



# Carbonization, differential preservation, and sampling biases in domestication studies: An erect knotweed (*Polygonum erectum* L.) case study



Natalie G. Mueller

Washington University in St. Louis, Department of Anthropology, United States

## ABSTRACT

Population morphometrics can be employed to explore the process of domestication, but only after accounting for biases introduced by taphonomic processes and sampling. For every cultivated plant, the challenges associated with carbonization, differential preservation, and sampling bias are different, as are the morphological characteristics of interest in domestication studies. This case study establishes correction factors and sampling parameters for assessing morphological indicators of domestication in erect knotweed (*Polygonum erectum* L.), an annual plant that was cultivated by Indigenous people in Eastern North America for about 2500 years. Documenting the unique domestication syndrome of erect knotweed creates three different sets of taphonomic and sampling problems that need to be addressed through experimentation and modeling: 1) Assess the morphometric effects of carbonization; 2) assess the effects of differential preservation; and 3) assess the effects of sampling error. The results of this study can be used by other analysts to identify domesticated assemblages of erect knotweed. These analyses also have implications for studies of plant domestication from the morphology of archaeological assemblages in general, especially when nominal variables are used to differentiate wild from domesticated populations (for example, the presence/absence of an abscission scar in cereals or the seed coat texture of chenopods).

## 1. Introduction

The analyses reported here were undertaken in the course of a study on erect knotweed (*Polygonum erectum* L.), an annual seed crop that was cultivated by ancient farmers in Eastern North America (ENA) from c. 3000–600 BP. Elsewhere, I have argued on the basis of population morphometrics that some assemblages of erect knotweed exhibit a unique domestication syndrome as a result of hundreds of generations of selection in agroecosystems (Mueller, 2017a, 2017b, 2017c). It is only possible to make such arguments, which are built on morphological comparisons of crop plants and their wild progenitors, after first exploring the many possible ways that archaeological assemblages may be altered and biased. For every cultivated plant, the challenges associated with carbonization, differential preservation, and sampling bias are different, as are the morphological characteristics of interest in domestication studies (Boardman and Jones, 1990; Smith, 2014; Wright, 2003, 2008; Yarnell, 1978). The purposes of this paper are 1) to provide guidelines for the identification of domesticated erect knotweed from its carbonized fruits; and 2) to address sampling and preservation issues common to all domestication studies that rely on the morphology of carbonized remains.

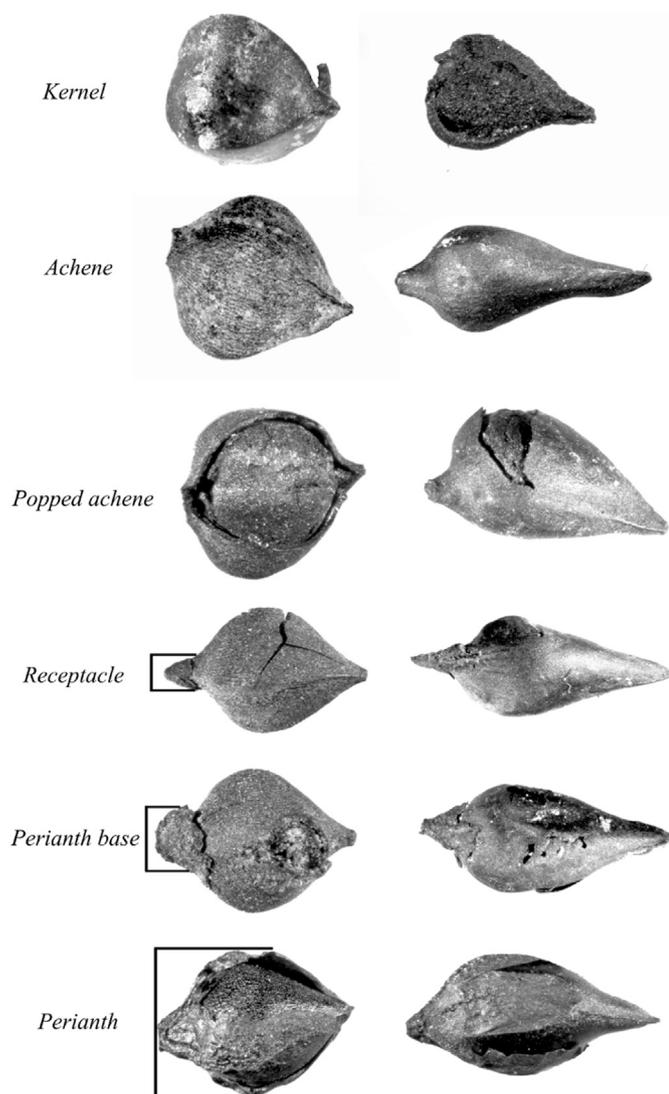
In ENA, an indigenous agricultural system began to develop c.

4000 BP. These early crops include some familiar plants: sunflowers (*Helianthus annuus* var. *macrocarpus* (DC.) Cockerell) and native squash varieties, such as acorn and crookneck squashes (*Cucurbita pepo* L. ssp. *ovifera* D.S. Decker), are among ENA's earliest domesticates (Crites, 1993; Kay et al., 1980). Sumpweed (*Iva annua* L. and its domesticated subspecies, *Iva annua* ssp. *macrocarpa* S.F. Blake) and goosefoot (*Chenopodium berlandieri* Moq. and its domesticated subspecies, *Chenopodium berlandieri* ssp. *jonesianum* Smith and Funk) were also domesticated by c. 3800 BP (Smith and Funk, 1985; Smith and Yarnell, 2009; Yarnell, 1972, 1978). By the Early Woodland period, c. 3000 BP, three new crops had entered the crop complex. These were maygrass (*Phalaris caroliniana* Walter), erect knotweed (*Polygonum erectum* L. and, by 2000 BP, its domesticated subspecies *Polygonum erectum* ssp. *watsoniae* N.G. Muell.), and little barley (*Hordeum pusillum* Nutt.) (Asch and Asch, 1978, 1985; Fritz, 1986, 1993; Mueller, 2017c; Simon and Parker, 2006). By the time of European colonization, several of these locally domesticated crops had apparently fallen out of use and were thus lost to history (Smith, 1992, 2006). Collectively, the native seed crops of ENA are referred to as the Eastern Agricultural Complex (EAC). EAC crops are commonly recovered from storage pits, hearths, and middens at archaeological sites spanning parts of modern day Arkansas, Illinois, Indiana, Iowa, Kentucky, Missouri, Ohio, and Tennessee (the “core

E-mail address: [ngmueller@gmail.com](mailto:ngmueller@gmail.com).

