

Experimenting with domestication: Understanding macro- and micro-phenotypes and developmental plasticity in teosinte in its ancestral pleistocene and early holocene environments



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ABSTRACT

Living representatives of the wild progenitors of domesticated species constitute a significant basis for morphological and genetic study of once ancestral plants and their early domesticated forms. However, plants, in part through phenotypic (developmental) plasticity, are well-known to directly respond to environmental changes creating phenotypic variability and new morphologies. Therefore, how the wild progenitors of domesticated species and their proto-crops may have responded to Late Pleistocene (LP) and early Holocene (EH) climatic conditions are important, yet little-studied issues. We grew the wild ancestor of maize, *Zea mays* ssp. *parviglumis* (Illis&Doebley), and maize in the lower atmospheric CO₂ and temperature characteristic of their ancestral LP and EH environments and studied key macro- and micro-traits important in the domestication process. Teosinte responded with some remarkable phenotypic changes including in key morphological traits in plant architecture, inflorescence sexuality, seed dormancy, and grain nakedness previously thought to be a result of domestication. An artificial selection experiment carried out on plastic maize-like traits in teosinte demonstrated their stability across generations that would have enabled early cultivators to cement the traits in all environments, as in modern maize. Our results arguably provide more faithful replicas of what the first teosinte collectors and cultivators exploited, and point to an alternative pathway to maize domestication not heretofore demonstrated in a crop plant. They demonstrate how experimental research informs current questions in domestication research and evolutionary biology more generally, while raising others that had not previously drawn attention.

1. Introduction and background to research

It is now little-disputed that agriculture arose in a number of different regions of the Old World and the Americas ca. 12,000–10,000 years ago during a profound period of environmental change as the Pleistocene epoch ended and transitioned to the Holocene (e.g., Piperno, 2011; Price and Bar Yosef, 2011; Larson et al., 2014). For a number of major crops and wild progenitor species, archaeobotanical and genetic research, including now ancient DNA work, have considerably elucidated areas of origins and timeframes of domestication, together with the genetic mechanisms that underwrote the phenotypic transformations from wild to domesticated species (e.g., Olsen and Wendel, 2013; Larson et al., 2014; Ramos-Madriral et al., 2016; Vallebuena-Estrada et al., 2016; Kistler et al., 2018). However, domestication research still must rely on imperfect morphological and genetic data. Archaeological sites dating to the periods when hunters

and gatherers became farmers are still few in number; preservation of macro-botanical plant remains (seeds, tubers) is poor in some regions and important data are missing in many others; and analyses of genetic changes associated with early periods of domestication are limited by the still sparse availability of ancient DNA. As a result, our current understanding of the morphological and molecular transformations that took place during domestication is based significantly on living wild ancestral species and their modern domesticates.

Furthermore, with regard to the association of, and interplay between, phenotypic (observable) and genetic traits integral to agricultural origin and domestication research, studies of many natural, and increasingly crop species, show that the environment can rapidly—in a single generation—influence some of those relationships by triggering a mechanism called phenotypic (developmental) plasticity (e.g., West-Eberhard, 2003; Gilbert and Epel, 2009; Gremillion and Piperno, 2009; Nicotra et al., 2010; Sultan, 2010, 2015; Beldade et al., 2011;

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Moczek et al., 2011; Chitwood et al., 2016; Chen et al., 2017; Gage et al., 2017; Mueller, 2017; Piperno, 2017). With this mechanism a single genotype directly responds to environmental variability and change by exhibiting multiple phenotypes through one of several available pre-adult developmental pathways. Traits generated in this manner can be both adaptive and heritable. Once highly controversial in evolutionary biology, in part because phenotypic change occurs in the absence of new mutations and therefore precedes genetic change, plasticity is now established as a mainstream concept in evolution and ecology, and is increasingly considered to be fundamental for understanding the genesis of phenotypes, both ancient and modern, and possibly even in human ancestors (e.g., Kuzawa and Bragg, 2012; Diggle and Miller, 2013; Standen et al., 2014; Wang et al., 2018; and Winter 2019).

Given the propensity of plants to exhibit plasticity, the current reliance on living plant examples in domestication research, and the far different environments that existed shortly before and at the origins of agriculture, we began a multi-year experiment in 2009. We grew the wild ancestor of maize, the teosinte *Zea mays* ssp. *parviglumis* (H.H. Iltis & Doebley) (hereafter, teosinte), in glass growing chambers where atmospheric CO₂ and temperature were lowered to the Late Pleistocene (LP) and early Holocene (EH) levels reconstructed for lowland Mesoamerica for ca. 16,000–9000 BP (all dates are in calibrated ¹⁴C years) (Piperno et al., 2015). Archaeobotanical evidence indicates maize domestication was well underway in maize's homeland in the Balsas watershed of Mexico by the end of that time frame (Piperno et al., 2009; Ranere et al., 2009; Van Heerwaarden et al., 2011).

In grow-outs carried out from 2009 to 2012, teosinte repeatedly exhibited considerable phenotypic plasticity in LP and EH conditions resulting in a number of remarkable characteristics, including maize traits in vegetative architecture (a single main stalk with a few, very short [often measureable in just mm] branches); inflorescence sexuality (branches tipped by female or mixed male-female ears instead of tassels, with the ear occurring directly on the main stalk, and a single tassel terminating the stalk); and seed maturation (synchronous) (Fig. 1). All were previously thought to have been domestication traits. These traits, controlled in part by the *teosinte branched 1* (*tb1*) gene, were among the most important in the domestication process, as they significantly increased harvesting efficiency and seed yield, the latter through the development of apical dominance (in which available

nutrients are directed efficiently to the main stalk, not dispersed throughout the plant) (Doebley et al., 1995; see Discussion section for further details). Furthermore, many plants grown in LP and EH conditions that did not exhibit the complete set of maize traits as listed above still were more maize-like than in modern teosinte, as they had shorter branches and fewer male inflorescences at the ends of branches (Piperno et al., 2015).

Our evidence indicated ancestral characteristics of crop plant progenitors aren't always predictable from living examples and that important maize traits may have been created by nature and already present in significant numbers in teosinte at initial human exploitation and cultivation. Our findings also indicated teosinte productivity (seed yield, biomass) was considerably lower during the LP than EH (Piperno et al., 2015). This finding supported hypotheses that pre-Holocene plant cultivation with much lower productivity, if attempted, may have been difficult to sustain as a successful strategy compared with full-time foraging, shedding light on debates concerning why persistent, archaeologically-detectable plant cultivation began when it did and is not evidenced before.

In order to further investigate the role of plasticity in maize domestication and the genetic basis of the phenotypic changes we observed in teosinte, we subsequently carried out gene expression (GE) work (RNA seq-whole transcriptome analysis) on the plants (Lorant et al., 2017). GE is how, when, and to what degree existing genes are expressed through changes in the amount of messenger RNA during transcription, when RNA molecules are written from a DNA template. An increase in expression for a gene during transcription simply means that gene is more active in underwriting its trait(s) and vice versa. It is increasingly shown in other taxa how GE is highly responsive to environmental change, often giving rise to new phenotypes (e.g., Beldade et al., 2011; Des Marais et al., 2013; Maurya et al., 2018). Our results indicated the substantial importance of GE in generating the plastic and other phenotypic responses we observed in teosinte in LP and EH conditions (Lorant et al., 2017).

