ARTICLE

# Turning Over a New Leaf: Experimental Investigations into the Role of Developmental Plasticity in the Domestication of Goosefoot (*Chenopodium berlandieri*) in Eastern North America

Megan E. Belcher[1] (iD), Daniel Williams[2], and Natalie G. Mueller[1]

[1]Department of Anthropology, Washington University, St. Louis, MO, USA, and [2]Greenstreet Growers Inc., Lothian, MD, USA
**Corresponding author:** Megan E. Belcher; Email: belcher.megan.e@gmail.com

## Abstract

In eastern North America, Indigenous peoples domesticated several crops that are now extinct. We present experimental data that alters our understanding of the domestication of one of these—goosefoot (*Chenopodium berlandieri*). Ancient domesticated goosefoot has been recognized on the basis of seed morphology, especially a decrease in the thickness of the seed coat (testa). Nondomesticated goosefoot also sometimes produces seeds that look similar or even identical to domesticated ones, but researchers believed that such seeds were rare (1%–3%). We conducted a common garden experiment and a series of carbonization experiments to better understand the determinants of seed polymorphism in archaeobotanical assemblages. We found that goosefoot produces much higher percentages of thin-testa seeds (mean 50% in our experiment, 15%–34% in free-living parent populations) than previously reported. We also found that cultivated plants produce more thin-testa seeds than their free-living parents, demonstrating that this trait is plastic in response to a garden environment. The carbonization experiments suggest that thin-testa seeds preserve under a larger window of conditions than thick-testa seeds, contrary to our expectations. These results suggest that (1) carbonized, phenotypically mixed assemblages should be interpreted cautiously, and (2) developmental plasticity and genetic assimilation played a role in the domestication of goosefoot.

## Resumen

En el este de América del Norte, los pueblos indígenas domesticaron varios cultivos que ahora están extintos. Presentamos datos experimentales que alteran nuestra comprensión de la domesticación de uno de estos: "goosefoot" (*Chenopodium berlandieri*). La antigua *Chenopodium berlandieri* domesticada ha sido reconocida sobre la base de la morfología de la semilla, especialmente una disminución en el grosor de la testa. La antigua *Chenopodium berlandieri* domesticada ha sido reconocida sobre la base de la morfología de la semilla, especialmente una disminución en el grosor de la testa. *Chenopodium berlandieri* no domesticada también a veces produce semillas que se ven similares o incluso idénticas a las domesticadas, pero los investigadores creían que tales semillas eran raras (1-3%). Llevamos a cabo un experimento de jardín común y una serie de experimentos de carbonización para comprender mejor los determinantes del polimorfismo de semillas en ensamblajes arqueobotánicos. Encontramos que *Chenopodium berlandieri* produce porcentajes mucho más altos de semillas de testa delgada (promedio 50% en nuestro experimento, 15-34% en poblaciones parentales de vida libre) de lo que se informó anteriormente. También encontramos que las plantas cultivadas producen más semillas de testa delgada que sus padres de vida libre, lo que demuestra que este rasgo es plástico en respuesta a un entorno de jardín. Los experimentos de carbonización sugieren que las semillas de testa delgada se conservan bajo una ventana de condiciones más grande que las semillas de testa gruesa, contrariamente a nuestras expectativas. Estos resultados sugieren que (1) los conjuntos carbonizados y fenotípicamente mixtos deben interpretarse con cautela, y (2) la plasticidad del desarrollo y la asimilación genética desempeñaron un papel en la domesticación de *Chenopodium berlandieri*.

In eastern North America, Indigenous peoples domesticated a suite of crops called the Eastern Agricultural Complex (EAC; Smith 2006). The EAC includes both extant and extinct domesticates, including native squashes (*Cucurbita pepo* ssp. *ovifera* D. S. Decker); two spring-maturing grasses, little barley (*Hordeum pusillum* Nutt.) and maygrass (*Phalaris caroliniana* Walt.); two members of the sunflower family, sumpweed (*Iva annua* var. *macrocarpa*) and sunflower (*Helianthus annuus* L.); goosefoot (*Chenopodium berlandieri* ssp. *jonesianum* Smith and Funk); and erect knotweed (*Polygonum erectum* ssp. *watsoniae* N. G. Muell.) (Asch and Asch 1977, 1985; Crites 1993; Decker 1988; Fritz and Smith 1988; Heiser 2008; Hunter 1992; Mueller 2017a; Smith 2014; Smith and Funk 1985; Yarnell 1972). This crop complex sustained Indigenous people in eastern North America from around 3900 BP to approximately 600 BP (Smith and Yarnell 2009). Although the timing of its first appearance is hotly debated, maize (*Zea mays*) from Mesoamerica was widely grown in eastern North America by AD 900 (Emerson et al. 2020; Hart et al. 2021; Simon et al. 2021). Maize, beans (*Phaseolus vulgaris*), squash, and sunflowers gradually became the dominant crop complex in this region, and people slowly stopped relying on the other EAC crops, although some were still cultivated as late as the fifteenth century AD (Fritz and House 2022). The EAC crops, excluding squash and sunflower, fell out of cultivation around the time of European contact, so there are only a few enigmatic ethnohistorical accounts of EAC cultivation, and their domesticated forms are thought to be extinct (Asch and Asch 1977). As a result, archaeologists rely on comparison between modern free-living[1] plants and archaeobotanical specimens to recognize domesticated assemblages in the archaeological record. Given that paleoethnobotanical assemblages found in eastern North America rarely consist of entire plants, most of the phenotypic criteria used to recognize plant domestication in this region are aspects of seed or fruit morphology.

To build these arguments about domestication from fruit/seed phenotype, paleoethnobotanists often use criteria of the domestication syndrome first established by Harlan et alia (1973), who argued that the selective pressures associated with planting and saving of seeds result in specific adaptations, some of which can be recognized in seeds and fruits. In this article, we focus on the reduction in germination inhibitors in one EAC crop—goosefoot (*C. berlandieri*). One of the key traits that has been used to argue for an extinct domesticated species of goosefoot in eastern North America is the thickness and texture of the seed coat (hereafter, testa), which mechanically restricts germination (Smith and Funk 1985). A loss or reduction of germination-inhibiting structures has been observed in many annual plants as they are domesticated. This phenomenon is thought to evolve in response to seed-saving and planting by ancient people, and by the relaxation of selective pressures associated with preexisting seed dispersal strategies (Spengler and Mueller 2019). A reduction in germination inhibitors can be recognized by a thinning of the seed or fruit coat, or by a reduction in seed polymorphism in favor of seed types with less robust seed protections (Mueller 2017b). Recent observations of free-living populations of goosefoot (Halwas 2017) suggest that this phenotype is more plastic than previous researchers appreciated. These observations prompted the two experimental studies reported here. The results of these experiments suggest refinements to the interpretation of ancient goosefoot and additions to the narrative of how this species was domesticated in eastern North America.

More generally, this study investigates the role of developmental plasticity in domestication (Piperno 2017; Zeder 2018). Developmental plasticity is the ability of a single genotype to exhibit phenotypic variation in response to environmental conditions. Plasticity can allow plants to take advantage of human-mediated environments (Herman and Sultan 2011), and ancient farmers could have used these plastic responses to their own advantage during the domestication process (Mueller 2017b, 2017c). Developmental plasticity can also complicate how paleoethnobotanists categorize archaeobotanical remains as domesticated or wild, because many domestication syndrome traits are plastic, including the prevalence of germination inhibitors (Mueller 2017b) and seed size (Gremillion and Piperno 2009). As Gremillion (1993a:147) stated in her discussion of

*Chenopodium* evolution under cultivation, "The rate and character of change in crop populations is the product of complex interactions between heritable traits and their phenotypic expression in particular environments."