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**Fig. 1.** Shows different preservation states for carbonized erect knotweed and achene dimorphism. Kernels are usually identified as erect knotweed by association – without associated achenes they are not diagnostic to species. They are of no known value for domestication studies. Well preserved achenes are the subject of morphometric analyses, but are less useful when they popped because their shape is then distorted to varying degrees. Achenes with receptacles (thickened portion of the stem to which the fruit adheres) and perianth base are another well-preserved form that can be analyzed in domestication studies. Corrections for comparing specimens with and without preserved receptacles are given in Table 2. The perianth is the outer part of the flower, which adheres to mature erect knotweed achenes but is almost always absent on carbonized achenes. The examples with most of their perianth preserved shown here are from my carbonization experiments; the rest of the pictured achenes are from archaeological assemblages.

area,” Fritz, 1993). Erect knotweed has been recovered from sites spanning the core area. It seems to have entered the crop complex in western Appalachia c. 3000 BP and was cultivated at least until c. 600 BP. Domesticated assemblages have been recovered from the Middle Woodland Walker Noe site, Kentucky (c. 2000 BP), and from Mississippian sites in Illinois, Missouri, and Arkansas (1000–600 BP) (Mueller, 2017c).

Erect knotweed produces *achenes*: one-seeded fruits with hard pericarps (fruit coats). Each plant produces two distinctly different types of achenes, a phenomenon known as fruit dimorphism (Fig. 1). Throughout the growing season, erect knotweed produces tubercled achenes, which have thicker pericarps and do not germinate readily (Mueller, 2017d). Beginning in mid-September, it begins to produce smooth achenes, which have thinner pericarps and almost always

germinate the spring after they are produced. This phenomenon is called germination heteromorphism, and is a form of evolutionary bet-hedging (Slatkin, 1974; Silvertown, 1984; Childs et al., 2010). The tubercled morphs persist in the seed bank, allowing populations to survive adverse growing seasons (Mueller, 2017d). Domesticated erect knotweed (*Polygonum erectum* ssp. *watsoniae* N.G. Muell.) differs from its wild progenitor in that 1) its achenes are larger and differently shaped and; 2) harvests from domesticated plants contain very few or no tubercled morphs (Mueller, 2017a, 2017b). Documenting this unique domestication syndrome creates three different sets of taphonomic and sampling problems that need to be addressed through experimentation and modeling.

- 1) *Assess the morphometric effects of carbonization.* Carbonization preserves plant parts which would otherwise be subject to decay by converting starch, protein, lignin and other constituents of plant tissues into inert compounds when plant parts are exposed to heat in an anoxic or near anoxic environment (Braadbaart et al., 2004). Unfortunately, the process also distorts the original shape and size of the seed. In order to compare carbonized assemblages to modern reference collections and desiccated archaeological assemblages, correction factors must be applied. For erect knotweed, only preliminary correction factors for achene length and width existed prior to this study (Asch and Asch, 1985:144). Changes in shape had never been investigated.
- 2) *Assess the effects of differential preservation.* An important part of the domestication syndrome in erect knotweed is the elimination of germination heteromorphism: domesticated erect knotweed produced almost no tubercled achenes, instead producing smooth achenes that germinate more reliably and contain less inedible material (Mueller, 2017d). Can the sample proportions (% smooth achenes) of carbonized assemblages be taken at face value, or is one morph more likely to be preserved via carbonization than the other?
- 3) *Assess the effects of sampling error.* Small sample size is a problem in and of itself for the study of variability in a population. It is rare for entire erect knotweed achenes to be preserved; most assemblages are made up of many kernels and fragments of pericarp (Fig. 1). Even if every effort is made to sample well-preserved contexts, it is rare to find a sample of > 50 well-preserved achenes from a single well-defined context (Mueller, 2017c). Estimates of population proportion derived from small samples have large margins of error, so clear guidelines are needed to recognize which assemblages are likely to be outside the natural range of variation after taking into consideration the uncertainty introduced by sampling error. This is a problem shared by any researcher who is attempting to differentiate between wild and domesticated populations on the basis of nominal variables (for example, the presence/absence of an abscission scar in cereals or the seed coat texture of chenopods).

The first two problems were addressed in a series of carbonization experiments. The effects of sampling error were explored probabilistically.

## 2. Carbonization background

Two variables are fundamental to any carbonization experiment: temperature and duration of exposure to heat. Different seeds and fruits will carbonize with more or less exposure to heat, depending on their physical and chemical characteristics. Märkle and Rösch (2008) tested the seeds or fruits of seven different taxa to determine minimum conditions for inducing carbonization and maximum conditions under which diagnostic attributes of the seed or fruit would be retained. Some taxa had very small temperature ranges in which they could be carbonized without disintegrating. Oil seed crops in particular had very specific necessary conditions for preservation (2008:S260). For example, Wright (2008:145) suggests that sunflower achenes are

unlikely to be preserved unless they are carbonized at temperatures somewhere between 300 and 440 °C. Below 300° they still contain enough uncarbonized material to be susceptible to decay, and above 440° they become more friable and less likely to survive. Several different varieties of wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.) have larger ranges of conditions that will lead to preservation by carbonization, both in terms of temperature and duration of exposure (Boardman and Jones, 1990:5). Their comparably large survival window may contribute to their abundance at archaeological sites. A seed or fruit may be preserved by carbonization, but the characteristics of interest may be destroyed or distorted. Braadbaart and van Bergen (2005) found that emmer wheat (*Triticum dicoccum* Schrank), bread wheat (*Triticum aestivum* L.), and durum wheat (*Triticum durum* Desf.) can be distinguished from one another on the basis of aspect ratio (called slenderness in their study). But the characteristic shapes of each type of wheat disappear when they are heated at 290 °C, making their identification to species impossible. For this study, I had to take into particular consideration the preservation of the pericarp, the characteristics of which are fundamental to the study of erect knotweed domestication. Temperature and duration of exposure needed to be set at levels that would consistently preserve the pericarp.

When plant tissues are carbonized, they generally shrink as water and oils evaporate and other tissue constituents change chemical structure. To account for this shrinkage, the use of carbonization correction factors is common, especially in domestication studies where seed and fruit size is often the object of study. For the EAC domesticates sunflower and sunflower, correction factors were derived from average shrinkage in carbonization experiments and applied as multipliers to the length and width of carbonized assemblages (Asch and Asch, 1978; Powell, 2000; Tarighat et al., 2011; Yarnell, 1972, 1978; Smith, 2014; Wright, 2008). This method is employed to approximate size change, even though experiments have shown that multiple factors, especially temperature and duration of heat exposure, affect post-carbonization size (Braadbaart and Wright, 2007). Temperature and duration of heat exposure lead to differential deformation, but few methods are available to reconstruct carbonizing conditions in order to take this variation into account (but see Braadbaart et al., 2004; Braadbaart and Wright, 2007). For erect knotweed, there is relatively small temperature window in which whole achenes will be preserved, but the carbonization corrections derived by these experiments should still be seen as preliminary estimates to be refined by future studies.

