to South America. Alternatively, it is conceivable that these varieties represent members of an ancient broad distribution of the *asiatica* subspecies across Africa, members of which were transmitted to the Americas along with other haplotypes within the *siceraria* group, as well as outside of these two recognized subspecies.

Another possibility involves the much-debated prospect of contact—and botanical exchange—between prehistoric South Americans and seafaring Polynesians (see Jones and Storey 2011 for an extensive review). Some of the most compelling evidence for such contact involves linguistic connections, especially in the case of the sweet potato, which goes by *kumara* and several cognates in both regions (reviewed and compiled in Clarke 2009). Oceanic drift of sweet potatoes to Polynesian islands from their native South America is plausible (Montenegro et al. 2008), but following a recent multidisciplinary evaluation of sweet potatoes in Polynesia and South America, Clarke (2009) believes that human-mediated dispersal by Polynesians making contact in coastal South America is much more likely. Notions of pre-Columbian contact between these regions have been hampered by arguments against diffusionism since the 1970s, and the lack of smoking gun evidence for a Polynesian presence in the Americas (Jones 2011; Jones and Storey 2011). However, a combined review of existing lines of research on the matter (Jones et al, Eds. 2011) concludes with the argument that “the most parsimonious explanation for the material, linguistic, biological, mythological, nautical, chronological, and physical anthropological evidence…is that Polynesians made pre-Columbian landfalls in the New World” (Jones et al. 2011:419).

Clarke and others (2006) recently began to assess bottle gourd genetic diversity in
Polynesia, where linguistic and morphological research has not been conclusive as to whether the region’s bottle gourds derived from Asian or New World predecessors (Clarke 2009). They conclude that plastid DNA evidence points to an Asian origin, which is consistent with our findings. However, they also identify a small number of nuclear loci at which African and New World-type alleles are present in Polynesian landraces, usually at low frequencies. Clarke ultimately suggests a dual origin, with Polynesian gourds hailing from both Asia and the Americas and hybridizing in the Pacific Islands (Clarke et al. 2006; Clarke 2009).

Bottle gourd movement from South America to Polynesia could have been natural or human-mediated. The nature of Pacific currents and gourds’ capacity for oceanic dispersal, combined with the fact that ancient (and modern) South Americans were using gourds to float fishnets that could, potentially, easily be lost to the ocean, provide a plausible scenario for dispersal by drift (Clarke 2009; Montenegro et al. 2008; Hudson 2004). However, the preponderance of evidence for pre-Columbian Polynesian contact in the Americas (Jones et al. 2011), and the probable cultural transmission of sweet potato germplasm during these exchanges (Clarke 2009), suggest that bottle gourd could have passed from South Americans to Polynesians during face-to-face interactions in coastal South America.

If gourds of Asian descent were already under cultivation in Polynesia, new South American varieties might have been grown only on a secondary or experimental basis, not as a primary container crop. Unless continual seed saving of newly introduced New World varieties allowed them to persist in the gardens of the South Pacific, the plastid lineage from the Americas could have died out while nuclear alleles were transmitted to established landraces via cross-pollination, leading to the modern genetic distribution. In a similar fashion, if human transmission brought gourds from the New World to the Pacific Islands, any linguistic signal
from South America would likely be lost against the already-established lexicon of local gourd cultivation.

Clarke (2009) advises that for a number of cultural and ecological reasons, we should not assume that bottle gourds were dispersed into Polynesia in the same manner as sweet potatoes; human and natural dispersal are both reasonable in this case. In addition, ocean currents could also carry gourds from Pacific Islands to the west coast of the Americas (e.g. Montenegro et al. 2006), and direct contact between Polynesian and New World cultures could certainly facilitate the movement of gourds in both directions. That is, if we invoke gourd transfer from the Americas to Polynesia (Clarke et al. 2006; Clarke 2009), we should not assume unidirectional transmission. It is easy to envision a direct exchange of germplasm during interpersonal contacts; Native Americans being near their cultivated plots, and Polynesians having traveled with gourds carrying fresh water and other supplies, or possibly serving in fishing technologies for food procurement on the journey. We cannot test these possibilities based on the present plastid dataset, but it will be worthwhile to conduct high-resolution nuclear DNA analysis to investigate a possible connection between the South Pacific and the asiatica-type South American landraces collected by Charles Heiser.

4. Materials and Methods

4.1. Sample Collection

We analyzed 41 modern samples, including 38 cultivars and landraces from Africa, Asia, the Americas, Europe, and Oceania, two wild L. siceraria accessions from Zimbabwe and Kenya, and one wild L. sphaerica accession from South Africa as an outgroup. We omitted four cultivars from final analysis due to poor provenance, as well as L. sphaerica, which we sequenced in case of need for an outgroup taxon. We also analyzed nine archaeological
specimens from sites throughout the Americas: Little Salt Spring in Florida, Spring Branch Shelter in eastern Kentucky, Alred and Putnam Shelters in the Ozarks, Tularosa Cave in New Mexico, Loreto Cave in Baja California, Mexico, Guila Naquitz in Oaxaca, Mexico, El Gigante in La Paz, Honduras, and Quebrada Jaguay in Arequipa, Peru. See Table 1, Figures 1 and 3, and Appendix for additional details on sample materials.

4.2. *Ancient DNA Authenticity*

We implemented standard protocols to prevent and detect contamination of ancient DNA extracts by modern sources (Gilbert et al. 2005). Ancient DNA isolation and library preparation were carried out in a dedicated, sterile facility in a building containing no molecular biology facilities. All work surfaces were regularly sterilized with bleach. Protective suits, masks, gloves, and footwear were worn at all times, and no supplies were carried into the ancient DNA lab from a building containing PCR facilities. Control negatives lacking tissue or template DNA were processed in parallel with ancient samples at all stages through indexing PCR to detect contamination. Following indexing PCR, all work was conducted in a modern molecular biology facility.

4.3. *DNA Extraction*

The majority of modern samples (excluding only 35B) were single seeds. We extracted DNA from modern seeds using Qiagen Plant DNEasy Mini Kits (Qiagen, Valencia, CA) according to the manufacturer’s protocol with a modified tissue disruption step. We used sterile razor blades to split the seeds and excise the embryos, discarding the outer tissue. We divided the embryos, reserving half for subsequent extraction, and grinding half directly in lysis buffer using sterile pellet pestles and a small amount of sterile sand as necessary. The lysis incubation and subsequent steps then proceeded as per the manufacturer’s protocol. Extraction success was
confirmed via PCR of the rpl32-trnL intergenic spacer using published primer sequences (Shaw et al. 2007). Sample 35B was a small piece of herbarium-preserved desiccated leaf tissue. For DNA extraction, we ground 50 mg of the leaf tissue directly in lysis buffer and proceeded as normal.

The archaeological accessions used in this study were desiccated (n=8) and waterlogged (n=1) rind fragments ranging from historic times to ca. 10,000 B.P. in age (Table 1). We carried out DNA isolation using the protocol developed previously for ancient bottle gourd rind (Erickson et al. 2005) with slight modification. We decreased the proteinase K concentration to 0.4 mg/mL, ground rind fragments at room temperature using a Micro-Dismembrator ball mill, and used between 50 and 200 mg of tissue per sample. We increased the volume of extraction buffer as necessary to ensure that powdered tissue remained in a fluid suspension during agitation. For the single waterlogged specimen from Florida, we gently ground the rind tissue by hand in a 2 mL tube using a sterile pellet pestle instead of using the ball mill. We eluted DNA in 100 µL Qiagen Buffer AE with 0.05% Tween to improve DNA recovery. Extraction success was verified via PCR and gel electrophoresis using the LS_INDEL1 and LS_SNP primers designed by Erickson and others (2005) for plastid DNA, and new primers targeting a short fragment of the BOP19_35 nuclear locus identified by Clarke and others (2006).

4.4. **Library Preparation, Target Capture, Sequencing, and Assembly**

We prepared barcoded DNA libraries for Illumina sequencing using the protocol described by Meyer and Kircher (2010). For modern accessions, we sheared DNA with 3 cycles of sonication for 7 minutes in a Biorupter sonicator using the highest energy setting. We used 50 µL template DNA in modern and ancient library preparation. For ancient libraries, we used Qiagen MinElute PCR Purification Kits for cleanup after blunt-end repair, as suggested in the
library protocol, because it retains shorter molecules than the SPRI bead cleanup described in the protocol. We used SPRI bead cleanup for all steps after adaptor ligation.

