



# Teosinte before domestication: Experimental study of growth and phenotypic variability in Late Pleistocene and early Holocene environments



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## ABSTRACT

Agriculture arose during a period of profound global climatic and ecological change following the end of the Pleistocene. Yet, the role of phenotypic plasticity – an organism's ability to change its phenotype in response to the environment – and environmental influences in the dramatic phenotypic transformations that occurred during plant domestication are poorly understood. Another factor possibly influential in agricultural origins, the productivity of crop plant wild progenitors in Late Pleistocene vs. Holocene environments, has received increasing attention recently and merits further investigation. In this study, we examined phenotypic characteristics and productivity (biomass, seed yield) in the wild progenitor of maize, the teosinte *Zea mays* ssp. *parviglumis* H.H. Iltis & Doebley, when it was first exploited and cultivated by growing it in atmospheric CO<sub>2</sub> concentrations and temperatures characteristic of the late-glacial and early Holocene periods. Plants responded with a number of attributes uncharacteristic of teosinte in today's environments, including maize-type traits in vegetative architecture, inflorescence sexuality, and seed maturation. Teosinte productivity was significantly lower in late-glacial compared with early Holocene and modern environments. Our evidence indicates that: a) ancestral biological characteristics of crop plant progenitors aren't always predicted from living examples, b) some important maize phenotypic traits were present at initial human exploitation and selection, and c) Pleistocene plant productivity should be considered a significant factor in the chronology of food production origins.

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## 1. Introduction

The development of agricultural societies made possible by plant and animal domestication was one of the most transformative events in human and ecological history. Agricultural beginnings can be traced around the world to 12,000–10,000 years ago (ka) during a time of profound global environmental change as the Pleistocene was ending and transitioning to the Holocene (e.g., Price and Bar-Yosef, 2011). From at least 40,000–12,000 ka, atmospheric CO<sub>2</sub> levels worldwide were as much as one-third lower (c. 180–235 ppmv) than early (12–10 ka) post-glacial levels (c. 265–270 ppmv) (Ahn et al., 2004). In many areas of the world, including the New World tropics where maize and other major crops were

domesticated, Late Pleistocene (c. 21–11 ka) temperature and annual precipitation were also significantly lower than early post-glacial levels by approximately 5–7 °C and 20–40%, respectively (Piperno et al., 2007; Hodell et al., 2008; Bush et al., 2009; Correa-Metrio et al., 2012). Temperature and atmospheric CO<sub>2</sub> were still lower than today by a few degrees Centigrade and about 130 ppmv at the beginning of the Holocene (Ahn et al., 2004; Correa-Metrio et al., 2012).

For a number of crop and wild progenitor species, recent research has considerably elucidated the genetic mechanisms that underwrote their phenotypic transformations from wild to domesticated status in those climate eras. Previously emphasized conventional assumptions for morphological change (e.g., that it was driven by human selection for rare mutants of single genes that were deleterious in wild plants and favorable in field environments, or selection for new, advantageous mutations that appeared post-cultivation) have for some domestication traits been supplanted by more complex processes. They include epistasis (when the phenotypic product of one gene depends on its interactions with

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other genes in the same plant), non-Mendelian inheritance, and changes in gene expression (such as how, when, and to what extent existing genes are expressed through changes in the amount of mRNA during transcription) (e.g., Nesbitt and Tanksley, 2002; Doebley, 2004; Studer and Doebley, 2011; Studer et al., 2011; Swanson-Wagner et al., 2012; Olsen and Wendel, 2013). Furthermore, in an increasing number of wild progenitors, pre-existing, non-deleterious genetic variation also called “standing” and “cryptic” genetic variation is being documented for major domestication traits, indicating genetic attributes for the traits were commonly available to the first plant cultivators (Lauter and Doebley, 2002; Nesbitt and Tanksley, 2002; Clark et al., 2004; Studer et al., 2011).

It is now also well-established through other research that the environment may influence some of these genetic–phenotypic relationships by directly inducing (triggering) phenotypic variability (e.g., West-Eberhard, 2003; Gilbert and Epel, 2009; Beldade et al., 2011; Palmer et al., 2012). Phenotypic plasticity – when a single genotype exhibits multiple phenotypes in response to environmental variability and change – is well-documented in plants, and there is increasing evidence that phenotypes generated in this manner can be both adaptive and inherited (e.g., West-Eberhard, 2003; Moczek, 2007; Gilbert and Epel, 2009; Nicotra et al., 2010; Sultan, 2010; Beldade et al., 2011; Moczek et al., 2011; Palmer et al., 2012). However, how natural- and human-mediated environmental change may have influenced the dramatic phenotypic transitions undergone by domesticated plants is a neglected area of domestication research.

It, therefore, becomes of considerable interest to ask if, during the late-glacial and early Holocene periods (c. 16–10 ka) when people first encountered, exploited, and cultivated many of the wild progenitors, the plants differed from modern wild populations, influencing crop plant evolution in ways that have been little considered. The last hunters and gatherers and first farmers worked with the phenotypes they saw, and it can be imagined they were attuned to and interested in the phenotypic variability they encountered on natural and cultivated landscapes. Unfortunately, chronologically-coarse and often geographically-uneven archaeobotanical records do not adequately capture the range of phenotypic attributes that early cultivators experimented with. Moreover, the macrofossils (seeds, fruits, stems) that can best inform this question are often poorly preserved and have as yet to be recovered from Late Pleistocene and early Holocene records for many wild progenitors and earliest cultivars, including *Zea* (e.g., Piperno et al., 2009; Piperno, 2011). Thus, modern representatives of crop plant ancestors constitute the basis for much of the morphological and genetic study of proto-domesticates and their wild ancestors.

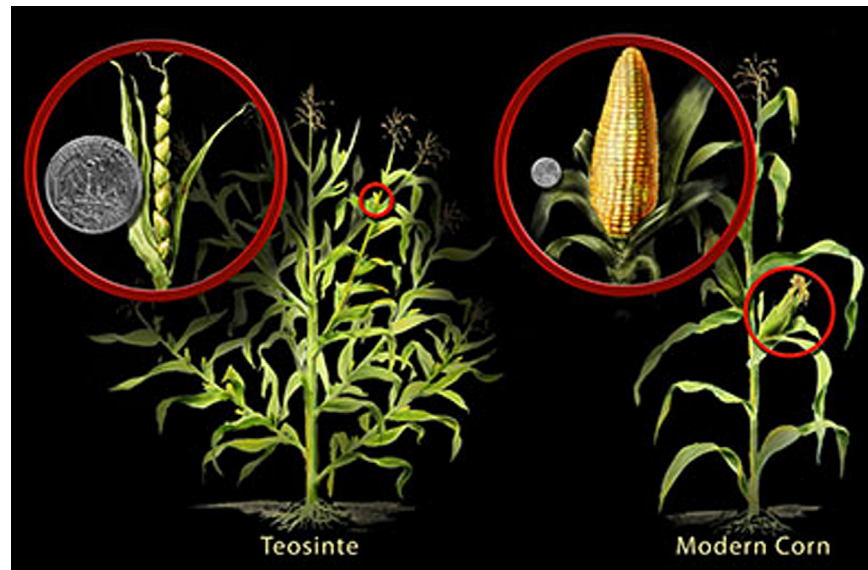
A related question of increasing interest concerns the productivity of wild progenitors of crops in Pleistocene environments before farming began. Plant growth is highly CO<sub>2</sub> dependent and low Pleistocene atmospheric CO<sub>2</sub> appears to have been a significant stress factor on many species, lowering their productivity because of its constraints on photosynthesis and water-use efficiency (Dippert et al., 1995; Sage, 1995; Cunliff et al., 2010; Gerhart and Ward, 2010; Cowling, 2011). It is increasingly seen as having been a major limiting factor for a pre-Holocene origin of agriculture (Sage, 1995; Richerson et al., 2001; Cunliff et al., 2008, 2010). Recent experimental studies on a number of C<sub>3</sub> and C<sub>4</sub> cereal crop progenitors, including wild maize – the teosinte *Zea mays* ssp. *parviglumis* H.H. Iltis & Doebley – found decreased biomass and/or photosynthetic activity when plants were grown in Pleistocene compared with Holocene CO<sub>2</sub> levels (Cunliff et al., 2008, 2010). Additional constraints on productivity during the Pleistocene may have included depressed temperatures and precipitation, but the impact of these factors has not been studied.