The chemical composition of seeds and fruits can also determine how they are affected by carbonization. For example, Braadbaart and Wright (2007) used an heirloom variety of sunflower seeds for their study because modern commercial varieties have been bred to increase oil content. This is problematic if the goal is to compare them to ancient varieties with lower oil content because lipids evaporate rather than carbonize. Fat content of the seed or fruit will thus affect how much it shrinks when carbonized (Braadbaart and Wright, 2007). Studying a closely related species of knotweed, Yurtseva (2001) has shown that the pericarp of the tubercled achenes is thicker than that of the smooth achenes partly because it is fortified with layers of wax, which are absent in smooth achenes. Wax (a lipid) is not at all likely to be preserved by carbonization, so tubercled achenes can be expected to shrink more than smooth ones. On the other hand, smooth morphs (since they are not protected by layers of moisture-retardant wax) are likely to have a higher water content. Since water evaporates during carbonization, this might offset the difference. Asch and Asch (1978:144), in what are only described as “preliminary experiments,” found that the length of both morphs decreased by 20% when carbonized, but that the width of smooth morphs was not affected, whereas the width of tubercled morphs also decreased by 20%.

### 3. Materials and methods

#### 3.1. Phase I

From observing archaeological collections, it is clear that many carbonized erect knotweed achenes become puffed or popped as they are subjected to heat (Fig. 1). Parts of their perisperm bubble through the pericarp, obscuring their shape and size. When this occurs, the pericarp usually splits into pieces and falls away from the kernel (Fig. 1). Pericarp fragments and kernels are commonplace in archaeological assemblages, but these fruit parts are not very useful for the purposes of identifying domestication syndrome in erect knotweed. The goal of Phase I was to determine the temperature threshold at which pericarps begin to fracture and disintegrate.

I carbonized batches of erect knotweed achenes to determine what temperature resulted in assemblages of undistorted achenes. I pulled four groups of five achenes each from herbarium specimens and removed their perianths (adhering flower parts, see Fig. 1) before photographing them. I then embedded them in quartz sand in aluminum containers, covering each achene with sand grains to provide near-anoxic conditions. I fired them at 250, 275, 300, and 325 °C for 30 min. All the achenes fired above 300 °C were puffed or popped, whereas none of the achenes fired at or below 300 °C were, so I set the temperature for the larger experiment at 300 °C. Phase I suggests that the window of sufficient conditions for achene preservation is quite narrow: any fruits subjected to temperatures above 300 °C are likely to be represented by kernels and pericarp fragments in archaeological samples.

#### 3.2. Phase II

The goal of Phase II was to determine how carbonization affected the size and shape of erect knotweed achenes in general, and if there were differential effects depending on 1) pericarp texture; 2) achene size; or 3) achene maturity. I selected 50 tubercled and 50 smooth morphs from a batch harvested at the Bellows Creek Farm, Jefferson County, MO, on October 30, 2015 (Mueller, 2017d). At the time of the experiment, these achenes had been in cold storage for about 9 months. Of these, 25 of each were mature and 25 were immature. Mature and immature in the context of this experiment refer to whether or not the pericarp could be deformed by pressing it with a metal tool, not to their viability. Greenhouse experiments have shown that “immature” achenes are often viable. Maturity still seems to be a reasonable adjective to describe this attribute, though, since the softness of the pericarp is developmental and the proportion of mature (hard pericarp) achenes increases as the season progresses (Mueller, 2017d). Each achene was given a unique number and stored in a separate, labeled capsule throughout the experiment.

I photographed each achene with its perianth intact (Fig. 1) because I also wanted to see what effect carbonization would have on perianth preservation. In archaeological assemblages of carbonized seeds, it is very uncommon for any part of the perianth to be preserved, yet it seemed likely that many erect knotweed achenes retained their perianth up until the time that they were carbonized for two reasons. First, in the only large, uncarbonized assemblage of archaeological erect knotweed available, the Whitney Bluff site assemblage, 75% retain some or all of their perianth. Second, it seems time consuming and pointless to remove the perianth from erect knotweed achenes without also fracturing or removing the pericarp. The tough pericarp presents more of a barrier to digestion than the open, papery perianth. If ancient farmers were successful in removing the pericarp, the perianth would also be eliminated as a matter of course, so there would be little reason to remove the perianth but not the pericarp. The most parsimonious explanation for the lack of perianths on carbonized archaeological specimens seemed to be that they are easily destroyed during carbonization.

**Table 1**  
Morphometric measurements.

Table 1 gives the formulas used to generate the morphometric measures. All image analysis was completed using ImageJ open source image analysis software.

Measurements	Description	Procedure/Formula
Area	# of pixels within the margins of object	Select image of achene, area measured in mm <sup>2</sup>
Length	#of pixels along major axis of selected object	Draw line to measure length in mm
Width	Number of pixels along minor axis of selected object	Draw line to measure length in mm
Shape Factors		
Aspect Ratio	Degree of elongation; major axis divided by (mean of) minor axis (axes). Axes derived from an ellipse fitted around the selected object.	[Major axis]/[Minor axis]
Roundness	Roughly inverse of aspect ratio, a measure of elongation of the major axis with respect to the object's area. Axis derived from an ellipse fitted around the selected object.	$4 \times ([Area]/(\pi \times [Major axis]))$
Circularity	Relationship between perimeter and area; degree of departure from a circle. 1.0 is a perfect circle, 0.0 is a straight line.	$4\pi \times [Area]/[Perimeter]^2$

After they were photographed, achenes were transferred to ceramic crucibles, covered in quartz sand, and fired for 30 min in a muffle furnace pre-heated to 300 °C. Each achene was then photographed again. Both pre- and post-carbonization images were subjected to the morphometric analysis to ascertain changes in both shape and size. Morphometric measures were taken using ImageJ open source image analysis software; formulas are given in Table 1.

### 3.3. Phase III

Given the major differences in the chemical composition and structure of the pericarps of smooth and tubercled morphs, I wanted to know if one was more likely to preserve via carbonization than the other. I carbonized 100 achenes of each morph, 50 each at 300 °C and 350 °C ( $n = 200$ ). I then counted how many of each type retained an intact pericarp, how many were popped, and how many pericarps had completely fractured leaving only a kernel.