Regardless of how plasticity is generated, in order to have evolutionary significance it requires a capacity to be passed from one generation to the next in the inducing environment and eventually stabilized (or fixed, also called canalized) so that it is present in all environments. It is not a given that plastic traits will be fixed, and the degree to which they can be and have been fixed through evolutionary



Fig. 1. A. A maize-like phenotype plant from the Late Pleistocene chamber. Like maize, it has a single tassel that terminates the main stem, female ears at the main stem (arrows) that terminate a few, very short lateral branches, and no secondary branching. The inset at the upper right is a close-up of one of the female ears, which, in contrast, in all respects is as found in normal teosintes from modern natural populations. B. Teosinte in the Modern Control Chamber. As in normal teosinte, it has many long, primary lateral branches (example, upper white arrow) terminated by tassels (black arrow) and secondary branching. Female ears, not yet developed, would be on secondary lateral branches at the location of the two bottom white arrows. From Piperno et al., 2015.

time has been a controversial issue in evolutionary biology (see Piperno, 2017 for discussion). When plastic traits are fixed it is often through a mechanism called genetic assimilation, whereby if the traits are exposed for a sufficient number of generations to the conditions that induce them, they may acquire through selection on standing (pre-existing) genetic variation, not new mutations or rare mutations usually deleterious to wild plants, the genetic characteristics allowing their stable growth in all environments (Waddington, 1953; Pigliucci et al., 2006; Piperno, 2017).

To first expeditiously study this issue at the genetic level without embarking on multi-generational grow-outs, we grew maize in EH vs. modern conditions and examined differential gene expression between it and teosinte in the contrasting conditions (Lorant et al., 2017). The data importantly indicated a substantial loss of plasticity occurred during maize domestication, as a large number of genetic loci, including several dozen with previous evidence of selection during domestication, that were differentially expressed in teosinte in EH vs. the modern environment were invariant in maize in the contrasting environments. This means genetic assimilation played a role in domestication and the first cultivators could have placed selection pressure on genetic mechanisms associated with GE levels that fixed the plastic maize-like and other responses in teosinte that had initially—without human influences—been engineered by nature (Lorant et al., 2017 and Piperno, 2017 for extended discussion). This is the first evidence for this process in domestication. Maize grown in EH conditions also exhibited significantly reduced plant height and fecundity (seed yield, cob size) compared with plants grown in modern conditions. In contrast, there was no variation in vegetative branching or inflorescence sexuality, as had been the case in teosinte, further indicating these traits have been fixed and are invariant in domesticated maize (Lorant et al., 2017).

We report here on our most recent experimental research with teosinte in which we carried out a multi-generational selection study to investigate through phenotypic analysis the issue of plasticity fixation and how persistent human cultivation of teosinte may have influenced plastic and other important traits we observed. We also present information on important phenotypic traits observed in teosinte and maize from all years of our work and not discussed in previous papers. They include flowering time, pollen and phytolith characteristics in teosinte and maize, and the surprising observation of open fruitcases (naked seeds) in teosinte. These are among the most important traits for archaeobotanical investigations of the maize domestication process and for understanding how maize adapted to different environments during its early dispersals.

2. Materials and methods

2.1. Plants utilized and growth conditions

Data presented here are results of experiments carried out with teosinte in 2009–2012, 2016, and 2017, and with maize in 2014. We grew four different natural Mexican populations of teosinte and three lines of inbred maize during their natural growing periods from July to December in five-gallon pots in two naturally-lighted glass environmental chambers housed at the Gamboa field station at the Smithsonian Tropical Research Institute in Panama (Table 1). One chamber was adjusted to either LP (ca. 16–13 ka) (20–22 °C, 200–220 ppmv) or EH (ca. 11–9 ka) temperature and CO₂ levels (23 °C, 260–265 ppmv) reconstructed from Mexican paleoecological sequences (Ahn et al., 2004; Piperno et al., 2007; Hodell et al., 2008; Bush et al., 2009; Correa-Metrio et al., 2012). Another chamber, the modern control (MCC), was at CO₂ and temperature levels characteristic of the central Balsas region of Mexico today (25 °C, 360–400 ppm). From nine to 12 plants were grown in each chamber. We repeated the experiment with teosinte in LP conditions three times from 2009 to 2011, and grew it in EH conditions in 2012, 2016, and 2017. In 2014, we grew maize in EH conditions. In all years, plants were germinated from seed in the chambers

so that all pre-adult development took place under the conditions being tested. For further details on the chambers and plants see Piperno et al. (2015) and Lorant et al. (2017). Plants were harvested upon maturity, dried, and transported to the laboratory for analysis.

3. Results

3.1. The artificial selection study

3.1.1. Stability of maize-like phenotype plants

As described above, we initially studied the significance of teosinte plasticity in maize evolution through a comparative gene expression study of teosinte and maize, with results indeed indicating a considerable loss of plasticity and a role of genetic assimilation in maize domestication (Lorant et al., 2017). Another, and much more time-consuming way, to study if plastic phenotypes are stable in the inducing environment and can evolve stability in any environment is to grow their progeny in the inducing vs. non-inducing conditions through successive generations. Hence, the plastic phenotypes are placed under artificial selection and specific traits can be directly observed during each year. We began such a selection experiment with teosinte, focusing on plants with the plastic maize-like traits in all of the following characteristics: vegetative architecture (a single main stalk with a few, very short [nearly un-measurable] branches); inflorescence sexuality (branches tipped by female or mixed male-female ears instead of tassels, with the ear occurring directly on the main stalk, and a single tassel terminating the stalk); and seed maturation (synchronous) (hereafter, called maize-like phenotype or MLP plants) (Fig. 1). We used seeds from MLPs originally induced from founder plants in LP conditions in 2009–2011 and planted them in EH vs. modern conditions in three subsequent years (Table 2) (i.e., 2009–2011 MLP seeds were grown in 2012; 2012 seeds in 2016; 2016 seeds in 2017, using a somewhat different design in 2017, explained below). MLP plants likely would have drawn the attention of teosinte cultivators because of their increased harvesting efficiency compared with normal teosintes, and MLP traits if selected on led to increased apical dominance and thus seed yield (see Introduction and Discussion). Although it is unknown how long it would take genetic assimilation to occur in plastic teosinte traits—if, as seems likely from our gene expression results, the plants were so inclined to be subject to this mechanism—and while only three years of artificial selection has been carried out thus far, the results are informative.

First, MLP seeds germinated and developed in every generation, showing their progeny are viable in past conditions. Moreover, plants from populations 2 and 3 became an MLP in one or two successive generations, respectively, in EH conditions following their original MLP-inducements from founder plants in LP conditions (Table 2; in this and tables following we show results for all plants grown so that the considerable variability between them in the same and different years can clearly be seen; this is plausibly the kind of variability the first teosinte cultivators saw). The results point to a stability of the maize-like traits in some teosinte genotypes from year to year in the physical environment that was typical of early cultivation for two thousand years. It also indicates that plastic responses for maize-like characters could be stable over the Pleistocene to Holocene transition.

Population 4 did not become an MLP in 2012 but then intriguingly when planted again in 2016 from 2010 seeds became MLPs in both EH and modern conditions. This suggests a capacity to be stable in any environment and, therefore, exhibiting a potential for genetic assimilation (Table 2). In 2017, the 2016 seeds from the modern chamber from population 4 were planted in modern conditions again (plant 3C MLP seeds from 2016 were not grown in 2017). No plant became an MLP indicating, not surprisingly after only a few generations, that genetic assimilation had not occurred. It also should be noted that because the population 4 plants may have had pollinators from different teosinte populations when grown with them in previous years, genetic

Table 1
Sources of the teosinte and maize seeds.