In order to experimentally investigate the phenotypic and productivity variability encountered by hunters-gatherers and proto-farmers, we grew maize's wild ancestor (hereafter ssp. *parviglumis*) in glass houses in which temperature and CO<sub>2</sub> levels were adjusted to those documented in Mesoamerica for the late-glacial segment (c. 16–11 ka) of the Late Pleistocene and the beginning of the Holocene (c. 11–10 ka) (e.g., Ahn et al., 2004; Piperno et al., 2007; Bush et al., 2009; Correa-Metrio et al., 2012). Our results address the possible multifactorial roles of phenotypic variability and plasticity, plant productivity, and environmental change in constructing theories for, investigating, and understanding maize domestication and perhaps crop plant origins more generally.

## 2. Teosinte, phenotypic plasticity, and environmental change: background and previous relevant research

Maize's wild ancestor appears to be a particularly suitable candidate with which to begin evaluating the links between phenotypic and environmental variability in domestication research. Archaeological and genetic evidence indicates that maize was domesticated in tropical southwestern Mexico, probably in the central Balsas River Valley region of Michoacán and Guerrero states, by 9000 BP (Matsuoka et al., 2002; Piperno et al., 2009; Ranere et al., 2009; Van Heerwaarden et al., 2011). Although not yet documented with archaeological data, the likelihood is that teosinte cultivation began at least 1000 years earlier (Wang et al., 2005), shortly after the Pleistocene ended and the climate and vegetation were in a state of considerable transformation. There are profound morphological differences between teosinte and maize in vegetative architecture and inflorescence sexuality that are the most dramatic known of any crop/progenitor pair, a factor that led to a century-long debate about maize's ancestry (discussed in Doebley, 2004). The differences are known to be in part underwritten by the gene *teosinte branched1* (*tb1*) through a gene expression change (change in the amount of mRNA) it mediates during early plant development (Doebley et al., 1995; Hubbard et al., 2002; Studer et al., 2011). Given this observation and reasons discussed below, a type of plasticity called developmental plasticity may have been relevant to teosinte and its domestication.

Developmental plasticity is the inherent capacity of organisms to rapidly produce phenotypic change through one of several available pre-adult developmental pathways in direct response to environmental perturbations and stress factors (e.g., West-Eberhard, 2003; Moczek, 2007; Fusco and Minelli, 2010; Gilbert and Epel, 2009; Phennig et al., 2010; Sultan, 2010; Beldade et al., 2011; Moczek et al., 2011). This capacity should be particularly important in plants, which cannot simply get up and move to places more to their liking when physical and biotic conditions become less favorable. Developmental plasticity is the integration of evolutionary developmental biology (evo-devo) with environmental influences in the determination of what causes phenotypic change. The environment can include factors emanating externally or from within the internal conditions of organisms. Developmental plasticity has become closely allied with the new field of ecological developmental biology (eco-devo or eco-evo-devo), which places particular emphasis on external environmental influences on phenotypes that broadly range from the physical environment to competitors and predators, and field rather than laboratory research (Gilbert and Epel, 2009; Sultan, 2010; Beldade et al., 2011). Developmental plasticity often gives rise to new phenotypes through changes in gene expression, which is known to be highly responsive to environmental perturbation (e.g., Gilbert and Epel, 2009; Beldade et al., 2011). New phenotypic variation can then be rapidly introduced without a corresponding genetic change (e.g., without the appearance or spread of a new mutation), and it can spread in populations if the inducing environment is



**Fig. 1.** The differences between teosinte and maize in branching architecture and inflorescence sexuality. Teosinte has many long primary lateral branches terminated by tassels, and secondary lateral branching. The female ears are located on the secondary lateral branches. Modern maize has a single main stem with a solitary tassel terminating it. There are few, very short primary lateral branches, and no secondary branching. The cobs are located at the ends of the short primary lateral branches in the positions occupied by tassels in teosinte. Credit: Nicolle Rager Fuller, National Science Foundation.

maintained over multiple generations. These points will be elaborated on further below.

Importantly, a developmentally plastic response in vegetative architecture and inflorescence sexuality takes place in teosinte today that adapts plants to their local environments. In good growing conditions (adequate sunlight, deep soils), the plant is tall – 2–3 m-high – with many long lateral branches tipped by tassels and secondary branches bearing female ears with a few small seeds (Fig. 1, left). These are the vegetative and floral characteristics normally associated with maize's wild ancestor both today and in the past. However, stressful or less optimal habitats today (shade, shallow soils, low moisture, crowding) induce a gene expression change that causes suppression of branch elongation during growth (Doebley et al., 1995; Hubbard et al., 2002; Whipple et al., 2011). The result is plants with maize-like attributes; namely, a few, dramatically shortened lateral branches tipped by female ears instead of tassels and a single tassel terminating the main stem (Fig. 1, right). These plants can also be very short (knee-high). It is reasonable to believe that teosinte plasticity today in sub-optimal growing conditions may be non-specific responses to a variety of environmental stresses/cues, and that past conditions such as low CO<sub>2</sub> and temperature may have been among them. In all environments, however, the domesticated maize exhibits a few, very short lateral branches terminating in large cobs instead of tassels, and has a solitary tassel at the top of a single main stem (Fig. 1, right). As in teosinte, these transformations are in part mediated by the gene expression and developmental changes discussed above (Doebley et al., 1995; Hubbard et al., 2002). Maize domestication then involved a loss of plasticity in these traits because maize vegetative architecture and floral sexuality are constitutively expressed regardless of the environment.

With a theory of maize evolution called “Catastrophic Sexual Transmutation”, Iltis (1983, 1987) first drew major attention to how the vegetative (also called branching) architecture and inflorescence traits of *Zea* discussed above were determined by mechanisms set in motion during early plant development. He emphasized how environmental factors, including cold growing seasons, were likely triggers, potentially producing a rapid phenotypic transformation from teosinte- to maize-type branching

and inflorescence sexuality without human involvement. A part of Iltis' theory that proposed the maize ear was derived from a feminized teosinte tassel is probably incorrect, as subsequent studies showed that when lateral branches were shortened and female ears were translocated to the maize cob position at the ends of the branches, they were of normal teosinte type with hard cupulate fruitcases and didn't possess characteristics, such as soft glumes, that would have been derived from tassels if the latter were involved (Doebley et al., 1995). Nonetheless, Iltis' focus on environmental influences, plasticity, pre-existing genetic variation, and the rapid, macro-evolutionary nature of such non-mutational phenotypic change was ahead of its time in domestication research and clearly warrants further attention.

Given both mechanisms for a direct environmental determination of phenotypic change, and a hypothesis for the involvement of such a mechanism in maize domestication, an important initial question for domestication studies becomes, “what is the sensitivity of teosinte and other crop progenitors to past environmental influences?”

### 3. Material and methods

#### 3.1. Experiment design

Data presented here are from grow-outs of *ssp. parviglumis* from four different natural Mexican populations and two lines of inbred teosinte during its natural growing period from July to December 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 late-glacial or early Holocene temperature, and CO<sub>2</sub> sub-ambient levels (Ahn et al., 2004; Piperno et al., 2007; Hodell et al., 2008; Bush et al., 2009; Correa-Metrio et al., 2012). The other was at modern CO<sub>2</sub> levels and temperatures characteristic of *ssp. parviglumis* environments today (Table 2). We repeated the experiment in late-glacial conditions three times from 2009 to 2011, and in 2012 conducted the experiment in early Holocene 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.

