Journal of Archaeological Science xxx (2011) 1–6

Contents lists available at SciVerse ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

Ancient DNA confirms a local origin of domesticated chenopod in eastern North America

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ARTICLE INFO

Article history:
Received 29 June 2011
Received in revised form
19 August 2011
Accepted 20 August 2011

Keywords:
Chenopodium
Plant domestication
Ancient DNA
Eastern North America

ABSTRACT

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

1. Introduction

Chenopod (*Chenopodium berlandieri* Moq.), a weedy relative of quinoa and spinach, was an extremely important starchy seed crop in North America from *ca.* 1800 B.C. until *ca.* A.D. 900 or later, when maize-based agricultural intensification began throughout much of the Southeast (Smith, 1989; Smith and Yarnell, 2009). During this period, chenopod was grown from modern-day Arkansas to eastern Kentucky, at latitudes from northern Alabama to central Ohio (Cowan, 1979; Fritz, 1984; Fritz and Smith, 1988; Smith, 1984). In some regions it persisted along with other pre-maize seed crops well after maize was established as the primary staple in the midcontinent (Fritz and Lopinot, 2007; Simon and Parker, 2006). Its cultivation helped facilitate population growth and human colonization of new parts of the landscape, which affected shifts in population pressure, political organization, and other important features of prehistoric society. Its modern and archaeological distributions, morphological characters, ecosystem dynamics, and prehistoric usage in the Eastern Woodlands have been extensively studied during the past several decades (Asch and Asch, 1977, 1985; Cowan, 1979, 1985; Fritz, 1984, 1990; Fritz and Smith, 1988; Gremillion, 1993a,b, 1998; Smith, 1984, 1987; Smith and Cowan, 1987; Smith and Yarnell, 2009; Yarnell, 1974). However, it is as yet unclear whether chenopod was domesticated locally in the Eastern Woodlands or introduced from Mesoamerica, where morphologically identical chenopods are cultivated today (Wilson, 1981).

Chenopod seeds of two different domesticated variants are found archaeologically in the Eastern Woodlands. One is darker in color and has a reduced testa, or outer seed-coat, and has been formally classified as an extinct cultivar, *C. berlandieri* ssp. *jonesianum* (Smith and Funk, 1985). The other is pale or horn colored because it lacks the typical hard, black, outer epidermal layer (Wilson, 1981), and has not been formally described as a novel taxon. The earliest known domesticated chenopods were recovered from the Riverton Site in southeastern Illinois, where pale and dark variants were both found dating to *ca.* 1800 B.C. Only very low level wild-type chenopod use is known from sites pre-dating Riverton (e.g. Asch and Asch, 1985).

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

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doi:10.1016/j.jas.2011.08.023

Mesoamerica and eastern North America until well after the appearance of chenopod in the latter.

Alternatively, chenopod may have been introduced into North America following domestication in Mesoamerica (Wilson, 1981, 1990). According to this hypothesis, the two modern Mexican cultivars, *huazontle* (or *huauzontle*) and *chia* (both *C. berlandieri* ssp. *nuttalliae* [Saff.] H.D. Wilson & Heiser), were introduced to eastern North America by the early fourth millennium B.P., when they first appear archaeologically (Smith and Cowan, 1987; Smith and Yarnell, 2009; Wilson, 1990). The Mexican origin hypothesis is rooted in the morphological similarity between the ancient eastern cultivars and the two modern Mexican cultivars: *Chia* seeds are essentially indistinguishable from ancient dark morph seeds (Wilson, 1990), and an articulated pale morph inflorescence with seeds from the Holman rockshelter in Arkansas was morphologically assigned as *huazontle* (Wilson, 1981). Once established in North America, *chia* and *huazontle* may have formed part of the basis for eastern agriculture as the dark and pale morph eastern chenopods, respectively.