### 3.4. Receptacle and perianth base

Two different types of well-preserved achenes occur. Type 1 consists of only the achene proper, and Type 2 consists of the achene and an adhering receptacle covered in remnants of the perianth – referred to here as the perianth base (Fig. 1). A receptacle is a thickened portion of a stem to which the fruit adheres. In the case of erect knotweed, it is usually covered by pieces of perianth (outer flower parts, commonly referred to as petals) that adhere to the mature fruit. Achenes lacking perianth bases will be, on average, shorter and smaller than those with preserved perianth bases, as well as differently shaped. I developed a set of correction factors for achenes missing their perianth base so that I could include both types in the morphometric analysis that were part of a larger study (Mueller, 2017c). The application of these correction factors increased the number of measurable archaeological achenes in the assemblages I analyzed from 747 to 1267, and can be employed in future morphological studies of this crop species in order to maximize sample size. To develop the corrections, I photographed and measured twenty achenes of both morphs from modern populations before and after removing their perianth base.

## 4. Results

### 4.1. Carbonization corrections

#### 4.1.1. Size

The effects of carbonization on the size of achenes are pronounced. By any measure, both morphs shrink significantly ( $p \geq 0.0001$  for change in area, length, width, and LXW for both morphs). But this effect is not uniform between the two morphs. Tubercled morphs shrink more, averaging only 70% of their previous area, whereas smooth

morphs average 76%. This difference is not a result of size differences between the two morphs: there is a very low correlation between original area and percentage remaining ( $r^2 = 0.106$ ). It is likely that differential shrinkage rate between the two morphs is caused by the composition of the pericarp itself. This conclusion is further supported by the fact that there are differences between how mature and immature achenes are effected by carbonization. In both morphs, soft, immature achenes whose pericarps haven't finished developing shrink less than fully developed achenes. The recommended correction factors reported in Table 2 are derived from the pooled effects on mature and immature achenes. They are thus probably conservative since ancient harvests taken in late October or early November, when the seeds are most abundant and easy to remove, are unlikely to have contained many immature achenes.

#### 4.1.2. Shape

Carbonization also affects achene shape. Differential effects between the two morphs are present but subtle. The aspect ratio of smooth morphs is not significantly different before and after carbonization ( $p = 0.8634$ ), whereas the aspect ratio of tubercled morphs decreases an average of 0.10, and this difference is significant ( $p = 0.0001$ ). This makes sense because the minor axis is differentially effected by carbonization between the two morphs (Table 3). The same is true of achene roundness: tubercled morphs roundness increases significantly, on average 0.50 (Table 4;  $p = 0.0001$ ), and smooth morphs do not change significantly one way or the other (Table 4;  $p = 0.2741$ ). But carbonization correction factors should not be used for aspect ratio or roundness because for both of these measures one standard deviation from the mean difference before and after carbonization includes both positive and negative values – i.e. shape, as measured by these descriptors, does not change in a predictable manor due to carbonization (Table 4).

The most sensitive measure of shape deployed here is circularity, because it measures the relationship between area and perimeter, rather than relying on single point-to-point distances across the shape. Probably due to its greater sensitivity, circularity is the most effective shape descriptor for quantifying the slight differences in shape that result from carbonization. The circularity of both morphs increases significantly ( $p = 0.0007$  for smooth morphs,  $p = 0.0004$  for tubercled morphs; Table 4). Change in circularity is correlated with original circularity (Fig. 2): the most circular achenes change shape less, whereas the least circular achenes gained the most circularity with carbonization. The effect is that the same assemblage of achenes is more homogenous with a smaller range of circularity values after carbonization than it was before carbonization. A simple additive correction factor using the average change in circularity overcorrects and yields a reconstructed mean that is too low (Table 5). A more accurate correction factor was derived from the equation of the least-squares regression line fit to a plot of post-carbonization circularity versus

**Table 2**

Recommended correction factors for archaeological assemblages of erect knotweed.

Table 2 recommended correction factors. Correction factors are only suggested for aspects of shape and size that change significantly and predictably (i.e. change is always in the same direction). See *Results* for more detailed discussion.

Corrections for changes due to carbonization				
Smooth			Tubercled	
Size	W/ perianth	No perianth	W/perianth	No perianth
Area	$A2 * 1.24 = A1$	$A2 * 1.18 = A1$	$A2 * 1.30 = A1$	$A2 * 1.22 = A1$
Length × Width	$LXW_2 * 1.20 = LXW_1$	$LXW_2 * 1.17 = LXW_1$	$LXW_2 * 1.29 = LXW_1$	$LXW_2 * 1.23 = LXW_1$
Shape				
Circularity	$C_1 = [(C_2 - 0.503) / -0.486] + C_2$			

Corrections for changes due to missing perianth base and receptacle				
Smooth			Tubercled	
Area	$A_{no\ receptacle} * 1.10 = A_1$		$A_{no\ receptacle} * 1.13 = A_1$	
Length	$L_{no\ receptacle} * 1.12 = L_1$		$L_{no\ receptacle} * 1.17 = L_1$	
Aspect ratio	$AR_{no\ receptacle} + 0.14 = AR_1$		$AR_{no\ receptacle} + 0.19 = AR_1$	
Roundness	$R_{no\ receptacle} - 0.03 = R_1$		$R_{no\ receptacle} - 0.10 = R_1$	

**Table 3**

Effects of carbonization on size.

N = 100		Smooth	Tubercled
Area	Mean ± Std. Dev.	3.12 ± 0.467	3.41 ± 0.489
	Std. Err. of Mean	0.0674	0.0692
Area carbonized	Mean ± Std. Dev.	2.37 ± 0.396	2.37 ± 0.367
	Std Err of Mean	0.0571	0.0518
% remaining	Mean ± Std. Dev.	0.76 ± 0.089	0.70 ± 0.080
	Std Err of Mean	0.0128	0.0113
Length	Mean ± Std. Dev.	3.12 ± 0.254	2.83 ± 0.183
	Std Err of Mean	0.0366	0.0259
Length carbonized	Mean ± Std. Dev.	2.81 ± 0.237	2.47 ± 0.193
	Std Err of Mean	0.0342	0.0273
% remaining	Mean ± Std. Dev.	0.90 ± 0.036	0.87 ± 0.042
	Std Err of Mean	0.0052	0.0060
Width	Mean ± Std. Dev.	1.6 ± 0.189	1.90 ± 0.226
	Std Err of Mean	0.0272	0.0319
Width carbonized	Mean ± Std. Dev.	1.43 ± 0.212	1.53 ± 0.171
	Std Err of Mean	0.0306	0.0241
% remaining	Mean ± Std. Dev.	0.89 ± 0.088	0.81 ± 0.080
	Std Err of Mean	0.0127	0.0114
L × W	Mean ± Std. Dev.	5.01 ± 0.807	5.37 ± 0.809
	Std Err of Mean	0.1164	0.1144
L × W carbonized	Mean ± Std. Dev.	4.02 ± 0.0730	3.79 ± 0.589
	Std Err of Mean	0.1054	0.0834
% remaining	Mean ± Std. Dev.	0.80 ± 0.089	0.71 ± 0.089
	Std Err of Mean	0.0129	0.0125

