MULTIDISCIPLINARY STUDIES IN SQUASH (*C. MAXIMA*) DOMESTICATION THROUGH EXPERIMENTAL, PHYSIOLOGICAL AND ARCHAEOBOTANICAL APPROACHES

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1 Introduction

Several authors suggested that South of Peru, Bolivia and NW Argentina (NWA) have been the area of domestication of *Cucurbita maxima* subsp. *maxima* Duch. ex Lam [1,2]. Based on genetic, archaeological and morphological studies, *Cucurbita maxima* Duch. ex Lam. subsp. *andreana* (Naudin) Filov was proposed to be its wild antecessor [3]. Archaeobotanical remains do not provide yet the whole evidence to confirm this domestication area of the species. However, previous studies carried on by one of the authors determined the chronological and spatial coexistence of wild and domesticated forms together with morphotypes of intermediate characters in archaeological sites of the Northwest of Argentina (NWA) as early as 2000 years BP [4]. Wild forms were characterized by micromorphological studies of pericarp remains, while the intermediate and domesticated forms by the same studies on seed and peduncle remains. The assemblage was preliminary proposed to be part of a complex weedy wild-domesticated [5] where genetic flux, introgression and hybridization might have been common processes, as it is observed in other modern species of the genus [6,7,8,9]. In order to test this hypothesis and to evaluate evolutionary pathways of the species under cultivation, the **objective** of this paper is to investigate the changes that occurred during the domestication of *Cucurbita maxima*, taking in account biometric, physiological and statistical analysis. The first ones were conducted on pericarps, peduncles and testa seeds in order to reconstruct size and shape evolution and its linkage with the second ones, which were address especially on dormancy. Modern and archaeological specimens were considered, as well as the spontaneous/wild and domesticated forms.

Fig. 1. Location of archaeological sites mentioned in the text. (see 2.2)

2. Materials

2.1 Current Material: it consisted of two sets of analysis. A first set comes from a an experimental plant field where crossings were conducted between domesticated (*C. maxima* subsp. *maxima*) and the spontaneous/wild form (*C. maxima* subsp. *andreana*) (Table 1), advancing F1 and F2 generations (Table 2). A second set of material (Table 3), consisted of specimens obtained from the commercial circuit, the Horticulture Chair of the FCAyF (UNLP), the researcher L. Ashworth (samples of subsp *andreana* corresponding to three populations obtained by outcrossing, autogamous and free collections). Physiological tests were performed on seeds on a total of 18 genotypes of the first set. Biometric studies were conducted on 82 peduncles , 67 pericarp fragments and 933 seeds of both sets.