An eastern origin seems more parsimonious than a Mexican origin considering the archaeological record, which reveals chenopod in wild, weedy, and domesticated forms in the mid-continent. The eastern archaeological record, however, does not preclude a Mesoamerican introduction. Early use of wild-type chenopod in eastern North America (Asch and Asch, 1985; Hollenbach and Walker, 2010) followed by the appearance of domesticates could reflect early low-level exploitation of a local variety preceding widespread displacement by Mexican cultivars.

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

2. Materials and methods

2.1. Sample collection

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

We processed 35 modern chenopod samples from throughout the U.S. and Mexico (Fig. 1, Table 1). We analyzed specimens from the following taxa (*sensu* the USDA PLANTS database [http://plants.usda.gov]):

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

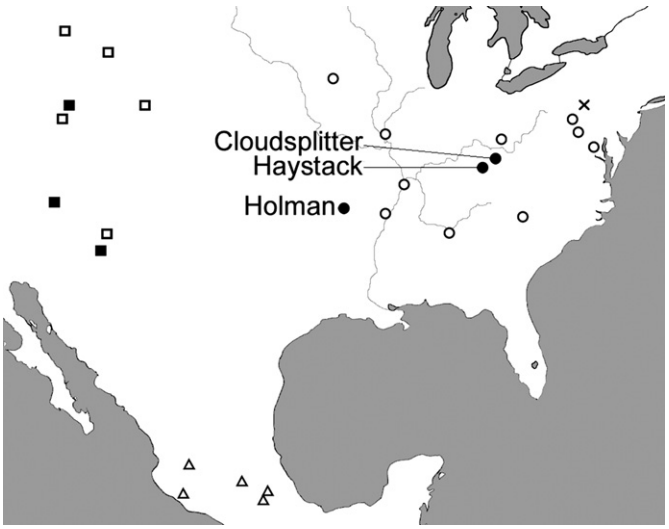

Fig. 1. Map showing locations of all modern and ancient samples. ○: Wild C. berlandieri from eastern North America, △: Huazontle (C. b. ssp. nuttalliae), the other potential parent population, □: Wild C. b. ssp. zschackei, ■: Wild C. b. var. sinuatum, x: C. album, ●: Archaeological samples.

C. berlandieri. ssp. *nuttalliae* is the potential Mexican progenitor to the prehistoric eastern cultivars, containing both *huazontle* and *chia* (Wilson, 1981). We analyzed six seeds, all *huazontle* collected in Mexico. *C. berlandieri* ssp. *zschackei* and *C. berlandieri* var. *sinuatum* are plains/western and southwestern U.S. wild types, respectively, with *C. berlandieri* var. *sinuatum* ranging into Mexico (Wilson and Heiser, 1979). We tested five seeds of ssp. *zschackei* and four of var. *sinuatum* from various western and southwestern locations. Finally, to provide an outgroup, we collected a single fresh *C. album* leaf sample in central Pennsylvania.

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

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

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

Finally, the Holman shelter is an Ozark Bluff Dweller site in Madison County, Arkansas, with occupation dating to *ca.* A.D. 1100 (Fritz, 1984; Fritz and Smith, 1988). This site yielded one of only a small number of articulated chenopod inflorescences, complete with pale seeds, from the Eastern Woodlands (Wilson, 1981). This specimen is remarkably preserved, and beautifully shows the

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Table 1
Geographic origin and observed haplotype (see Fig. 2) of all modern and ancient samples. In accession number column, superscript numbers indicate the source of material.