**Table 1**  
Sources of the teosinte seeds.

	Accession	Origin	Plant name	Elevation asl
Population 1	PI 384062	Mexico, Guerrero State East side of highway, 1 mi s of Palo Blanco, Latitude: 17° 25 min 0 s N (17.41666667), Longitude: 99° 30 min 0 s W (–99.5)	B-K4	1350 m
Population 2	PI 384063	Mexico, Mexico State West side of road, 4 km s of Valle de Bravo, Latitude: 18° 50 min N (18.83333333), Longitude: 100° 10 min 0 s W (–100.16666667)	B-K7	1300 m
Population 3	PI 384071	Mexico, Guerrero State Iguala–Arcelia Rd. 103 km from Iguala Latitude: 18° 20 min N (18.33333333), Longitude: 100° 19 min 0 s W (–100.31666667)	Wilkes 10	1100 m
Population 4	PI 566692	Mexico, Michoacán State Km 43 Rd. Zitacuaro to Tuzantla 19° 4 min 0 s N (19.06666667), Longitude: 100° 25 min 0 s W (–100.41666667)	Collected by J. Sanchez and G. Wilkes	850 m
RIMPA 0064	Ames 28408 06ncao01 SD	Pedigree Beadle & Kato: Site 4		
RIMPA 0065	Ames 28409 06ncao01 SD	Pedigree Wilkes: Site 6		

**Table 2**  
Temperatures in the chambers (°C).

Year		Range	Range		
		<b>Late Glacial Chamber</b>		<b>Modern Control Chamber</b>	
2009	Mean	21.3	20.3–23.5	25.5	24.4–28.0
	Mean Min	19.6	17.9–20.2	24.0	22.6–25.8
	Mean Max	27.8	21.4–33.6	31.3	25.7–36.3
2010	Mean	22.5	20.6–29.7	26.1	24.1–29.3
	Mean Min	18.7	17.3–24.5	24.2	23.0–24.9
	Mean Max	30.3	24.4–44.7	31.4	24.8–45.1
2011	Mean	20.1	18.1–22.7	23.2	21.5–31.2
	Mean Min	15.5	14.5–16.3	19.8	18.5–22.7
	Mean Max	30.2	21.8–37.2	32.7	25.2–48.8
		<b>Early Holocene Chamber</b>		<b>Modern Control Chamber</b>	
2012	Mean	23	17.2–41.9	24.8	21.43–34.16
	Mean Min	15.9	9.3–24.4	17.9	16.0–26.5
	Mean Max	34.0	22.2–45.7	36.2	23.85–53.12

### 3.2. Plant descriptions

Non-inbred teosinte seeds were provided by the USDA North Central Regional Plant Introduction Station located in Ames, Iowa. They are from previous collections made by various investigators in four different localities in the states of Guerrero, Michoacán, and Mexico and belong to four discrete populations (Table 1). In the first grow-out in 2009, three different populations, labeled Nos. 1–3, were planted; a fourth population was added in 2010 and 2011, and included in the 2012 study. Collected from altitudes between 850 and 1350 masl, they provide a good representation of the elevational range of *ssp. parviglumis* today. There was no evidence for maize introgression in the source plants and none were collected from around existing maize fields. One of the populations, from 1 mile south of Palo Blanco in Guerrero, rarely hybridizes with maize and is thought to be the least similar to maize of the annual teosintes (Wilkes, 1977). Inbred teosinte seeds (RIMPA 0064 and 0065, listed as *ssp. parviglumis* Ames 28408 and 28409 in the USDA Grin data base), that became available to us for the 2012 study were supplied by Jeffrey Ross-Ibarra.

### 3.3. Growing conditions

The two naturally lighted glass-houses each have a c. 27 m<sup>3</sup> internal volume (Fig. 2). Light intensity in the houses is about 80% of

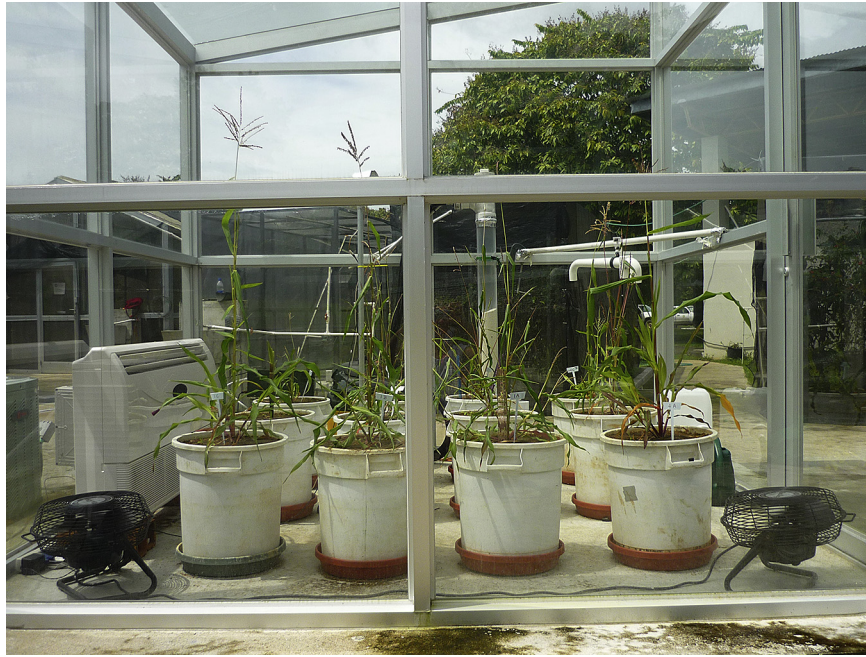
the natural solar irradiation. Split air-conditioning units provided temperature control. In the sub-ambient chambers, CO<sub>2</sub> was lowered using a CO<sub>2</sub> scrubber consisting of an acrylic column filled with soda-lime, and air-pump and dust-bag of an industrial vacuum cleaner. Operation of the CO<sub>2</sub> scrubber was under feedback control via a Vaisala CO<sub>2</sub> analyzer and a Campbell datalogger. CO<sub>2</sub> values were logged every minute and overall mean values were computed for the full growth period. The CO<sub>2</sub> analyzer had a resolution of 10 ppmv and average daily CO<sub>2</sub> concentration generally varied between 200 and 220 ppmv. In the chamber adjusted to late-glacial conditions, average CO<sub>2</sub> and temperature levels over the three growth periods were from 203 to 216 ppmv and 20 to 22.5 °C, respectively (average CO<sub>2</sub> in 2009 was 216 ppmv; in 2010, 203 ppmv; in 2011, 215 ppmv). Table 2 contains average diurnal, maximum (daytime), and minimum (night), chamber temperatures. Modern ambient CO<sub>2</sub> in the experiment area ranged from 360 ppmv in 2009 to 410 ppmv in 2012.

The 2012 grow-out was conducted at targeted, sub-ambient early Holocene temperature and CO<sub>2</sub> levels (c. 23 °C, 260–265 ppm) (Table 2). Daily average CO<sub>2</sub> toward the end of the grow-out became lower due to lower morning values, with the result that at the point when every plant in the early Holocene chamber was mature, CO<sub>2</sub> for the grow-out averaged to 252 ppmv. Average CO<sub>2</sub> was 258 ppm by the time seven of the 13 plants, including the two maize-like phenotypes grown from MLP seeds (discussion below), were mature; and 255 ppmv when nine plants, including one of the inbred MLPs (discussion below), had matured. The remaining plants grew a small amount in stature during their last few weeks of growth. Therefore, phenotypic and yield results, including the induced maize-like phenotypes, reflect CO<sub>2</sub> levels very close to the targeted value.