We used in-solution RNA hybridization to enrich DNA libraries (Gnirke et al. 2009; MycroArray, Ann Arbor, MI). We designed bait libraries to target the entire plastid LSC region based on the published cucumber plastid genome (Plader et al. 2007). In addition, we used PCR and Sanger sequencing to produce approximately 20kb of bottle gourd plastid sequence data for bait design, targeting hypervariable intergenic regions where bait molecules based on cucumber data may be ineffective. Finally, we used the published cucumber nuclear genome (Huang et al. 2009) to design bait molecules targeting roughly 900kb of putatively neutrally evolving nuclear regions across all seven cucumber chromosomes. We enriched and sequenced plastid and nuclear regions simultaneously. Comparison between reads mapping to targeted versus untargeted components of the cucumber plastid genome indicates approximately 2000% enrichment of targeted regions in both modern and ancient libraries.

We pooled and sequenced all libraries in parallel using paired-end 150bp reads on an Illumina HiSeq platform at University of California, Berkeley. We demultiplexed individual sample reads, merged forward and reverse reads, and performed read quality control after Kircher (2012). A small number of reads failed to merge, and these were discarded from the dataset.

To develop an accurate bottle gourd LCS reference sequence, we used MIA (Green, www.sourceforge.net/projects/mia-assembler) to map reads from sample Ls6A to the cucumber LSC (see Appendix Table A.1), iteratively correcting the reference based on the consensus sequence and remapping until the program failed to further refine the reference. We edited the provisional reference sequence by integrating the bottle gourd Sanger sequence data used in bait
library design, and by closing gaps when possible via manual read alignment. This yielded a LSC reference sequence with only three small ambiguous regions of 10, 38, and 104bp when aligned to the cucumber plastid genome; an estimated 99.8% complete LSC sequence. We used BWA (Li and Durbin 2009) to assemble modern sample reads to the new LSC reference sequence, and Samtools (Li et al. 2009) to produce a consensus sequence for each LSC assembly. We visually scanned the modern LSC assemblies for regions with indels that mapped incorrectly, and corrected the consensus sequences as necessary.

For ancient samples, we used MIA (Green, www.sourceforge.net/projects/mia-assembler) to map sequence reads to the new LSC reference, invoking the ancient DNA substitution matrix. We generated consensus sequences with a minimum coverage of two independent reads and minimum consensus identity of 80% to combat artifactual results driven by ancient DNA damage and sequencing error. Ancient samples yielded LSC sequences ranging from 10% to 96% complete. We retained only those samples with at least 50% coverage of the LSC for analysis.

4.5. **Phylogenetic and Phylogeographic Analysis**

We used Mafft (Katoh et al. 2002) to align all LSC consensus sequences, and used JModelTest (Posada 2008; Guindon and Gascuel 2003) to select the HKY + G nucleotide substitution model. We assumed the Bayesian skyride coalescent model (Minin et al. 2008) due to its flexibility. In BEAST (Drummond and Rambaut 2007), we ran two independent Markov chain Monte Carlo simulations, sampling every 10,000 states, until all parameters reached effective sample size of at least 200, and visually verified MCMC convergence using Tracer (approximately 50,000,000 iterations; Rambaut and Drummond 2007). The initial 10% of samples were discarded as burn-in, and we combined the remaining posterior samples from the
two chains. TreeAnnotator was used to summarize the maximum clade credibility (MCC) tree (Figure 3.4).

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Chapter 4: Experimental Investigation of Pathogenic Stress on Phytolith Formation in Wild-Type Gourds (*Cucurbita pepo* var. *texana*)

**Chapter Abstract**

Silica phytoliths that form in plant tissues are useful to archaeologists because of their diagnostic value and longevity in ancient deposits. Paleoecology, site formation processes, plant domestication, and other topics are routinely addressed using phytolith assemblages, especially when macrobotanical remains are not well preserved. However, little research has been conducted to document the effects of ecological variables on phytolith formation. Here, we investigate the effects of mosaic virus and bacterial wilt disease on diagnostic scalloped phytoliths in the rind of a wild-type gourd. We observe a minimal shift in phytolith size distribution between control plants and individuals with mosaic virus. However, we observe a notable difference between plants with bacterial wilt disease and control plants, with diseased individuals carrying a greater proportion of large-diameter scalloped phytoliths. This and similar phenomena could potentially confound archaeological interpretations of phytolith assemblages, and we suggest that the effects of this and other ecological variables should be studied in a diverse range of taxa.

**Keywords:** Phytoliths, Silica, *Cucurbita pepo*, Bacterial wilt disease, Mosaic virus
1. Introduction

Silica phytoliths derived from plant tissues receive considerable attention from archaeobotanists because of their diagnostic potential and durability in the archaeological record. Paleoecology, site use dynamics, plant domestication, and other topics are frequently addressed using phytolith assemblages (Ball et al. 1999; Piperno and Stothert 2003; Piperno 2006, 2009; Piperno et al. 2009; Strömberg 2004; Tsartsidou et al. 2008; Whang et al. 1998; Zhang et al. 2010), especially when the macrobotanical record is too sparse for in-depth analysis. Phytoliths have proven to be useful components in archaeobotanical analyses, but little work has focused on ecological and functional variation in phytolith production and morphology.

For seeds, wood, and other plant organs and tissues that commonly occur in the archaeological record, the relationships between anatomy, morphology, and diverse factors and influences—such as age and functional status, moisture stress, nutrient availability, pathogens, and temperature—have been extensively studied (Baas 1982, 1986; Baas et al. 2003; Bell 1959; Carlquist 1975; Endress et al. 2000; Koehl 1996; Miner et al. 2005; Noshiro and Baas 2000; Stuessy 2009). This has illustrated the full range of variation under normal ecological and developmental conditions. The same level of experimental treatment has not yet been widely applied to phytolith development and morphology (with a few notable exceptions [e.g. Whang et al. 1998; Stuessy 2009:189]), leaving a gap in our understanding of archaeological phytolith assemblages.

Silica occurs commonly in two forms in plants: 1) soluble monosilicic acid (Si(OH)$_4$) taken up through groundwater and distributed throughout organs and tissues, and 2) polymerized silica that exists in several forms in plant tissues (Cseke and Kaufman 1999; Simpson and Volcani 1981). The latter includes silica deposits that collect in the intercellular spaces of
subepidermal tissues or form within the cell walls, especially those of epidermal cells, after infiltration of the microfibril structure by silica gel (SiO$_2$·nH$_2$O). Silica also commonly occurs as isolated isotropic forms known as “silica bodies” in the grasses and sedges, and as “stegmata” for other taxa (Mauseth 1988:34). These develop within the lumens of parenchyma and other cells after the dissolution of all organelles during programmed cell death (Simpson and Volcani 1981). In grasses this occurs in specialized “silica cells” (Cseke and Kaufman 1999; Epstein 2009; Evert 2006; Lanning and Eleuterius 1989; Metcalf 1960; Prychid et al. 2004; Simpson and Volcani 1981).

Silica phytoliths and other types of plant crystals (Cutler et al. 2008:100; Judd et al. 2002:86; Simpson 2006:410) comprise one class of plant ergastic substances—cellular exudates and other materials that are secondarily deposited and not actively metabolized, existing largely as storage reserves or wastes (Judd et al. 2002; Simpson 2006). The presence and form of these materials is highly subject to internal and external influences on plant growth. While silica is not strictly essential for most plants (exceptions include the horsetails, Equisetum [Currie and Perry 2007]), it helps to mediate a wide range of biotic and abiotic stresses in diverse plant taxa. For example, silica contributes strength and support to stems and leaves, alleviates metal toxicity, deters herbivory, and inhibits fungal and bacterial pathogenic infections via physical and chemical mechanisms (Agarie et al. 1996; Cai et al. 2008; Cherif et al. 1994; Cooke and Leishman 2011; Cseke and Kaufman 1999; Epstein 2009; Evert 2006; Fauteux et al. 2005; Fauteux et al. 2006; Iwasaki and Matsumura 1999; Kaufman et al. 1981; Kaufman et al. 1985; Liang et al. 2007; Massey and Hartley 2006; Samuels et al. 1991; Savvas et al. 2009; Simpson 2006).