change in circularity ( $C_1 - C_2$ ) (Fig. 2). I solved the equation of the least squares regression line for change in circularity (the unknown variable for archaeological assemblages):

$$C_2 = 0.503 - 0.486 * (C_1 - C_2) \rightarrow C_1 - C_2 = (C_2 - 0.503) / -0.486$$

The expected change in carbonization for a particular achene can be calculated and applied to its post-carbonization circularity to reconstruct its original circularity using the following formula:

$$C_1 = [(C_2 - 0.503) / -0.486] + C_2$$

I checked this correction factor using the known values for the pre-carbonization assemblage. It calculated the mean circularity of the assemblage to within 0.01 of the real value (Table 5).

#### 4.2. Receptacle and perianth base

There was a significant difference between the size of the perianth

**Table 4**

Effects of carbonization on achene shape.

N = 100		Smooth	Tubercled
Aspect ratio ( $AR_1$ )	Mean ± Std. Dev.	1.94 ± 0.254	1.46 ± 0.169
	Std. Err. of Mean	0.0366	0.0239
Aspect ratio carbonized ( $AR_2$ )	Mean ± Std. Dev.	1.93 ± 0.368	1.57 ± 0.194
	Std Err of Mean	0.0531	0.0275
$AR_1 - AR_2$	Mean ± Std. Dev.	0.01 ± 0.250	-0.10 ± 0.138
	Std. Err. of Mean	0.0361	0.0195
Roundness ( $R_1$ )	Mean ± Std. Dev.	0.52 ± 0.063	0.69 ± 0.076
	Std. Err. of Mean	0.0091	0.0107
Roundness carbonized ( $R_2$ )	Mean ± Std. Dev.	0.54 ± 0.092	0.64 ± 0.082
	Std. Err. of Mean	0.0133	0.0116
$R_1 - R_2$	Mean ± Std. Dev.	0.00 ± 0.070	0.05 ± 0.062
	Std. Err. of Mean	0.0102	0.0088
Circularity ( $C_1$ )	Mean ± Std. Dev.	0.46 ± 0.086	0.41 ± 0.073
	Std. Err. of Mean	0.0124	0.0103
Circularity carbonized ( $C_2$ )	Mean ± Std. Dev.	0.52 ± 0.060	0.61 ± 0.055
	Std. Err. of Mean	0.0087	0.0078
$C_1 - C_2$	Mean ± Std. Dev.	-0.10 ± 0.113	-0.20 ± 0.071
	Std. Err. of Mean	0.0164	0.0100

bases between the two morphs: the smooth morph perianth base accounts for a smaller percentage of total achene area than does the perianth base of the tubercled morph, and is smaller in absolute terms (Table 6). Not surprisingly, removing the perianth base also changes the shape of the achene (Table 6). Here, aspect ratio and roundness show more clear differences than does circularity because the shape of the achene mostly changes in that its major axis is reduced when the perianth base is removed. Recommended correction factors are given in Table 2.

#### 4.3. Preservation of perianth

The perianth is almost never preserved on archaeological carbonized specimens. At the outset of this experiment I considered it likely that the perianth was destroyed by carbonization. Results suggest that, to the contrary, the perianth is not at all likely to be destroyed by carbonization, at least under the conditions used in this study. Of 100 achenes carbonized in Phase III, 36 retained their entire perianth after carbonization, and 60 retained parts of it. Only 4 completely lacked a perianth as a result of carbonization. It is possible that a different firing temperature or duration would destroy the perianth, or that taphonomic processes after carbonization removed the perianths from

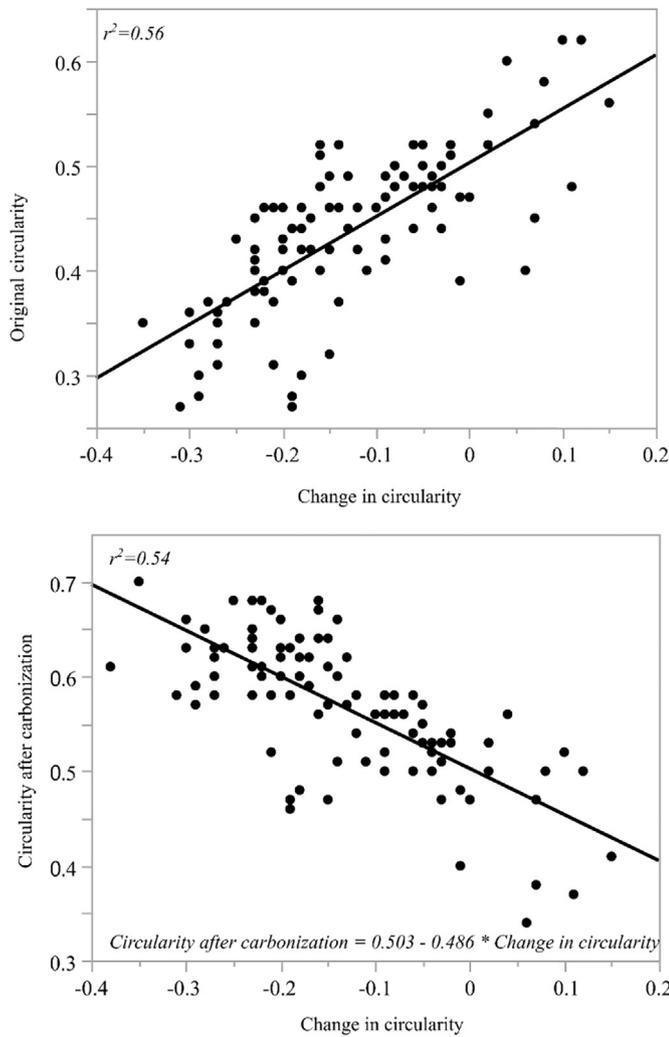


Fig. 2. Shows the strong correlations between achene circularity and change in circularity. The equation of the line fit to the correlation between post-carbonization circularity and change in circularity was used to derive the correction factor recommended for achene shape (Table 2; Table 5).