### 3.4. Sample size and sampling

In the 2009–2011 grow-outs, from nine to 12 plants were grown in each chamber in five-gallon pots in natural top soil from a local orchard without fertilizers. Three pots for each population were planted with three seeds each to allow for non-germinating seeds; after germination one plant per pot was allowed to grow to maturity. In the 2012 grow-out, the sub-ambient and Modern Control Chambers had the following: four plants grown from seeds of maize-like phenotypes that were induced in the sub-ambient





**Fig. 2.** The Sub-ambient chamber set at late-glacial conditions with teosinte.

chambers from 2009 to 2011 (plants 3C-2009, 4C-2010, 1A-2011, 2B-2011); one plant each from the two different inbreds, and two replicate plants (sub-ambient) or one plant (modern control) from the four different populations of the founder seed collections (in one sub-ambient pot with seeds from founder population 2, germination didn't occur). Pots were watered from two to four times per week. During the five to six month growth period various aspects of plant development were recorded at least once a week, such as height, branch length and number, and inflorescence

characteristics. After plants matured they were harvested and transported to the lab where additional descriptions and measurements were made (e.g., seed number/size/weight; biomass). In order to take into account differences in plant height a ratio of branch length to plant height was used to determine final branch length at maturity, called here relative branch length. Biomass was measured after plants were air dried on the sum of four component parts; seeds, leaves, other vegetative parts (stems, leaf sheaths, ear bracts) and roots.



**Fig. 3.** A. A Maize-like phenotype plant from the Late Glacial 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. B. Teosinte in the Modern Control Chamber. Like in modern natural populations, it has many long, primary lateral branches (example, upper white arrow) terminated by tassels (black arrow). Female ears, not yet developed, would be on secondary lateral branches at the location of the two bottom white arrows.

## 4. Results

### 4.1. Phenotypic changes in branching architecture and inflorescence sexuality in late-glacial environments

We observed major phenotypic differences between the plants from the sub-ambient chamber adjusted to late-glacial conditions and modern control chamber. In every grow-out some of the plants in the chamber with late-glacial conditions (hereafter, referred to as the LGC) were complete maize-like phenotypes in branching architecture and inflorescence sexuality; like maize, they had a few, very short (non-measure-able) lateral branches tipped by female ears instead of tassels, that were attached directly to a single main stem tipped by a tassel (Fig. 3A) (hereafter, these plants with maize-type branching and inflorescence sexuality traits are referred to as “maize-like phenotypes” or MLPs). A total of six plants out of 33 from all the grow-outs combined representing every population studied had these characteristics (one plant from population 3 in 2009; one from pop. 4 in 2010; and one each from pops. 1 and 2, plus two from pop. 3 in 2011). Female ears of these plants, although translocated to the ends of the primary branches at the main stems and thus in positions homologous to where tassels are located in teosinte and cobs in maize, were of normal teosinte type. They were typically composed of 5–12 hard, disarticulated, cupulate fruitcases (consisting of a kernel enveloped by a glume and rachid) subtended by vegetative bracts (Figs. 3 and 4). In contrast, the maize-like phenotypes did not occur in any grow-out in the modern control chamber (hereafter, MCC), where plants were much like those seen in natural environments today; they had many long lateral branches terminated by tassels and secondary branches terminated by female ears (Fig. 3B).

The maize-like phenotypes also exhibited a seed maturation strategy characteristic of maize, with most of their seeds maturing at the same time. In contrast, in other plants in the LGC and MCC, as in the wild, seeds matured sequentially from the tips of the branches to the base over a period of about two months, requiring several “harvest” trips to collect them before they began to fall off the plant soon after maturation. Overall in the three grow-outs, LGC plants had fewer (Table 3) and shorter lateral branches (mean relative branch length of  $0.37 \pm 0.03$  in the LGC vs.  $0.42 \pm 0.03$  in the MCC). Furthermore, comparing branch length with the sexuality of the flower terminating the branches, it can be seen the two traits are highly correlated. Average relative branch length when terminated by tassels tended to be longer than when terminated by mixed male–female inflorescences (mixed M–F) (explained below), and were significantly longer than those terminated by

females (Fig. 5). Branches tipped by mixed male–female inflorescences were significantly longer than branches terminated by female ears, which were always on the shortest branches on the plant. The data provide significant support to arguments that branch length strongly influences floral sexuality (Iltis, 1983). Another finding relating to floral sexuality is that a nearly complete feminization of the primary lateral branches occurred in the LGC, where inflorescences were almost always either completely feminine or mixed M–F (Fig. 6). Only one lateral branch out of a total of 128 in the LGC had a tassel. Therefore, LGC plants exhibited significant similarities to maize regardless of whether they became the complete, “maize-like phenotypes” in branching and inflorescence sexuality.

**Table 3**

Mean branch and node number.

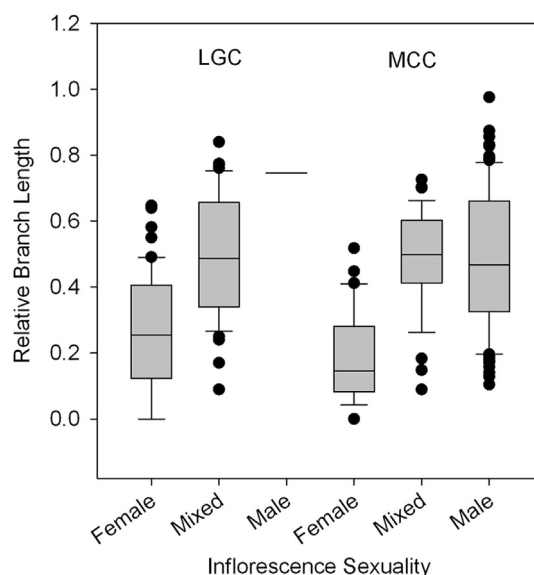
	LGC or EHC		MCC	
	Branches	Nodes	Branches	Nodes
Mean 2009	$4 \pm 1.2$	$5 \pm 1.1$	$5 \pm 1.2$	$9 \pm 2.3$
Mean 2010	$4 \pm 0.9$	$6 \pm 0.9$	$7 \pm 1.7$	$8 \pm 1.6$
Mean 2011	$4 \pm 1.3$	$5 \pm 1.3$	$6 \pm 2.1$	$11 \pm 1.5$
<b>Mean 09–11</b>	<b><math>4 \pm 0.2</math></b>	<b><math>5 \pm 0.2</math></b>	<b><math>6 \pm 0.3</math></b>	<b><math>9 \pm 0.6</math></b>
Mean 2012	$5 \pm 1.8$	$7 \pm 1.9$	$10 \pm 2.1$	$13 \pm 5.7$

Means  $\pm$  SD, Mean 09–11  $\pm$  SEM. For branch No. in MCC vs. LGC 09–11  $P < 0.001$ ; For node No. in MCC vs. LGC 09–11  $P < 0.001$ ; For branch No. in LGC 09–11 vs. EHC 2012  $P = 0.127$  (not significant); For node No. in LGC 09–11 vs. EHC  $P = 0.006$ ; For branch No. in EHC vs. MCC 09–11  $P = 0.042$ ; For node No. in EHC vs. MCC 09–11  $P = 0.042$ . Statistical significance tested by the Mann–Whitney rank sum test. The LGC is the chamber adjusted to late-glacial conditions in years 2009–11 and the EHC is the chamber adjusted to early Holocene conditions in 2012.