Modern taxon	Sampling location	Haplotype	Acc. No.
C. berlandieri, ENA	Calhoun Co., IL	A	PI 608030[a]
C. berlandieri, ENA	Cherokee Co., SC	B	82A[b]
C. berlandieri, ENA	Cullman Co., AL	B	105[b]
C. berlandieri, ENA	Fulton Co., PA	B	39[b]
C. berlandieri, ENA	Fulton Co., PA	B	40[b]
C. berlandieri, ENA	Mississippi Co., AR	B	57[b]
C. berlandieri, ENA	Mississippi Co., MO	B	96[b]
C. berlandieri, ENA	Mississippi Co., MO	B	94A[b]
C. berlandieri, ENA	Mississippi Co., MO	B	94S[b]
C. berlandieri, ENA	Mississippi Co., MO	B	95A[b]
C. berlandieri, ENA	Mississippi Co., MO	B	95C[b]
C. berlandieri, ENA	Pike Co., OH	B	92C[b]
C. berlandieri, ENA	Pike Co., OH	B	92E[b]
C. berlandieri, ENA	Pike Co., OH	B	92G[b]
C. berlandieri, ENA	Washington Co., MD	B	89B[b]
C. berlandieri, ENA	Washington Co., MD	B	90C-1[b]
C. berlandieri, ENA	Washington Co., MD	B	90C-3[b]
C. b. ssp. *nuttalliae*	Colima, Mexico	C2	668[c]
C. b. ssp. *nuttalliae*	Puebla, Mexico	C2	PL 568155[a]
C. b. ssp. *nuttalliae*	Jalisco, Mexico	C3	PL 433230[a]
C. b. ssp. *nuttalliae*	Jalisco, Mexico	C3	PL 433229[a]
C. b. ssp. *nuttalliae*	Mexico, Mexico	C3	PL 568156[a]
C. b. ssp. *nuttalliae*	Puebla, Mexico	C3	PL 433231[a]
C. b. ssp. *zschachei*	Catron Co., NM	C1	878[c]
C. b. ssp. *zschachei*	Fremont Co., ID	C1	622[c]
C. b. ssp. *zschachei*	Fremont Co., WY	C1	862[c]
C. b. ssp. *zschachei*	Jackson Co., CO	C1	637[c]
C. b. ssp. *zschachei*	Utah Co., UT	C1	882[c]
C. b. var. *sinuatum*	Wasatch Co., UT	A	AMES 27372[a]
C. b. var. *sinuatum*	Grant Co., NM	B	845[c]
C. b. var. *sinuatum*	Apache Co., AZ	C1	874[c]
C. b. var. *sinuatum*	Coconino Co., AZ	C1	865[c]
C. album	Centre Co., PA	A	C.a.2[d]
Ancient sample	**Site location**	**Haplotype**	**Estimated age**
Cloudsplitter	Menifee Co., KY	B	1800 B.C.
Haystack	Powell Co., KY	B	A.D. 400–700
Holman	Madison Co., AR	B	A.D. 1100

[a] -USDA NPGS.
[b] -Bruce Smith, Smithsonian Institution.
[c] -Eric Jellen, Brigham Young University.
[d] -collected by Logan Kistler.

compaction and terminalization of the fruiting structure associated with seed crop domestication. As indicated above, this specimen has been assigned previously as *huazontle* based on morphology (Wilson, 1981), but other researchers have suggested that it instead represents a distinct lineage native to the Eastern Woodlands (Smith and Yarnell, 2009). The University Museum at the University of Arkansas provided seeds from this specimen for analysis.

2.2. DNA amplification and sequencing

We selected Chloroplast DNA intergenic spacers for analysis. Certain chloroplast regions have a rapid rate of evolution in comparison to other genetic loci, and are informative in studies of closely related plant taxa (Shaw et al., 2005,; Shaw et al., 2007). Chloroplast DNA is also present in greater copy-number than nuclear loci, and therefore has a significantly improved chance of survival in ancient materials. Finally, like mitochondrial DNA, cpDNA is maternally inherited in most plant taxa (Schlumbaum et al., 2008), which simplifies the phylogenetic analysis.

Using a small number of modern samples, we screened eight cpDNA intergenic spacer regions (Shaw et al., 2005; Shaw et al., 2007) for polymorphisms fixed between the two potential parent populations. Of these, trnQ-5′rps16 proved the most informative, and offered the best clustering of informative sites to target using new primers in ancient accessions. This spacer is located on the long single copy region of the chloroplast genome, and was shown to provide high phylogenetic resolution within several divergent angiosperm lineages (Shaw et al., 2007). The trnQ-5′rps16 spacer of 697 bp (aligned length) was sequenced in all modern samples (Genbank Accession no. JN646817-JN646851).