Table 5  
Tests of circularity correction factor.

Table 5 reports the results of tests on two different types of correction factors for changes in achene circularity due to carbonization: a linear function correction, and an additive correction factor. The linear function correction is able to more accurately predict the real values for the assemblage before carbonization from the post carbonization measurements.

Actual metrics	Reconstructed metrics	
	$C_1 = [(C_2 - 0.503) / -0.486] + C_2$	
		Smooth $C_1 = C_2 - 0.10$ Tubercled $C_1 = C_2 - 0.20$
Mean	0.435	0.437
Min	0.62	0.68
Max	0.23	0.29
Range	0.39	0.38

carbonized archaeological specimens. Still, the fact that perianth fragments are extremely rare on carbonized archaeological specimens and are almost universally retained on experimentally carbonized achenes suggests that perianth removal may have been a processing task prior to and separate from pericarp fracturing or removal.

Table 6  
Effects of perianth base and receptacle size and shape.

N = 20		Smooth	Tubercled
Area (A <sub>1</sub> )	Mean ± Std. Dev.	2.42 ± 0.264	2.93 ± 0.298
	Std Err of Mean	0.0836	0.0942
A <sub>no perianth base</sub>	Mean ± Std. Dev.	2.19 ± 0.252	2.53 ± 0.219
	Std Err of Mean	0.0797	0.0693
% remaining	Mean ± Std. Dev.	0.90 ± 0.029	0.87 ± 0.031
	Std Err of Mean	0.009	0.0099
A <sub>1</sub> -A <sub>no perianth base</sub>	Mean ± Std. Dev.	0.23 ± 0.071	0.40 ± 0.116
	Std Err of Mean	0.0226	0.0368
Length (L <sub>1</sub> )	Mean ± Std. Dev.	2.94 ± 0.201	2.68 ± 0.098
	Std Err of Mean	0.0635	0.031
L <sub>no perianth base</sub>	Mean ± Std. Dev.	2.58 ± 0.152	2.23 ± 0.132
	Std Err of Mean	0.048	0.0417
% remaining	Mean ± Std. Dev.	0.88 ± 0.024	0.83 ± 0.044
	Std Err of Mean	0.0077	0.0139
L <sub>1</sub> -L <sub>no perianth base</sub>	Mean ± Std. Dev.	0.36 ± 0.085	0.45 ± 0.124
	Std Err of Mean	0.0268	0.0393
Aspect ratio (AR <sub>1</sub> )	Mean ± Std. Dev.	2.12 ± 0.270	1.46 ± 0.118
	Std Err of Mean	0.0853	0.0373
AR <sub>no perianth base</sub>	Mean ± Std. Dev.	1.99 ± 0.194	1.27 ± 0.090
	Std Err of Mean	0.0612	0.0285
AR <sub>1</sub> -AR <sub>no perianth base</sub>	Mean ± Std. Dev.	0.14 ± 0.137	0.19 ± 0.104
	Std Err of Mean	0.0434	0.0328
Roundness (R <sub>1</sub> )	Mean ± Std. Dev.	0.48 ± 0.055	0.69 ± 0.056
	Std Err of Mean	0.0175	0.0178
R <sub>no perianth base</sub>	Mean ± Std. Dev.	0.51 ± 0.048	0.79 ± 0.058
	Std Err of Mean	0.0153	0.0184
R <sub>1</sub> -R <sub>no perianth base</sub>	Mean ± Std. Dev.	-0.03 ± 0.028	-0.10 ± 0.057
	Std Err of Mean	0.0088	0.0179
Circularity (C <sub>1</sub> )	Mean ± Std. Dev.	0.49 ± 0.055	0.46 ± 0.051
	Std Err of Mean	0.0175	0.0161
Circularity <sub>no perianth base</sub>	Mean ± Std. Dev.	0.57 ± 0.061	0.57 ± 0.091
	Std Err of Mean	0.0193	0.0287
C <sub>1</sub> -C <sub>no perianth base</sub>	Mean ± Std. Dev.	-0.08 ± 0.080	-0.11 ± 0.092
	Std Err of Mean	0.0252	0.0290

4.4. Differential preservation of smooth and tubercled pericarps

At 300 °C, smooth morphs were slightly more likely to fracture (Fig. 3). At 350 °C, the difference in preservation between the two morphs increased greatly: only 4/50 smooth achenes remained intact, compared to 27/50 tubercled achenes (Fig. 3). We can thus expect tubercled morphs to be preserved in carbonized assemblages in greater numbers than smooth morphs. Until methods for reconstructing the carbonizing conditions that effected particular assemblages improve, it will be impossible to correct for this differential preservation quantitatively. Qualitatively, this result makes the existence of carbonized assemblages composed primarily of smooth morphs all the more compelling evidence of domestication: it is highly unlikely that such assemblages are the result of a preservation bias. On the contrary, this indicator of domestication is evident *in spite of* a preservation bias that would tend to obscure it.

5. Sampling bias

5.1. Accurately estimating population proportion

Population proportion is the percentage of a given population that has a particular nominal (non-continuous) characteristic. Sample proportion is the percentage of a given sample that has that characteristic. In the case of erect knotweed domestication, the characteristic of interest is the percentage of a given harvest made up of smooth morphs. I sampled populations of erect knotweed between mid-October and mid-November over two growing seasons in order to estimate the range of normal population proportions of smooth morphs for erect knotweed (Table 7; see also Mueller, 2017d). There was a large range of sample proportions in modern erect knotweed, from 29 to 72% smooth morphs at sample sizes varying from 814 to 3011 achenes. Fig. 4 shows the