Tillering, the production of branch-like organs at the bottom of plants, also differs significantly in modern teosinte and maize (e.g., Hubbard et al., 2002; Whipple et al., 2011). Teosinte may typically tiller, particularly when grown at temperate latitudes, whereas

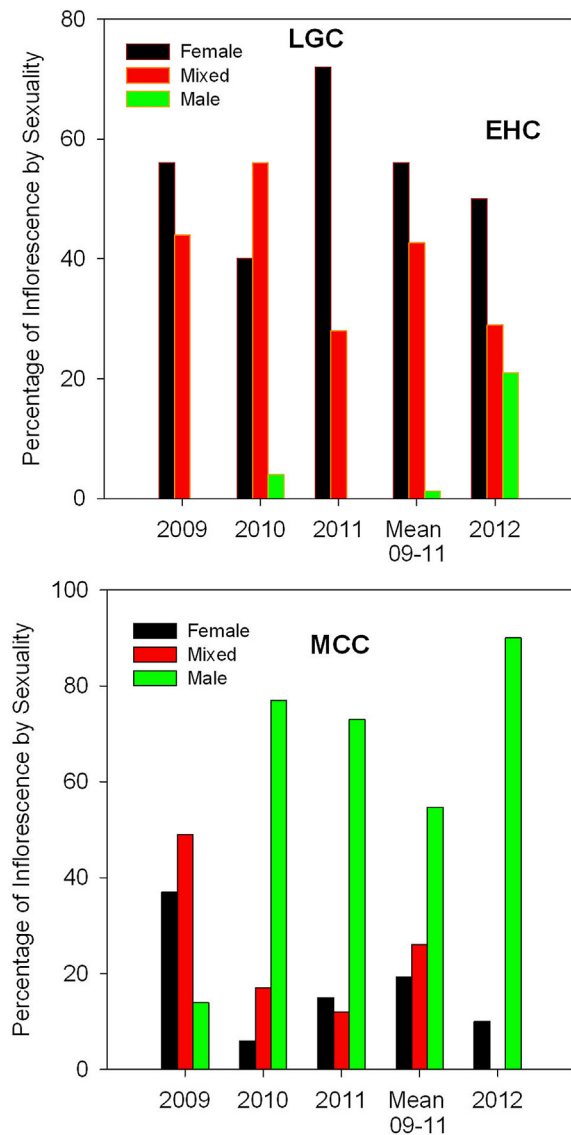


**Fig. 4.** Seeds enclosed in hard cupulate fruitcases (glume + rachid) from a maize-like phenotype plant that were removed from the vegetative bracts.



**Fig. 5.** A box plot of relative branch length vs. inflorescence sexuality of primary lateral branches for data from 2009 to 2011 combined for the Late Glacial Chamber and Modern Control Chamber. Notes: for MCC,  $P < 0.05$  for mixed vs. female,  $P > 0.05$  for mixed vs. male,  $P < 0.05$  for male vs. female. For LGC,  $P < 0.05$  for mixed vs. female,  $P > 0.05$  for mixed vs. male,  $P < 0.05$  for male vs. female. Statistical significance analyzed by the Kruskal–Wallis one way analysis of variance on ranks and the Dunn’s method for all pairwise multiple comparison procedures. No tassels occurred in the LGC in 2009 and 2011 and one occurred in the LGC in 2010.





**Fig. 6.** The percentages of inflorescences of all sexualities terminating primary lateral branches in the sub-ambient (top) and Modern Control Chambers (bottom) for the years 2009–2012. The LGC is the chamber adjusted to late-glacial conditions in years 2009–11 and the EHC is the chamber adjusted to early Holocene conditions in 2012.

maize less commonly does so. This attribute was highly variable in the grow-outs. Many plants in both chambers produced tillers in 2010, few in either chamber had them the other two years. A number of factors are thought to control tillering in *Zea* and other grasses, including the identified domestication genes associated with branching and inflorescence sexuality (Hubbard et al., 2002; Doust, 2007; Whipple et al., 2011). One out of the six maize-like phenotypes had tillers, suggesting an association between genes thought to control tillering and environmental cues, as would be expected (Doust, 2007; Whipple et al., 2011), although sample size may not be large enough to make robust correlations. Feminization in the LGC was also true of many tillers, all but two of which were male in the MCC.

The mixed male–female inflorescences (mixed M–F), which commonly terminated lateral branches in the LGC and also occurred in the MCC, contained seeds positioned proximally to the lateral branch attached to male flowers terminating the branch (Fig. 7). In both chambers they are most typically present on

branches of intermediate length. They have been briefly noted by other researches in  $F_2$  populations of maize/teosinte hybrids and in plants in the wild growing under sub-optimal conditions where branch shortening has occurred (Doebley et al., 1990). Also of interest is that mixed M–F seeds often were not enclosed in vegetative bracts and were completely exposed, unlike normal female ears (Fig. 7, compare with Fig. 3A).

#### 4.2. Other phenotypic characteristics in the late-glacial environment

Another major difference between the chambers was the short stature of the plants in the LGC, which on average were less than half the height of the MCC plants (Mean:  $94 \pm 4.6$  cm in the LGC vs.  $226 \pm 10.4$  cm in the MCC) (Fig. 8). There were no apparent differences in germination ability as most seeds in the LGC (Mean: 84%) and MCC (Mean: 82%) readily germinated in every grow-out.

#### 4.3. Phenotypic plasticity and variability in the early Holocene environment

Teosinte foragers probably transitioned into persistent cultivators around the beginning of the Holocene, by c. 10.5–10 ka, when Greenland ice core data and paleoenvironmental reconstructions from Mesoamerican lakes indicate that atmospheric  $CO_2$  and annual temperature were still depressed by more than 100 ppmv (at c. 260–265 ppmv) and about two degrees (at c. 23 °C) compared with conditions experienced by modern teosinte (Ahn et al., 2004; Wang et al., 2005; Piperno et al., 2007, 2009; Bush et al., 2009; Correa-Metrio et al., 2012). We conducted a grow-out at these and modern control conditions using the following: seeds from four maize-like phenotype plants that were induced in the LGC from 2009 to 2011; founder seeds from the four original ssp. *parviglumis* populations; and seeds from two inbred lines of teosinte (plants self-pollinated for multiple generations so that they have the same genotype) (see Methods: Sample size and sampling for more details). Most seeds in both chambers germinated readily again. In the chamber adjusted to early Holocene conditions (hereafter, EHC), two out of four plants grown from maize-like phenotype seeds were MLPs with uniform seed maturation again. Branches of the other two, while longer than in the MLPs, continued to be feminized; i.e., terminated by either completely female ears or mixed male–female flowers. Three out of four of these plants, including the two MLPs, were short-statured (52–79 cm-high; the other reached 152 cm). Both lines of inbreds also became short-statured (83–84 cm-high), maize-like phenotypes in the EHC. Responses of plants grown in the EHC from founder seeds were more variable. Five of the seven had lateral branches terminated by female ears or mixed male–female flowers and were short-statured (55–129 cm-high), as was common in late-glacial conditions in 2009–2011. The other two had long lateral branches terminated by tassels, as normal in modern conditions; one of these was tall (181 cm) (Fig. 8 for mean height data for all plants). None of the founder seed plants became MLPs.

In summary, in early Holocene conditions, maize-like phenotypes were both reproduced in a second generation from first generation, induced MLP plants, and induced from seeds of long-branched, tall teosinte. A few plants from founder seeds responded to the conditions with attributes typical of modern-day plants; however, most continued to differ from modern plants in the same vegetative, inflorescence, and stature traits shown to differ in late-glacial environments (Figs. 6, 8 and 9; mean relative branch length for all plants in the EHC was  $0.39 \pm 0.06$ ). In contrast, no maize-like phenotypes occurred in the modern control chamber. Plants were tall with many branches or branch nodes, and almost all flowers

terminating lateral branches were tassels (Figs. 6, 8 and 9) (Table 3). Many plants still had under-developed branches without flowers and branch nodes without branches when the grow-out was halted after six months. It is unclear what caused these features.

#### 4.4. Plant productivity in late-glacial and early Holocene environments

Previous experimental research on a range of C<sub>3</sub> and C<sub>4</sub> crop plant progenitors including wild barley (*Hordeum spontaneum* K. Koch), foxtail millet (*Setaria viridis* (L.) P. Beauv.), and ssp. *parviglumis* showed that photosynthesis and biomass increased when CO<sub>2</sub> was raised from 180 ppmv, representing its lowest level of the last glacial period at c. 20 ka, to early Holocene levels of 270 ppmv (Cunniff et al., 2008, 2010). Teosinte seed yield and weight were not reported. Our results also indicate that teosinte productivity is significantly lower under reduced temperature and CO<sub>2</sub>. Seed yield, fruitcase weight (kernel plus surrounding glume and rachid), and biomass in 2009–11 were higher in the MCC than LGC by an average of 85%, 99%, and 618%, respectively (Table 4; Fig. 10). In early Holocene conditions, seed yield, weight, and biomass increased by 180%, 206%, and 249% respectively, over those in late-glacial conditions. Fruitcase size varied little between the chambers in all years and conditions, ranging between about 6.5 and 7.5 mm in length as in natural teosinte populations today.