	Accession	Origin	Plant Name	Elevation asl
Teosinte Population 1	PI 384062	Mexico, Guerrero State East side of highway, 1 mi s of Palo Blanco, Latitude: 17 deg 25 min 0 s N (17.41666667), Longitude: 99 deg 30 min 0 s W (-99.5)	B-K4	1350m
Teosinte Population 2	PI 384063	Mexico, Mexico State West side of road, 4 km s of Valle de Bravo, Latitude: 18 deg 50 min N (18.83333333), Longitude: 100 deg 10 min 0 s W (-100.16666667)	B-K7	1300m
Teosinte Population 3	PI 384071	Mexico, Guerrero State Iguala-Arcelia Rd. 103 km from Iguala Latitude: 18 deg 20 min N (18.33333333), Longitude: 100 deg 19 min 0 s W (-100.31666667)	Wilkes 10	1100m
Teosinte Population 4	PI 566692	Mexico, Michoacán State Km 43 Rd. Zitacuaro to Tuzantla 19 deg 4 min 0 s N (19.06666667), Longitude: 100 deg 25 min 0 s W (-100.41666667)	Collected by J. Sanchez and G. Wilkes	850m
RIMMA 1	Ames 19288, Oh43	Ohio, United States		
RIMMA 19	PI 550473, B73	Iowa, United States		
RIMMA 140	NSL 30053, W22	Wisconsin, United States		
RIMMA 809	PI 558532, Mo17	Missouri, United States		

Table 2

Year	MLP Plant Seeds Utilized
2009	3C, MLP from Founder Plant in LPC
2010	4C, MLP from Founder Plant in LPC
2011	1A, MLP from Founder Plant in LPC
2011	2B, MLP from Founder Plant in LPC
2012	3C-2009, in EHC, not in MCC
2012	4C-2010, not MLP in EHC or MCC
2012	1A-2011, not MLP in EHC or MCC
2012	2B-2011, in EHC, not in MCC
2016	3C-2012, in EHC, not in MCC
2016	3C-2012, in EHC, not in MCC
2016	3C-2012, not MLP in EHC or MCC
2016	2B-2012, not MLP in EHC or MCC
2016	2B-2012, not MLP in EHC or MCC
2016	2B-2012, not MLP in EHC or MCC
2016	4C-2010, in EHC and MCC
2016	4C-2010, in EHC and MCC
2016	4C-2010, in EHC and MCC
2017	9 plants grown from 4C-2010 from MCC in the MCC again; no MLPs were produced.

Notes: Each row represents a plant grown. Plant numbers denote founder populations with descriptions in Table 1 (e.g., 1 is founder population 1, etc.). Letters after the numbers denote seeds from that particular plant of the replicates grown from each population in previous generations that were planted.

Plants in green indicate they became maize-like phenotypes in subsequent generations of grow-outs. EHC = early Holocene chamber; LPC = Late Pleistocene chamber; MCC=modern control chamber.

mixing among plants may have occurred, possibly resulting in the non-MLP phenotypes in 2017, since plastic responses can vary among genotypes of a single species. This factor may relate to other plants not becoming MLPs in successive generations. However, growing plants of different genotypes together may better simulate early cultivated fields.

We will further explore these issues in future grow-outs.

It is also important to note that plants that did not become MLPs in every generation (e.g., 1A, 4C, 2B) continued nonetheless, as in the 2009–2011 grow-outs in LP conditions (see Piperno et al., 2015), to often have in EH conditions some of the maize-like traits of the MLP

Table 3
Occurrence and percentages of open fruitcases in the Grow-Outs.

MCC	Non- MLP	
Year	Plant ID	%OFC
2009	1A	1
2009	1B	2.2
2009	1C	0
2009	2A	1.4
2009	2C	20.4
2009	3A	0.7
2009	3B	2.9
2009	3C	0.5
2010	1A	0
2010	1B	0
2010	1C	7.1
2010	2A	3.3
2010	2B	0.6
2010	3A	1.3
2010	3B	0
2010	3C	3.1
2010	4A	0
2010	4B	0
2010	4C	0.9
2011	1A	1
2011	1B	0
2011	1C	2.7
2011	2A	0.6
2011	2B	7.4
2011	2C	1.6
2011	3A	3.7
2011	3B	0.3
2011	3C	0
2011	4A	0.5
2011	4B	0
2011	4C	0
Mean		2.0

EHC	Plant ID	%OFC
2012	3C-2009	0
2012	4C-2010	0.7
2012	1A-2011	4.2
2012	4A	6.4
Mean		2.8

2016	3C-2009	0
2016	2B-2011	0
2016	2B-2011	2.9
Mean		0.9

LPC	Non- MLP	
Year	Plant ID	%OFC
2009	1A	1.8
2009	1B	2.6
2009	1C	0
2009	2A	0
2009	2C	0.8
2009	3A	0
2009	3B	0
2010	1A	0.7
2010	1B	1.8
2010	1C	2.9
2010	2A	9.4
2010	2B	1.4
2010	3A	1.2
2010	3B	0
2010	3C	2.4
2010	4A	0.4
2010	4B	5.1
2011	1B	1.4
2011	1C	0
2011	2A	25
2011	2C	7.1
2011	3B	25
2011	4A	32.7
2011	4B	26.8
2011	4C	18.8
Mean		6.7

EHC	Plant ID	%OFC
2012	4C-2010	2.1
2012	1A-2011	9.5
2012	1A	0.7
2012	1B	0.5
2012	2A	45.8
2012	3A	0.5
2012	3B	10.7
2012	4A	5.1
2012	4B	1.8
Mean		8.5

2016	3C-2009	0
2016	2B-2011	0.3
2016	2B-2011	0.4
2016	2B-2011	49.8
Mean		12.6

LPC	MLP	
Year	Plant ID	%OFC
2009	3C	7.9
2010	4C	1.7
2011	1A	5.3
2011	2B	78.3
2011	3C	2.4
Mean		19.1

EHC	MLP	%OFC
2012	3C-2009	0
2012	2B-20 1	44.8
Mean		22.4

EHC	MLP	%OFC
2016	3C-2012	0.4
2016	3C-2012	1
2016	4C-2010	1.1
2016	4C-2010	0.5
2016	4C-2010	0.2
Mean		0.60

MCC	MLP	%OFC
2016	4C-2010	3.1
2016	4C-2010	1.3
2016	4C-2010	10
Mean		4.8

Year	Selection Study MLPs	% OFC
2009	3C	7.9
2010	4C	1.7
2011	1A	5.3
2011	2B	78.3
Mean		23.3

2012	3C-2009, in EHC	0
2012	4C-2010	2.1
2012	1A-2011	9.5
2012	2B-2011, in EHC	44.8
Mean		14.1

2016	3C-2012, in EHC	0.4
2016	3C-2012, in EHC	1
2016	3C-2012	0
2016	2B-2012	0.3
2016	2B-2012	0.4
2016	2B-2012	49.8
2016	4C-2010, in EHC and MCC	1.1, 3.1
2016	4C-2010, in EHC and MCC	0.5, 1.3
2016	4C-2010, in EHC and MCC	0.2, 10
Mean		6.0, 4.8

2017	4C-2016	7.8
2017	4C-2016	14.6
2017	4C-2016	2.4
2017	4C-2016	0.8
2017	4C-2016	3.9
2017	4C-2016	9.6
2017	4C-2016	18.2
2017	4C-2016	1.3
2017	4C-2016	1.2
Mean		6.6

In 2017 plants were grown in the MCC

complex, such as female or mixed male-female flowers at the ends of branches instead of tassels and/or shorter branches than in modern conditions (data not shown). This again points to a stability of the maize-like plastic responses. Plants in EH conditions also continued each year to have shorter stature than in modern conditions.

3.1.2. Teosinte open fruitcases

One of the most crucial phenotypic traits selected by teosinte cultivators was that which uncovered the fruitcase composed of a glume and rachid (the latter the future cob cupule) that completely surrounds the teosinte grain, creating “naked grains” through rachid shallowing and narrowing (Doebley et al., 1995, Fig.7 in Iltis, 2000). The *teosinte glume architecture 1* (*tga1*) gene controlled this process, also reducing in maize cobs the heavy lignification and silicification characteristic of teosinte glumes and rachids that allows phytoliths from it and maize cobs to be distinguished (Doebley et al., 1995; Dorweiler and Doebley,

1997; Wang et al., 2005; Piperno, 2006:61–63). Indeed, *tga1* was the first genetic locus demonstrated to regulate control over phytolith deposition (Dorweiler and Doebley, 1997). However, unlike with other domestication genes, the maize allele of *tga1* has not been found in molecular work on teosinte (e.g., Wang et al., 2005), and the 1971 expedition organized by Beadle that examined numerous teosintes growing naturally in maize's homeland (called the “Teosinte Mutation Hunt”) revealed no open fruitcases or soft glumes (see Iltis, 2000; Wang et al., 2005). It is therefore thought the mutation that produced the maize allele, *Tga1*, occurred post-cultivation or is at least a rare variant today in teosinte (Wang et al., 2005).