In some taxa, the presence and deposition of silica has been studied in relation to growth
conditions and environmental stressors. Soluble silica is highly bioactive in its role as a stress mediator. Microarray-based analysis of the Arabidopsis transcriptome revealed that in plants with powdery mildew infections, the presence of silicon altered the expression of a large set of genes in order to facilitate a systemic defense response (Fauteux et al. 2006; and see Heidrich et al. 2011, Bhattacharjee et al. 2011). It has also been shown to protect against fungal infection by forming a physical barrier against the invading fungal hyphae (Samuels et al. 1991). Silica is similarly linked with stress mediation in numerous other taxa, and is used in commercial agriculture due to its broad-spectrum effectiveness in combating biotic and abiotic stressors.

Polymerized silica is traditionally considered to be a deterrent to herbivory, acting as a mechanical defense or physical barrier that strengthens tissues and abrades the mouthparts and digestive anatomy of herbivores (Cseke and Kaufman 1999; Epstein 2009; Evert 2006). Relationships between herbivory and silica deposition have been investigated directly in some grasses (e.g. Massey and Hartley 2006; McNaughton and Tarrants 1983). For example, silica in grass tissues has been shown to negatively impact the growth rate of herbivores, possibly by decreasing the nutritional availability of nitrogen-based compounds (Massey and Hartley 2006).

In rice, distinctive phytoliths that form within the lumens of bulliform cells—specialized cells involved in water storage (Evert 2006)—have been shown to assume different shapes under paddy versus dry field cultivation (Fujiwara 1993). Similarly, silica deposition patterns have been used archaeologically to infer irrigation in ancient deposits containing wheat phytoliths (Rosen and Weiner 1994), and phytoliths in wheat and barley have been experimentally shown to display distinct morphologies under varying conditions of moisture availability (Madella et al. 2009). Given the relationships between environmental variation and plant anatomy, and between silica and stress mediation, it is likely that ecological variables affect silica deposition and
phytolith morphology in a wide range of plant taxa.

Our study begins to explore the effects of disease, one such external variable, on phytolith production and development. We concentrate on distinctive scalloped phytoliths that occur in the rinds of wild-type gourds (*Cucurbita pepo* L. var. *texana* (Scheele) D. Decker [Cucurbitaceae]).

2. Materials and Methods

2.1 Sample Collection and Phytolith Extraction

At the end of the 2009 growing season, we harvested mature fruits from *C. pepo* var. *texana* plants grown at the Rock Springs Agricultural Experiment Station at The Pennsylvania State University in Centre County, PA. We harvested test fields grown under controlled conditions as part of a study on disease transmission, resistance, and virulence. Plants received only rainwater after a single watering at the time of transplanting, and fields were fertilized at 50% of the recommended level for commercial squash production before fine tilling and transplanting. The diseased plants in the study acquired their pathogens naturally, not through inoculation. We chose to use wild-type plants rather than cultivars largely to avoid the immense morphological variation found in *C. pepo* cultivars, and to avoid any influence of human selection pressures on our results. In other words, wild plants provide a more homogenous population without carrying the genetic and phenotypic legacies of human selection, allowing for a more controlled experiment.

We grouped plants into three categories: healthy individuals that had been sprayed to prevent insect herbivory, individuals affected by mosaic virus, and individuals affected by bacterial wilt disease. Mosaic virus comprises a group of viral pathogens carried by aphids that tend to spread aggressively when introduced in Cucurbitaceae crop fields. Afflicted plants can
remain viable and produce fruits, although fruit size, yield, and quality may suffer dramatically (Fletcher et al. 2000). Bacterial wilt disease, caused by *Erwinia tracheiphila* bacteria carried by cucumber beetles (*Acalymma vittatum* [F.] and *Diabrotica undecimpunctata howardi* [Barber]), leads to water deprivation via blockage of the xylem, usually resulting in vine collapse and plant death (Sasu et al. 2010).

In preparation for analysis, we bisected the fruits longitudinally using a coping saw and removed the seeds and fleshy middle mesocarp. This left only the hard outer layers of the rind, which contain the diagnostic scalloped phytoliths at the hypoderm interface in the outer mesocarp (Barber 1909; Hayward 1938). We excised a 1 cm² section of rind from near the equator of each specimen for phytolith extraction. We extracted phytoliths from the rind tissue samples using a chromic-sulfuric acid wet oxidation procedure based on a protocol described by Pearsall (2000).

We placed the rind fragments in 15 ml glass centrifuge tubes, and added 10 ml chromic-sulfuric acid (VWR cleaning solution, #2150-32). We left the samples uncovered at room temperature in a fume hood, and agitated them occasionally for 48 hours to digest organic material. We then centrifuged the samples at 60 x g rcf for 5 minutes, and decanted the supernatant. We cleaned the samples by washing twice with distilled water and once with absolute ethanol in their original tubes, with centrifugation as before between each wash. We mounted samples in rubber grommets affixed to microscope slides with rubber cement, allowed the ethanol to dry, and then removed the grommets, leaving a standardized area (11 mm diameter) of dry-mounted phytoliths for quantification. We measured the scalloped phytoliths in a standardized subsample of each slide (2 transects across the center of the phytolith cluster) using a compound microscope at 200x with an eyepiece micrometer. Most phytoliths were essentially
spherical, but for slightly aspherical examples, we recorded the longest axis. Measures are reported in length, which equates to diameter in spherical examples.

2.2 Statistical Analysis

We compared the phytolith assemblages from the three populations based on grouped measurement data using descriptive statistics and a G-test for goodness of fit (log-likelihood ratio test). We used phytoliths from the healthy fruits to generate expected values against which we compared the two diseased populations. The log-likelihood ratio approximates the chi-square distribution with large sample sizes, but the G-test is preferable when classes number greater than five and expected frequencies are greater than or equal to three (Sokal and Rohlf 1995).

We generated a dataset from 45 fruits comprising a total phytolith count of n = 1072. We grouped phytoliths into size classes for analysis. Since the sum of the expected values must equal the sum of the observed values, the expected values were generated as percentages from the healthy distribution to standardize the expected and observed data. Each disease population was compared independently to the healthy distribution resulting in a G-statistic using the equation: \[ G = 2 \sum^{a} p_i \ln \left( \frac{p_i}{\hat{p}_i} \right) \]; where \( a \) = number of classes and \( p = \) phytolith count. Our expected values were based on an independent (extrinsic) dataset, so our degrees of freedom were equal the number of size classes minus one in each case (df = \( a – 1 \)).

3. Results

We generated data for 1072 phytoliths from 45 fruits (Figure 4.1). We recorded a mean phytolith length among all samples of 53.4 µm, with a range from 25 to 105 µm. The mean length was 53.2 µm among the healthy population, 51.7 µm among the mosaic virus population, and 58.5 µm among plants with bacterial wilt disease. The mosaic virus population closely resembles the healthy population, but with slight negative shift in the distribution. The bacterial
The dimodal distribution of the wilted population may represent normal fruits that matured before disease contraction (the lower peak), and fruits that exhibit the effects of the bacterial wilt disease (the higher peak). The wilt population carries a mean length of 5.3 µm greater than the healthy population, as well as an increase in the mode length from 50 µm to 60 µm. G-tests for goodness of fit showed that the distributions of both diseased populations deviated significantly (p < 0.001) from the healthy population. We did not note systematic morphological differences other than size between the test populations, although the wilt population tentatively appears to contain a greater proportion of aspherical phytoliths than normal. This could be due to constriction of large phytolith formation by the hypodermal cell layers, forcing additional silica deposition and phytolith enlargement to occur tangential to the fruit surface.
4. Discussion

Our results indicate that both mosaic virus and bacterial wilt disease can significantly alter the production of phytoliths in wild-type gourds. The effects of the mosaic virus appear to be less pronounced, and little is known about the relationships between plant silica and viral pathogens. It is likely that the observed slight decrease in phytolith size results from a decrease in fruit volume driven by the disease, given that fruit and phytolith size are correlated in Cucurbita spp. (Piperno and Stothert 2003). The viral distribution resembles the healthy distribution in our wild-type test population, but with a slight negative shift. However, mosaic virus can substantially decrease cultivar fruit size (Fletcher et al. 2000), and further experimentation may reveal important changes in phytolith size among diseased domesticated gourds.