Here, we present our findings from two experiments. First, we used a common garden experiment to explore the effect of the growth environment on seed phenotype. A common garden experiment takes individuals from different populations and grows them in a single experimental space (de Villemereuil et al. 2016). Common garden experiments help us better understand if phenotypic differences observed between populations are developmental (plastic) or heritable (fixed). We collected seeds from wild populations of goosefoot in Illinois and Missouri and grew them in a common garden experiment to observe any phenotypic differences between two local populations, and between the parent populations and their cultivated offspring. Although we collected various kinds of phenotypic data, here we will focus on the proportion of thin-testa seeds (hereafter, "red morphs") in a random subsample of the harvest (sample proportion). We also conducted a series of carbonization (experimental burning) experiments on goosefoot seeds to see if carbonization differentially affects thin- versus thick-testa seeds.

## The Red Morph Problem Revisited

Each goosefoot plant produces a range of seed colors with varying testa thickness. The most prevalent are black and red, although it is possible to break down a harvest into more nuanced color categories. Lighter-colored seeds have a thinner testa (Halwas 2017; Smith 1985:202) with a smoother surface texture, although the diagnostic alveolate (honeycomb) texture of *C. berlandieri* and its close relatives is still sometimes visible (Figure 1). Smith's (1985) measurements of testa thickness in modern, free-living populations have generally been used for comparison with archaeobotanical specimens. He found that the red morphs from the three populations he measured overlapped in testa thickness with domesticated archaeobotanical specimens (~5–30 μm), whereas black morphs had testas that measured 40–90 μm—much thicker than those of the archaeobotanical specimens he was studying. Smith went on to describe what he called "the red morph problem":

> Establishing the presence of a thin-testa domesticated variety of *Chenopodium* in archaeobotanical assemblages is obviously complicated by the ability of nondomesticated chenopod populations to produce small frequencies of red-morph fruits which are sometimes indistinguishable from the fruits produced by domesticated stands of *Chenopodium* [Smith 1985:122–123].

However, the prevalence of red morphs in harvests from nondomesticated *Chenopodium* plants was thought to be very low under most circumstances.

This belief rests on limited observations of free-living goosefoot phenotypes by pioneering North American paleoethnobotanists in the 1970s and 1980s. Asch and Asch (1977) published their observations of *C. bushianum* (now considered a subspecies of *C. berlandieri* by the *Flora of North America*; Clemants and Mosyakin 2003) populations in the lower Illinois River valley in late October. They collected seeds from a population located on mudflats, which were barely a meter tall (which is short for goosefoot plants in this season) and from a nearby stand of taller plants growing in partial shade on higher ground. Seeds from the mudflat population had "reddish brown, translucent seed coats," whereas harvests from the higher-ground population consisted of mostly "black and opaque" seeds (Asch and Asch 1977). Asch and Asch stated that the higher-ground plants had already senesced and dispersed most of their seeds by mid-November, and the mudflat plants were still green, suggesting that they were likely younger plants that emerged after spring floods receded. Asch and Asch later referred to these observations when they concluded that "the conditions under which the red morph predominates in the wild are unusual ones" (Asch and Asch 1977:21)—since their 1977 publication, they had only found one other goosefoot population in which red morphs "comprised most of the seeds" (Asch and Asch 1985:180). Building on their research, Smith (1985) collected seeds from four free-living populations. Smith (1985:122) found that red morphs will "represent only 1–3% of a population's fruit production" apart from rare situations that may encourage a higher production of red morphs.
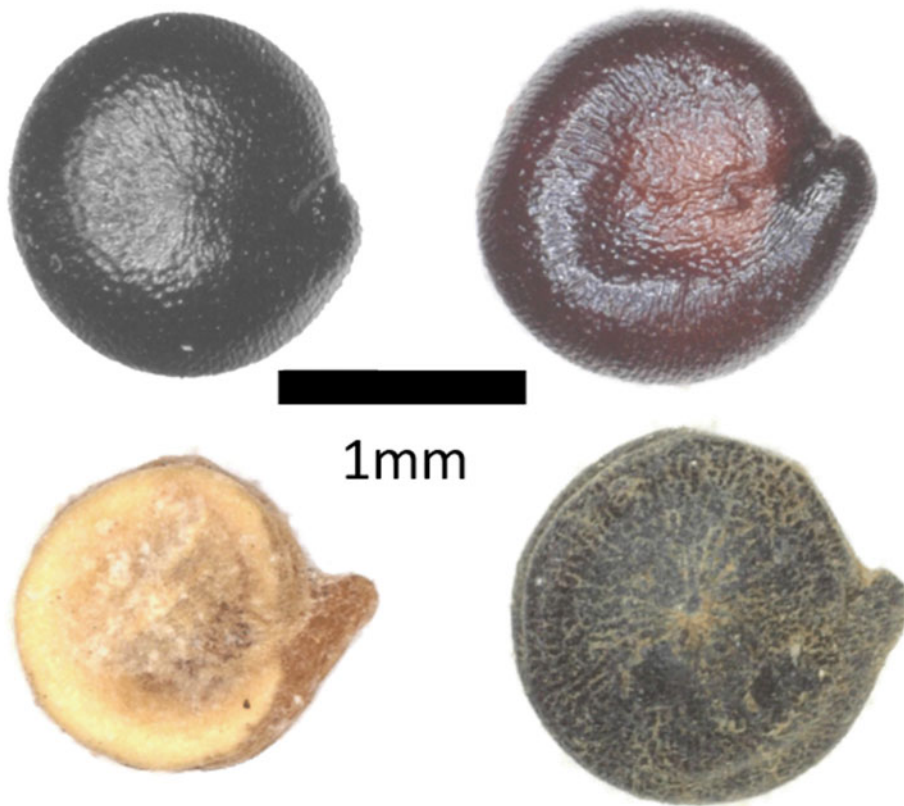
**Figure 1.** *Chenopodium berlandieri* polymorphic seeds and domesticated landraces: (*top left*) black, thick-testa morph; (*top right*) red, thin-testa morph, seeds from modern population CB004 (see text for details). Pericarps have been removed. Note the characteristic alveolate (honeycomb) texture on both morphs. *Bottom left*: typical pale-domesticated-variety seed from an intact inflorescence at Holman rockshelter, Arkansas (see Fritz 1984; Wilson 1981). *Bottom right*: A typical *C. berlandieri* ssp. *jonesianum* seed from the Edens Bluff bag, Arkansas (Fritz 1986; Fritz and Smith 1988). (Color online)