It is of considerable note that we observed open teosinte fruitcases (OFs) in both modern and simulated past conditions in all of our grow-outs (Table 3). In these fruits there clearly is a shallowing or reduction of the rachid that is now too small to house the kernel, exposing it (Fig. 2, compare with Fig. 3). The outer glume is also pushed apart from

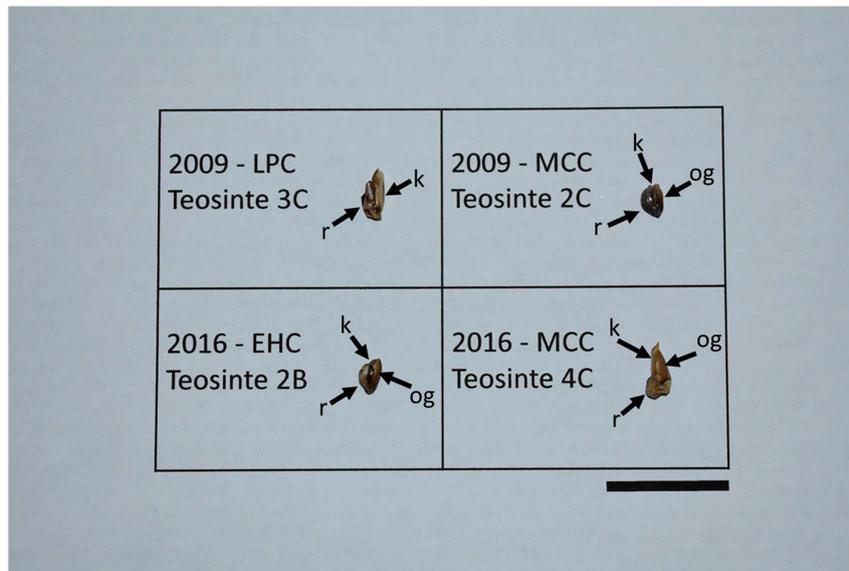


Fig. 2. Open fruitcases in teosinte revealing exposed kernels. Arrows point to: og (outer glume), r (rachid), k (kernel).



Fig. 3. Normal teosinte fruitcases from an ear grown in our experiments. In all respects including their phytolith assemblages, they are the same as expected in natural populations of teosinte today. The kernels are completely enveloped by the outer glume (og) and rachid (r) of the fruitcase, so are not exposed. From Piperno et al., 2015.

the rachid. The hardness of the rachids and outer glumes does not seem to be affected, indicating a still substantial lignification and silicification, borne out by our phytolith analysis (below). A number of interesting patterns in OFC distributions can be seen. In the 2012–2017 selection experiment plantings (Table 3, right), population 2 plants continually produced very high OFC percentages from generation to generation demonstrating stability in this trait in some genotypes. Furthermore, among all plants, non-MLPs in LP and EH conditions had higher mean and maximum percentages of OFCs than non-MLPs in the modern control chamber (MCC). MLPs in the late Pleistocene chamber (LPC) always had higher percentages than their paired plants in the MCC (e.g., 2009 3C MLP in LPC vs. 3C non-MLP in MCC, etc.) (Table 3). Among all MLPs in the grow-outs, only one plant out of 15 did not produce them. The data suggest the genetic mechanisms that underwrite the OFs and other maize-like traits observed may be linked. More discussion of these patterns is in the final section.

As mentioned, phytoliths from the glumes and rachids of teosinte fruitcases and the glumes and cupules of maize cobs are differentiable,

providing a means to distinguish them in archaeological sequences from Mesoamerica where teosinte is native (Piperno, 2006:61–63). We examined phytoliths from three open fruitcases grown in LP and EH conditions in 2011, 2012, and 2016 to ascertain if the same was true in these fruits. Phytolith production was high and phytoliths typical of normal fruitcases that distinguish them from maize were commonly found in the glumes and rachids of all the fruits (Fig. 4). This finding goes along with their sustained hardness. Therefore, although it has become doubtful that macrobotanical remains of fruits such as these can be considered a post-cultivation domestication trait, their phytoliths should still indicate teosinte and not maize presence. As expected, fruitcases from plants grown in our study that were not open (Fig. 3) had phytolith assemblages the same as in natural populations studied (data not shown).

3.1.3. Seed yield and weight, and tillering influences

We examined seed yield and weight in the selection study. Because the propensity of teosinte plants to tiller (develop extra branches and flowers at ground level from underground nodes) may have a significant influence on seed yield, we also examined this aspect. Although more planting generations are needed to assess the robustness of the patterns, some plants (1A, 2B) showed significant increases in seed yield irrespective of tiller influence in subsequent generations of planting, while in 3C and 4C plants there was no evident trend unless tillers were produced (Table 4). Although tillers are typically thought to be largely disadvantageous to farmers in way of their negative effect on planting density and competition for resources, and genes that reduced their production were selected during maize domestication and improvement (Whipple et al., 2011), tillers always significantly increased seed yield in our grow-outs. This is mainly a result of the tiller flowers that are normally male also being feminized in a maize-like way in EH conditions (and modern conditions in 2016), leading to more seed production.

3.2. Other important traits and their characteristics

3.2.1. Flowering (tassel initiation) time, pollen characteristics, and tassel branch number in teosinte and maize

In *Zea* as in other taxa, flowering time shifts are important factors adapting plants to local environmental factors and climate changes, importantly including temperature and precipitation, and today in maize land races the time to flower varies greatly (Buckler et al., 2009).

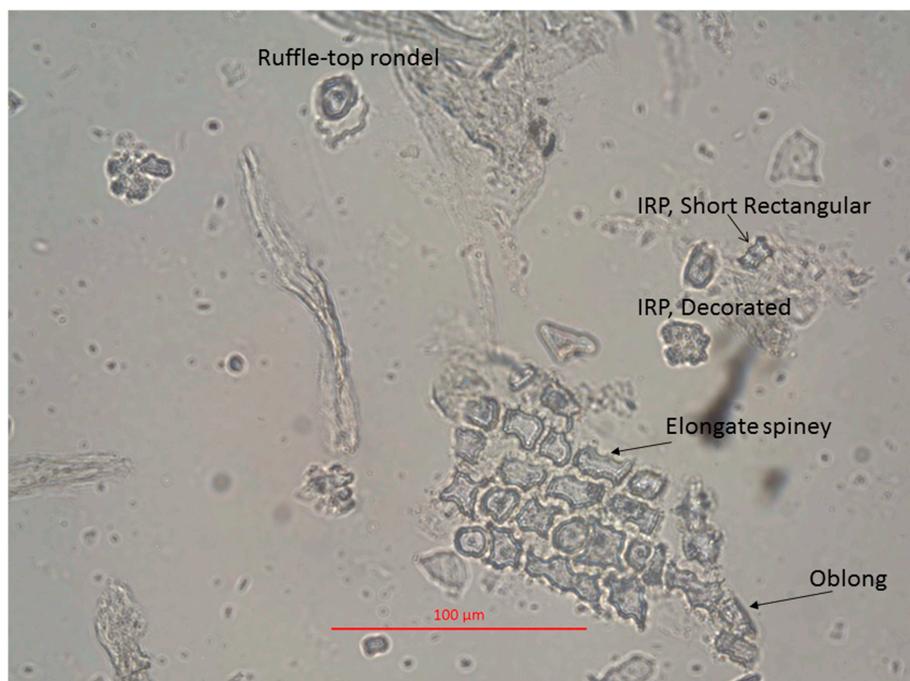


Fig. 4. Phytoliths from the glumes and rachids of open fruitcases. The epidermis is heavily silicified as in normal fruitcases and resulting phytoliths are the same as found in them, and that distinguish teosinte and maize (see Piperno, 2006:61–63). Scale bar = 2 cm.

