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ORIGINAL ARTICLE

Multidisciplinary studies in *Cucurbita maxima* (squash) domestication

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Abstract Plant domestication is a complex process in which natural and cultural factors play important roles delimiting evolutionary pathways of plants under cultivation. In order to deal with and understand the changes generated during this process, multi-disciplinary research is required, especially when a full picture of the domestication history of a taxon is to be assessed. We present here some advances in the study of *Cucurbita maxima* (squash) domestication from an integrated perspective, including experimental, morphometric and archaeobotanical approaches, which are discussed in the light of new data from physiological analyses. Modern material includes plants obtained from experimental fields, derived from crosses between domesticated (*C. maxima* ssp. *maxima*) and spontaneous/wild forms (*C. maxima* ssp. *andreana*), resulting in F1 and F2 generations. The archaeobotanical material includes remains recovered from sites in southern Peru and northwest Argentina ranging in date from 3,000 to 800 BP. Morphological and anatomical analyses were conducted on seeds, pericarps and peduncles (the stem of the flower or fruit) for reconstructing squash size and shape evolution under domestication. The results suggest the presence of hybrid forms, mainly from the earlier sites, but also from more recent ones. As expected, a linear evolutionary pathway was not found. Diversity and multiple crossings seem to have been a constant in squash cultivation over time, emphasising the role of gene flows between domestic and wild variants in the domestication process. Finally, we hypothesize the possible linkage between past gene flow and different dormancy patterns as part of management practices, allowing the maintenance of squash populations adapted to different environmental conditions.

Keyword Domestication · Cucurbita maxima · Squash cultivation · Hybridisation/crossing · South America

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Introduction

Along with *Zea mays* (maize, sweetcorn) and various species of *Phaseolus* (beans), *Cucurbita* spp. (squashes, gourds or marrows) have been part of the crop plants and diet of American societies for thousands of years. According to Lira et al. (2016), *Cucurbita* includes 20 taxa and 15 species, and the earliest evidence for its domestication dates to 9,000 BP from southwestern Central America and to 10,000 BP from southwestern Ecuador. In recent years, advances in the study of *Cucurbita* domestication have been made mainly concerning *C. argyrosperma, C. pepo* and *C. moschata* (Decker and Wilson 1986; Cowan and Smith 1993; Cowan 1997; Smith 1997, 2006; Hart 2004; Piperno and Stothert 2003; Kistler et al. 2015), while the history of the widespread *C. ficifolia*

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is unknown (Lira et al. 2016). Despite their equal relevance, the southern members of the genus have been less studied and these are the focus of this paper.

Several authors have suggested that southern Peru, Bolivia and northwest Argentina (NWA) were the areas of domestication of *Cucurbita maxima* ssp. *maxima* Duch. ex Lam (Millán 1945; Whitaker and Bemis 1964; Nee 1990). Based on genetic, archaeological and morphological studies, *C. maxima* ssp. *andreana* (Naudin) Filov. was proposed as its wild ancestor (Sanjur et al. 2002; Kates et al. 2017). Archaeobotanical remains do not yet provide enough evidence to confirm this part of South America as the domestication area of the species. However, previous studies carried out by one of the authors of this work have determined the chronological and spatial coexistence of wild and domesticated forms together with intermediate morphotypes in archaeological sites of NWA as early as 2,000 BP (Lema 2011, 2015). Wild forms were characterized by micromorphological studies of pericarp remains, while for intermediate and domesticated forms, pericarp, seed and peduncle remains were studied (Lema et al. 2008; Lema 2010). The assemblage was preliminarily considered to be part of a wild-weedy-domesticated complex (Lema 2011, 2015), where genetic flux, introgression and hybridisation might have been common processes, as can be observed in other modern *Cucurbita* species (Nee 1990; Wilson et al. 1994; Ellstrand et al. 1999; Montes Hernández and Eguiarte 2002; Lira et al. 2016).

Earlier investigations have thus contributed to advance the hypothesis that *C. maxima* domestication in the Andean area was a process in which hybridization, introgression and gene flow played important evolutionary roles. In this sense, the objective of this paper is to integrate experimental, morphometric and archaeobotanical analyses carried out on *C. maxima* seeds, peduncles and pericarps in order to test this hypothesis through the reconstruction of size and shape evolution and its linkage with diversification processes under cultivation; a few of these data were partially published before in Martínez et al. (2015). We finally discuss our results in the light of the latest advances in physiological analysis from an integrated perspective.