Smith and Funk (1985) formally described an extinct domesticated subspecies, *C. berlandieri* ssp. *jonesianum*, on the basis of two characteristics of its seeds that differed from its closest modern relative, *C. berlandieri*: (1) dominance of seeds with a thin (<20 μm), smooth testa (seed coat); and (2) a truncate seed margin with prominent "beak" (see images in Gremillion 1993a). This formal botanical designation was based on a type specimen from Ash Cave, Ohio, which is an assemblage of approximately 25,000 seeds, and it also characterizes an assemblage of approximately 50,000 seeds from Russell Cave, Alabama—both of which are approximately 2,000 years old (Smith 1985; Smith and Funk 1985). Older assemblages from the western front of the Appalachian Mountains in Kentucky, such as those from Cloudsplitter Rockshelter and Newt Kash Shelter, both of which were dated to about 3400 BP, also exhibit this seed phenotype (Gremillion 1993a; Smith and Cowan 1987). *C. berlandieri* ssp. *jonesianum* also appears at Edens Bluff and White Bluff, Arkansas, both of which are approximately 2,000 years old (Fritz and Smith 1988). However, *C. berlandieri* ssp. *jonesianum*, in the strict sense, is only one of at least two domesticated phenotypes in the archaeological record of eastern North America. The other has been called a pale or naked variety because it entirely lacks an outer epiderm, giving it a translucent, golden-yellow appearance similar to quinoa. This phenotype has been recovered from several rockshelters in Arkansas dating between 1400 and 800 BP, and at the Riverton site, Illinois (Fritz 1986; Fritz and Smith 1988; Smith and Yarnell 2009; Figure 1). Pale-seeded *Chenopodium* was also recovered from Terminal Late Archaic layers at Cold Oak Rockshelter, in Kentucky, and directly dated to 2600 BP (Gremillion 1988). The assemblage of 540 uncarbonized pale-variety seeds recovered at Riverton is remarkable in that it is roughly 1,000 years

older than other occurrences of pale-variety *Chenopodium*, pushing back the evolution of this domesticated form to approximately 3800 BP (Smith and Yarnell 2009).

Most of the assemblages originally used to describe the two domesticated phenotypes discussed above and illustrated in Figure 1 come from large desiccated or mineralized assemblages in clear storage contexts. This makes them unlike the vast majority of archaeobotanical specimens in this region, which are carbonized (preserved via burning) and which come from a variety of contexts, rarely in concentrations large or homogenous enough to describe as a seed cache. Although a high proportion of thin, smooth testa seeds is only one-half of the domestication syndrome described for *C. berlandieri* ssp. *jonesianum* (Smith and Funk 1985), in practice, it is a more useful criterion than aspects of seed margin shape, because the shape of the seed margin is frequently impossible to characterize in carbonized specimens. Moreover, until recently, testa thickness could only be measured with a scanning electron microscope. As a result, it is rarely undertaken—and then, only on a subset of all *Chenopodium* seeds recovered from a given site or context. Our own experience as analysts suggests that testa texture and estimated thickness are the most expedient and frequently preserved criteria to employ when categorizing fragmentary carbonized specimens as domesticated or wild for the purposes of standard archaeobotanical reporting. For example, Simon and Parker's masterful summary of hundreds of such reports sums up Late Woodland chenopod assemblages in this way: they are "morphologically mixed and contain both thick-testa *wild* forms and thin-testa *domesticated* seeds, as well as specimens that appear intermediate between the two" (Simon and Parker 2006:228; emphasis added). Here, we can see how thin-testa and thick-testa seeds are generalized as domesticated and wild, respectively.

Halwas's (2017) survey of modern free-living goosefoot populations suggests that it is not necessary to invoke domestication to explain the presence of thin-testa seeds in archaeobotanical assemblages. She found a range of seed polymorphism among free-living *C. berlandieri* populations. Thin-testa, light-colored seeds comprised up to 30% of the seed harvested from wild goosefoot populations in her survey (Halwas 2017:202). Halwas also conducted a common garden experiment with seeds from these wild populations and found that the common garden populations had lighter-colored seeds, thinner testas, and larger perisperm diameters, on average, compared to their free-living parent populations (Halwas 2017:203). But Smith's (1985) and Asch and Asch's (1977, 1985) earlier observations have established the idea among a generation of researchers in eastern North America that red morphs are rare occurrences among modern free-living goosefoot populations. This key observation provided the basis for modern comparison and a way for archaeologists to theorize about past goosefoot populations. For example, Gremillion (1993b) used this idea to support her theory that ancient *Chenopodium* populations existed as a crop/weed complex where domesticated goosefoot and a companion *Chenopodium* weed evolve together in human-disturbed environments. Gremillion (1993b:506) argues that this crop/weed complex could explain the seed polymorphism and variation in morphological traits present in archaeological goosefoot, and she pointed out other instances of seed polymorphism from *Chenopodium* species in the Andean altiplano that are now well known as examples of crop/weed complexes (Bruno 2006; Bruno and Whitehead 2003; Langlie et al. 2011; Wilson 1990). An alternative explanation, based on Halwas's data, is that a polymorphic assemblage of seeds in the archaeological record could come from a single population of cultivated, but genetically wild, plants.

A preponderance of thin-testa seeds in ancient *Chenopodium* assemblages has been explained as evidence of domestication by archaeobotanists, but there are other ways to explain their presence on any given goosefoot plant or in the archaeological record. They may be a plastic response to the growth environment that serves as a bet-hedging strategy to deal with an unpredictable growth environment or a means of transgenerational signaling (Mueller 2017b, 2017c). A simpler explanation is that they are simply seeds harvested at an earlier stage of development, and that, given more time, they would have developed into thick-testa seeds. We investigate these possibilities using data from our common garden experiment.

## Previous Carbonization Experiments with *Chenopodium*

Testa texture and thickness are not always observable in carbonized assemblages because *Chenopodium* seeds often rupture when burned (Wright 2003). Fractured testa halves frequently disarticulate, leaving

many *Chenopodium* assemblages so fragmentary that it has been necessary to use seed number estimate (SNE) methods similar to the minimum number of individuals (MNI) metric used in osteology and zooarchaeology (Lopinot 1984). As Fritz and Smith (1988:10) observed, "The task of recognizing [pale variety thin-testa seeds] once they have been carbonized . . . may require searching through hundreds or even thousands of specimens to find the few that survived relatively intact." Smith spoke from experience: he sorted through an estimated 49,650 ruptured seeds in the carbonized Russell Cave assemblage to find a mere 581 unruptured *C. berlandieri* ssp. *jonesianum* specimens (Smith 1992:101–102). Consequently, we also wanted to understand how carbonization might be biasing these assemblages in favor of one seed phenotype or another.

Carbonization protects seeds from the typical decomposition processes of organic materials over time and increases the likelihood that they will be preserved for hundreds or thousands of years. Differences in necessary conditions for carbonization may lead to preservation bias in the archaeological record (Märkle and Rösch 2008; Mueller 2017d; Mueller et al. 2022; Wright 2003, 2008). In cases where seed polymorphism is reduced or eliminated during domestication, differences in carbonization requirements between seed morphs need to be understood in order to interpret carbonized assemblages. We undertook exploratory experiments to see if thin- and thick-testa morphs have different potentials to be well preserved via carbonization. Mueller's (2017d) carbonization experiments with *Polygonum erectum* showed that seeds with more robust protections were more likely to be preserved. We expected the same would be true of *Chenopodium*: that the thin-testa morph would be systematically underrepresented in the archaeological record.

There are several general expectations for morphological change associated with carbonization: seeds may or may not pop, swell, or distort, leading to an increase or reduction in diameter or thickness. Due to these changes, seeds may or may not maintain enough of their qualitative morphology to be diagnostic. Consequently, specific circumstances need to be met in order to produce identifiable and fully carbonized *Chenopodium* specimens. Furthermore, to assess domestication status, seeds must be well preserved enough to observe the shape of the seed margin and/or the texture and thickness of the testa. However, previous studies have mainly focused on establishing how seed size changes with carbonization, not elucidating the conditions under which specimens will be well preserved. For example, Smith (1985) did not include specific information about duration, temperature, or oxidation, but he reported that carbonization resulted in an average reduction in maximum diameter of 5% (1985:66). Wright's (2003) carbonization experiment tested both dried and "fresh" (higher moisture content) goosefoot samples. She found that fresh, carbonized specimens proved too distorted to be measured, so only the dried specimens were measured, yielding shrinkage rates of 14% for length (diameter through the "beak") and 8% for width (diameter perpendicular to length; Wright 2003:581).