Bacterial wilt disease attacks cucurbits by obstructing the vascular system, leading to severe water distress. Cucumber beetles introduce the bacteria while feeding on plant tissues, which then proliferate in the plant xylem and secrete an exopolysaccharide matrix that inhibits fluid dynamics (Sasu et al. 2010). Deprived of water, the vines begin to wilt as early as a week after exposure to the bacteria, and the entire plant collapses and dies shortly thereafter (Figure 4.2).

It is plausible that disruption of soluble silica movement via bulk flow is somehow linked to passive changes in silica deposition in the rind, leading to the abnormal phytolith assemblage derived from the wilt population. However, given the diverse roles of silicon in mediating the stress of fungal and bacterial attacks (Cooke and Leishman 2011; Fauteux et al. 2005; Cherif et
Figure 4.2. Above, healthy *C. pepo* var. *texana*. Below, the effects of an aggressive bacterial wilt infection.

al. 1994; Samuels et al. 1991), it is also possible that disease contraction, and/or the onset of symptoms, triggers changes in the movement and deposition of silica by the gourds as part of an evolved physiological response to cope with the infection. It is difficult to use only the current data to understand the effects of the wilt disease in detail, but we propose two “active response” hypotheses that can be tested with further research:

First, the plants may produce larger phytoliths in the rind in order to strengthen herbivory defense in diseased individuals. That is, plants that will soon collapse and die due to bacterial wilt disease might be evolved to react in ways that enhance their seeds’ chances for survival and
germination after plant death, including a change in silica allocation. Indeed, researchers have observed increased production of cucurbitacin—an intensely bitter secondary compound that functions as a chemical defense against predation (Cseke and Kaufman 1999)—in direct response to herbivory in stressed plants (Tallamy and Krischik 1989). When herbivory defense is physiologically prioritized under stress, it is therefore conceivable that phytolith production could be correspondingly affected.

Furthermore, phytolith production and rind lignification (i.e. sclerification [Cutler et al. 2008]) appear to strictly covary under the control of the Hard Rind (Hr) genetic locus (Piperno et al. 2002). This suggests that they may have evolved under shared selection against herbivory. This does not directly support an herbivory-defense hypothesis for increased phytolith size in diseased plants, but it does implicate herbivory defense in the production of scalloped phytoliths in *Cucurbita* rinds.

Second, it is plausible that an increase in soluble silica bioactivity as part of a stress response may lead to changes in polymerization and deposition of silica phytoliths. Soluble silica is thought to play a role in primary signal transduction to induce stress responses, possibly acting as a ligand capable of binding with a variety of organic compounds (Epstein 2009; Fauteux et al. 2005). In this capacity, silicon acts as a broad-spectrum signaling molecule that helps trigger a variety of localized responses to stress, which ultimately affect systemic defense strategies within the plant (Fauteux et al. 2005). Polymerization of silica during these processes is not reversible, and deactivates it as a mitigating agent in certain stress responses (Samuels et al. 1991). Therefore, a continual supply of silica is necessary to maintain the benefits it confers on plants under stress. It is possible that increased binding activity related to stress leads to changes in the plants’ requirements for soluble silica and in silica allocation after bioactive
polymerization, resulting in the observed effect on phytolith size.

While it is difficult to use only the current dataset to discuss the specific relationships between bacterial wilt disease and phytolith size, these hypotheses provide some plausible scenarios for further investigation. In the future, it will be useful to gather more detailed data on the timing of disease contraction relative to the effects on phytolith formation, and to use high-resolution *in situ* anatomical analysis to study the changes that occur in diseased plants at the sites of phytolith formation. In addition, it might be informative to analyze the expression and activity of the specialized silicon transporter genes recently identified in *Cucurbita* (*CmLsi*) genes, (Mitani et al. 2011; Mitani-Ueno et al. 2011) relative to wilt disease activity, in order to begin understanding molecular mechanisms responsible for the observed effects. Similarly, a more comprehensive understanding of the *Hard Rind* locus (Piperno et al. 2002) could be informative as to rind strengthening and silicification in general.

5. **Conclusion**

It is critical to understand and quantify how normal ecological variation might influence phytolith formation. By extensively documenting the range of environmentally driven variation in phytoliths, it may be possible to strengthen archaeological phytolith analyses by quantitatively integrating background information on the normal variables affecting phytolith morphology. For example, domesticated *Cucurbita* spp. produce larger phytoliths, on average, than their wild counterparts, making changes in scalloped phytolith size over time a potential indicator of domestication (Piperno and Stothert 2003). However, our experimental data indicate that a wild population affected by bacterial wilt disease could produce a disproportionate number of large phytoliths, potentially skewing the phytolith assemblage of a wild population toward the domesticated end of the size spectrum. By fully explicating this and similar relationships,
however, it may be possible to integrate margins of error and confidence intervals, for example, to quantitatively mitigate any effects introduced by confounding ecological variables during analysis.

Our study provides data on the relationship between a common bacterial pathogen and phytolith morphology in wild-type gourds. Given the degree to which cellular anatomy and plant physiology are affected by numerous variables, phytolith production is likely influenced by a wide range of additional environmental factors. It is crucial to further explore these relationships in order to understand the normal range of variation in phytolith morphology.

References


Chapter 5: Continuing Research

Ongoing research on these topics and related issues will continue to improve our understanding of plant domestication in the New World specifically, and the broader cultural and biological phenomena associated with domestication at large. Biomolecular research in archaeology is itself a very new field, and as ancient DNA recovery, sequencing, and analytical techniques rapidly improve, these techniques are primed to play a central role in domestication studies moving forward. As yet, only a small number of studies focusing in the Americas have utilized these techniques to study plant domestication (Jaenicke-Depres et al. 2003; Erickson et al. 2005; Lia et al. 2007; and see Palmer et al. 2012a), and the bottle gourd study described in Chapter 3 represents one of the first uses of so-called next-generation sequencing of ancient DNA to directly investigate crop plant movement, evolution, and domestication (see also Palmer et al. 2012b; Ávila-Arcos et al. 2011). Below, I propose several lines of continuing research.

In addition, I would like the phytolith experiment described in Chapter 4 to function as a proof-of-concept that normal ecological variables have the potential to affect phytolith morphology in ways that may confound archaeological phytolith analyses. Phytoliths are routinely studied as a component of archaeobotanical assemblages, and in recent decades, a number of substantial claims have been made regarding early crop domestication based on phytolith morphology alone (e.g. Piperno et al. 2009). However, such inferences have drawn some skepticism in the absence of corroborating macrobotanical evidence for two key reasons (for an in-depth review of debates, see Shillito 2012): phytoliths may tend to be taxonomically shoehorned into a finite set of user-biased identifications, and insufficient background information on ecological and functional variation exists to confidently interpret atypical forms as products of human selective influence. The former issue can be addressed using exhaustive
survey of background vegetation and rigorous study of the taxonomic value of various phytolith forms, employing blind tests to assess diagnostic potential. The latter must be experimentally treated on a per-taxon basis, and below I propose a number of potentially worthwhile areas of experimental study. In addition, I propose the application of improved imaging techniques in experimental phytolith research to reduce potential protocol bias and better assess phytolith morphology on a quantitative basis.

1. Eastern North American Plant Domestication

1.1. Chenopodium domestication genetics

At least three independent domestication events have occurred within the genus *Chenopodium*, including two within the species *C. berlandieri*, respectively representing the extinct eastern North American cultivars and the Mexican lineage containing *chia* and *huazontle* (see Chapter 2). In addition, the chenopod crop complex of South America contains the well-known quinoa (*C. quinoa*), and a lesser-known taxon restricted to the Andean Altiplano called *cañihua* (or *kañawa*, *C. pallidicaule*) that is sometimes described as a “rustic domesticate” (Bruno 2006). That is, it carries typical morphological and physiological characteristics of a wild plant (asynchronous maturation, self-dispersal, fruits paniculate instead of compact and terminal) but is cultivated and essentially functions culturally as a domesticated taxon (Gade 1970; Bruno 2006). In several separate parts of their range (Figure 5.1), chenopods are nutritious, storable, and productive crop plants that are apparently well-suited to a cultivated environment and, ultimately, domestication. Additional research is ongoing to elucidate some additional phylogenetic details of domestication across the genus (e.g. Walsh and Emshwiller 2011). Higher-resolution molecular analysis of archaeological chenopods might be informative...
as to details such as the timing, location, the extent and nature of selection pressures introduced by humans during early cultivation, and the level of differentiation between the pale- and dark-morph cultivars in the Eastern Woodlands. It is worth investigating whether the \textit{C. b. ssp. jonesianum} description of ancient thin-testa chenopod (Smith and Funk 1985) should be amended to include the eastern pale \textit{huazontle}-like form, especially in light of the fact that the two comparable extant cultivars in Mexico are classified together in \textit{C. b. ssp. nuttalliae} (Wilson 1981, 1990).