**Table 4**  
Seed yield and other seed data.

	Total# viable seeds		Mean# viable seeds per plant		Total weight g		Total# seeds not pollinated or developed	
	LGC or EHC	MCC	LGC or EHC	MCC	LGC or EHC	MCC	LGC or EHC	MCC
2009	1260	2223	140	247	47.74	107.88	230	277
2010	2470	2642	225	240	113.11	136.76	626	986
2011	1479	4766	123	397	76.13	225.50	946	5085
Total	5209	9631			237	470	1802	6348
<b>Mean 09–11</b>	<b>1736 ± 372</b>	<b>3210 ± 787</b>	<b>163 ± 31.4</b>	<b>295 ± 51.2</b>	<b>79 ± 19</b>	<b>157 ± 35</b>	<b>601 ± 207</b>	<b>2116 ± 1498</b>
2012	5631		433	—	242.25	—	3929	—

Data are ± SEM; For LGC vs. MCC seed number and seed weight 2009–2011,  $P < 0.001$ .

Statistical significance tested by the Mann–Whitney rank sum test. Data were not compiled for the MCC in 2012 because branches commonly did not develop flowers (see text).

The LGC is the chamber adjusted to late-glacial conditions in years 2009–11 and the EHC is the chamber adjusted to early Holocene conditions in 2012.

We did not measure photosynthesis or transpiration rate. With relation to overall seed viability, we recorded the number of fertile and un-pollinated/undeveloped seeds for each plant (Table 4). The latter are characterized by being completely white in color in contrast to the black or other pigmentations on seeds that have been pollinated and are fertile. There was no evidence for a higher proportion of defective seeds in the LGC or EHC than MCC. We did not attempt to formally calculate pollen productivity. However, in addition to a reduction in the overall number of tassels, tassels had noticeably fewer main tassel branches in the LGC and EHC, all of which suggest that pollen grain number was substantially lower than in the MCC and probably than in today's natural environments.

## 5. Discussion

### 5.1. Phenotypic plasticity and variability

Our research is one of the first attempts to examine the roles of plasticity, variability, and the external environment in the plant

domestication process. Two increasingly important areas of research closely linked to these themes, developmental plasticity and ecological developmental biology (eco-devo) (West-Eberhard, 2003; Gilbert and Epel, 2009), are key components of the New Biology and Extended Modern Evolutionary Synthesis, representing the integration of different disciplines and sub-fields not previously a part of the Modern Synthesis and now thought necessary to understand the generation of variation and evolutionary change (e.g., Members of the National Research Council, 2009; Pigliucci and Muller, 2010; Wake, 2010). Extending these concepts to domestication research allows anthropologists to become fully engaged in what almost certainly will become important components of the New Modern Evolutionary Synthesis.

We demonstrated here major phenotypic changes in one generation produced solely through the manipulation of environmental conditions. It is reasonable to expect that the variation we observed existed in the past climate eras simulated in this experiment; the same branching, inflorescence, and stature phenotypic responses to limiting growing conditions we observed in our study are seen today in ssp. *parviglumis* growing in sub-optimal habitats (Doebley et al., 1995; Whipple et al., 2011), suggesting they are generalized responses to a variety of limiting growing conditions. We also note that a flower feminization response to low temperature has been recorded in previous experimental work with *Zea* and

a variety of other plant taxa (Richey and Sprague, 1932; Heslop-Harrison, 1957), further indicating the importance of temperature to floral sexuality. The observation in our 2012 study that plants grown in the modern control chamber from seeds of induced, maize-like phenotypes reverted back to tall plants with many branches/branch nodes and branches tipped predominantly by tassels, and that inbred teosinte seeds behave differently in the two environments—becoming maize-like phenotypes in the early Holocene conditions — is further evidence of a plasticity response to the environmental differences.

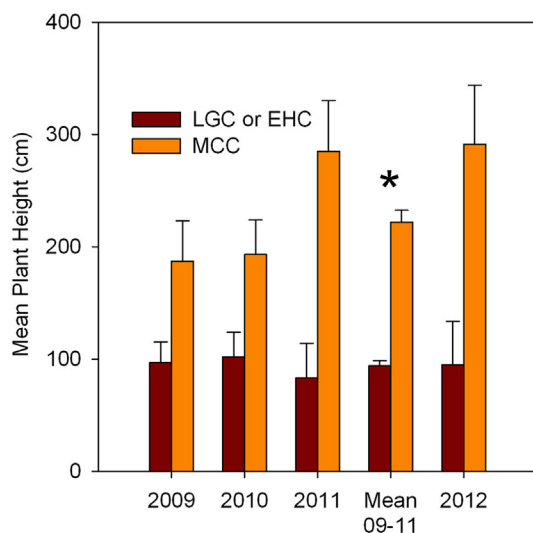
The data indicate that ssp. *parviglumis* phenotypes first exploited and then initially cultivated by human populations differed substantially from modern plants with a considerable number possessing maize-type attributes in important vegetative architectural and inflorescence sexuality traits, and in seed maturation, the latter also apparently influenced by environmental factors. The implications of our data for understanding teosinte exploitation and domestication are varied, especially as the kind of detailed phenotypic information retrieved from this study will not be easily recovered from early records from maize's homeland due to the





**Fig. 7.** Right, a mixed male–female inflorescence with a row of kernels positioned proximal to the lateral branch (bottom arrow) and male flowers terminating the structure (top arrow). The seeds are not enveloped by vegetative bracts as occurs in normal teosinte female ears on secondary lateral branches (see Fig. 3). Left, a close-up of the kernels.

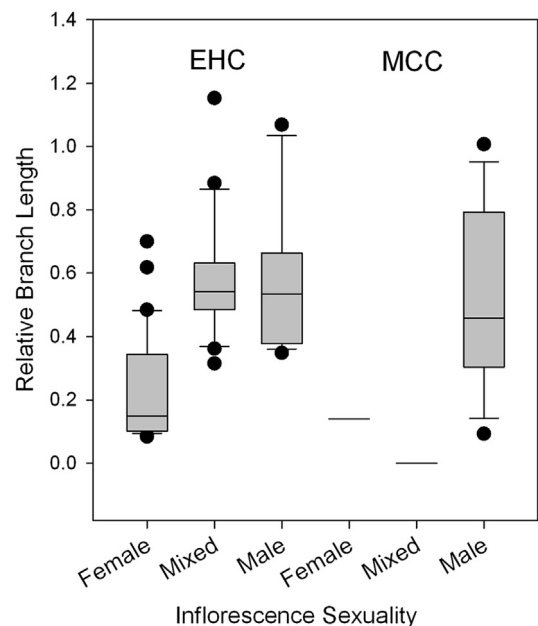
poor preservation of macro-plant remains (seeds, stems, flowers) (Piperno et al., 2009; Piperno, 2011) and scarcity of sites occupied during the relevant time periods (Ranere et al., 2009; Kennett, 2012).