Wright (2003:581) also found an increase of about 14% in thickness (width of seed margin) and determined that overall goosefoot is more likely to swell due to carbonization than other small-starchy seeds that were tested. Perhaps this explains why so many ruptured *Chenopodium* seeds are found in the archaeological record. There have also been two carbonization experiments on quinoa (López et al. 2011; Nordstrom 1990), which generally replicated Smith's (1985) and Wright's (2003) observations that well-preserved, carbonized *Chenopodium* seeds undergo slight shrinkage in diameter and an increase in thickness. Although these previous experiments provided useful starting points for temperatures and durations to use, none of them assessed differential preservation between seed morphs during carbonization.

## Materials and Methods

### Garden Experiment

Seed for this experiment came from two populations of *C. berlandieri* harvested by Mueller in 2017. Seed lots were numbered by population and gathered from at least 10 plants, or all plants if the population was less than 10. CB002 was harvested on October 17 on the Big River, in Jefferson County, Missouri. CB004 was harvested on November 6 on the Mississippi River, in Madison County, Illinois. Both populations were diffuse stands of small to medium (0.25–1.50 m) plants growing in partial shade on sandy riverbanks. Plants in both populations were not senesced and still had flowers present when harvested (Figure 2).

**Figure 2.** Free-living parent populations: (*left*) CB002, (*right*) CB004. Note that parent population plants were not senesced when harvested. Compare with offspring in garden environment illustrated in Figure 3. (Photographs by Natalie G. Mueller.) (Color online)

In the spring of 2019, a 1.5 mL random sample of seeds from each seed lot was cold stratified in distilled water for one month at 4°C (March 20–April 23, 2019) to begin the germination process. Next, seeds were lightly pressed into moist potting soil—one seed per cell in 72 cell trays—and placed on a mist bench in the greenhouse at Washington University under light for 12 hours/day. After approximately six weeks, on June 6, 2019, Williams transplanted 10 seedlings from each population into a raised bed at the Center for American Archaeology in Kampsville, Illinois (Figure 3). Ten plants (replicants) from each *C. berlandieri* population were planted at intervals of 16.5 cm in a raised bed

**Figure 3.** Experimental garden: (*top left*) an example of a fully senesced plant harvested on October 28; (*top right*) an example of a plant harvested before senescence on November 15; (*bottom*) a view of the garden environment in mid-August. The *Chenopodium* plants are on the left with the white isolation bags on their branches. (Photographs by Natalie G. Mueller.) (Color online)

measuring 1.2 m², split into four quadrants to distribute plants from both populations evenly across the bed, and they were watered as needed for two weeks after transplanting. Soil in the raised beds was a mixture of commercial topsoil and compost. Plants were surrounded by an electric fence to prevent predation, and the bed was kept weeded so that there was no competition from other plants. Each plant was measured and scored for phenology on a weekly basis from June to October of 2019. Mueller harvested plants as they senesced, beginning on October 28, 2019. She considered plants with dry and brittle leaves and stems and with no remaining flowers to be senesced (Figure 3). On November 16, Mueller harvested all remaining plants, including those that were not yet senesced, because the first heavy snow was forecast. This procedure serendipitously resulted in a sample of five harvests from senesced plants and five harvests from not yet senesced plants from each population.

Mueller harvested by cutting off the entire plant at ground surface, transported them back to the lab in individual labeled paper bags, and dried them for several days. After drying, Mueller hand-stripped the seeds, then weighed the dried and stripped plant (minus the roots) to provide a final overall measurement of plant size. The seeds, including abundant chaff, were thoroughly dried on custom drying

racks for two weeks, then weighed and stored in paper bags at 0°C. In fall 2020, Belcher cleaned each sample by threshing and winnowing. Threshing is the process of removing seeds from other plant material, or chaff (e.g., leaves, flowers, stems). To thresh, each individual harvest was rubbed between gloved hands to release the seeds and pulverize the chaff. This step facilitates the subsequent separation of the seeds from chaff via winnowing. A custom vacuum winnower was used for this process (Mueller et al. 2019). After cleaning the seeds, we recorded the mass (g) for each sample, then used a sample splitter to obtain a random subsample for sorting.

Testa thickness is correlated with seed color: lighter-colored seeds have thinner testas (Halwas 2017; Smith 1985). Because we needed to characterize thousands of seeds, we sorted them by color (red vs. black) as an expedient proxy for testa thickness, which can only be directly measured after dissecting individual seeds. We determined a sample proportion of red morphs for each plant by sorting 300 seeds into red and black categories under low magnification (10×–40×, see Figure 1). We determined sample proportions for 20 experimental plants and two parent populations, for a total of 6,600 sorted seeds. We used $t$-tests to determine whether mean sample proportions were significantly different between populations or between plants that were harvested before versus after senescence. We also compared the sample proportions of experimental replicants to those of their parent populations.

### Carbonization Experiment

We conducted exploratory experiments to determine the time and temperature conditions required to carbonize goosefoot seeds while maintaining their diagnostic morphological characteristics. Belcher sorted seeds from population CB002 by color, then placed them in the preheated Thermolyne muffle furnace under approximately 1 cm of sand in a small crucible. After the amount of time at temperature specified in Table 1, she assessed each seed for carbonization and preservation. Seeds that were fully carbonized with their seed margin intact were labeled "carbonized and well-preserved." Seeds categorized as "carbonized and popped" were fully carbonized but had a ruptured seed margin (Figure 5). These would be impossible to assess for margin shape, and popping might also lead to the disarticulation and loss of the testa. Finally, each seed was carefully opened using a razor blade to assess whether the seeds were fully carbonized. A seed that is incompletely carbonized is less likely to be preserved in the archaeological record.

### Results

### Growth Experiment

Our two free-living populations had sample proportions of 15% (CB002$_O$) and 34% (CB004$_O$) red morphs (see Supplemental Table 1), which much higher than the 1%–3% estimate used by archaeobotanists to characterize a typical wild assemblage (Smith 1985) and similar to Halwas's (2017:202) survey, where she found a maximum of 30% lighter-colored seeds in free-living populations. We found that the overall sample proportion of red morphs for our garden populations was even higher, at 50%. The two experimental populations did not have significantly different sample proportions (CB002$_O$, $n = 10$, mean = 49%, std. dev. = 0.15; CB004$_O$, $n = 10$, mean = 50%, std. dev. = 0.09; $t$-test,