Flowering times are also closely associated with traits such as seed set/ yield and plant height (Craufurd and Wheeler, 2009). We observed significant differences in flowering time in both teosinte and maize grown in LP and EH environments compared with in modern conditions. In teosinte, mean flowering time was 25 and 19 days earlier in LP and EH, respectively, than in modern conditions (Table 5, earlier time designated with a +). Teosinte in the 2012 selection experiment flowered 30 days earlier and in 2016 just 12 days earlier. It is unclear what this considerable difference means at this point, especially as all MLPs were grown in the MCC as well. In maize, flowering was later in EH than modern conditions by an average of 12 days, reflecting a longer vegetative phase. The data suggest teosinte adapted to lower CO₂ and temperature in the past by shortening its vegetative phase, while maize may have done the opposite.

We previously showed that teosinte and maize pollen overlap considerably in size and do not exhibit differences morphologically, making it difficult to differentiate wild and domesticated maize where teosinte is native (Holst et al., 2007). In order to examine if past environmental conditions could have lessened or enhanced differences among or between them, we analyzed pollen from teosinte in LP and EH conditions and maize pollen from a number of inbreds in EH conditions, comparing pollen characteristics with those in the MCC (Table 6). Pollen from the main tassel was sampled and 50 grains mounted in silicone oil were analyzed. Results indicate there is little difference in mean and maximum size in teosinte pollen grown in LP and EH conditions compared with in the MCC. Pollen size in LP, EH, and modern conditions overlaps with that previously recorded from non-manipulated modern plants collected from a number of Mexican natural populations (Table 1 and SI Table 6 in Holst et al., 2007). An exception is plant 4C from 2010, a MLP that produced a large maize-like grain 138 μm in maximum size in LP conditions.

In the maize inbreds, very large grains in mean and maximum size occurred in population 2 plants in EH conditions and in population 2 and others in the MCC, reaching 148 μm and more in maximum size in both chambers (Table 4). It is unknown if the large sizes are due to the inbred nature of the maize. Some plants (3A-4B) had sizes typical of some non-manipulated traditional maize races from Mexico (SI Table 4 in Holst et al., 2007) in both EH and modern conditions. Also

importantly, morphological characteristics (surface texture and sub-exine traits) of teosinte and maize pollen in LP and EH were the same as in natural populations of *Zea*. Both teosinte and maize pollen were larger than most species of *Tripsacum* and differentiable from the entire genus in morphology, as in previous work with plants collected from their natural habitats (see Holst et al., 2007).

A phenotypic trait related to flowering that differed substantially in teosinte and to a much lesser extent in maize in the contrasting growing environments was tassel branch number. In teosinte there were far fewer tassel branches in LP and EH conditions (Table 7 and Table Notes, Fig. 5). It is likely this resulted in considerably less pollen production per plant in the LP and EH chambers compared with the MCC. In maize, average tassel branch number in the MCC was 2.8 ± 1.9 compared with 2.0 ± 1.7 in the LP chamber, with many plants having one fewer branch in the LP chamber than in the MCC.

4. Discussion

In this and previous research (Piperno et al., 2015), we studied collections of whole plant specimens of teosinte grown in its ancestral environments. They arguably are more faithful replicas of ancient examples than living representatives. Our results strongly suggest that teosinte foragers and cultivators saw and worked with significantly different phenotypes than those collected and utilized today in archaeobotanical and genetic research, and saw a greater amount of phenotypic variability than uncovered in modern teosinte. Teosinte responses to the simulated past conditions were likely due in significant part to the high amount of standing genetic variation it possesses (Lauter and Doebley, 2002; Doebley, 2004), which offered pre-existing mutations sensitive to environmental cues, a major fueler of plasticity and other changes through gene expression. Some of the pre-existing variation mediates maize-like traits in branching and inflorescence sexuality uncovered in teosinte in our study (Lauter and Doebley, 2002).

The key traits differing from modern teosinte that were likely common in Late Pleistocene and early Holocene populations are: 1) vegetative architecture, with a few, short branches and a single main stalk, 2) inflorescence sexuality, with the short primary branches being

Table 4
Teosinte seed yield, weight, and tillering Influences.

Year	MLP Plants Utilized	Total # viable seeds		Total weight g	
		LGC or EHC	MCC	LGC or EHC	MCC
2009	3C	126		5.22	
2010	4C	358		17.77	
2011	1A	19		0.52	
2011	2B	23		0.89	
2012	3C-2009	18		1.03	
2012	4C-2010	141		8.42	
2012	1A-2011	752, 1420 T		27.79, 50.72 T	
2012	2B-2011	29		1.10	
2016	3C-2012	85, 220 T		4.02, 10.48 T	
2016	3C-2012	157, 285 T		6.84, 11.83 T	
2016	3C-2012	156, 289 T		6.30, 11.54 T	
2016	2B-2012	234, 584 T		7.22, 17.60 T	
2016	2B-2012	183, 455 T		6.85, 16.94 T	
2016	2B-2012	249, 570 T		8.72, 19.77 T	
2016	4C-2010	159, 444 T	95, 225 T	6.74, 18.40 T	5.06, 11.70 T
2016	4C-2010	156, 427 T	109, 236 T	7.57, 22.68 T	4.74, 10.03 T
2016	4C-2010	159, 426 T	133, 330 T	8.32, 21.36 T	6.25, 16.00 T
2017	4C-2016		231		10.2622
2017	4C-2016		423		22.0474
2017	4C-2016		299,537 T		16.02, 30.01 T
2017	4C-2016		329, 468 T		19.22, 27.21 T
2017	4C-2016		51 T		2.52 T
2017	4C-2016		122, 250 T		7.41, 14.37 T
2017	4C-2016		11 T		0.78 T
2017	4C-2016		197, 476 T		10.75, 25.76 T
2017	4C-2016		364, 680 T		18.10, 32.24 T

Notes: T= Seed yield including tillers; no T indicates plants did not produce tillers. Viable seeds were those that developed into normal fruitcase phenotypes (i.e. did not show pollination incompatibility such as white color).

Table 5

Year	Plant ID	LPC or EHC		MCC		
		Days to TI	SD	Days to TI	Mean	SD
2009–2011, LPC	Teosinte	73.4	15.2	98.3	+ 24.9	27.8
2012, EHC	Teosinte, non-selection	74.1	19.0	93.0	+ 18.9	
2012, EHC	Teosinte selection	82.8	17.8	112.3	+ 29.5	17.0
2016, EHC	Teosinte selection	81.3	4.0	93.1	+ 11.8	9.1
2014, EHC	Maize	66.4	7.2	54.9	– 11.5	4.5

Notes: TI - tassel initiation. The 2012 teosinte non-selection plants represent plants grown from founders, not MLPs induced in 2009–2011. LPC = Late Pleistocene chamber, EHC = early Holocene chamber.

tipped by female ears located directly on the main stalk, or female or mixed male-female ears near the main stalk, and a single tassel terminating the stalk, 3) synchronous seed maturation, 4) presence of open fruitcases, 5) shorter plant height, 6) earlier flowering time, and 7) fewer tassel branches with probable lower overall pollen production. Nos. 1–3 are clearly a result of developmental plasticity and they along with No. 4 are maize traits previously believed to result from domestication and among the major morphological differences separating teosinte and maize. In the light of these data, their domesticated status if recovered in early archaeobotanical records should no longer be assumed.

Key traits that appear to not have much differed are pollen size and

morphology in teosinte and maize, and phytolith characteristics in teosinte fruitcases. Pollen size in the maize inbreds studied, though sometimes larger than in traditional modern landraces in both modern and EH conditions, did not exhibit trends for larger or smaller size nor a different morphology in EH conditions. It appears that archaeobotanists and paleoecologists can be confident that studies of these micro-fossils using modern reference collections will lead to valid results. Other important wild-type traits found in teosinte today such as disarticulating ears did not differ in our grow-outs.

Our artificial selection experiment demonstrated that MLPs induced in Late Pleistocene and early Holocene conditions from modern natural populations can be stable from one generation to the next in those

Table 6
Pollen size in teosinte grown in late pleistocene and early holocene environments.