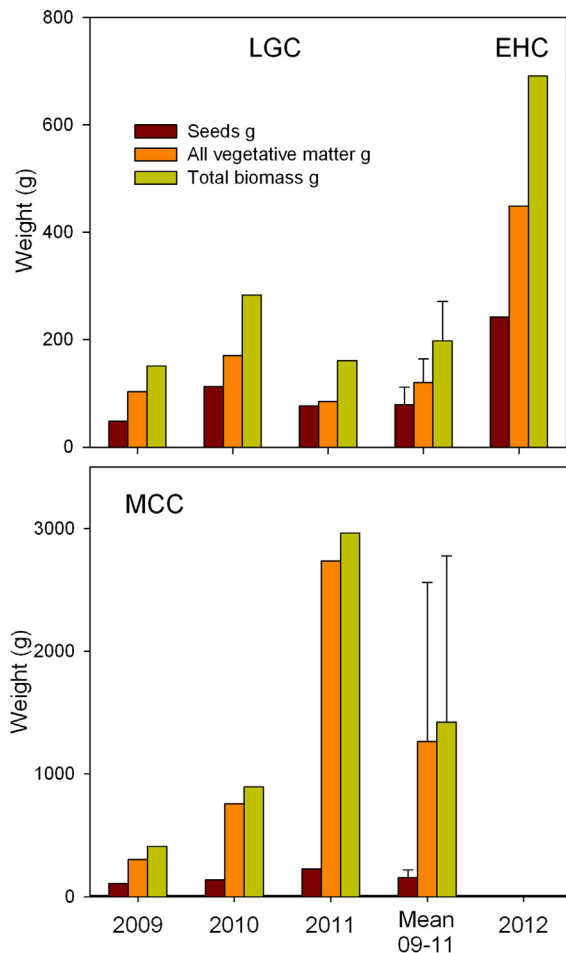
**Fig. 8.** Plant height. Notes: \* $P < 0.001$  for LGC vs. MCC mean 2009–2011;  $P = 0.495$  (not significant) for EHC 2012 vs. LGC 09–11;  $P < 0.001$  for EHC 2012 vs. MCC 09–11;  $P = 0.004$  for EHC 2012 vs. MCC 2012. Statistical significance tested by the Mann–Whitney rank sum test. The error bars represent SD for each individual year and the SEM for all years. The LGC is the chamber adjusted to late-glacial conditions in years 2009–11 and the EHC is the chamber adjusted to early Holocene conditions in 2012.

For example, plants in our study with maize-like branching, inflorescence, and seed maturation traits were easier and more efficient to harvest because they have more compact clusters of female ears located in an easily visible position on the main stem, and most seeds could be collected with a single harvest effort and minimal seed loss. 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 components of the domestication syndrome—the group of phenotypic characteristics that were key to initial wild to domesticated transitions (e.g., Olsen and Wendel, 2013). Moreover, the branch transformations resulted in an increase of an important feature called apical dominance that led to a greater concentration of nutrients in the single main stem, to where female ears were translocated, eventually permitting the development of large seeds and cobs of maize (Doebley et al., 1997). Because the maize-like plants had visible and highly desirable traits with obvious advantages, it is reasonable to expect they would have gained the attention of early collectors and then cultivators, and the latter likely would have sought to increase their numbers through replanting. Their pre-existing availability may have hastened the successful establishment of *Zea* cultivation once initiated. However, indications of short-branched *Zea* with maize-like floral sexuality in archaeobotanical records would no longer automatically point to domestication of these traits, particularly during early cultivation phases.

Other interesting examples of phenotypic variability occurring in this study and potentially relevant to the past are the mixed male–female inflorescences, many of which had fruitcases lacking the tightly surrounding vegetative bracts found in normal female ears (Fig. 7). Exposed fruitcases such as these may also have been considerably easier to harvest and turn into foods. A possible significance of these flowers in the maize domestication process has not to our knowledge been explored and may deserve consideration.



**Fig. 9.** A box plot of relative branch length vs. inflorescence sexuality of primary lateral branches for data from 2012. For EHC,  $P < 0.05$  for mixed vs. female,  $P > 0.05$  for mixed vs. male (not significant),  $P < 0.05$  for male vs. female. Statistical significance analyzed by the Kruskal–Wallis one way analysis of variance on ranks and the Dunn's method for all pairwise multiple comparison procedures.



**Fig. 10.** Plant biomass. Notes:  $P < 0.001$  for LGC vs MCC total mean biomass for 2009–2011. Statistical significance tested by the Mann–Whitney rank sum test. Error bars represent the SD.

## 5.2. The possible role of plasticity in maize domestication

To move forward with a more complete understanding of these questions requires a better understanding of the links between genotype, phenotype and the environment. Among the important avenues of research will be investigating the genetic underpinnings, inheritance, and molecular mechanisms of plasticity; i.e., if there is underlying genetic variation in the phenotypic responses that we observed to the experimental past environments and how it responds to selection, together with whether gene expression changes were involved in the plasticity we recorded. As discussed above, teosinte plasticity in branching and floral sexuality today, and the differences in these traits between teosinte and maize, are known to be in part mediated by expression of the *tb1* gene, a transcriptional factor that represses bud outgrowth and increases apical dominance resulting in a maize-like phenotype (Doebley et al., 1995; Hubbard et al., 2002). Increase in *tb1* expression accounts for about 35–50% of the lateral branch number/length and inflorescence sexuality changes accounting for the teosinte to maize transition. It was recently determined that an allele of *tb1* with a transposon insertion called “*Hopscotch*” that enhances the gene expression for short-branch development was selected during maize domestication (Studer et al., 2011). *Hopscotch* is not fixed (present) in all modern maize landraces, suggesting other factors also control plant architecture. It is present as pre-

existing genetic variation in some ssp. *parviglumis* populations, suggesting it was available to the first teosinte cultivators (Studer et al., 2011). The relationship between *Hopscotch* and phenotypic plasticity described here is unknown and should be investigated.

Therefore, questions for future work are many and include whether an environmental induction (upregulation) of *tb1*-associated gene expression contributed to the changes we observed and what other factors might be involved. Whole transcriptome expression studies (RNAseq) on the plants examined here are in progress. They will provide a greater understanding of how the environmentally-induced phenotypic responses we observed might be coupled with genetic change, and establish an excellent foundation to explore the connections between ancestral plasticity and domestication.

In summary, it appears that teosinte foragers and early cultivators worked with wild *Zea* phenotypes considerably different than the modern ssp. *parviglumis* presently used as the baseline for the domestication process in genetic and archaeological research. Pre-cultivation availability of maize-like plants may have increased the speed of the selection process leading to the fixation of the traits, no matter what genetic process resulted in the constitutive expression of the phenotypes in all environments. Considering that i) the first few thousand years of the Holocene in maize's homeland were not climatically stable, but rather had abrupt 200–400 year-long reversals in annual precipitation and temperature (c. 2 °C lower) (Bernal et al., 2011), and ii) that until the Industrial Revolution, Holocene atmospheric CO<sub>2</sub> was more than 100 ppmv lower than today, it would be wise to consider when in the Holocene teosinte became the tall, long-branched plant we observe today.

Finally, although our experiment has opened a wider window onto the range of phenotypic variation that teosinte collectors and early cultivators probably saw, there remains much to be learned from experimental and archaeological study. For example, the artificial selection process that led from an ear of teosinte to a maize cob is still little understood, and we don't have a good idea of what early domesticated maize ears were like. The earliest known cobs dating to 6.7–6.2 ka from Mexico and Peru that survived for archaeological retrieval only because they came from sites located in arid environments outside of the origin area and ecological contexts of maize domestication, are already genetically and phenotypically well-advanced (Piperno and Flannery, 2001; Grobman et al., 2012). A major consequence of a long domestication process indicated for maize is that considerable genetic and possibly phenotypic variation was probably lost. Crop progenitors faced new environments when taken from their native habitats to plots prepared for them, and although the latter are characterized as largely benign compared with natural environments, this does not mean plasticity responses and the expression of new variation would have ceased (Schlichting, 2008). This could be especially true in out-crossing plants with high cryptic or standing genetic variability, such as ssp. *parviglumis* (Lauter and Doebley, 2002).