**Table 1.** Summary of Carbonization Experiments.

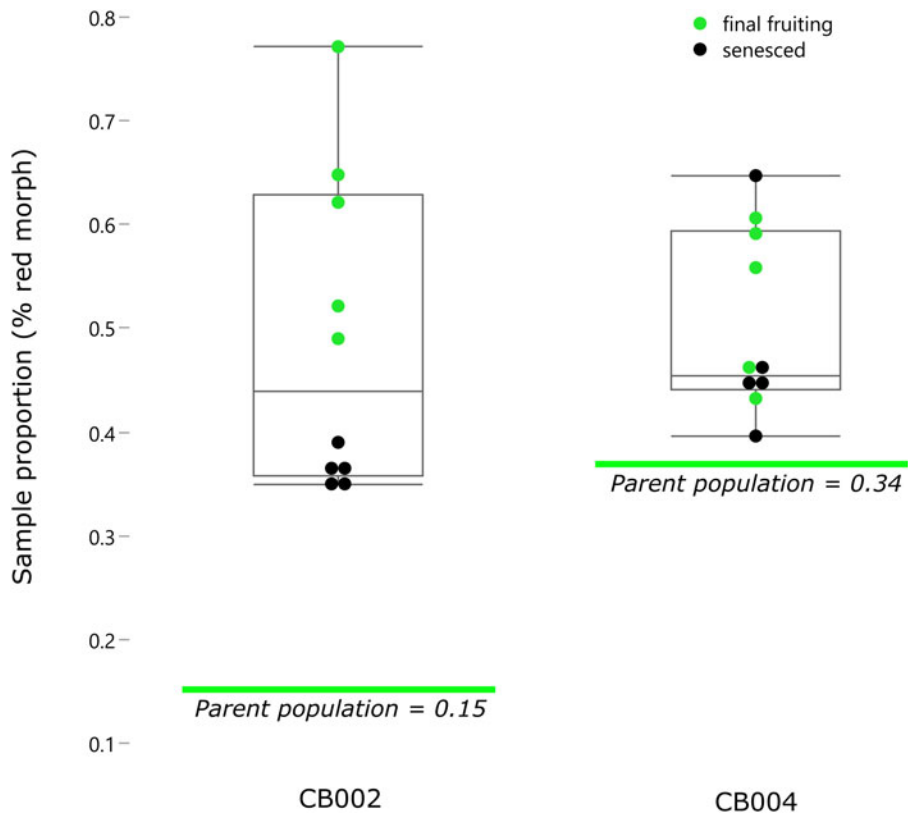| Temp. | Minutes | Sample Size ($n =$ number of seeds) | Red Morph (% carbonized and well preserved) | Red Morph (% carbonized and popped) | Red Morph (% not fully carbonized) | Black Morph (% carbonized and well preserved) | Black Morph (% carbonized and popped) | Black Morph (% not fully carbonized) |
|---|---|---|---|---|---|---|---|---|
| 250°C | 25 | 10 | 30 | 20 | 50 | 20 | 50 | 10 |
| 300°C | 10 | 10 | 20 | 0 | 80 | 80 | 20 | 0 |
| 300°C | 15 | 10 | 100 | 0 | 0 | 0 | 100 | 0 |
| 350°C | 10 | 10 | 90 | 10 | 0 | 20 | 80 | 0 |
| 400°C | 5 | 20 | 100 | 0 | 0 | 10 | 90 | 0 |

**Figure 4.** Distributions of sample proportions of red morphs in our study. Boxes show the interquartile range; the center line is the median.



**Figure 5.** An example of a "popped" and carbonized seed from one of our carbonization experiments. Most of the seeds labeled "popped" broke into two pieces (two halves of the testa) and left the inner perisperm and embryo exposed. Granules of sand are attached to the exposed inner parts of the seed. (Color online)

$p = 0.7828$), but both had much higher mean sample proportions of red morphs than their parent populations. Statistical tests could not be performed to compare parent population to garden population, given that each parent population is a sample of one, but in both cases, the sample proportion of the free-living parent population was *lower than the entire range of variation* for the harvests from its offspring in the garden (Figure 4).

Phenological stage seems to play a role in determining red morph proportion (Figure 4). Senesced plants, whether they were harvested late or early, produced relatively low thin-testa morph proportions

(mean = 42%, std. dev. = 0.09) when compared to proportions from plants harvested during their final fruiting stage (mean = 57%, std. dev. = 0.10; *t*-test, *p* = 0.0025). This supports the hypothesis that red morphs are immature or juvenile seeds. Note that the two free-living parent populations produced fewer thin-testa morphs than their garden-grown offspring despite the fact that neither was senesced when the seeds were harvested (Figure 4). If all other things were equal, they should have had relatively high proportions of thin-testa morphs, like garden plants harvested before senescence, but this was not the case.

### Carbonization Experiment

Contrary to our expectations, we found that thick-testa morphs are less likely to be completely carbonized and well preserved than thin-testa morphs (Table 1). The range for percentage of well-preserved, carbonized thin-testa morphs was 20%–100% across all experiments, whereas for thick-testa morphs it was 0%–80%. Thick-testa morphs are more likely to rupture when carbonized, making it less likely that they will be observable in carbonized archaeobotanical assemblages. No single experiment yielded a 100% carbonized, well-preserved sample of both morphs. For thin-testa morphs, treatments at temperatures 300°C–400°C yielded 90%–100% fully carbonized, well-preserved seeds. This range of treatments, capable of creating well-preserved assemblages of red morphs, cumulatively only preserved 0%–20% of black morphs. Black morphs were best preserved by a treatment of 10 minutes at 300°C, which was not sufficient to carbonize 80% of red morphs, suggesting that all carbonized assemblages are biased but not necessarily always in the same direction.

### Discussion

This study adds new dimensions to the story of goosefoot domestication in eastern North America and has implications for how analysts interpret goosefoot assemblages, especially carbonized ones. The simplified narrative of goosefoot domestication generally agreed upon by archaeologists in eastern North America is that past peoples gathered the seeds of wild goosefoot either in their natural habitats or as an encouraged weed in cultivated fields (Anderson 1952; Asch and Asch 1977; Mueller et al. 2021; Rindos 1984; Smith 1992; Struever and Vickery 1973). Goosefoot was domesticated because of selective pressures exerted by human actions and relaxation of selective pressures that affect free-living populations, although domesticated populations were never completely isolated from wild and weedy ones (Asch and Asch 1977; Gremillion 1993a, 1993b; Halwas 2017). The plant domestication process is often simplified as linear in the archaeological literature, with archetypal wild and domesticated seed morphs that have clear, identifiable traits. Any seeds that do not fit these criteria are the intermediate forms that represent a point somewhere between wild and domesticated. For our character of interest, it has long been clear that "there is no evidence that seed coat thickness was reduced in a gradual, linear fashion in the past" (Gremillion 1993a:160). Even so, paleoethnobotanists assign labels such as "domesticated," "cultivar," "intermediate," "weed," and "wild" to seed types in carbonized or desiccated forms, seemingly as a shorthand or heuristic device (Asch and Asch 1977; Fritz and Smith 1988; Gremillion 1993b; Patton and Curran 2016; Smith 1984, 1985; Smith and Yarnell 2009).

Our results suggest that this kind of characterization is obscuring our understanding of *Chenopodium* domestication, because these plants are incredibly plastic and produce a spectrum of seed types. First, the idea that only 1%–3% of seeds harvested from free-living goosefoot will be red morphs must be laid to rest, along with the associated interpretations of individual seeds with thin testas as domesticated types. Our data demonstrate that nondomesticated plants can produce up to 77% red morphs when cultivated (Figure 4). No garden-grown plant produced less than 35% red morphs. Our small sample from free-living populations produced 15%–34% of this seed type (Figure 4). Unexpectedly, our carbonization experiments also showed that red morphs are likely to be *overrepresented* in carbonized assemblages compared to black morphs. This means that mixed, carbonized assemblages, even with a very high proportion of red morphs, cannot be interpreted as evidence of a domesticated subspecies. In addition, it is time to retire the "intermediate" or "weed" category from *Chenopodium* analyses (e.g., Fritz 1986:83; Gremillion 1993b:499; Patton and Curran 2016:143; Smith and Yarnell 2009:6564). This category is based on two observations that appear to be invalid: (1) that thin-testa morphs are rare in harvests from nondomesticated plants and (2) that

there is a clear break in testa measurements between thin-testa morphs (<20 μm) and thick-testa morphs (>40 μm). Although we did not measure testa thickness for this study, Halwas's (2017:201) sample, which included all seed phenotypes, varied continuously between 25 μm and 47 μm.