Cucurbita maxima in the south-central Andes

Cucurbita maxima ssp. *andreana* has a herbaceous and deciduous peduncle; its small smooth-surfaced fruit is bitter due to the presence of cucurbitacins and becomes lightweight at maturity when the mesocarp is reduced (Millán 1945; Ashworth 1997). *C. maxima* ssp. *maxima*, in contrast, has a more robust corky peduncle that remains attached to the heavy, fleshy and sweet fruit that has different colours, shapes and sizes, as well as wartiness and lobes. Both the fruit and the seeds are consumed in several ways (Millán 1945; Lira Saade 1995). Bigger seeds and fruits, the absence of cucurbitacins and tendril detachment are usually mentioned as the main results of human selection in cucurbits (Smith 1997, 2006; Kistler et al. 2015; Lira et al. 2016). In the case of *C. maxima* ssp. *maxima*, archaeobotanical analysis of dry macroremains recovered from several archaeological cave sites in Pampa Grande in the province of Salta, NWA, dated to 1,700 BP, allowed the recognition of different morphotypes. Those intended for food had thin pericarps to facilitate consumption, while those intended for both food and for use as containers (similarly to *Lagenaria*) had thickened and lignified rinds and may possibly also represent the development of landraces with longer storage capability of the fruit (Lema 2011). Previous studies carried out on these same remains highlighted the diversity of shapes and the possible presence of several varieties or landraces of the *maxima* subspecies from this archaeological site (Whitaker 1983). In this sense, the proposal of Decker-Walters and Walters (2000) that certain varieties could have developed through selection among populations of *C. maxima* ssp. *andreana* with a certain degree of introgression appears interesting for our research.

Prolonged seed dormancy is usually considered as an undesirable trait for a crop, therefore its absence is one of the main characters of domestication in many annual plants (Hillman and Stuart Davies 1990; Smith 2006; Fuller 2012). In the case of the two *C. maxima* subspecies, information about dormancy is scarce and has not been a subject of systematic investigation. Therefore, the influence of dormancy on seeds and embryos was studied by some of the authors of this paper, using parameters such as temperature, hormones, light, scarification and diffusion of water through the seed coat. Even though the study of the regulation of seed dormancy is still in progress, it is possible to confirm that: (1) Dormancy is present in the seeds of wild *Cucurbita maxima* ssp. *andreana* and lasts for several months; (2) In contrast, seeds of domesticated ssp. *maxima* show a reduction or loss of this barrier to rapid germination; (3) Hybrids between the two subspecies have variable dormancies; (4) The varying degrees of dormancy in seeds of the wild and domesticated *C. maxima* may be regulated by many physiological and environmental factors (Martínez et al. 2016). As mentioned above, these results will be evaluated in the discussion below, on the basis of morphometric and statistical analysis from an integrated perspective.

Material

For the modern material, two sets of specimens were used in this study. The first came from experimental fields where crosses were made between the domesticated *C. maxima* ssp.

maxima and wild *C. maxima* ssp. *andreana* forms, producing F1 and F2 generations (Fig. 1; Tables 1, 2). A second set of modern material consisted of specimens obtained from commercial sources, the Horticulture Chair of the FCAyF (UNLP) and from the researcher Ashworth, which were samples of ssp. *andreana* corresponding to three populations obtained by outcrossing, autogamous and free pollination (Table 3). Altogether, morphometric studies were done on 82 peduncles, 67 pericarp fragments and 1,242 seeds from both sets (Tables 1, 2, 3).

The archaeobotanical material consisted of desiccated macroremains which were recovered from archaeological sites in south-central Peru and NWA (Fig. 2). Among the earliest sites were (1) Cerro Lampay, (2) Pampa de los Perros and (3) Bandurria, corresponding to the Archaic period (ca. 3,000–2,000 BP). Samples dated to the Early or Formative period (ca. 2,000–1,200 BP) sites are: (4) SSalLap 20, (5) Pampa Grande (Fig. 3) and (6) Los Viscos. Finally, the Late period (ca. 1,200–800 BP) is represented by the following sites: (7) Bebe de la Troya, (8) Las Champas and (9) Lorohuasi. The total number of analysed archaeobotanical remains was nine peduncles, 60 pericarp fragments and 132 seeds (Table 4). The archaeological complex of Pampa Grande consists of a series of caves (Los Aparejos, El Litro, Caverna II, Caverna IV), to which we refer in the results.

Methods

Morphometric and statistical analyses were done by using measurements obtained with digital calipers. The lengths, widths and thicknesses of the seeds were measured. These data were used to estimate the sizes and shapes of the seeds. The size was estimated by the geometric mean

Fig. 1 Parental material of experimental crosses. Accession numbers, wild/spontaneous, **a** 130; **b** 140; **c** 160. Domesticated; **d** 93; **e** 56; **f** 153

Table 1 Parental genotypes and F1 experimental crosses

Female parent	Male parent	ssp. *maxima*			ssp. *andreana*		
	Code / accession, type of remains	93, "Abobrinha de tronco verde" Brazil	56, "Queensland blue", S Africa	153, "Zapallo gris Plomo", Argentina	160, Córdoba, Argentina	140, Santiago del Estero, Argentina	130, Entre Ríos, Argentina
ssp. *maxima*	93, 1; 5; 20				20, F1 (D×W)	3; 20, F1 (D×W)	1; 20, F1 (D×W)
	56, 1; 3; 15				30, F1 (D×W)	2; 60, F1 (D×W)	2, 1 (D×W)
	153, 2; 4; 15				1, F1 (D×W)		1; 15, F1 (D×W)
ssp. *andreana*	160, 3; 4; 45	1; 20, F1 (W×D)	1; 30, F1 (W×D)	1, F1 (W×D)			
	140, 3; 4; 15	1; 6, F1 (W×D)	1; 30, F1 (W×D)	1; 45, F1 (W×D)			
	130, 3; 2; 30	1; 20, F1 (W×D)	1, F1 (W×D)	1; 15, F1 (W×D)			