Year	Plant				Year	Plant				Year	Plant				
	2010	Teosinte	Mean	Maxium		Minimum	2016	Teosinte	Mean		Maxium	Minimum	2014	Maize	Mean
MCC	1A		76.07	92.04	63.72	MCC	3C-1	59.1	72	48	MCC	1-A	115.17	166.08	94.08
	1B		75.94	95.58	64.9		3C-2	54.87	66.24	48		1-B	111.02	147.84	92.16
	1C		74.66	90.86	53.1		3C-3	62.63	77.76	49.92		2-A	105.63	120	95.04
	2A		78.81	99.12	59		2B-1	56.62	68.16	39.36		2-B	121.6	159.36	100.8
	2B		69.83	80.24	53.1		2B-2	48.87	62.4	38.4		3-A	91.59	122.88	67.2
	3A		67.97	93.22	51.92		2B-3	62.51	72.96	54.72		3-B	98.86	140.16	90.24
	3B		77.21	90.86	61.36		4C-1	68.75	86.4	55.68		4-A	89.63	111.36	72.96
	3C		71.94	87.32	54.28		4C-2	68.65	86.4	54.72		4-B	87.97	117.12	69.12
	4A		83.07	112.1	62.54		4C-3	70.23	86.4	58.56		Mean	102.69	135.6	85.2
	4B		69.37	83.78	57.82		Mean	61.4	75.4	49.7					
	4C		63.26	74.34	53.1						EHC	1-A	88.94	96	69.12
	Mean		73.5	90.9	57.7							1-B	90.84	104.64	75.84
												2-A	142.64	173.76	97.92
	LPC	1A		67.85	83.78	53		3C-1	65.93	77.76	46.08		2-B	133.28	148.8
1B			80.76	101.48	63.72		3C-2	70.34	83.52	57.6		3-A	105.83	133.44	81.6
1C			70.88	81.42	55.46		2B-1	66.84	78.72	56.64		3-B	90.21	128.64	69.12
2A			73.5	107.38	50.74		2B-2	71.39	83.52	57.6		4-A	93.81	123.84	72.96
2B			78.56	94.4	60.18		2B-3	64.51	84.48	54.72		4-B	96.82	132.48	70.08
3A			76.45	107.38	59		4C-1	68.1	83.52	52.8		Mean	105.3	130.2	81.24
3B			76.69	107.38	55.46		4C-2	66.28	89.28	56.64					
3C			83.11	100.3	59		4C-3	74.5	94.08	57.6					
4A			82.67	92.04	70.8		Mean	68.4	83.8	54.7					
4B			74.31	99.12	54.28										
4C			87.6	138.06	61.36										
Mean			77.5	101.2	58.5										

Notes: Teosinte plants in green are maize-like phenotypes.

Table 7
Number of tassel branches in teosinte in the Late Pleistocene growing chamber (LPC) compared with the modern control chamber (MCC).

Plant	MCC		LPC	
	# Primary Branches	# Secondary Branches	# Primary Branches	# Secondary Branches
1A	10	many	5	1
1B	11	many	5	3
1C	8	3	6	6
2A	8	many	1	0
2B	9	many	4	0
3A	15	many	6	2
3B	15	many	4	1
3C	10	~5	4	1
4A	8	many	10	~11
4B	10	many	4	0
4C	11	many	9	4
Mean	10.5		5.3	

Notes: The primary tassel on the main stalk was evaluated. Many = > 15. The grow-out year is 2010. In EH conditions (year 2012), not shown, primary branch number was 4.1 ± 3.5 with 0 secondary branches on all but two plants, with 4 on one of them and Many on the other. In the MCC in 2012 there were obviously many more primary and secondary branches than in the EHC judging from photographs we took of the plants in which the branches were too dense to count.

ancestral environments. We believe these plants would have drawn attention and selection pressure from teosinte cultivators due to their increased harvesting efficiency. For example, with their maize-like branching and inflorescences, MLPs have more compact clusters of female ears located in an easily visible position on the main stem and synchronous seed maturation, meaning seeds could be collected with a single harvest effort and minimal seed loss. In modern teosintes, seeds matured sequentially over a period of a few months, required several harvesting periods over that time, and fell off easily shortly after maturation if not collected (Piperno et al., 2015 and our observations in

Mexican natural stands). Harvesting effectiveness is a central trait influencing cereal collection and cultivation strategies, a point underscored by the fact that traits associated with harvesting, such as reduced stem/branch number and uniform seed maturation, are key components of the domestication syndrome (e.g., Olsen and Wendel, 2013).

Our associated gene expression (GE) study indicates a sound underlying genetic basis for the plastic and other phenotypic changes we observed, with environmentally-responsive gene expression changes often playing a major role. For example, genes known to underwrite vegetative architecture, inflorescence sexuality, plant height, biomass, and seed yield in *Zea* were differentially expressed in teosinte in EH vs. modern conditions, indicating they mediated the differences we observed in those traits in the contrasting environments (Lorant et al., 2017; Piperno, 2017). Our GE results also indicate EH conditions with their lower temperature and CO₂ were more stressful for plants than modern conditions. In fact, less optimal or stressful growing environments today in the Balsas watershed (shade, shallow soils, low moisture) still induce gene expression change in *tb1*, and/or genes in the *tb1* regulatory network, and developmental plasticity that results in what we called here the MLP phenotype (Doebley et al., 1995; Whipple et al., 2011) (Fig. 6). Furthermore, our GE data indicating a substantial loss of plasticity occurred during maize domestication dovetail with our preliminary artificial selection findings on the multi-generational stability of MLP progeny (and often, stability of individual traits of the MLP complex in progeny that didn't become full MLPs) that was necessary for the plastic traits to have eventually occurred. Considering that: 1) during the first few thousand years of the Holocene in maize's homeland temperature and atmospheric CO₂ were considerably lower than today's, and 2) until the Industrial Revolution, Holocene atmospheric CO₂ was still more than 100 ppmv lower than at present, it is interesting to consider when in the Holocene teosinte became the plant we typically observe today.

Therefore, the combined data indicate the first cultivators through artificial selection could have fixed (cemented) plastic phenotypes that were created by nature in the lower than today's LP and EH CO₂ and temperature conditions. If true, this represents an alternative pathway to maize domestication not heretofore demonstrated in a crop plant. It is currently unknown how long GA would take in teosinte and further



Fig. 5. Comparison of tassel branching in teosinte in Late Pleistocene (left) and modern (right) conditions. There are far fewer branches in the LP conditions.



Fig. 6. A maize-like phenotype plant growing in the central Balsas watershed on a hillslope in shallow, dry soil. In all respects it is the same phenotype as the MLP plants in this study. Photo: Anthony J. Ranere.

work is needed to assess the meaning of the MLPs that intriguingly grew even in the modern environment in 2016. Interestingly in this regard, in a southwest annual USA grass, GA for leaf stomatal conductance took place in just seven years of growth under the inducing high CO₂ air (Grossman and Rice, 2014). A process related to GA called genetic accommodation retains plasticity. Because one would expect that farmers desired to reduce plasticity for favored traits, and gene expression

evidence indicates they did so, genetic assimilation was probably considerably more influential in domestication.

In view of the profound environmental changes that took place around the world at the origins of cultivation and domestication, the natural environment would have been a powerful force if plasticity was an intrinsic component of various wild progenitors, as it appears to be in teosinte. Recent work has established the explanatory potential of developmental plasticity in crop plant evolution in another region of the world, eastern North America (Mueller, 2017). Fruit morphology in the wild progenitor of *Polygonum erectum* L. (erect knotweed) is a plastic trait that responds to growing season differences to produce morphs that can be of a wild or cultivated/domesticated morphology. Moreover, their experiments have shown that transplantation of wild knotweed from its natural river bank environment to gardens quickly transforms the plant's architecture from a herb to a small shrub with more branches and thus higher seed yield (Mueller et al., 2017). This research shows how simply moving wild plants to human-created, enriched habitats in the same climate can quickly drive morphological changes intrinsic to domestication. It would seem that plasticity-mediated pathways should now be considered in crop plant evolution more widely. Considerable study of other domesticates and, importantly also, persistently cultivated plants that for unclear reasons are resistant to genetic and phenotypic change, is needed before the role of plasticity in crop plant evolution more generally can be properly assessed (see Piperno, 2017 for current examples of other crops where plasticity occurs or is possibly influential).