We have used "free-living" rather than "wild" in this article in recognition of that fact that all *Chenopodium* on the landscape in eastern North America is weedy, both in the ecological sense and in the sense that is has been evolving in anthropogenic landscapes for thousands of years (Harlan and De Wet 1965). It is impossible to know whether the free-living plants that we have studied and cultivated, or those analyzed by Asch and Asch (1977, 1985), Smith (1992), Patton and Curran (2016), and Halwas (2017) have the same evolutionary history. Part of the explanation for the divergent observations that researchers have made over the years (Asch and Asch 1985; Halwas 2017; Patton and Curran 2016; Smith 1992) could be the remarkable plasticity of this species. But we may also be blindly sampling from a mix of feral descendants of domesticated plants, those whose ancestors were never cultivated, and members of both groups that have since hybridized with each other and other *Chenopodium* species, of which there are many. Only genetic analysis can begin to tease out these differences in lineage and their influence on phenotype.

We are emphatically not suggesting that there is no domesticated goosefoot in the archaeological record of eastern North America. Well-preserved (mostly uncarbonized) assemblages in the Ozarks, in Appalachia, and at Riverton do not look like any assemblage of seeds from modern populations of *C. berlandieri* that we have ever seen. Moreover, there appear to be multiple different landraces of domesticated goosefoot. Examples include the famous assemblage stored in a beautiful woven bag at Edens Bluff, Arkansas, which is made up of relatively uniform uncarbonized goosefoot seeds with thin, black, faintly honeycomb-pitted testas (Figure 1; Fritz 1986:85). The relative homogeneity of this assemblage is apparent even in comparison to other domesticated assemblages: it had the second-lowest coefficient of variation for seed diameter among the 18 assemblages that Gremillion (1993a) compared. This uniformity does not occur in modern free-living populations that we have seen or that others have reported. A later assemblage from nearby Holman Rockshelter is made up of seeds with smooth, golden testas, the so-called pale variety, entirely different from the Edens Bluff landrace (Fritz 1984; Wilson 1981; see Figure 1). This seed color is rare in modern populations, and no one has yet documented seeds from free-living plants that lack an outer epiderm. Consequently, although thin-testa goosefoot seeds *are* characteristic of this species' domesticated forms, the presence of this phenotype alone in a mixed assemblage—especially a carbonized one—is not necessarily indicative of domestication or any evolutionary change in that direction. Rather, goosefoot produces polymorphic seeds under a variety of conditions, and it is *the loss of this plasticity,* evident in internally homogenous but unique assemblages like those at Edens Bluff and Holman, that is the true marker of domestication.

If variation in red morph proportion is not a reliable indicator of evolution toward a domesticated form, or of the presence of distinct weed populations, then what does it reflect? Our research shows that senesced goosefoot plants have more thick-testa seeds than plants that have not yet senesced (Figure 4). Consequently, a mixed assemblage with a high proportion of thin-testa morphs may be interpreted as a harvest taken before senescence, which is an agricultural practice with potential evolutionary effects. Over the course of many generations, such a harvesting practice could have selected for populations that matured simultaneously if the seeds of early senescing plants were disadvantaged in subsequent plantings by having a higher proportion of dormant seeds. Such a harvest strategy could also facilitate the switch from a preexisting seed dispersal strategy, likely endozoochory (dispersal in animal feces) in the case of goosefoot. By beating herbivores to the harvest, humans can insert themselves into the life cycle of annual plants as seed dispersers, relaxing selective pressures to maintain tough seeds that can survive digestion (Spengler and Mueller 2019).

Our most significant result is that the garden environment caused the goosefoot plants in our experiment to produce more thin-testa morphs than their free-living parent populations. Red morph proportions in cultivated populations were *universally* higher than those of free-living parent populations, and Halwas's (2017) study demonstrated the same phenomenon. These data suggest that something about cultivation or the garden environment triggers goosefoot plants from many different local

populations to produce more red morphs. Follow-up experiments will be necessary to determine what it is about the garden environment that elicits this response from goosefoot. For now, we can say that goosefoot responds to cultivation in a way that would have been immediately beneficial to ancient farmers. It may have been partly this plasticity that encouraged people to deepen their relationship with this plant.

## Conclusions

We used common garden and carbonization experiments to explore the determinates of goosefoot seed morphology in the archaeological record in order to better understand the domestication of this species. Our data demonstrate that nondomesticated goosefoot produces a much higher proportion of thin-testa red morphs (an average of 50% in our garden experiment, 15%–34% in free-living parent populations) than the previously accepted estimate of 1%–3% (Smith 1985). Within the experiment, the proportion of red morphs is significantly higher in harvests taken from plants before senescence, supporting an interpretation of these seeds as differentially developed. However, this did not hold true when comparing free-living plants with their garden-grown offspring. Harvests from cultivated plants, whatever their phenological stage when harvested, contained more thin-testa morphs than those from their free-living parent populations, even though the latter were not senesced when harvested (Figure 4). This suggests that, overall, the garden growth environment is the best predictor of red morph prevalence, although more harvests of free-living plants could strengthen this conclusion.

Our observations do not support previous interpretations of phenotypically mixed *Chenopodium* assemblages as deriving from distinct domesticated versus wild/weedy populations (Gremillion 1993b; Patton and Curran 2016; Simon and Parker 2006; Smith and Yarnell 2009). Such assemblages could just as easily have come from a single population, or even a single plant. We also showed that thin-testa morphs are more likely to be preserved via carbonization than thick testa ones. Analysts should therefore exercise caution interpreting carbonized assemblages, given that red morphs are likely to be overrepresented wherever they occur. We suggest that mixed, carbonized assemblages of goosefoot, even with a very high proportion of red morphs, are better interpreted as harvests taken from free-living plants before they were completely senesced or from a cultivated population rather than as evidence for domestication per se. Yet by drawing on studies of well-preserved, desiccated assemblages, we can see that domesticated assemblages of goosefoot do occur, and that their hallmark is *a loss of seed polymorphism*.

Seed polymorphism in wild goosefoot is an example of a plastic trait that allows weedy plants to adapt swiftly to environmental variation (Herman and Sultan 2011; Mueller 2017b; Zeder 2018). Mueller and colleagues (2017) have previously argued that plasticity could have been an important prerequisite for domestication if it allowed early cultivators to see immediate benefits from their interventions. For example, by harvesting free-living goosefoot before it was completely senesced or cultivating it in settings similar to our raised bed, ancient people would have obtained a harvest that was immediately both more palatable and more likely to germinate when planted—without any selection for domesticated traits. Genetic assimilation is the fixation of a plastic trait so that it occurs irrespective of environmental triggers. When human-constructed niches are predictable, stable, and maintained for many generations, they are suitable places for (1) the fixation of plastic traits that confer advantages within the constructed niche and (2) the loss of plasticity through genetic assimilation (Pigliucci et al. 2006). We can see this outcome in some of the best-preserved assemblages of ancient domesticated goosefoot (Figure 1), making this species one of a growing list where genetic assimilation must be considered as a potentially important part of the domestication process (Lorant et al. 2017; Matesanz and Milla 2018; Ménard et al. 2013; Mueller et al. 2023).

to them and the many other researchers who have carefully and thoughtfully attended to goosefoot domestication in eastern North America over the years.