Figure 5.1. Independently domesticated chenopod complexes in the Americas for potential research on parallel evolution in non-cereal grain crops. Map outline after Erickson et al. (2005).

In addition, within quinoa, the lone commercial cultigen in the group, efforts are being made to document genetic diversity and functionality, especially with an eye toward sustainable production and conservation (Jarvis et al. 2008; Christensen et al. 2007; Gonzalez et al. 2011). Comparative functional and conservation genetic studies across extant cultivars—and eventually applied to archaeological specimens—might be very informative as to the genus’s apparent genetic disposition toward domestication, and also the nature of parallel morphological evolution in independent domesticated lineages of a given taxon. Plant genera in which multiple domestication events have occurred are relatively rare (see also, e.g., beans [\textit{Phaseolus} spp.],
wheats \([Triticum\ spp.]\), gourds/squashes \([Cucurbita\ spp.]\), cotton \([Gossypium\ spp.]\) and rices \([Oryza\ sativa\ sspp.]\), Murphy 2007; Decker-Walters et al. 2002; Sanjur et al. 2002; Londo et al. 2006; Willcox 2005; Palmer et al. 2012b), and the chenopods provide a potentially valuable model for studying these processes in a non-cereal grain crop.

1.2. \textit{Gourd/Squash} (\textit{Cucurbita spp.}) \textit{evolution and domestication}

A group of five domesticated plant species was cultivated in eastern North America beginning in the Late Archaic Period: chenopod, sunflower, sumpweed, gourd/squash, and bottle gourd (see Chapter 1). All five cultigens were present in appreciable quantities in Early Woodland deposits and paleofeces in the Mammoth Cave System (Yarnell 1974a, 1974b; Watson 1974; Gardner 1987; Watson and Kennedy 1997), and inter-site analysis of relative abundance throughout the Eastern Woodlands reveals a fairly rapid increase in total usage during the Middle Woodland, around 2,000 B.P. (see Smith 1989; Milner 2004:87). Of the five, chenopod, sumpweed, and sunflower are well-accepted to be indigenous, locally domesticated crop plants (see Chapter 2; Smith 2006a; but see Lentz et al. 2001, 2008; Tarighat 2011 for ongoing arguments regarding sunflower domestication in Mexico). Regarding bottle gourd, the specific details of its origins in the midcontinent are unclear, but could lie in Florida, Central America, both, or neither (see Chapter 3).

Gourd/squash, as discussed in Chapter 1, first appears throughout parts of Florida, the midcontinent, and the Southeast Coastal Plain with thin rinds and small seeds characteristic of wild plants (Fritz 1999; King 1985; Newsom et al. 1993; Crook 2009). The first evidence for domestic forms of \textit{Cucurbita} in eastern North America occurs at the Phillips Spring site in south-central Missouri (Kay et al. 1980), where several gourd seeds exceed size thresholds used to discriminate wild from domestic specimens (Smith 2006b). Smith (2006a) argues that a “well
documented gradual increase in seed size” ensues, providing strong evidence for local
domestication and crop improvement. Some molecular studies have offered tentative support for
this model, but have not been entirely conclusive (Decker-Walters et al. 1993, 2002; Emshwiller
2006).

An alternative hypothesis argues for domestication of a wild gourd in northeastern
Mexico, *C. pepo* ssp. *fraterna*, followed by introduction to the Eastern Woodlands (Sanjur et al.
2002). This model does not have archaeological or molecular support, but should be tested
directly. This is especially true in light of the fact that *Cucurbita*, like *Lagenaria*, seems to have
undergone a level of population disruption and range contraction during the Holocene (e.g.
Decker-Walters et al. 2002), and we cannot necessarily make secure assumptions about the
provenance and continuity of modern populations. A fairly straightforward experimental design
should prove informative as to the origins of domesticated *C. pepo* in the Eastern Woodlands,
further enhancing our understanding of the emergence of domesticated forms in this region.

In addition, techniques optimized for analyzing *C. pepo* in eastern North America can be
extending to several other difficult questions regarding domestication and evolution within the
genus. For example, wild progenitors are unknown for *C. pepo* ssp. *pepo* and *C. moschata*
cultivar lineages (Nee 1990; Ferriol and Picó 2004), the former likely originating in southern
highland Mexico (Smith 1997a; Decker-Walters et al. 2002), and the latter possibly having an
ancient circum-Caribbean distribution and multiple domestications followed by wild-population
collapse during the Holocene (Whitaker et al. 1957; Pickersgill 1969; Smith 1997b; Ferriol and
Picó 2004; Dillehay et al. 2007; Newsom and Scarry 2012). Furthermore, the archaeological and
paleontological record at Florida wet sites reveals large amounts of *Cucurbita* spp. material as
early as *ca.* 31 kya, being recovered from preserved mastodon dung dating of this age (Newsom
2006; Newsom and Mihlbachler 2006; Newsom et al. 1993; Newsom and Scarry 2012; Decker and Newsom 1988; Newsom lab unpublished data). This material provides an exciting opportunity to study Cucurbita populations into the Pleistocene, including those before and after the extinction of the large-mammal dispersers, profound ecological shifts, and the arrival of humans in the region. In addition, it is worth evaluating the variety of Cucurbita taxa utilized by prehistoric Native Americans in Florida—which may include C. pepo, C. okeechoboeensis, and C. moschata, and which are difficult to discriminate based on seed morphology alone (Newsom et al. 1993; Newsom and Scarry 2012)—and further assessing the possibility of Cucurbita cultivation or other population management in prehistoric Florida, especially in light of the taxonomic, ecological, and cultural parallels with Lagenaria.

1.3. Maygrass (Phalaris caroliniana Walter)

One additional component of Eastern Woodlands agriculture that may benefit from biomolecular research involves the archaeological presence of small starchy-seeded plants whose remains do not carry morphological signs of domestication, but which appear to have been cultivated and utilized alongside the domesticated taxa (Fritz 1990, 1995; Smith 2006a). Maygrass is one such taxon that dominates some assemblages from the Early Woodland Period into Mississippian times, frequently alongside chenopod and the other native cultigens (e.g. Yarnell 1974; Cowan 1978, 1985; Asch and Asch 1985; Gremillion 1993; Fritz and Lopinot 2007). Modern maygrass is most prevalent in the Mississippi Valley region, but it occurs archaeologically as far east as the Cumberland Plateau in eastern Kentucky, illustrating a significant range extension in the past that has largely been attributed to human intervention (Cowan 1978). Fritz (2011) indicates that recent work with maygrass has fostered “greater appreciation of its geographic range, longevity, and centrality as a component of indigenous
eastern North American agricultural systems.” However, its macrobotanical remnants do not show clear morphological signs of domestication, and maygrass therefore tends to receive somewhat less attention as a major crop plant than its counterparts with such modifications (Fritz 2011; Smith 2006c).

An inherent shortcoming of seed morphological analysis is its inability to detect many of the processes accompanying the emergence of domesticated forms. Whereas domestication often involves seed size increase, discernable loss of dispersal mechanisms, reduction in germination dormancy and associated structures, and other seed-based morphological changes, it also entails changes in plant architecture, synchronization of fruit maturation for efficient harvesting, biochemical changes to increase palatability, and other improvements for human cultivators that are not reflected by seed morphology (Harlan et al. 1973; Smith 2006c). Therefore, it may be premature to reject a taxon as a domesticate solely on the basis of seed morphology, particularly in a case like maygrass where its archaeological importance and cultivated status are well-documented.