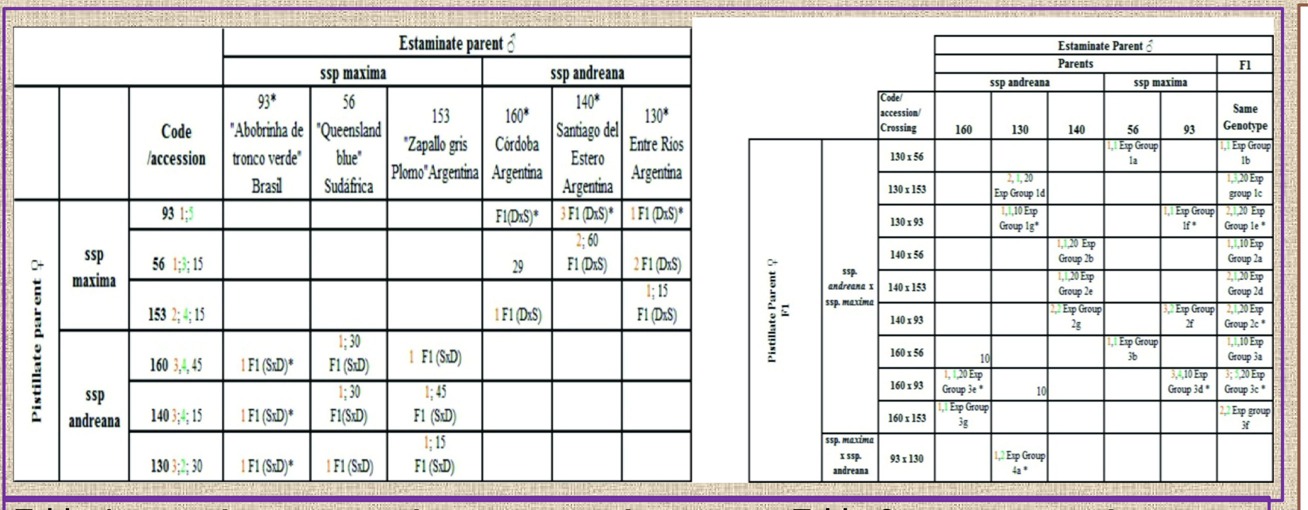


Table 1: Parental genotypes and F1 experimental crossings. **Table 2:** F2 experimental crossings. Numbers in color represent quantity of measured specimens: red=peduncle, green=pericarps, black=seed. Asterisk indicate the material used for the physiological tests and the code corresponding to the names under which the specimens are mentioned in Figure 10-11.

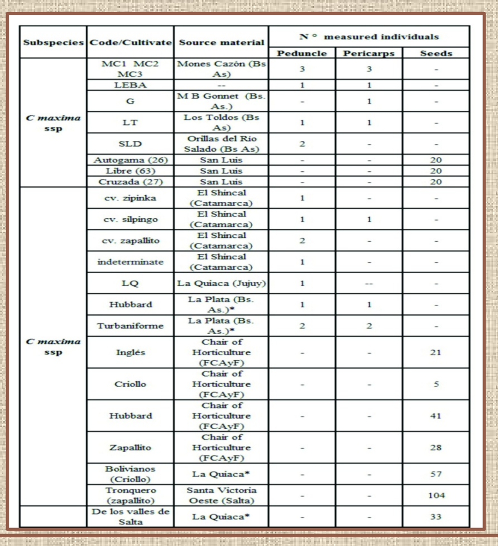


Table 3: reference material. *Indicates obtained from commercial circuit.

Fig. 2. View of the experimental fields

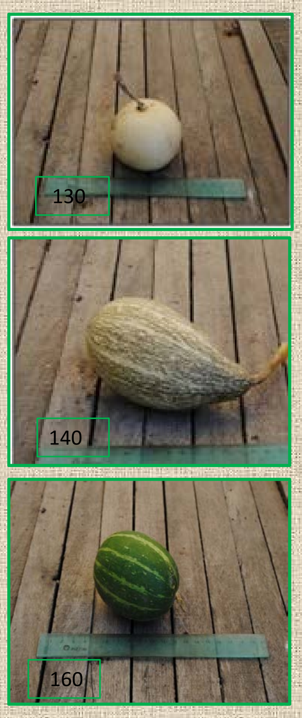


Fig. 3. Parental material of experimental crossings: wild/spontaneous=130,140,160 . Domesticated=93. TS of 93

	Perú			Argentina					
	Bandurria	Cerro Lampay	Pampa de los Perros	Los Viscos	SSalLap20	Pampa Grande	Las Champas	Bebé de La Troya	Lorohuasi
Seeds	1	3	3	1	3	9	6	100	6
Peduncles	-	-	-	-	-	9	-	-	-
Pericarps	-	-	-	-	-	60	-	-	-

Table 4: Archaeobotanical material analyzed.

Fig. 4 Arqueological seed, peduncle and pericarps from Pampa Grande

2.2 Archaeobotanical material: it was recovered from archaeological sites of the south-central of Peru and NWA. Among the first, sites were (1) Cerro Lampay, (2) Pampa de los Perros and (3) Bandurria, which corresponds to the Archaic period (ca. 3000-2000 AP). Among the second, there are samples of Early or Formative period (ca. 2000-1200 AP; sites: (4) SSalLap 20, (5) Pampa Grande and (6) Los Viscos) and Late period (ca. 1200-800 AP; sites: (7) Bebe de la Troya, (8) Las Champas and (9) Lorohuasi) (see Fig. 1) Number of remains analyzed were 9 peduncles, 60 pericarp fragments and 132 seeds (Table 4).

3. Methods

3.1 Physiological Analysis: They were performed on seeds and embryos using the following parameters:

1) Temperature (T°C): seeds were placed to germinate at 16°, 20° and 28°, and alternating between them.
2) Hormones: seeds were treated with abscisic acid (0, 0.1, 1 and 10 uM) and gibberellins (0, 0.1, 1, 10 uM)
3) Light : absence and presence of light.
4) Scarification: mechanical abrasion of the seed coat
5) Diffusion of water through the seed coat: tests using a staining technique safranina 50%.