## Note

1. "Free-living" denotes "not intentionally cultivated" (Wilson 1990). We use "free-living" instead of "wild" because we cannot know if the populations on the landscape today are wild or feral descendants of cultivated populations, and because all *C. berlandieri* populations in this region have been growing in anthropogenic ecosystems for hundreds—if not thousands—of years.

## References Cited

Anderson, Edgar. 1952. *Plants, Man and Life*. Missouri Botanical Garden, St. Louis.

Asch, David L., and Nancy B. Asch. 1977. Chenopod as Cultigen: A Re-evaluation of Some Prehistoric Collections from Eastern North America. *Midcontinental Journal of Archaeology* 2(1):3–45.

Asch, David L., and Nancy B. Asch. 1985. Prehistoric Plant Cultivation in West Central Illinois. In *Prehistoric Food Production in North America*, edited by Richard. I. Ford, pp. 149–203. Museum of Anthropology, University of Michigan, Ann Arbor.

Bruno, Maria C. 2006. A Morphological Approach to Documenting the Domestication of *Chenopodium* in the Andes. In *Documenting Domestication: New Genetic and Archaeological Paradigms*, edited by Melinda A. Zeder, Daniel G. Bradley, Eve Emshwiller, and Bruce D. Smith, pp. 32–45. University of California Press, Berkeley.

Bruno, Maria C., and William T. Whitehead. 2003. *Chenopodium* Cultivation and Formative Period Agriculture at Chiripa, Bolivia. *Latin American Antiquity* 14(3):339–355.

Clemants, Steven E., and Sergei L. Mosyakin. 2003. *Chenopodium*. In *Flora of North America*, Vol. 4, edited by Flora of North America Editorial Committee, pp. 294. Oxford University Press, Oxford.

Crites, Gary D. 1993. Domesticated Sunflower in the Fifth Millennium B.P. Temporal Context: New Evidence from Middle Tennessee. *American Antiquity* 58(1):146–148.

Decker, Deena S. 1988. Origin(s), Evolution, and Systematics of *Cucurbita pepo* (Cucurbitaceae). *Economic Botany* 42(1):4–15.

de Villemereuil, Pierre, Oscar E. Gaggiotti, Médéric Mouterde, and Irène Till-Bottraud. 2016. Common Garden Experiments in the Genomic Era: New Perspectives and Opportunities. *Heredity* 116(3):249–254.

Emerson, Thomas E., Kristin M. Hedman, Mary L. Simon, Mathew A. Fort, and Kelsey E. Witt. 2020. Isotopic Confirmation of the Timing and Intensity of Maize Consumption in Greater Cahokia. *American Antiquity* 85(2):241–262.

Fritz, Gayle J. 1984. Identification of Cultigen Amaranth and Chenopod from Rockshelter Sites in Northwest Arkansas. *American Antiquity* 49(3):558–572.

Fritz, Gayle J. 1986. Prehistoric Ozark Agriculture: The University of Arkansas Rockshelter Collections. PhD dissertation, Department of Anthropology, University of North Carolina, Chapel Hill.

Fritz, Gayle J., and John H. House. 2022. Native Crops on the Threshold of European Contact: Ritual Seed Deposits at Kuykendall Brake, Arkansas. *Southeastern Archaeology* 41(2):121–141.

Fritz, Gayle J., and Bruce D. Smith. 1988. Old Collections and New Technology: Documenting the Domestication of *Chenopodium* in Eastern North America. *Midcontinental Journal of Archaeology* 13(1):3–27.

Gremillion, Kristen J. 1988. Changing Roles of Wild and Cultivated Plant Resources among Early Farmers of Eastern Kentucky. *Southeastern Archaeology* 17(2):140–157.

Gremillion, Kristen J. 1993a. The Evolution of Seed Morphology in Domesticated *Chenopodium*: An Archaeological Case Study. *Journal of Ethnobiology* 13:149–169.

Gremillion, Kristen J. 1993b. Crop and Weed in Prehistoric Eastern North America: The *Chenopodium* Example. *American Antiquity* 58(3):496–509.

Gremillion, Kristen J., and Dolores R. Piperno. 2009. Human Behavioral Ecology, Phenotypic (Developmental) Plasticity, and Agricultural Origins. *Current Anthropology* 50(5):615–619.

Halwas, Sara J. 2017. Domesticating *Chenopodium*: Applying Genetic Techniques and Archaeological Data to Understanding Pre-Contact Plant Use. PhD dissertation, Interdiciplinary Program, Departments of Biological Sciences and Anthropology, University of Manitoba, Winnipeg, Manitoba, Canada.

Harlan, Jack R., and J. M. J. De Wet. 1965. Some Thoughts about Weeds. *Economic Botany* 19:16–24.

Harlan, Jack R., J. M. J. De Wet, and E. Glen Price. 1973. Comparative Evolution of Cereals. *Evolution* 27(2):311–325.

Hart, John P., William A. Lovis, and M. Anne Katzenberg. 2021. Early Maize in Northeastern North America: A Comment on Emerson and Colleagues. *American Antiquity* 86(2):425–427.

Heiser, Charles B. 2008. The Sunflower (*Helianthus annuus*) in Mexico: Further Evidence for a North American Domestication. *Genetic Resources and Crop Evolution* 55:9–13.

Herman, Jacob J., and Sonia E. Sultan. 2011. Adaptive Transgenerational Plasticity in Plants: Case Studies, Mechanisms, and Implications for Natural Populations. *Frontiers in Plant Science* 2:1–10.

Hunter, Andrea A. 1992. Utilization of *Hordeum pusillum* (Little Barley) in the Midwest United States: Applying Rindos' Co-evolutionary Model of Domestication. PhD dissertation, Department of Anthropology, University of Missouri, Columbia.

Langlie, BrieAnna S., Christine A. Hastorf, Mario C. Bruno, Marc Bermann, Renée M. Bonzano, and William C. Condarco. 2011. Diversity in Andean *Chenopodium* Domestication: Describing a New Morphological Type from La Barca, Bolivia 1300–1250 B.C. *Journal of Ethnobiology* 31(1):72–88.

López, Laura M., Aylen Capparelli, and Axel Emil Nielsen. 2011. Traditional Post-Harvest Processing to Make Quinoa Grains (*Chenopodium quinoa* var. *quinoa*) Apt for Consumption in Northern Lipez (Potosí, Bolivia): Ethnoarchaeological and Archaeobotanical Analyses. *Archaeological and Anthropological Sciences* 3:49–70.

Lopinot, Neal H. 1984. Archaeobotanical Formation Processes and Late Middle Archaic Human-Plant Interrelationships in the Midcontinent, U.S.A. PhD dissertation, Department of Anthropology, University of Illinois, Champaign-Urbana.

Lorant, Anne, Sarah Pedersen, Irene Holst, Matthew B. Hufford, Klaus Winter, Dolores Piperno, and Jeffrey Ross-Ibarra. 2017. The Potential Role of Genetic Assimilation during Maize Domestication. *PLoS ONE* 12(9):e0184202. https://doi.org/10.1371/journal.pone.0184202.