It may be feasible, however, to conduct a study comparing genetic diversity of modern wild maygrass throughout its range with well-preserved archaeological specimens to test for the occurrence of a bottleneck or other population genetic signals that may be consistent with domestication. For example, certain suites of nuclear alleles might have been driven to fixation under selection, which we would not expect to have occurred in the case of genetic drift alone during a range extension. This will be a difficult question to address, but potentially very worthwhile in terms of identifying an important domestic taxon for the first time by means other than morphological analysis.
2. Bottle Gourds

The study described in Chapter 3 confirms that prehistoric New World bottle gourds originated in Africa, and that their complex biogeographic history includes independent domestication within the New World. However, there are several ways to expand on this study to better understand bottle gourd evolution and domestication. For example, it remains unclear where in the New World domesticated bottle gourd morphotypes emerged, and on how many occasions. In addition, we have not addressed the questions of gourd domestication in the Old World, where at least two separate domestic lineages have arisen, one of which spread across Eurasia, the South Pacific, and possibly as far as South America (Decker-Walters et al. 2001; Heiser 1973, 1989; Whitaker 1971). Furthermore, we still have no useful analog for a wild L. siceraria population, and therefore have difficulty assigning archaeological rind and seed materials as wild or domestic.

2.1. Nuclear DNA analysis

It will be worthwhile to analyze several nuclear loci to gain a more complete picture of the phylogenetic and biogeographic history of bottle gourds than that provided by the maternally inherited single-locus plastid genome, especially among modern samples. During our bottle gourd study, we targeted approximately 900kb of neutrally evolving sequence based on the cucumber genome in addition to the plastid LSC. At the time, cucumber (Cucumis sativus) was the closest nuclear genome available for bait design, but the newly sequenced watermelon (Citrullus lanatus) appears to be a better model for Lagenaria. A substantial number of sequence reads from modern samples map successfully to parts of the watermelon nuclear genome at sufficient depth for analysis, and preliminary examination reveals some potentially informative loci. Reads from separate samples cluster in certain genomic regions, apparently
indicating that RNA hybridization was successful in enriching DNA libraries at a number of nuclear sites; however, it did so unpredictably, given that the watermelon and cucumber genomes are quite different from one another. I am currently working to identify informative nuclear loci for further analysis.

Ancient samples appear to have yielded very few nuclear reads, which is not surprising in light of low initial copy number, DNA loss during library preparation, and poor nuclear bait specificity. However, I successfully PCR-amplified short nuclear fragments from all ancient extracts, suggesting that after analyzing the modern dataset, it may be possible to target specific informative regions for PCR and Sanger sequencing in archaeological samples.

2.2. Morphometric analysis

Another useful line of research, especially in terms of understanding the domestication and movement of bottle gourds within the New World, is morphometric seed and rind analysis. As discussed previously, a fundamental shortcoming of morphological analysis in gourds is the lack of sufficient wild materials to develop methods of confidently delineating wild forms from domesticates. However, as discussed briefly in Chapter 3, the abundant bottle gourd remains in early Florida deposits might represent some proportion of wild materials. It might be possible to use techniques similar to those previously applied to infer possible management of *Cucurbita* (Newsom et al. 1993; Newsom and Scarry 2012; Cowan and Smith 1993) to assess intra-population variability that might be indicative of wild vs. domestic morphology in gourd seeds. Numerous modern cultivar populations are available for comparative analysis, and may be useful for developing predictions regarding morphological variance in wild and domesticated plants. In addition, similar studies with *Cucurbita* might provide a baseline model from which to proceed with morphometric analysis. However, it will be useful to update the measurement and
analytical techniques to detect more subtle aspects of morphological variation than can be captured by relying primarily on length and width measures, for example. If these techniques are successful, we might be able to use certain rich archaeobotanical assemblages as analogs for wild populations.

Furthermore, given the huge amount of seed morphological variation within the group, some of which seems to be suggestive of ancestry (Heiser 1973; Decker-Walters et al. 2001), it may be feasible to detect multiple domestic lineages in the Americas based on independent paths of seed evolution. For example, the small, non-descript seeds—in terms of the ornamentation commonly observed in cultivar seeds—in early Florida and midcontinental deposits seem superficially like they might be distinct from much more robust contemporaneous examples from Mesoamerica (e.g. Kay et al. 1980; Whitaker et al. 1957; Cutler 1975; Newsom and Scarry 2012). This could potentially support a model for gourd domestication involving separate lineages in eastern North America and in Central America, but requires detailed investigation.

Much of gourd seeds’ variation can be captured in the two-dimensional outline (see Heiser 1973; Whitaker 1971), which opens the possibility of using well-scaled photographs from archaeological literature to begin a study of seed morphological variation across numerous New World sites. I am currently collecting photographs of archaeological seeds for this purpose, and will shortly begin digitizing their shapes and exploring methods of two-dimensional analysis that might capture informative aspects of variation.

2.3. *Archaeological bottle gourd DNA in the Old World*

Bottle gourds had dispersed to parts of east Asia by *ca.* 11,000 B.P. (Figure 5.2), where they remained a more-or-less continual a part of the cultural flora (for a review, Fuller et al. 2010). They were widely distributed throughout much of the Roman Empire (Figure 5.2), where
Schlumbaum and others (2011) point out that “the ancient authors distinguish between the narrow, long-fruit cultivars that were used as vegetables and the broad, short-fruit cultivars that were used for a variety of purposes such as containers or as swimming aids.” A recent study (Schlumbaum et al. 2011) reports preliminary molecular research on archaeological gourds of the Roman Empire, finding that at the plastid markers analyzed previously by Erickson and other (2005), they carry a haplotype consistent with modern Eurasian cultivars. However, given the limits of these plastid sites (see Chapter 3), it will be worthwhile to analyze these ancient European examples at higher resolution in an effort to better understand the movement of gourds into Europe out of Africa, and the emergence of domesticated lineages leading up to this time period. A number of well-preserved waterlogged gourd samples are also known from early deposits in China, Thailand, and Japan (Fuller et al. 2010). Analysis of these specimens will help further elucidate the nature of the early worldwide bottle gourd dissemination.

Figure 5.2. Archaeological bottle gourds from Old World sites. Site data after Richardson 1972, Fuller et al. 2010, and Schlumbaum et al. 2011. *Spirit Cave, Thailand: This is an estimated date from an associated stratum. The earliest directly dated gourd remains from east Asia are at 9,000-8,500 B.C. in Japan.
3. **Phytolith Analysis**

3.1. *Sources of variation*

A small number of previous studies have assessed the impact of normal ecological variables on silica deposition, and abundant research has revealed silica’s role in broad-spectrum biotic and abiotic stress mediation (e.g. Epstein 2009; Cooke and Leishman 2011). However, these relationships play little if any role in archaeological phytolith analyses, which leaves the possibility for premature interpretations based on poorly understood sources of variation. Our *Cucurbita* phytolith study is the first to experimentally assess normal sources of variation specifically as they might confound archaeological phytolith analysis. Previous studies have focused on the ability to infer fine-scale activities such as crop irrigation (Fujiwara 1993; Rosen and Weiner 1994), and have not yet addressed background variation. I suggest that a wide range of additional studies should be conducted to investigate the possible effects of environmental variables on phytolith morphology.

With horticultural and agronomic research underway across a huge range of taxa at numerous institutions, procuring materials for experimental studies via collaboration with crop researchers is quite feasible. This is the most cost- and time-effective way to acquire materials for analysis. Several classes of variation and specific ecological variables might be worth assessing in detail for their potential effects on phytolith formation. These include, but are certainly not limited to: water stress, nutrient availability, light conditions and light competition, daily and seasonal temperature fluctuation, a variety of bacterial, fungal, and viral pathogens, ambient humidity, and more.

As discussed briefly in Chapter 4, it may also be fundamentally problematic to use
modern populations to establish quantitative morphological thresholds for use with archaeological materials. Hather (1994:2) addresses this “archaeobotanical species problem,” pointing out that narrow interpretation of archaeological materials based on the characteristics of modern counterparts may lead to unwarranted interpretations, potentially ignoring phenotypic plasticity, hybridization, range movements, and other, largely ecological processes. Hather’s discussion focuses mainly on taxonomic assignments of archaeological macroremains based on modern reference materials, but his underlying concern extends to assignment of wild vs. domestic plants, and to phytolith assemblages.