Numbers in colour represent quantity of measured specimens; red, peduncles; green, pericarps; black, seeds. The codes or accession numbers correspond to the ones under which the specimens are mentioned in Figs. 8, 9

D domesticated, *W* wild

Table 2 F2 experimental crosses; for further explanations, see Table 1

Female parent F1		Male parent F1									
		ssp. *andreana*			ssp. *maxima*		ssp. *maxima* × *andreana*			ssp. *andr.* × *maxima*	F1
	Code / accession	160	140	130	56	93	93×130	93×140	93×160	160×93	Same genotype
ssp. *andr.* x *maxima*	130×56			10	1; 1; 10						1; 1; 10
	130×153			2; 1; 20							1; 1; 20
	130×93			1; 1; 30		1; 1; 20					2; 1; 20
	140×56		1; 1; 20								1; 1; 10
	140×153		1; 1; 20								2; 2; 20
	140×93		2; 2			3; 2					2; 2; 20
	160×56	10			1; 1						1; 1; 10
	160×153	1; 1; 10									2; 2; 20
	160×93	1; 1; 20				3; 4; 20					3; 5; 20
ssp. *max.* x *andreana*	93×130			1; 2		20					6
	93×140										6
ssp. *andr.*	130						6				
	140							6			
	160								6	6	
ssp. *maxima*	93						20			6	

(GM), the arithmetic measurement of the original variables on a logarithmic scale of length, width and thickness (Jungers et al. 1995). Because principal component analysis showed that thickness was not important for shape variation, this last variable was calculated in terms of the length/width ratio (Corruccini 1995; Jungers et al. 1995). Coefficient of variation (CV) was calculated for both the GM and length/width ratio.

For the peduncles, an average of two measurements of the basal diameter was calculated for each specimen and processed by statistical analyses.

The thickness of the pericarps was measured following Cowan and Smith (1993).

Results

For the seeds, the geometric mean (GM) differentiated wild from domesticated forms, but the F1 and F2 generations had intermediate values, mainly F2 (Fig. 4a). Archaeobotanical remains from the Archaic and Formative periods had a range of values matching those of the F1 and F2 (hybrids) as well as the domesticated forms, but those from later periods matched mainly with the domesticated forms and only partially with the F1 and F2 ones. Archaeological seeds showed a non-uniform trend towards an increase in size with time (Fig. 4a, b). Most of the seeds had values of coefficient of

Table 3 Modern reference material. Asterisk indicates specimens obtained from commercial sources

Code/cultivate	Source material	No. measured individuals		
		Peduncle	Pericarp	Seed
C. maxima ssp. *andreana*				
MC1, MC2, MC3	Mones Cazón (Bs. As.)	3	3	–
LEBA	–	1	1	–
G	M B Gonnet (Bs. As.)	–	1	–
LT	Los Toldos (Bs. As.)	1	1	–
SLD	Shore of Salado river (Bs. As.)	2	–	–
Autogamous (26)	San Luis	–	–	20
Free (63)	San Luis	–	–	20
Outcrossing (27)	San Luis	–	–	20
C. maxima ssp. *maxima*				
cv. zipinka	El Shincal (Catamarca)	1	–	–
cv. silpingo	El Shincal (Catamarca)	1	1	–
cv. zapallito	El Shincal (Catamarca)	2	–	–
Indeterminate	El Shincal (Catamarca)	1	–	–
LQ	La Quiaca (Jujuy)	1	–	–
Hubbard	La Plata (Bs. As.)*	1	1	–
Turbaniforme	La Plata (Bs. As.)*	2	2	–
Inglés	Chair of Horticulture (FCAyF)	–	–	21
Criollo	Chair of Horticulture (FCAyF)	–	–	5
Hubbard	Chair of Horticulture (FCAyF)	–	–	41
Zapallito	Chair of Horticulture (FCAyF)	–	–	28
Bolivianos (Criollo)	La Quiaca*	–	–	57
Tronquero (zapallito)	Santa Victoria Oeste (Salta)	–	–	104
From Salta Valleys	La Quiaca*	–	–	33