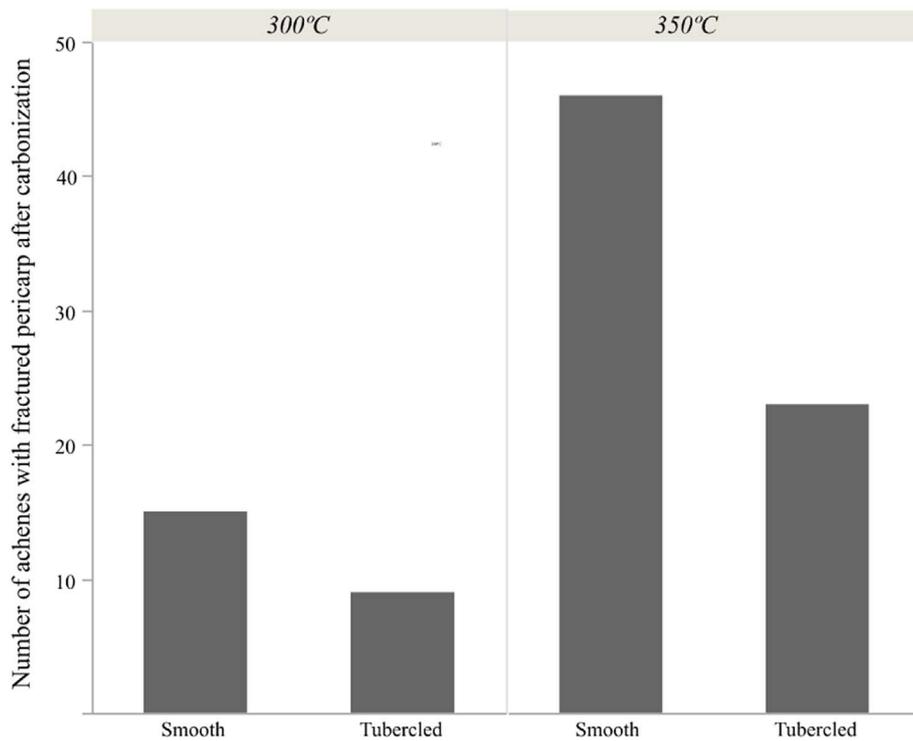


Fig. 3. Shows the differential preservation of smooth and tubercled morphs after 30 min at 300 °C and 350 °C, respectively (n = 50 for each treatment).

sample size necessary to estimate population proportion with a 5% margin of error at 95% confidence for different population proportions. It is more difficult to accurately predict population proportions close to 50/50 than it is to estimate highly skewed population proportions, but the sample sizes necessary for estimates at this level of accuracy even for skewed populations are larger than most of the archaeological samples of erect knotweed available.

Archaeologists cannot dictate sample size but must instead account for the error and uncertainty inherent to our small samples. I calculated the margin of error for different sample sizes at 95% confidence for three hypothetical populations of erect knotweed: 1) an assemblage made up of 90% smooth morphs, which is outside of the natural range of variation and constitutes evidence for domestication; 2) an assemblage made up 75% smooth morphs, which is just over the highest

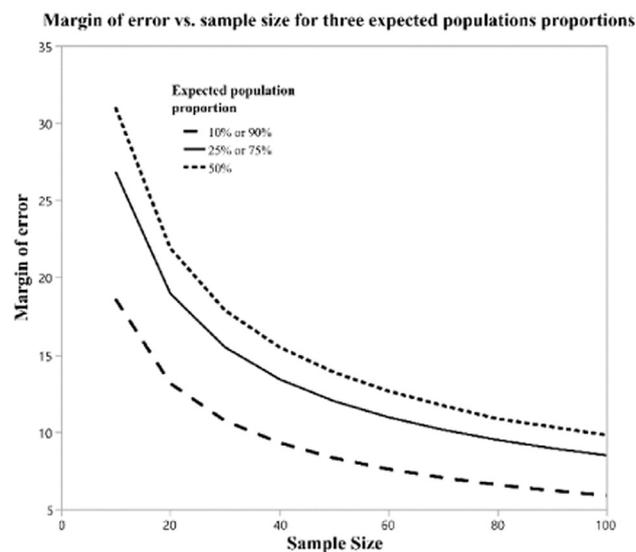
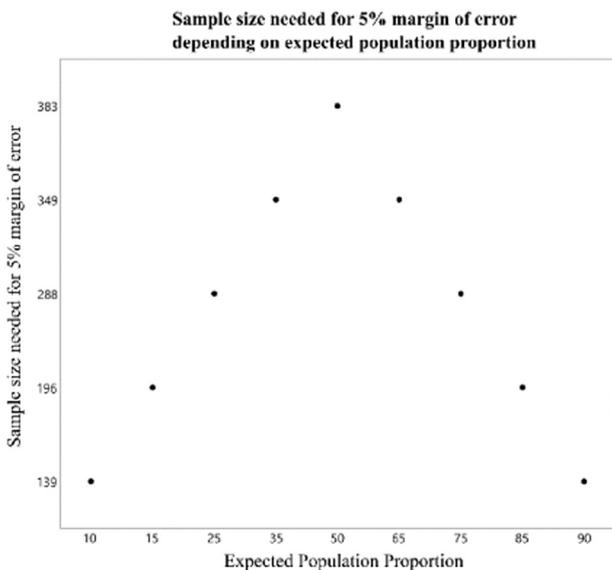


Fig. 4. These two charts are visualizations of the probabilistic relationship between population proportion and sample size. For both graphs, confidence is set at 95%. Left: It is more difficult to estimate population proportion accurately from a sample the closer the population proportion is to 50/50. If the true population proportion is skewed towards one or the other type (90% or 10% in this chart), it is relatively easy to estimate. Right: Since the ideal sampling conditions cannot always be met, the graph on the right shows the margins of error for different sample sizes given different true population proportions (which are unknown for archaeological assemblages). The maximum population proportion of smooth morphs observed in modern wild populations is 72%. Example: With a sample of 30, the margin of error for a population proportion of 75% is 15.49. In other words, we cannot be confident that a sample (n = 30) with a proportion of 90% smooth morphs is outside of the range of natural variation. At n = 40, these two hypothetical populations (75% and 90%) still have slightly overlapping expected sample proportion ranges: 61–89% and 81–99%, respectively. However, if we have a sample of 40 with a proportion > 89% smooth morphs, we can be confident that it is outside the natural range of variation. There is some overlap in expected ranges even with large samples of > 100, which makes it hard to draw definite conclusions about the domestication syndrome of archaeological samples, even large ones, with sample proportions in the high 70s or low 80s, like the Westpark assemblage (see text).

observed wild population proportion; and 3) an assemblage that is 50% smooth morphs and 50% tubercled morphs, normal for wild erect knotweed (Fig. 4). Given the large margins of error, we can expect samples of ten from all three hypothetical populations to overlap. That is, even if all ten in the sample are smooth morphs, the sample could very easily come from a normal wild population (Fig. 4). Because of the dynamic relationship between observed sample proportion, sample size, and margin of error, I decline to set a sample proportion above which an assemblage is domesticated and below which it is wild. The sample proportion should be assessed along with assemblage size for each individual case, as, for example, in the following case study (see also Mueller, 2017c).