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

We extracted DNA from modern single seeds following a modified CTAB protocol (Doyle and Doyle, 1987). We incubated seeds in 500 μL of CTAB extraction buffer with 0.02 g polyvinylpyrrolidone and 2.5 μL β-mercaptoethanol at 55 °C for 1 h, agitating them periodically. We then ground seeds in the buffer using pellet pestles, adding small amounts of sterile sand as necessary to assist with grinding. We added 700 μL chloroform, centrifuged, and isolated the aqueous phase. We incubated this on ice with 0.08 volumes 7.5 M ammonium acetate and 0.54 volumes of isopropanol for 45 min. The mixture was centrifuged and supernatant was discarded, and DNA was washed once with 70% ethanol and once with 95% ethanol, then allowed to dry completely. DNA was resuspended overnight in TE buffer.

We extracted DNA from archaeological seeds using Qiagen DNEasy Plant Mini kits [Qiagen, Valencia, CA] according to the manufacturer's protocol, except that seeds were ground in Qiagen buffer AP1 using pellet pestles before the initial incubation period. DNA was eluted in 100 μL Qiagen buffer AE. We observed severe PCR inhibition in dark morph seeds, probably caused by lignin and/or other compounds in the epidermal tissue. To counteract this, the testa was removed from dark morph specimens and DNA was extracted directly from the seeds' internal tissues.

For modern seeds, we PCR-amplified the trnQ-5′rps16 spacer using primers trnQ (5′-GCG TGG CCA AGY GGT AAG GC-3′) and rps16 × 1 (5′-GTT GCT TTY TAC CAC ATC GTT T-3′) (Shaw et al., 2007). Amplification took place in 25 μL reactions comprising: 2.5 mM $MgCl_2$, 75 μM each dNTP, 0.8 μM each primer, 0.1 ng BSA, 1.25 units taq DNA polymerase, and 2 μL template DNA. We used the rpl16 PCR program designed by Shaw et al. (2005) with an optimized annealing temperature of 63 °C.

We used the two newly designed primer pairs to target informative regions in trnQ-5′rps16: trnQA (5′-TCA TCC CGG CAA AGA AMG T-3′) and rps16A (5′-ATC CCT AAG AAA TAC AAA TCC AT-3′); and trnQB (5′-TCA AAT GAA AGG AAG ATA AGT GTT-3′) and rps16B (5′-CCG GGA TGA ATA AAA AAA KAA CTA-3′). Amplification took place in 25 μL reactions comprising, for trnQA-rps16A: 2 mM $MgSO_4$, 250 μM each dNTP, 1 μM each primer, 50 μg RSA, 1.25 units PLATINUM taq DNA polymerase, and 11.5 μL template DNA. For trnQB-rps16B, $MgSO_4$ was decreased to 1.5 mM and template DNA was increased to 12 μL. We found that using a large amount of template DNA from ancient seeds improved PCR product yield and decreased non-specific amplification. We used the following PCR program for both primer pairs: A 2-min denaturation step at 94 °C was followed by 45 cycles of 45 s denaturation at 94 °C, 45 s annealing at 59 °C for the A primers and 48 °C for the B primers, 90 s extension at 68 °C, and

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a 5-min final extension step at 68 °C. PCR using the A primers more frequently produced a product suitable for sequencing, and both fragments could be amplified in one accession from each site.

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

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

2.3. Phylogenetic analysis

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

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

3. Results

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

ML and MP analyses result in similar, well-supported phylogenies with identical branching order (Fig. 2). Modern samples cluster into three distinct clades with little variation within each clade: The domesticated Mexican samples and most wild eastern North American samples fall into two distinct clades separated by four single nucleotide polymorphisms (SNPs). One eastern specimen carries a third, distinct haplotype. All ancient samples carry the eastern haplotype. Median-joining network analysis supports the close evolutionary relationship between the ancient samples and the modern eastern populations (Fig. 2).