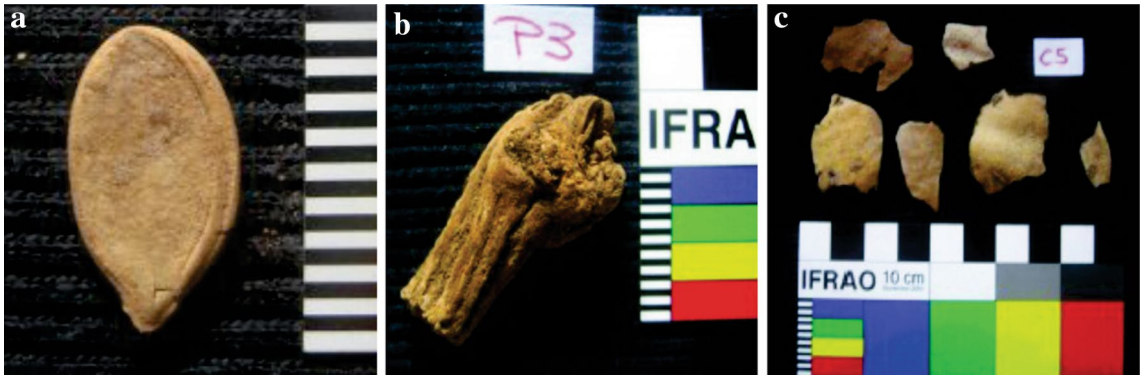

Fig. 2 Archaeobotanical remains from the Pampa Grande site. **a** Seed; **b** peduncle (fruit stalk); **c** pericarp

variation (CV) between 10 and 15, but material from the Archaic period showed high CV values, which decreased to values resembling those of F2 in material from later periods (Fig. 5). Variability in the archaeological assemblages was not similar to that seen in modern cultivars with extreme values alone (in Fig. 5 e, f), nor with all the analysed cultivars taken as a whole (Fig. 5d), making probable the presence of more than one cultivar in the ancient assemblages. Despite this, there was a tendency towards a reduction in variability of size through time.

Regarding shape analysis, the length/width index was not useful for differentiating the modern genotypes among them, but it allowed the differentiation between the modern genotypes and the archaeological seeds (Fig. 6a, b). A great

Fig. 3 Map, showing locations of the archaeological sites mentioned in the text. 1, Cerro Lampay; 2, Pampa de los Perros; 3, Bandurria; 4, SSalLap 20; 5, Pampa Grande; 6, Los Viscos; 7, Bebe de la Troya; 8, Las Champas; 9, Lorohuasi

variability of shapes was recorded among Archaic specimens, becoming less in the material from the Formative and Late periods, in which the variability was similar (Fig. 7). More elongated seeds were common in the Formative period material, while in the Late period specimens the length/width index remained within the values of Archaic seeds. Despite the fact that a general trend towards more elongated seeds through time was detected, this was not absolutely true for seeds from Late period archaeological sites. The CV showed high shape variability during the Archaic period, even higher than in modern domesticated seed assemblages, but decreasing during the Formative period towards values close to those of modern F2 genotypes, and slightly increasing in the Late period material, in which the variability approximated to the values of modern domesticated forms (Fig. 7). It therefore seems that a tendency towards a reduction in shape variation and an increase in length can be assumed for the material from the Archaic-Formative transition, but not for the Late period, when it is partially maintained.

In the peduncles, the basal diameters clearly differentiated between modern wild/spontaneous and domesticated forms. F1 specimens were grouped according to their female parent. F2 ones mainly occupied an intermediate position between wild/spontaneous and domesticated forms. The archaeological remains from the cave sites of Pampa Grande coincided with F2 and partly with F1 (domesticated × wild) and with domesticated forms (Fig. 8).

The analysis of the pericarps showed no distinctive values among *C. maxima* subspecies, but there was a difference in thickness values between the two, which was only seen in F2 material. Archaeological pericarps have a wide distribution within the values of current material measurements, which reaffirms the idea that a variety of fruit forms were used and managed at the archaeological site of Pampa Grande (Fig. 9).

Discussion and conclusions

Our results show that the analysed archaeological remains correspond to a post-domestication stage in the history of *C. maxima* cultivation. The general trend after the first domestication of *C. maxima* ssp. *maxima* appears to have been, first, the presence of new seed shapes and sizes during the Archaic period, while maintaining hybrid populations all the time. Later, during the Formative period, the variability in shape and size was reduced and longer seeds appear together with those present in earlier times. Finally, during the Late period these tendencies were partially reverted. After the Archaic period, the presence of hybrid

Table 4 Archaeobotanical remains analysed, numbers and sites

	Peru			Argentina					
	Bandurria	Cerro Lampay	Pampa de Los Perros	Los Viscos	SSal Lap20	PampaGrande	Las Champas	Bebé de La Troya	Lorohuasi
Seed	1	3	3	1	3	9	6	100	6
Peduncle	–	–	–	–	–	9	–	–	–
Pericarp	–	–	–	–	–	60	–	–	–