Open fruitcases represent another crucial maize trait that appears to have been present in LP and EH teosinte populations. Why they were found in many plants we grew, occurred in nearly all the MLPs, and had the highest overall frequencies in LP and EH conditions (Table 3) is possibly explainable by recent genetic findings by Studer et al. (2017). They found that the gene *tga1*, known to control the opening of the teosinte fruitcase, is itself directly regulated by another gene, *tb1*, known to in part underwrite the MLP traits we observed. It is then possible that *tb1* increased the gene expression of *tga1*, resulting in the open fruitcases. Work will be undertaken to directly measure *tga1* activity in these fruits.

Our seed yield results require further work with additional planting generations to establish if trends seen for yield increases in some plants across generations are robust. However, the beneficial effects of tillers on plant seed yield are clear.

Flowering time adaptations in maize were crucial for dispersals into more temperate elevations and latitudes (e.g., Buckler et al., 2009; Swarts et al., 2017), and in teosinte presumably for adaptations to LP and EH conditions. Maize flowered later in EH than in modern conditions while teosinte flowered earlier in LP and EH conditions than in modern. Earlier flowering in teosinte probably contributed to those plants having much shorter heights in LP and EH conditions (Piperno et al., 2015). Later flowering may have contributed to the higher number of incompletely developed kernels and lower seed yield in maize grown in EH than in modern conditions (Lorant et al., 2017). The finding that tassel branch and probably pollen numbers were reduced in teosinte in LP and EH conditions may have implications for ancient pollen records with respect to making *Zea* pollen more difficult to find during those periods. Maize had slightly less branching in EH conditions. Macrobert et al. (2017) showed that maize plants with few tassel branches produced less pollen than those with more branches. Low pollen yield was also associated with low grain yield in their study.

Our combined results add to the evidence for the prominence of regulatory genes, gene expression, and gene interactions in domestication origins (e.g., Hufford et al., 2012; Meyer and Purugganan, 2013; Stitzer and Ross-Ibarra, 2018). Our work also relates to how long “domestication” took. The domestication of some crops appears to have been a more protracted process than was previously thought, as archaeobotanical and ancient DNA records show the fixation of the complement of traits observed in major domesticated cereals, including maize, and some legumes took thousands of years (e.g., Piperno, 2011; Larson et al., 2014; Ramos-Madrugal et al., 2016; Vallebuena-Estrada et al., 2016; Kistler et al., 2018). On the other hand, a pre-cultivation availability of maize-like traits in teosinte may have increased the speed of the selection process for those traits, while also hastening the development of apical dominance that would significantly increase maize cob size and seed yield.

Finally, Darwin's recognition of the value of domestication as a model of evolution forever thrust plant and animal domesticates into the forefront of biological science. He would likely follow with interest an active debate in evolutionary biology regarding broadening the Modern Synthesis (MS) through new or renewed emphases on elements of what is called an Extended Modern Synthesis (ES) (Piperno, 2017 for a review). Important ES elements include developmental plasticity, niche construction, and epigenetic inheritance. In a paper that was ahead of its time, Hugh Iltis (1983), focusing on the profound differences in vegetative architecture and inflorescence sexuality between teosinte and maize that were among the major phenotypic changes we observed in teosinte in simulated past climates, first proposed the importance of environmental influences on development and phenotype, and genetic assimilation in plant domestication. Following Iltis and a review of other intriguing older research and suggestions they raised on the potential relevance of developmental plasticity to domestication (Gremillion and Piperno, 2009), our studies have initiated its empirical examination in crop plant evolution. Clearly, however, ES and standard MS concepts and mechanisms shouldn't be de-emphasized with respect to one another when investigating domestication or evolutionary change more generally, as mechanisms stressed by each were likely operating. In domestication both conscious and unconscious selection directed at mutations for favorable traits that probably were not plastic occurred in a standard Mendelian manner (Meyer and Purugganan, 2013; Olsen and Wendel, 2013; Larson et al., 2014). New mutations and standing genetic variation were both involved, and moreover, the genetic process leading to GA following plastic change follows standard Darwinian theory (Piperno, 2017).

In conclusion, our results demonstrate how experimental research can shed light on important questions in domestication research and evolutionary biology more generally, while raising a number of others not previously considered. Because loss of plasticity in major crops may limit their ability to adjust to future environmental change (Gage et al., 2017; Kusmec et al., 2018), understanding plasticity in teosinte and

what remains of it in maize may aid geneticists and breeders in better understanding and adapting their responses to future climates.

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References

- Ahn, J., et al., 2004. A record of atmospheric CO₂ during the last 40,000 years from the Siple Dome, Antarctica ice core. *J. Geophys. Res.* 109, D13305 8 pp.
- Beldade, P., Mateus, A.R.A., Keller, R., 2011. Evolution and molecular mechanisms of adaptive developmental plasticity. *Mol. Ecol.* 20, 1347–1363.
- Buckler, E., et al., 2009. The genetic architecture of maize flowering time. *Science* 325, 714–718.
- Bush, M.B., et al., 2009. Re-evaluation of climate change in lowland Central America during the last glacial maximum using new sediment cores from lake petén Itzá, Guatemala. In: Vimeaux, F., Sylvestre, F., Khodri, M. (Eds.), *Past Climate Variability in South America and Surrounding Regions*. Springer Science & Business Media B.V., pp. 113–128.
- Chen, Y.H., et al., 2017. Back to the origin: in situ studies are needed to understand selection during crop diversification. *Fron. Ecol. Evol.* <https://doi.org/10.3389/fevo.2017.00125>.
- Chitwood, D.H., et al., 2016. Climate and developmental plasticity: Interannual variability in grapevine leaf morphology. *Plant Physiol.* 170, 1480–1491.
- Correa-Metrio, A., et al., 2012. Rapid climate change and no-analog vegetation in lowland Central America during the last 86,000 years. *Quat. Sci. Rev.* 38, 63–75.
- Craufurd, P.Q., Wheeler, T.R., 2009. Climate change and the flowering time of annual crops. *J. Exp. Bot.* 60, 2529–2539.
- Des Marais, D.L., Hernandez, K.M., Juenger, T.E., 2013. Genotype-by-environment interaction and plasticity: exploring genomic responses of plants to the abiotic environment. *Annu. Rev. Ecol. Evol. Syst.* 44, 5–29.
- Diggle, P.K., Miller, J.S., 2013. Developmental plasticity, genetic assimilation, and the evolutionary diversification of sexual expression in *Solanum*. *Am. J. Bot.* 100, 1050–1060.
- Doebley, J., 2004. The genetics of maize evolution. *Annu. Rev. Genet.* 38, 37–59.
- Doebley, J., Stec, A., Gustus, C., 1995. *Teosinte branched1* and the origin of maize: evidence for epistasis and the evolution of dominance. *Genetics* 141, 333–346.
- Dorweiler, J., Doebley, J., 1997. Developmental analysis of *teosinte glume architecture1*: a key locus in the evolution of maize (Poaceae). *Am. J. Bot.* 84, 1313–1322.
- Gage, J.L., et al., 2017. The effect of artificial selection on phenotypic plasticity in maize. *Nat. Commun.* 8, 1348.
- Gilbert, S.F., Epel, D., 2009. *Ecological Developmental Biology: Integrating Epigenetics, Medicine and Evolution*. Sinauer Associates, Inc., Sunderland, MA.
- Gremillion, K.J., Piperno, D.R., 2009. Human behavioral ecology, phenotypic (developmental) plasticity, and agricultural origins: insights from the emerging evolutionary synthesis. In: In: Cohen, M.N. (Ed.), *Rethinking the Origins of Agriculture*. *Curr. Anthropol.* vol 50. pp. 615–619.
- Grossman, J.D., Rice, K.J., 2014. Contemporary evolution of an invasive grass in response to elevated atmospheric CO₂ at a Mojave Desert FACE site. *Ecol. Lett.* 17, 710–716.
- Hodell, D.A., et al., 2008. An 85-ka record of climate change in lowland Central America. *Quat. Sci. Rev.* 27, 1152–1165.
- Holst, I., Piperno, D.R., Moreno, E., 2007. Identification of teosinte, maize, and *Tripsacum* in Mesoamerica by using pollen, starch grains, and phytoliths. *Proc. Natl. Acad. Sci. U.S.A.* 104, 17608–17613.
- Hufford, M.B., et al., 2012. Comparative population genomics of maize domestication and improvement. *Nat. Genet.* 44, 808–811.
- Iltis, H.H., 2000. Homeotic sexual translocations and the origin of maize (*Zea mays*, Poaceae): a new look at an old problem. *Econ. Bot.* 54, 7–42.
- Iltis, H.H., 1983. From teosinte to maize: the catastrophic sexual transmutation. *Science* 222, 886–894.
- Kistler, L., et al., 2018. Multiproxy evidence highlights a complex evolutionary legacy of maize in South America. *Science* 362, 1309–1313.
- Kusmec, A., de Leon, N., Schnable, P.S., 2018. Harnessing phenotypic plasticity to improve maize yields. *Front. Plant Sci.* <https://doi.org/10.3389/fpls.2018.01377>.
- Kuzawa, C.W., Bragg, J.M., 2012. Plasticity in human life history strategy. *Curr. Anthropol.* 53, S369–S382.
- Larson, G., Piperno, D.R., et al., 2014. Current perspectives and the future of domestication studies. *Proc. Natl. Acad. Sci. U.S.A.* 111, 6139–6146.
- Lauter, N., Doebley, J., 2002. Genetic variation for phenotypically invariant traits detected in teosinte: implications for the evolution of novel forms. *Genetics* 160, 333–342.
- Lorant, A., et al., 2017. The potential role of genetic assimilation during maize domestication. *PLoS One.* <https://doi.org/10.1371/journal.pone.0184202>.
- Macrobert, J., Minnaar-Ontong, A., Labuschagne, M., 2017. Effect of the few-branched-1 (*Fb1*) tassel mutation on performance of maize inbred lines and hybrids evaluated under stress and optimum environments. *Maydica* 62, 1–10.
- Maurya, J.P., et al., 2018. Environmentally Sensitive Molecular Switches Drive Poplar Phenology. <https://doi.org/10.3389/fpls.2018.01873>.