## 5.2. Case study: the Westpark assemblage

The largest archaeological assemblage that I analyzed came from Feature 300 of the Westpark site, IL. Westpark is a multi-component site that was occupied from at least c. cal. 650–1100 CE ((Lopinot et al., 1991; Powell, 2000; Mueller, 2017c). A direct date on the analyzed knotweed yielded a date of cal. 1037–1183 CE (uncal. 910 BP +/- 20). Feature 300 was an irregular oval shaped pit. The bottom 2–5 cm of the pit contained a homogenous mass of erect knotweed kernels and achenes. For my initial morphometric analysis, I randomly sub-sampled 100 achenes from the tens of thousands that were recovered from this context. These achenes are significantly larger, on average, than my modern comparative sample (Mueller, 2017c). The proportion of smooth morphs in this first sub-sample was 81%, somewhat outside of the natural range of variation, but within the 95% confidence estimate for a population proportion of 75% with a sample of 100 (Fig. 4). I took advantage of this unusually large assemblage to independently confirm the confidence and accuracy estimates generated mathematically. An additional 20 random samples of 10 yielded sample proportions ranging widely from 30 to 100% smooth morphs – exactly as predicted if the true population proportion was ~75% smooth morphs. Ten random samples of 20 yielded sample proportions of 70–90%, again as predicted by the laws of probability.

For the entire sample of 500 that this process yielded, the Westpark assemblage sample proportion was 78% smooth morphs. Since a sample of 500 is more than is required for a 5% margin of error no matter what the true population proportion (Fig. 4), this means that the Westpark assemblage has at least a slightly higher proportion of smooth morphs than has been observed in a wild population: somewhere between 73 and 83% smooth morphs. Considering that smooth morphs are likely to be systematically underrepresented in carbonized assemblages due to differential preservation (Fig. 3), this sample proportion constitutes equivocal evidence for the effects of human selection at the Westpark site. Taken together with the fact that the Westpark achenes are significantly larger than is normal for erect knotweed, and come from a context that clearly indicates large scale cultivation (Powell, 2000), a strong case can be made for domestication at this site.

## 6. Conclusions

The analyses reported here were conducted primarily to facilitate the study of a particular crop: erect knotweed. But the issues raised pertain to other studies of plant domestication using archaeological assemblages in several ways. Previous studies have shown that carbonization can affect the shape and size of seeds differently depending on the composition of the seed, duration of exposure to heat, and fire temperature. Carbonization can also lead to differential preservation, biasing the archaeological record and sometimes eliminating the very characters necessary for recognizing a species or domesticated subspecies. Both of these effects present problems for the study of domestication through morphology. But in this case, the narrow window of conditions under which the pericarp is preserved helps narrow down the possible effects of carbonization on erect knotweed

achenes. Experiments showed that the pericarp of erect knotweed achenes, especially on smooth morphs, is unlikely to survive intact at temperatures above 300 °C. This means that carbonization corrections derived from low-temperature fires (300 °C in this study) are likely to be accurate for all well-preserved archaeological assemblages, because specimens burned in hotter fires are probably only preserved in the form of kernels. Such triangulation may be possible for other crop species as well.

Variation in the effects of carbonization has led some researchers to call for the use of nominal (present/absent) characteristics to argue for domestication (Wright, 2003). The state of the art in cereal domestication studies is to use the presence or absence of non-shattering inflorescences as an indicator of domestication, either along with seed size or independently of it (e.g. Fuller et al., 2009; Tanno and Willcox, 2012). For chenopods, seed coat texture and margin configuration are crucial nominal characteristics for identifying taxa and for distinguishing between wild and domesticated assemblages (Bruno, 2006; Fritz and Smith, 1988), and some researchers have used the sample proportions of wild and domesticated types to argue for divergent management practices (and thus crop evolution) between communities (Gremillion, 1993). For erect knotweed, the nominal characteristic of interest is the proportion of smooth morphs in a population. Because wild erect knotweed also produces smooth morphs, their presence alone does not suggest domestication. While this may seem like a problem unique to erect knotweed, it is not. The characteristics associated with domestication (testa characteristics and margin configuration for chenopods, tough rachises in cereals) are also present at low frequencies in wild populations, and become more common over time as selective pressures associated with cultivation begin to leave their mark.

For each domesticate, it is necessary to determine what sample proportion (at what sample size) is necessary to recognize an assemblage that is outside the range of variation for wild plants. In some cases, this will probably involve first establishing what that range of natural variation is. The characterization of erect knotweed variability presented here and elsewhere (Mueller, 2017d) should be considered preliminary. Harvest proportions of smooth morphs varied from 29%–72% across two growing seasons and two populations – such variance requires explanation. The same problem applies to another EAC crop, goosefoot (*C. berlandieri*) in ENA. The syndrome of domestication includes, among other characters, a reduction in testa thickness. Specimens exhibiting this characteristic are similar to “red morphs” – a thin-testa seed type present in small proportions on wild plants (Fritz and Smith, 1988). Very few studies have attempted to quantify the frequency of red morphs in wild populations. Asch and Asch (1985: 179–180) state that “most wild plants have at least a few” of this type, but they also encountered a population in which the red morph predominated. Their subsequent harvests convinced them that this condition was rare and probably resulted from unusual growing conditions. Smith (1985:122) made a collection of 5736 fruits from four different populations. Red morphs constituted 1–3% of these samples. As in the case of erect knotweed smooth morphs, a much larger sampling effort could be made to determine what genetic or environmental factors govern the production of red morphs.

For wheat and barley, even if we assume that wild populations originally had negligible proportions of domesticated types on the theoretical grounds that such a trait would be maladaptive (Hillman and Davies, 1990:167), changes over time in the population proportion of brittle to tough rachises are used to track the process of domestication (i.e. Asouti and Fuller, 2012, 2013). The same is true for rice; for example, a shift from 27.3% domesticated type to 38.8% domesticated type over ~300 years at one site has been reported as evidence of the process of domestication (Fuller et al., 2009). Such subtle shifts in population proportion are only detectable with very large samples (> 100) that are at least arguably drawn from the same living population. Aggregating many small samples from across an entire site

to estimate population proportion may lead to large sampling errors. The more evenly mixed an assemblage is between domesticated and wild types, the greater the margin of error (Fig. 4) and potential for mistaking sampling error for a real trend, and the larger the sample needed to recognize subtle changes in proportion confidently.

The correction factors and sampling models presented here should be of use to paleoethnobotanists who wish to assess where their assemblages of erect knotweed fall on the continuum between wild and domesticated. I expect that both experimental corrections and assessments of variability in wild populations will be refined and amended by future studies, but the analyses presented here should allow researchers to track the process of erect knotweed domestication in the archaeological record, thus adding to our knowledge of agricultural practice across ancient ENA.

### Conflict of interest

The author declares that she had no conflict of interest.

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