Using *Cucurbita* scalloped phytoliths as an example, there have been a number of events over the last several millennia that could have driven changes making modern wild populations inappropriate morphological baselines for ancient materials. For example, wild *Cucurbita* are evolved for dispersal by large mammals (Barrow 2000; Janzen and Martin 1982; Newsom and Mihlbachler 2006), including megafauna that faced extinction moving toward the Holocene. With primary disperser extinction, we might predict a selective shift favoring smaller fruits, being more easily disseminated and broken open to facilitate seed release by alternative means. Because scalloped phytolith diameter is closely correlated with fruit size (Piperno and Stothert 2003), we might then expect a gradual decrease in wild phytolith size, such that ancient wild phytoliths better adapted for megafaunal dispersal could fall within the size ranges of modern domesticated examples. This would be problematic for ancient phytolith analyses based on modern size thresholds for obvious reasons. In addition, the combined ecological effects of keystone species (e.g. Proboscidean) extinction and Holocene climate change in the range of *Cucurbita* would have been profound, and could have introduced any number of new selection pressures to affect phytolith evolution directly, or via changes in fruit morphology.
Climate change could also have more direct physiological effects on phytolith formation. For example, photosynthetic efficiency is closely linked with temperature, largely due to activity of the enzyme RuBisCO (ribulose-1,5-bisphosphate carboxylase oxygenase) (e.g. Taiz and Zeiger 2010). In taxa with a C3 photosynthetic pathway (like *Cucurbita*), this enzyme catalyzes the linkage between carbon dioxide and the biomolecules of the photosynthetic dark reactions, ultimately initiation the assimilation of atmospheric carbon into sugar molecules. RuBisCO activity increases with temperature up to a point, but then photosynthetic efficiency declines as the enzyme’s oxygenase function begins and carbon fixation slows.

This marks a process called photorespiration, which is a waste of energetic resources from the plant’s perspective, and which many warm-climate plants have evolved adaptive mechanisms to minimize. As temperatures warmed during the Holocene, it is conceivable that *Cucurbita* photosynthetic efficiency—especially in tropical climates—could have declined as the result of higher average temperatures and an increase in the frequency and intensity of photorespiration. This would result in a net loss of photosynthate production over the growth cycle of the plant, which may then need to sacrifice the extent of energetic commitment to certain processes. If this had any effect on fruit growth, phytolith morphology could easily be an indirect target. These scenarios are hypothetical, but are meant to illustrate that we should not assume morphological consistency over a period of time such as the Holocene.

3.2. *Analytical techniques*

Phytolith analysis begins with extraction of phytoliths directly from plant tissues (usually for comparative purposes), from archaeological sediments in the manner of pollen, or from the surfaces of artifacts such as ceramics and groundstone tools (e.g. Pearsall 1982; Rovner 1983; Piperno 2006). In plant tissues, phytoliths can be extracted by dry-ashing, burning tissues to fine
ash and then extracting phytoliths through brief acid, water, and ethanol washes; or wet-ashing, digesting organic tissue in strong acid and washing of the remaining phytoliths with water and ethanol (Pearsall 2000; Piperno 2006). Wet-ashing is the only method widely used in archaeological phytolith recovery. Isolated phytoliths are mounted on standard glass slides and analyzed under compound light microscopy, where they are identified by phytolith taxonomic indices and reference collections. Identifications are based on a combination of discrete and continuous characters, and statistical analyses may be used to strengthen identifications.

As mentioned, criticisms have been leveled at the diagnostic value of archaeological phytoliths from the perspective of insufficient user-bias controls and replicability (e.g. Rovner 1990; 2004; and see Shillito 2012). Much of this uncertainty could be eliminated—or possibly validated—with higher-resolution imaging and analytical techniques. For example, it is widely cited that phytoliths from domesticated maize cobs are reliably distinguishable from not only other wild grasses, but also from teosinte, their wild progenitor. This practice has detractors, however, who argue that poor experimental design created crippling flaws in the study that purports to have developed a reliable maize phytolith typology, which could not be replicated (Rovner 2004).

However, a well-designed study using a more replicable means of measurement and character observation, and a more complete range of related species, might be informative as to the diagnostic potential of phytoliths these taxa under typical growth conditions. Scanning electron microscopy is a simple and effective means of capturing high-resolution images of phytolith-sized objects (i.e. a few to a few hundred microns).

Phytoliths form in three-dimensional cellular matrices, and diagnostic measurements in multiple dimensions are typically taken by manipulating the orientation of phytoliths by agitation.
of the coverslip (see Piperno 2006). An SEM-based alternative is to generate multiple images from different angles, which can be compiled using imaging software to generate an effective 3D model. Several of these models per taxon—which could be generated rapidly with a series of optimized protocols—would provide an excellent set of materials with which to assess the diagnostic potential of, for example, maize vs. teosinte phytoliths. One especially promising approach might be landmark-based analysis of phytolith shape, simultaneously analyzing the relative positioning and informative value of a large number of user-identified or CPU-generated surface sites attached to each phytolith model. This would be similar to ongoing landmark-based analysis for studying the evolution of craniofacial morphological variation relative to genetic ancestry (e.g. Liberton 2012; Shriver 2012). With phytoliths, this technique is conceptual at present, but I feel that this and other highly quantitative approaches to phytolith morphological characterization may prove useful to archaeobotanists.

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Appendix: Supplemental information on samples used in Chapters 2 and 3

1. *Chenopodium* samples

1.1. *Site descriptions for archaeological chenopods*

**Cloudsplitter Rockshelter**

Cloudsplitter Rockshelter is located in Menifee County, Kentucky, in the uplands of the Red River Basin on the Cumberland Plateau (Cowan et al 1981; Cowan 1985). The site is a protected dry shelter with complex stratigraphy and good organic preservation of plant remains. Initial occupation at Cloudsplitter began during the Early Archaic Period, sometime prior to 9,000 B.P. Middle Archaic use was sparse, with few radiocarbon dates and no cultural materials indicative of Middle Archaic activity. Two subsequent, apparently discrete occupations took place at Cloudsplitter, in the Late Archaic (*ca.* 4500-3000 B.P) and Early Woodland (*ca.* 2800-2300 B.P.) Periods. Chenopods first appear in the Late Archaic occupation layers (Smith and Cowan 1987). Material used in Chapter 2 was from FS#1120, part of the site’s Archaic component.

**Haystack Rockshelter**

This site is another Cumberland Plateau dry rockshelter, located in Powell County, Kentucky (Cowan 1979). Occupation at Haystack was shorter than that at Cloudsplitter, and the site’s prehistoric usage was restricted to the Late Woodland Period. Cowan (1979) believes that Haystack was probably occupied somewhere between A.D. 400 and 700. In spite of serious damage to the site by looters, Haystack has yielded an abundance of desiccated organic materials, including the remains of several native cultigens. Several pale morph specimens were analyzed from FS#3, all yielding identical trnQ-5’rps16 sequences (see Chapter 2).
Holman Shelter

Holman Shelter is an Ozark Bluff Dweller site located on the King’s River in Madison County, Arkansas (Gilmore 1931; Fritz 1984; Wilson 1981; Fritz and Smith 1988). Its well-preserved botanical assemblage was one of the first that allowed Melvin Gilmore (1931) to identify several potential native cultigens. The evolution of crop chenopods in the Eastern Woodlands has been studied extensively using materials from Holman and other Ozark shelters (see Fritz 1986). One of the most complete chenopod specimens from eastern North America, an articulated inflorescence complete with many pale-morph fruits, was recovered from Holman shelter. Morphological analysis of this specimen led Wilson (1981) to suggest that it represented introduced *huazontle* from Mexico. This specimen (Cat. # 32-22-3) was sampled for Chapter 2.