Märkle, Tanja, and Manfred Rösch. 2008. Experiments on the Effects of Carbonization on Some Cultivated Plant Seeds. *Vegetation History and Archaeobotany* 17(1):257–263.

Matesanz, Silvia, and Rubén Milla. 2018. Differential Plasticity to Water and Nutrients between Crops and Their Wild Progenitors. *Environmental and Experimental Botany* 145:54–63.

Ménard, Léa, Doyle McKey, Gilda S. Mühlen, Bruno Clair, and Nick P. Rowe. 2013. The Evolutionary Fate of Phenotypic Plasticity and Functional Traits under Domestication in Manioc: Changes in Stem Biomechanics and the Appearance of Stem Brittleness. *PLoS ONE* 8(9):e74727. https://doi.org/10.1371/journal.pone.0074727.

Mueller, Natalie G. 2017a. An Extinct Domesticated Subspecies of Erect Knotweed in Eastern North America: *Polygonum erectum* L. ssp. *watsoniae* (Polygonaceae). *Novon* 25(2):166–179.

Mueller, Natalie G. 2017b. Documenting Domestication in a Lost Crop (*Polygonum erectum* L.): Evolutionary Bet-Hedgers under Cultivation. *Vegetation History and Archaeobotany* 26(3):313–327.

Mueller, Natalie G. 2017c. Evolutionary "Bet-Hedgers" under Cultivation: Investigating the Domestication of Erect Knotweed (*Polygonum erectum* L.) using Growth Experiments. *Human Ecology* 45(2):189–203.

Mueller, Natalie G. 2017d. Carbonization, Differential Preservation, and Sampling Bias in Domestication Studies: An Erect Knotweed (*Polygonum erectum* L.) Case Study. *Journal of Archaeological Science: Reports* 13:303–311.

Mueller, Natalie G., Gayle J. Fritz, Paul Patton, Stephen B. Carmody, and Elizabeth T. Horton. 2017. Growing the Lost Crops of Eastern North America's Original Agricultural System. *Nature Plants* 3(17092):1–5.

Mueller, Natalie G., Steven T. Goldstein, Damaris Odeny, and Nicole Boivin. 2022. Variability and Preservation Biases in the Archaeobotanical Record of *Eleusine coracana* (Finger Millet): Evidence from Iron Age Kenya. *Vegetation History and Archaeobotany* 31(3):279–290.

Mueller, Natalie G., Elizabeth T. Horton, Megan E. Belcher, and Logan Kistler. 2023. The Taming of the Weed: Developmental Plasticity Facilitated Plant Domestication. *PLoS ONE* 18(4):e0284136. https://doi.org/10.1371/journal.pone.0284136.

Mueller, Natalie G., Robert N. Spengler III, Ashley Glenn, and Kunsang Lama. 2021. Bison, Anthropogenic Fire, and the Origins of Agriculture in Eastern North America. *Anthropocene Review* 8(2):141–158.

Mueller, Natalie G., Andrea White, and Peter Szilagyi. 2019. Experimental Cultivation of Eastern North America's Lost Crops: Insights into Agricultural Practice and Yield Potential. *Journal of Ethnobiology* 39(4):549–566.

Nordstrom, Carol. 1990. Evidence for the Domestication of *Chenopodium* in the Andes. *UC Berkeley McCown Archaeobotany Laboratory Reports* 19. https://escholarship.org/uc/item/39w7x4dz, accessed September 6, 2023.

Patton, Paul E., and Sabrina Curran. 2016. Archaic Period Domesticated Plants in the Mid-Ohio Valley: Archaeobotanical Remains from the County Home Site (33at40), Southeastern Ohio. *Midcontinental Journal of Archaeology* 41(2):127–158.

Pigliucci, Massimo, Courtney J. Murren, and Carl D. Schlichting. 2006. Phenotypic Plasticity and Evolution by Genetic Assimilation. *Journal of Experimental Biology* 209(12):2362–2367.

Piperno, Dolores R. 2017. Assessing Elements of an Extended Evolutionary Synthesis for Plant Domestication and Agricultural Origin Research. *PNAS* 114(25):6429–6437.

Rindos, David. 1984. *The Origins of Agriculture: An Evolutionary Perspective*. Academic Press, Orlando, Florida.

Simon, Mary L., Kristin M. Hedman, and Thomas E. Emerson. 2021. Interpreting Isotopic and Macrobotanical Evidence for Early Maize in the Eastern Woodlands: A Response to Hart and Colleagues. *American Antiquity* 86(2):428–430.

Simon, Mary L., and Kathryn E. Parker. 2006. Prehistoric Plant Use in the American Bottom: New Thoughts and Interpretations. *Southeastern Archaeology* 25(2):212–257.

Smith, Bruce D. 1984. *Chenopodium* as a Prehistoric Domesticate in Eastern North America: Evidence from Russell Cave, Alabama. *Science* 226(4671):165–167.

Smith, Bruce D. 1985. *Chenopodium berlandieri* ssp. *jonesianum*: Evidence for a Hopewellian Domesticate from Ash Cave, Ohio. *Southeastern Archaeology* 4(2):107–133.

Smith, Bruce D. 1992. *Rivers of Change: Essays on Early Agriculture in Eastern North America*. Smithsonian Institution, Washington, DC.

Smith, Bruce D. 2006. Eastern North America as an Independent Center of Plant Domestication. *PNAS* 103(33):12223–12228.

Smith, Bruce D. 2014. The Domestication of *Helianthus annuus* L. (Sunflower). *Vegetation History and Archaeobotany* 23(1):57–74.

Smith, Bruce D., and Wesley C. Cowan. 1987. Domesticated *Chenopodium* in Prehistoric Eastern North America: New Accelerator Dates from Eastern Kentucky. *American Antiquity* 52(2):355–357.

Smith, Bruce D., and Vicki A. Funk. 1985. A Newly Described Subfossil Cultivar of *Chenopodium* (Chenopodiaceae). *Phytologia* 57(7):445–448.

Smith, Bruce D., and Richard A. Yarnell. 2009. Initial Formation of an Indigenous Crop Complex in Eastern North America at 3800 B.P. *PNAS* 106(16):6561–6566.

Spengler, Robert N., and Natalie G. Mueller. 2019. Grazing Animals Drove Domestication of Grain Crops. *Nature Plants* 5(7):656–662.

Struever, Stuart, and Kent D. Vickery. 1973. The Beginnings of Cultivation in the Midwest-Riverine Area of the United States. *American Anthropologist* 75(5):1197–1220.

Wilson, Hugh D. 1981. Domesticated *Chenopodium* of the Ozark Bluff Dwellers. *Economic Botany* 35:233–239.

Wilson, Hugh D. 1990. Quinua and Relatives (*Chenopodium* sect. *Chenopodium* subsect. *Cellulata*). *Economic Botany* 44:92–110.

Wright, Patti J. 2003. Preservation or Destruction of Plant Remains by Carbonization? *Journal of Archaeological Science* 30(5):577–583.

Wright, Patti J. 2008. Understanding the Carbonization and Preservation of Sunflower and Sumpweed Remains. *Midcontinental Journal of Archaeology* 33(2):139–153.

Yarnell, Richard A. 1972. *Iva annua* var. *macrocarpa*: Extinct American Cultigen? *American Anthropologist* 74(3):335–341.

Zeder, Melinda A. 2018. Why Evolutionary Biology Needs Anthropology: Evaluating Core Assumptions of the Extended Evolutionary Synthesis. *Evolutionary Anthropology* 27(6):267–284.