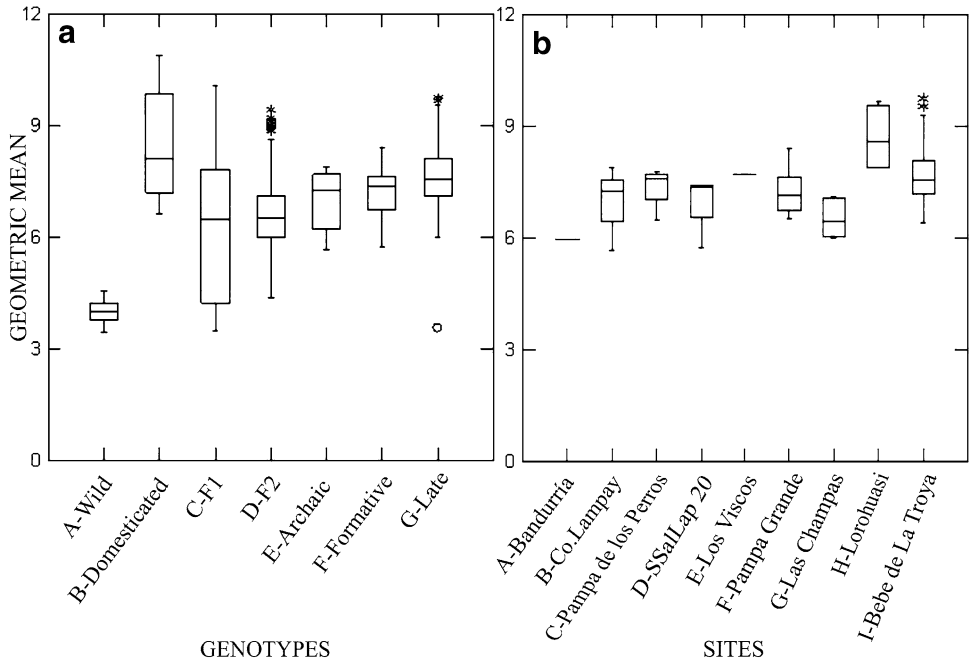

Fig. 4 Geometric means (GM) of seed sizes. **a** A–D, wild, domesticated, F1 and F2 genotypes of modern material; E–G, archaeobotanical remains arranged by chronological periods; **b** archaeobotanical remains by sites, from the earliest to the latest

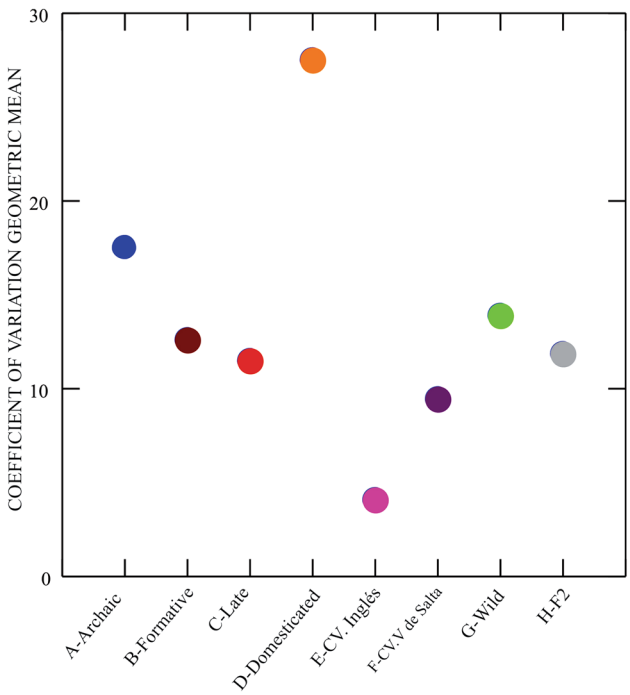

Fig. 5 Coefficients of geometric mean size variation of the archaeobotanical seeds. A–C, arranged by chronological periods; D, reference domesticated genotypes; E, F, cultivars with extreme values; G, wild; H, F2 generation

forms still persists, but their numbers decrease in the archaeobotanical record. The analysis of modern peduncles allowed us to corroborate that the archaeobotanical remains from the Pampa Grande caves with intermediate characters are likely to correspond to hybrid specimens. Quantitative characters of pericarps did not show a great diagnostic value in identifying domesticated, wild/spontaneous or hybrid forms. However, the study of pericarp anatomy allowed us to confirm the presence of the ssp. *andreana* in Pampa Grande (Lema 2011, 2015). Taking into account that presumably seed and fruit size increases are linked, the trend towards larger seeds could also be the result of selection for larger fruit (Cowan 1997; Fuller 2012).