## 5.3. Plant productivity

Previous experimental research indicated significant decreases in biomass and seed yield in a range of C<sub>3</sub> and C<sub>4</sub> wild progenitors of crops at the Last Glacial Maximum (20 ka) CO<sub>2</sub> level of 180 ppmv, one of the lowest in the history of land plant evolution (Leakey and Lau, 2012), compared with their growth in Holocene pre-industrial CO<sub>2</sub> levels of 270–280 ppmv (Cunniff et al., 2008, 2010). Seed yield, which was not studied in teosinte, increased in other C<sub>4</sub> species and by even greater amounts in the C<sub>3</sub> plants at 280 ppmv. Photosynthesis itself responded little in teosinte to increased CO<sub>2</sub>. However, increasing CO<sub>2</sub> to 270 ppmv significantly lowered the transpiration rate in teosinte and other C<sub>4</sub> species, suggesting that glacial period

water limitation was exacerbated by low CO<sub>2</sub> and an improvement in plant water balance was an important factor that would have improved teosinte and other C<sub>4</sub> plant growth and productivity during the early Holocene (Cunniff et al., 2008, 2010).

Teosinte grown in this study at late-glacial levels of 206–215 ppmv CO<sub>2</sub> and reduced temperature also responded with significantly poorer productivity compared with early Holocene and modern levels, concordant with previous results. The biomass and seed yield reductions measured here are more pronounced than in the Cunniff et al. research for teosinte and other C<sub>4</sub> wild progenitors. The results provide additional evidence that Pleistocene environments imposed physiological stress factors affecting growth of C<sub>4</sub> as well as C<sub>3</sub> plants, and indicate that low temperature was another inhibitory factor on plant growth. Clearly, an important consideration in assessing plant response is the inclusion of temperature reduction. It is known that different abiotic factors have interacting effects on plant growth that may not be predictable when one is studied alone, thus lowering CO<sub>2</sub> and temperature simultaneously probably provides a more realistic test of glacial environments (Cowling and Sage, 1998; Shaw et al., 2002; Ward et al., 2008; Leakey and Lau, 2012). In a previous experiment on C<sub>3</sub> (*Abutilon*) and C<sub>4</sub> (*Amaranthus*) plant responses to Pleistocene-like temperature and CO<sub>2</sub> combined, the expected, large growth advantage in biomass measured in the C<sub>4</sub> over the C<sub>3</sub> plant when grown in low CO<sub>2</sub> alone was considerably attenuated when temperature was simultaneously lowered (Ward et al., 2008). This finding further indicates that low Pleistocene temperatures had significant negative effects on growth of C<sub>4</sub> annuals such as teosinte.

It is important to point out a possible bias in our and other experiments when modern plants are grown for a single generation—that they do not take into account evolutionary responses to the past conditions being tested. Multi-year artificial selection studies that measure adaptive responses to Pleistocene factors are rare but instructive. Ward et al. (2000) found that a C<sub>3</sub> annual, *Arabidopsis*, partially ameliorated the negative effects of low (200 ppmv) CO<sub>2</sub> on its biomass and seed yield by lengthening the vegetative growth phase. Single generation experiments may then overestimate negative responses. However, it is reasonable to conclude from low CO<sub>2</sub> and/or temperature experiments carried out to date, together with simulation and modeling research on ecosystem vegetation response to Pleistocene atmospheres and climate (e.g., Cowling, 2011) and studies on Late Pleistocene fossil plants that had undergone evolutionary responses to glacial conditions (Ward et al., 2005; Gerhart et al., 2011), that Pleistocene CO<sub>2</sub>, temperature, and probably precipitation were significant limiting factors on plant growth compared with the following Holocene. Because plants were adequately watered in this study, teosinte growth improvement in early Holocene and modern conditions may have been underestimated.

#### 5.4. Plant productivity, climate change, and agricultural origins

The role of climate change in agricultural origins has long been a contentious issue in anthropology. The synchronous beginning of this most fundamental economic transition during the first few thousand years of the Holocene in Mesoamerica, South America, the Near East, and China (e.g. Price and Bar-Yosef, 2011) leads some scholars, including ourselves, to conclude that the Pleistocene–Holocene climate and ecological transition resulted in common underlying influences on people's decision to become farmers (e.g., Sage, 1995; Richerson et al., 2001; Piperno, 2006, 2011; Cunniff et al., 2008, 2010). Others have viewed this as an overly deterministic process inadequately focused on socio-cultural factors (see Piperno and Pearsall, 1998; Price and Bar-Yosef, 2011 for reviews

and examples). However, when considered in a broader evolutionary and ecological context, the physical environment becomes not a simplistic, single-factor or “prime mover” explanation, but instead a necessary component of cardinal questions about interactions between humans, their environments, and resource sets at the transition from foraging to farming.

Our data support hypotheses and previous results bearing on them which suggest that a persistent cultivation of plants in the pre-Holocene era may have been difficult to sustain in the face of low yields of the wild progenitors in Pleistocene environments (Sage, 1995; Richerson et al., 2001; Cunniff et al., 2008, 2010). Neither archaeological nor paleoecological data provide estimates of the relative productivities of crop plant progenitors in pre-Holocene and Holocene environments, making experimental research particularly important for assessing the issue. The archaeological record does bear direct witness to the onset of cultivation and domestication, and notably, although empirical data are both rapidly accumulating and being refined around the world, they continue to indicate that plant food production was initiated, or at least sustained to the point when it becomes recognizable and subsequently results in domestication, when the Holocene began (e.g., Piperno, 2011; Zhao, 2011; Asouti and Fuller, 2012; Willcox, 2013). Experimental productivity and archaeological data can therefore be used jointly to test hypotheses that limitations on plant growth during the Pleistocene contributed significantly to the chronology of the rise of agriculture, and as research progresses hypotheses evaluations will become more robust.

Plant productivity issues are of importance for other hypotheses of agricultural origins. For example, under the assumptions of optimal foraging – specifically, the diet breadth model – from the field of human behavioral ecology (e.g., Kennett and Winterhalder, 2006; Piperno, 2006; Gremillion and Piperno, 2009), increases in teosinte seed yield and, probably, population density during the early Holocene would have increased its encounter rate, handling (collecting) time, resource ranking, and overall foraging efficiency, likely making it more attractive and ultimately adaptively advantageous to human populations who were evaluating and choosing from the new assortments of resources available to them. This is one pathway by which previously un- or little-utilized resources become objects of human attention. Furthermore, it is likely that potential plant foods differed in their productivity responses regionally and around the world at the beginning of the Holocene, and this contributed to the intensification of use of certain species at the expense of the many others that did not become components of food producing strategies, as well as to regional chronological trends and differences in agricultural development (early vs. middle Holocene food production origins). Yield increases would have also enhanced opportunities for food surpluses and storage, which in turn may have led to human settlement stability and population growth (Cunniff et al., 2010).

Reaching a better understanding of how end-Pleistocene environmental shifts affected resource quality together with the choices people made when they became farmers will involve extending experimental studies to more plant species. Study has so far been limited to cereal wild progenitors, but there are numerous other important ancestral species still common on landscapes today and available for study. In the New World they include five different wild squash (*Cucurbita*) species and wild legumes such as *Phaseolus* common and lima beans, all of which are annuals that were grown from and for their seeds and should provide rich opportunities for experimental research. Ancient DNA research has exposed the sometimes incomplete and biased views of domestication history that result from relying on the genetics of modern domesticated species and their wild ancestors (e.g., Larson et al., 2007; Roullier et al., 2013). It is clear that we need to also better



understand the interplay between past ecology, climate, human environmental modification, and plant development and phenotypic responses, together with their complex feedbacks. In all fields concerned with understanding past biota, reconstructions of vegetation and plant responses to past environmental change have significantly depended on modern-day species characteristics and ecological processes, and rarely provide information on whole plant attributes of individual species in past era. Thus, experimental work of this kind may assume increasing importance. Because the possible range of future plant phenotypic responses may be significantly mediated through gene expression and plasticity, studies such as these also may be important for assessing the effects of global environmental change.

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