3.2: Morphometric and statistical analysis: all measures were obtained by digital caliper

Seeds: Length, width and thickness were measured. Data were partitioned into a size and a shape component. The size was estimated by the Geometric Mean (GM) (arithmetic measure of the original variables in a logarithmic scale) of length, width and thickness. As Main Component Analysis showed that thickness was not significant for shape, this last variable was calculated in terms of the length/width ratio [9]. Variation coefficient (VC) was calculated for both variables.

Peduncles: an average between two measures of the basal diameter was calculated for each specimen and processed with statistical software [10].

Pericarps: thickness (height) was measured following [11].

4. Results

4.1 Physiological Analysis: *C. maxima* subsp. *andreana* presents dormancy at an optimum temperature (28 °C), while subsp. *maxima* lacks this condition, germinating immediately after harvest. Differences in dormancy were observed between different *andreana* accessions: 130 could be considered wild, and 140 and 160 spontaneous (Fig. 5). In the case of F1, the germination and growth pattern resembles that of pistillate parent; while in the case of F2 they have a certain percentage of germination and acquire a tendency of behavior towards domesticated forms (Fig 6).

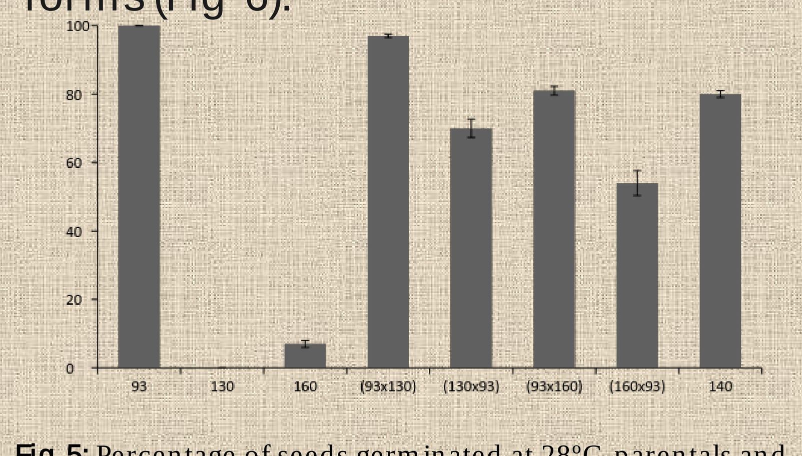


Fig. 5: Percentage of seeds germinated at 28°C, parentals and F1.

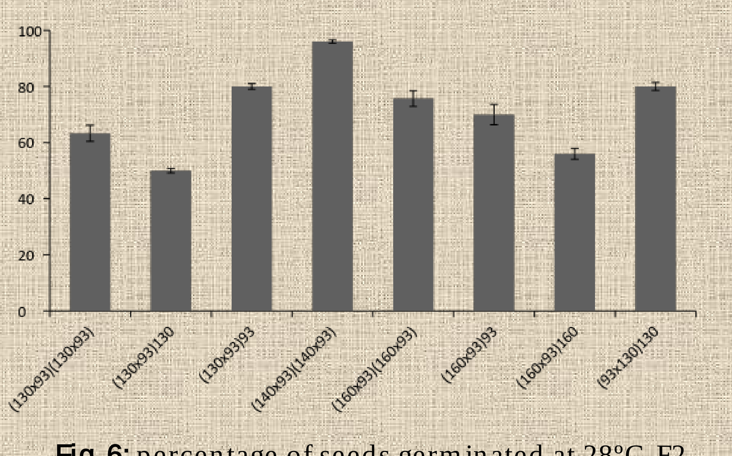


Fig. 6: percentage of seeds germinated at 28°C, F2.

Fig. 7: Embryos germination of *C. maxima* subsp. *andreana* (1) and subsp *maxima* (2) after hormone treatments (0.1uM ABA)

As regards hormone treatments wild show a greater sensitivity to abscisic acid and gibberellins than domesticated form. One factor that indeed influenced on dormancy was the presence of the seed coat. Naked embryos germinated immediately after imbibition indicating that dormancy is imposed by seed covers. This inhibition may be due to the presence of an inhibitor such as abscisic acid (Fig. 7).

4.2 Biometric analysis

4.2.1 Seeds: GM differentiated wild from domesticated forms, but F1 and F2 have intermediate values (Fig. 8a). Archaeobotanical remains from the Archaic and Formative periods has a range matching with F1, F2 (hybrids) and domesticated forms, and those from Late periods with domesticated forms. There is a trend to increase size along time (Fig 8a,b). VC shows high variability during Archaic period, which diminishes to values approx. to F2 during later periods (Fig. 8c).