From these results and others obtained previously by our team work, we can say that our initial hypothesis was confirmed, since hybridization, introgression and gene flow were part of the post-domestication history of *C. maxima* cultivation from the Archaic to Late periods, with different intensities. With the presence of seeds, rinds and peduncles, the Pampa Grande site allows us to obtain a more complete picture of early squash cultivation, where domesticated and wild/spontaneous *C. maxima* subspecies coexisted with hybrid forms resulting from crosses between the two. This can be seen in modern examples of conscious and unconscious practices of human selection which allow genetic interchange between weedy, wild and domesticated forms (Montes Hernández and Eguiarte 2002; Lira et al. 2016). Gene flow is a relevant evolutionary mechanism which produces micro-evolutive processes with long term consequences, which can be the creation of new forms such as domesticated species, cultivars, weeds, or the extinction of others such as wild ancestors (Wilson et al. 1994; Ellstrand et al. 1999; Lira et al. 2016), when F1 generations

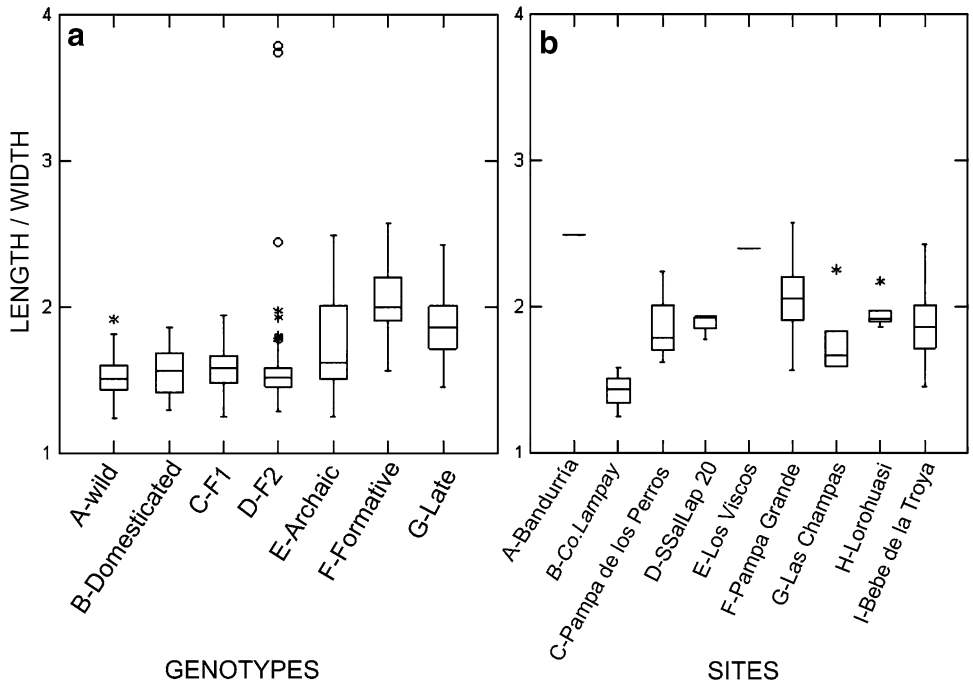

Fig. 6 Seed shapes. **a**, A–D, wild, domesticated, F1 and F2 genotypes of modern material; E–G, archaeobotanical remains arranged by chronological periods; **b**, archaeobotanical remains by sites, from the earliest to the latest

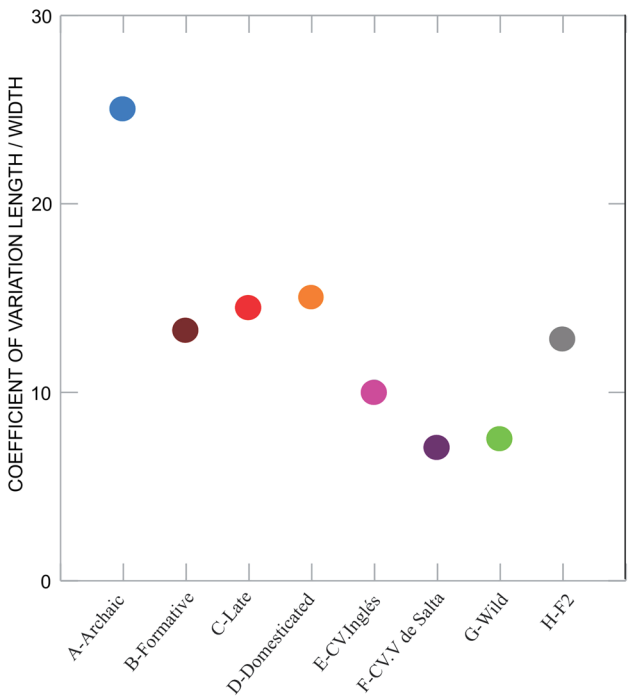

Fig. 7 Coefficient of length/width variation of the archaeobotanical seeds, modified from Martínez et al. (2015). A–C, arranged by chronological periods; D, reference domesticated genotypes; E, F, cultivars with extreme values; G, wild; H, F2 generation

are fertile, which has been confirmed for the *C. maxima* ssp. *andreana*—ssp. *maxima* complex.