- Meyer, R.S., Purugganan, M.D., 2013. Evolution of crop species: genetics of domestication and diversification. *Nat. Rev. Genet.* 14, 840–852.
- Moczek, A.P., et al., 2011. The role of developmental plasticity in evolutionary innovation. *Proc. Roy. Soc. B* 278, 2705–2713.
- Mueller, N.G., 2017. Documenting domestication in a lost crop (*Polygonum erectum* L.): evolutionary bet-hedgers under cultivation. *Veg. Hist. Archaeobotany* 26, 313–327.
- Mueller, N.G., et al., 2017. Growing the lost crops of eastern North America's original agricultural system. *Nat. Plants* 3, 17092.
- Nicotra, A.B., et al., 2010. Plant phenotypic plasticity in a changing climate. *Trends Plant Sci.* 12, 684–692.
- Olsen, K.M., Wendel, J.F., 2013. A bountiful harvest: genomic insights into crop domestication phenotypes. *Annu. Rev. Plant Biol.* 64, 47–70.
- Pigliucci, M., Murren, C.J., Schlichting, C.D., 2006. Phenotypic plasticity and evolution by genetic assimilation. *J. Exp. Biol.* 209, 2362–2367.
- Piperno, D.R., 2006. *Phytoliths: a Comprehensive Guide for Archaeologists and Paleocologists*. AltaMira, Lanham, MD.
- Piperno, D.R., 2011. The origins of plant cultivation and domestication in the new world tropics: patterns, process, and new developments. *Curr. Anthropol.* 52 (S4), S453–S470.
- Piperno, D.R., 2017. Assessing elements of an extended evolutionary synthesis for plant domestication and agricultural origin research. *Proc. Natl. Acad. Sci. U.S.A.* 114, 6429–6437.
- Piperno, D.R., et al., 2007. Late Pleistocene and Holocene environmental history of the Iguala valley, central Balsas watershed of Mexico. *Proc. Natl. Acad. Sci. U.S.A.* 104, 11874–11881.
- Piperno, D.R., et al., 2009. Starch grain and phytolith evidence for early ninth millennium B.P. Maize from the central Balsas river valley, Mexico. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5019–5024.
- Piperno, D.R., et al., 2015. Teosinte before domestication: experimental study of growth and phenotypic variability in Late Pleistocene and early Holocene environments. *Quat. Int.* 363, 65–77.
- Curr Anthropol Price, D., Bar-Yosef, O. (Eds.), 2011. *The Origins of Agriculture*, vol. 52 New Data, New Ideas Supplement 4.
- Ramos-Madrigal, J., et al., 2016. Genome sequence of a 5,310-year-old maize cob provides insights into the early stages of maize domestication. *Curr. Biol.* 26, 3195–3201.
- Ranere, A.J., Piperno, D.R., Holst, I., Dickau, R., Iriarte, J., 2009. Pre-ceramic human occupation of the Central Balsas Valley, Mexico; cultural context of early domesticated Maize and squash. *Proc. Natl. Acad. Sci. USA* 106, 5014–5018.
- Standen, E.M., Du Trina, Y., Larsson, H.C.E., 2014. Developmental plasticity and the origin of tetrapods. *Nature* 513, 54–58.
- Stitzer, M.C., Ross-Ibarra, J., 2018. Maize domestication and gene interaction. *New Phytol.* 220, 395–408.
- Studer, A.H., Wang, H., Doebley, J., 2017. Selection during maize domestication targeted a gene network controlling plant and inflorescence architecture. *Genetics* 207, 755–765.
- Sultan, S.E., 2010. Plant developmental responses to the environment: eco-devo insights. *Curr. Opin. Plant Biol.* 13, 96–101.
- Sultan, S.E., 2015. *Organism and Environment*. Oxford University Press, Oxford.
- Swarts, K., et al., 2017. Genomic estimation of complex traits reveals ancient maize adaptation to temperate North America. *Science* 357, 512–515.
- Vallebuena-Estrada, M., et al., 2016. The earliest maize from San Marcos Tehuacan is a partial domesticate with genomic evidence of inbreeding. *Proc. Natl. Acad. Sci. U.S.A.* 113, 14151–14156.
- Van Heerwaarden, J., et al., 2011. Genetic signals of origin, spread, and introgression in a large sample of maize landraces. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1088–1092.
- Waddington, C.H., 1953. Genetic assimilation of an acquired character. *Evolution* 7, 118–126.
- Wang, H., et al., 2005. The origin of the naked grains of maize. *Nature* 436, 714–719.
- Wang, M., Stidham, T.A., Zhou, Z., 2018. A new clade of basal Early Cretaceous pygostylian birds and developmental plasticity of the avian shoulder girdle. *Proc. Natl. Acad. Sci. U.S.A.* 115, 10708–10713.
- West-Eberhard, M.J., 2003. *Developmental Plasticity and Evolution*. Oxford University Press, Oxford.
- Whipple, C.J., et al., 2011. Grassy Tillers1 promotes apical dominance in maize and responds to shade signals in the grasses. *Proc. Natl. Acad. Sci. U.S.A.* 108, E506–E512.
- Winter, K., 2019. Ecophysiology of constitutive and facultative CAM photosynthesis. *J. Exp. Bot.* <https://doi.org/10.1093/jxb/erz002>.