4. Discussion

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

However, given that a progenitor population is expected to carry greater overall genetic diversity than its domesticated subset, the modern wild plants might represent feral derivatives of the ancient crop. This is not likely to be true, since we sampled modern chenopods from outside the known range of prehistoric use, including materials from Pennsylvania, Maryland, and South Carolina. This sampling helps control for the possibility of feral populations, assuming that domesticated chenopods would not have spread prolifically and displaced local wild varieties outside their range of cultivation. Further, the use of wild chenopod at *ca.* 6000 B.C. in Illinois and even earlier in Alabama (Asch and Asch, 1985; Hollenbach and Walker, 2010) suggests that native chenopod populations significantly pre-date cultivation. The broadly-sampled modern materials from eastern North America therefore most likely represent native, wild populations, rather than naturalized escaped cultivars.

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

The pre-maize eastern North American agricultural complex consisted of at least four domesticated plants, as well as a handful of others that were cultivated but show no morphological signs of domestication. Those that were domesticated include chenopod, sunflower, sumpweed, and gourd/squash (*Cucurbita pepo* L.) (Fritz, 1990, 1995; Smith, 2006). Reportedly cultivated plants whose archaeological remains show no recognized signs of domestication

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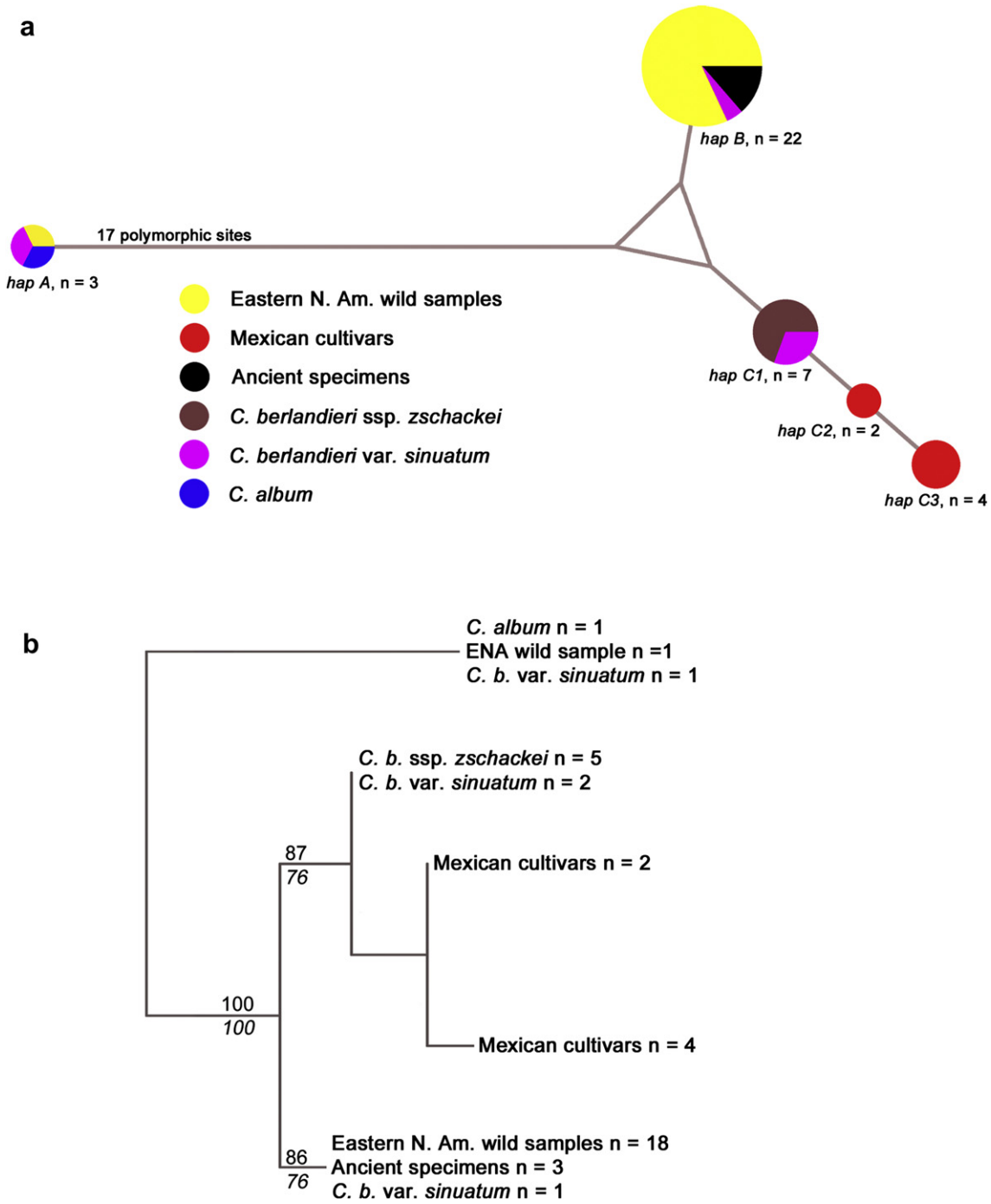