Facing scenarios like this one, where gene flow is present in several species and subspecies of *Cucurbita*, reproductive isolation is an essential mechanism for the fixation of domesticated traits (Kistler et al. 2015; Lira et al. 2016). In the case of *C. maxima*, this isolation was probably geographical, and it would then have allowed further crossings and the creation of fertile hybrids, when both subspecies were growing nearby each other. The origin of the domesticated subspecies should thus be looked for in the earliest archaeological records such as those analysed here, or from other areas. In the case of the material from the Pampa Grande caves, the high diversity shown by the archaeobotanical remains of the domesticated subspecies makes us consider that crossing and gene flow within the wild-weedy-domesticated complexes could have been a strategy to generate diversity in both selection pathways previously detected, either as fruits used as containers or as fruits used only for consumption (Lema 2011). Taking this into account we can hypothesize that hybrid forms were not eradicated, but were cultivated, or a combination of both, and that gene flow was intentional and controlled with the aim of generating diversity in squashes and to enlarge the pool of selection. Post-domestication gene flow from wild ancestors or related wild or weedy taxa has also been detected in other domesticated

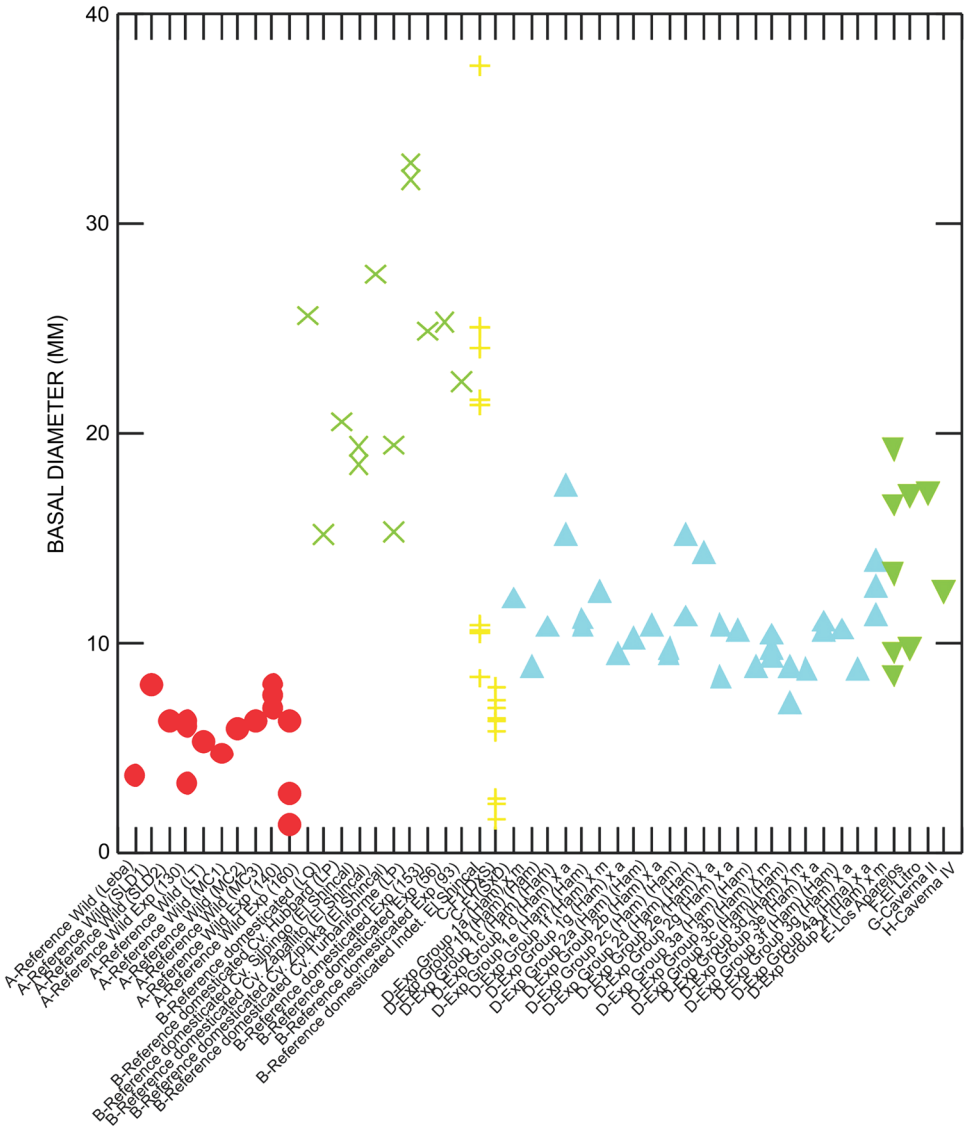

Fig. 8 Basal diameters of peduncles (fruit stalks) in mm, in archaeological and modern material. Red dots, wild; green crosses, domesticated; yellow plusses, F1; light blue triangles up, F2; green triangles down, archaeological; reference names in Tables 1, 2, 3 and 4

plants such as maize, contributing to its present diversity (Matsuoka et al. 2002). These observations have led us to consider introgression as a mechanism that allowed recovery of diversity after domestication (Hufford et al. 2012). Introgression, gene flow and genetic drift are being considered in several cases of crop domestication (Fuller 2012; Aguirre-Dugua and González-Rodríguez 2016; Allaby et al. 2016; Casas et al. 2016; Vibrans 2016).