1.2. Modern chenopods

Modern seed samples used in the chenopod study came from three sources. Dr. Bruce Smith (National Museum of Natural History, Smithsonian Institution) provided samples of eastern free-living chenopod populations collected during harvesting trips in the mid 1980’s. Information of specific populations and catalog numbers corresponding to Chapter 2, Table 1, can be found in Smith (2007:165-172). Dr. Eric Jellen (Brigham Young University) provided western and Mexican free-living and cultivar chenopod samples acquired during collection trips during 2004 and 2008 as part of his ongoing research on *Chenopodium* systematics and genetics. The USDA National Plant Germplasm System North Central Regional Plant Introduction Station provided additional accessions. Catalog numbers corresponding to Chapter 2, Table 1, can be accessed via the USDA Agricultural Research Service, Germplasm Resources Information Network (www.ars-grin.gov/npgs).
2. *Lagenaria* samples

2.1. Site descriptions for archaeological gourds

**Little Salt Spring**

Little Salt Spring is a large sinkhole near the gulf coast of Florida that has collected and preserved organic archaeological materials via anaerobic waterlogging since Paleoindian times (Clausen et al 1979). The site provides several early radiocarbon dates for human occupation in Florida, including evidence of human hunting of an extinct megafaunal giant land tortoise around the Pleistocene-Holocene transition. This site has yielded several bottle gourd fragments. The sample utilized in this study, recovered during recent excavations on a submerged ledge 27m beneath the current water level, is the earliest directly dated gourd in the Americas.

**Guila Naquitz**

Guila Naquitz is a dry cave site in Oaxaca, Mexico, that has yielded abundant archaeobotanical material from early strata, including the earliest directly dated maize macrofossils (Flannery 1986; Smith 1997; Piperno and Flannery 2001). This site has contributed substantially to our understanding of crop plant evolution and agricultural development in Mesoamerica, and has been especially informative as to early *Cucurbita* gourd use, yielding the earliest and among the most extensive *Cucurbita* assemblages outside of Florida (Whitaker and Cutler 1971; Smith 1997). Bottle gourd materials are not as abundant as *Cucurbita* specimens at Guila Naquitz, forming only 10% of all Cucurbitaceous remains (Whitaker and Cutler 1971), but they do occur in the same early deposits, directly dating to nearly *ca.* 10,000 B.P. (Erickson et al 2005). It has been suggested that the climate of the cool uplands in the area of Guila Naquitz is better suited for *Cucurbita* cultivation, making this the preferred container crop for the area
(Whitaker and Cutler 1971). A similar argument is sometimes made for *C. argyrosperma* (*C. mixta*) in the Southwestern U.S.

**Quebrada Jauay**

Quebrada Jauay is a coastal Peruvian site with components spanning from the Terminal Pleistocene, *ca.* 13,000 B.P., to *ca.* 8,000 B.P. (Sandweiss et al 1998). Excavations at the site have been informative as to the extensive exploitation of marine resources in the region, yielding abundant zooarchaeological materials indicative of subsistence based on fishing. The Early Holocene component of the site yielded three bottle gourd fragments. The use of gourds as fishnet floats in coastal Peru is known to extend back an estimated four thousand years (Hudson 2004), and it is conceivable that gourd fragments at Quebrada Jauay represent an earlier manifestation of this practice.

**El Gigante**

El Gigante is a well-preserved, deeply stratified cave site in Honduras with occupation levels beginning by *ca.* 11,000 B.P. and continuing to roughly *ca.* 1,800 B.P. (Scheffler 2008). The site contains abundant botanical remains, including delicate textiles, masticated “quids” of maguey fibers, an extensive assemblage of morphologically variable maize, and a number of Cucurbitaceous specimens. Several bottle gourd rind fragments and a small number of seeds were recovered during excavations at El Gigante.

**Spring Branch Rockshelter**

Investigations are ongoing at this multi-component rockshelter site in McCreary County, eastern Kentucky (Pollack [ed.] 2012 in press). The site has dry, deep cultural deposits, but has been heavily disturbed by looting. The gourd fragment analyzed in Chapter 3, and another gourd fragment AMS-dated by the Kentucky Archaeological Survey, originate from the site’s Middle
Woodland component. Bottle gourds are reportedly the only cultivated plants represented in the botanical assemblage, which also contains chestnut and hazelnut debris, cane fragments—a common building material—and a small assortment of other wild taxa (Jack Rossen, personal communication).

**Alred and Putnam Shelters**

These are two of several Ozark rockshelter sites excavated by the University of Arkansas Museum between 1929 and 1934 whose botanical remains were analyzed at length by Gayle Fritz (1986) as part of an effort to understand the development and nature of prehistoric agriculture in the area. The Alred assemblage yielded ca. 110 gourd seeds and seven rind fragments, and the rind fragment used in Chapter 3 is the earliest directly dated *Lagenaria* specimen from a site north of Florida. Putnam produced over five hundred bottle gourd seeds and seven rind fragments, and represents a much later Ozark gourd assemblage than Alred.

**Tularosa Cave**

This dry cave site in New Mexico yielded a large, well-stratified, and well-preserved botanical assemblage from a period of occupation dating to ca. 2,200-800 B.P. (Cutler 1952; Cutler and Whitaker 1961). The maize assemblage was sufficient for extensive morphological analysis, and the site also yielded *Cucurbita* and *Lagenaria* gourds, beans, cotton, wild-type sunflower, and more.

**Loreto Cave**

Finally, Loreto Cave, a site in the mountains of southern Baja California, produced a small botanical assemblage consisting of *Lagenaria* (25 rind fragments and one seed), and also including a single true calabash (*Crescentia cujete*) rind fragment. Maize cobs are also mentioned in a description of the botanical assemblage (Whitaker 1957) but are not treated in
detail. The site represents a fairly short period of occupation by Native Americans who left nearby Spanish missions due to “a great deal of unrest” created there by “epidemics or related factors” (Whitaker 1957). “Related factors,” in this case, possibly stemmed from the fact that Spanish colonizers were not known for their humanitarian treatment of American Indians. This occurred in the first half of the 18th century, which is consistent with the AMS date acquired for the Loreto Cave specimen used in Chapter 3.

2.2. Modern gourds

Modern gourd seeds—and one leaf sample—for molecular analysis were provided by a number of organizations and individuals. See Table A.1 for sample details.

Table A.1. Modern gourd samples used for analysis in Chapter 3. Source information: USDA – USDA Agricultural Research Service, National Plant Germplasm System; DDW – Deena Decker-Walters, the Cucurbit Network; IU – Indiana University Herbarium; MB – Mike Burtenshaw, The Open Polytechnic of New Zealand; BDS – Bruce Smith, National Museum of Natural History, Smithsonian Institution. *Indicates wild L. sphaerica collected in South Africa. This accession was excluded during analysis. **Indicates cultivar names of uncertain geographic origin. These accessions were excluded during analysis.

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MB191 New Guinea MB MB191

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Ls10c *Nigerian Saybo DDW TCN 1042

Ls10d *Oval Basket Japan DDW TCN 1046

Ls10e Malaysia BDS BDS 111

Ls10f Thailand BDS BDS 188

Ls10g Hong Kong USDA PI 419215

Ls10h Israel USDA PI 487482

Ls10i Greece USDA PI 491252

References


Logan Jonas Kistler  

Curriculum Vitae

2012-  Postdoctoral Scholar, Dept. of Anthropology, The Pennsylvania State University
Ph. D.  Anthropology, The Pennsylvania State University, defended April 27, 2012
M.A.  Anthropology, The Pennsylvania State University, August 2009
B.A.  Anthropology, Sociology, magna cum laude, University of Kentucky, May 2007

Publications
2009  Contributor to Macrobotanical Analysis:
Crook, MR. Bilbo (9Ch4) and Delta (38Ja23): Late Archaic and Early Woodland Shell Mounds at the Mouth of the Savannah River. *Occasional Papers in Cultural Resource Management* 17.

Selected Honors, Grants, and Awards
2010, 2011  Pennsylvania State University Dept. of Anthropology Hill Fellowship
2010  Southeastern Archaeological Conference Student Paper Competition Award
2009-2012  National Science Foundation Graduate Research Fellowship
2009  Cave Research Foundation Graduate Research Grant
2007-2008  Pennsylvania State University Graduate Fellowship

Selected Invited Presentations
2012  Henry ER, **Kistler L**. Site-Selection, Use, and Reuse of the LeBus Circle – an Early-Middle Woodland Period Earthwork in Central Kentucky. Theoretical Archaeology Group, Buffalo NY.
2011  **Kistler L**. *Utilizar de ADN Antiquo: Algunas Aplicaciones y Desafíos*. Workshop in Environmental Archaeology, CISAT, Holguín, Cuba.
2009  **Kistler L**. Cave Archaeology and Ancient DNA. Penn State Anthropology 083S, Freshmen Seminar.