Fig. 2. a): Median-joining network from a 291 bp alignment of the trnQ-rps16x1 spacer in modern and ancient samples. Circles represent unique haplotypes, and circle size is proportional to the number of individuals carrying each haplotype. Colors indicate the samples carrying each haplotype, and slices are proportional to the number of specimens represented. The length of lines connecting the haplotypes is not proportional to genetic distance, and all lines except where noted otherwise represent a single mutation. Internal connections between lines represent putative ancestral states. b): Tree showing branching order from ML and MP analyses. Trees were estimated using *Beta vulgaris* as an outgroup; this branch has been removed here. Bootstrap support values are given for ML (above line) and MP (italics, below line) analyses. Ancient specimens from all three sites carry the characteristic haplotype of wild chenopods in eastern North America. Two specimens, one each of *C. berlandieri* and *C. b.* var. *sinuatum*, cluster with *C. album*.

include maygrass (*Phalaris caroliniana* Walter), knotweed (*Polygonum erectum* L.), and little barley (*Hordeum pusillum* Nutt.) (Fritz, 1995; Smith, 2006). Chenopod and these presumably non-domesticated plants were used for their starchy seeds, sunflower and sumpweed for their oily seeds, and squash for its seeds and flesh. Along with recent genetic and archaeological literature on sunflower, sumpweed, and gourd/squash (*Cucurbita pepo* L.) (Asch and Asch, 1985; Cowan, 1997; Crites, 1993; Decker-Walters et al., 1993; Harter et al., 2004; Wills and Burke, 2006), our findings here provide compelling support for the development of an entirely indigenous agricultural complex in eastern North America. The crop complex developed from a diverse group of weedy taxa within the context of a mainly hunting and gathering lifestyle, and came to dominate Eastern Woodland archaeobotanical assemblages during the Woodland Period (Milner, 2004; Smith and Yarnell, 2009; Yarnell, 1974).

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

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

Acknowledgments

We thank Bruce Smith and Eric Jellen for providing modern chenopod samples. Additional samples were provided by the North Central Regional Plant Introduction Station, part of the United States National Plant Germplasm System, USDA. We also thank Kris Gremillion for providing the Haystack materials, the University Museum at the University of Arkansas for allowing sampling of the Holman specimen, and the William S. Webb Museum of Anthropology and U.S. Forest Service for allowing sampling of the Cloudsplitter assemblage. Funding and other support for this research was provided by Lee Newsom, specialist in paleoethnobotany and director of the Pennsylvania State University Environmental Archaeology Lab. Additional funding for the research was provided by the Cave Research Foundation, and CodonCode aligner software was provided through a license grant by CodonCode Corporation.

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Please cite this article in press as: Kistler, L., Shapiro, B., Ancient DNA confirms a local origin of domesticated chenopod in eastern North America, Journal of Archaeological Science (2011), doi:10.1016/j.jas.2011.08.023