Physiological studies confirm the loss of seed dormancy in domesticated specimens and also that hybridisation generates multiple responses (Martínez et al. 2016). This led us to propose as a hypothesis that the presence of hybrid forms in the archaeobotanical record also reflects ancient plant populations with differences in dormancy. The basis of this hypothesis is that practices tending to maintain populations with different dormancies could be interpreted as a strategy to reduce the potential risks of having ones with similar maturities, keeping a source of diversity of populations adapted to different environmental conditions. This strategy is crucial when different altitudinal microenvironments are managed, a recurrent scenario in modern and ancient Andean communities. There are parallels to be seen in the cultivation of other *Cucurbita* species, for example in Mexico (Lira et al. 2016). These kinds of crop practices could also guarantee an adaptation capacity to random rainfall due to the increase in homogeneity and germination speed in domesticated *Cucurbita* spp. mentioned by Lira et al. (2016).

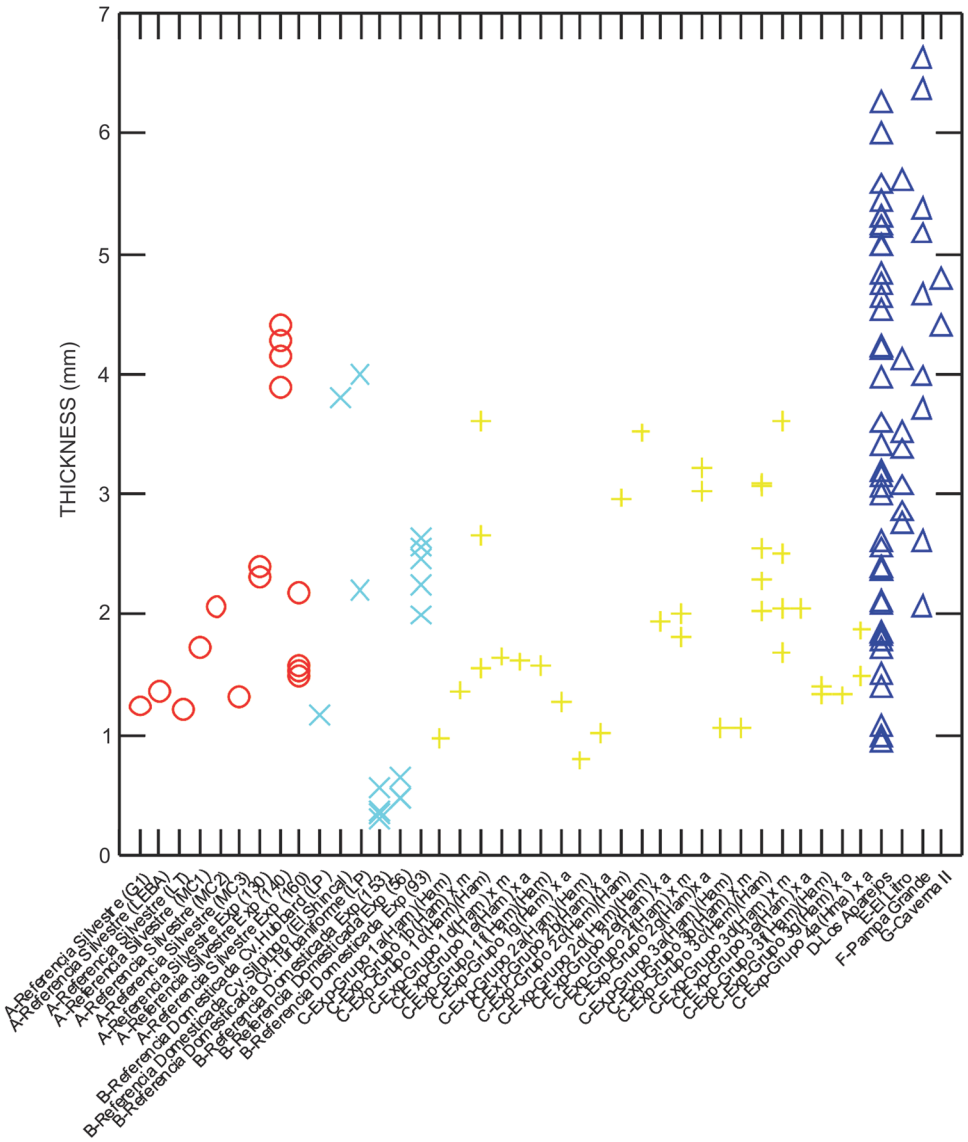

Fig. 9 Pericarp thicknesses in archaeological and modern material, in mm. Red circles, wild; light blue crosses, domesticated; yellow plusses, F2; blue triangles, archaeological; reference names in Tables 1, 2, 3 and 4

In sum, these various approaches, carried out by a multidisciplinary research group, have allowed us to obtain a more comprehensive picture of *C. maxima* cultivation history and to propose new areas for future investigation.

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