Shape differentiated current from archaeological material, but not the current material itself (Fig. 9a,b). More elongated seeds were common during the Formative. VC showed high shape variability during the Archaic diminishing in later periods towards values approx. to F2 (Fig. 9c).

4.2.2 Peduncles: basal diameter clearly differentiated between wild/spontaneous, domesticated, F1 and F2 forms. Archaeological remains coincided with the F2, partly with F1 (DxS) and domesticated forms (Fig. 10).

4.2.3 Pericarps: analysis showed no distinctive values between subspecies. Archaeological pericarp have a wide distribution throughout the gradient of current material measures, which reaffirms the proposal of use and management of a variety of forms of fruits in the archaeological site of Pampa Grande (Fig. 11).

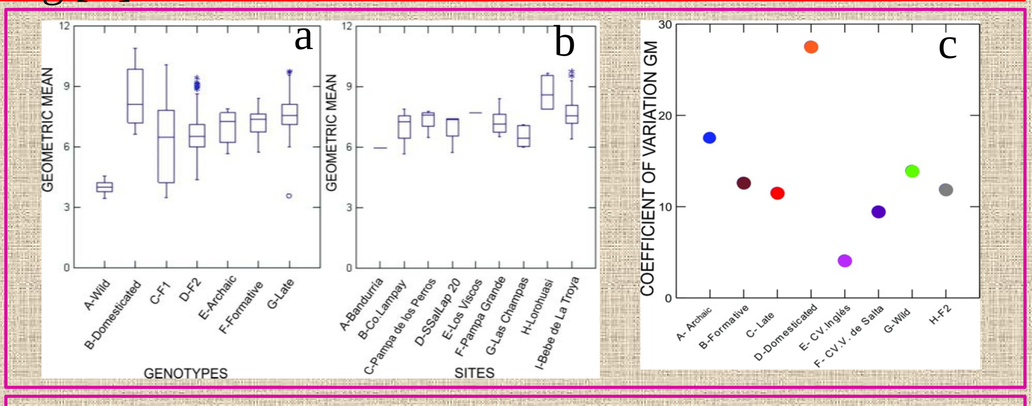


Fig. 8. Seed size. **a.** Wild and domesticated (A-B), F1 and F2 genotypes of current material (C-D), archaeobotanical remains arranged chronologically (E-G), **b.** Sites sorted from the earliest to the latest, **c.** VC of the GM by site chronology (A-C); of extreme reference cultivars (E,F), of wild (G) and domesticated genotypes (D) and F2 generation (H).

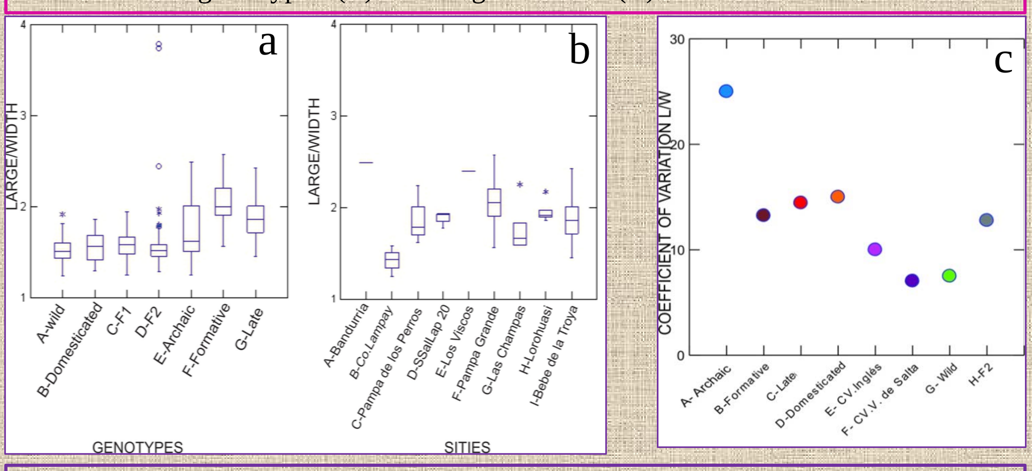


Fig. 9. Seed shape. **a.** Wild, domesticated, F1 and F2 genotypes of current material (A-D), archaeobotanical remains arranged chronologically (E-G); **b.** Sites sorted from the earliest to the latest, **c.** VC by site chronology (A-C); of extreme reference cultivars (E,F), of wild (G) and domesticated genotypes (D) and F2 generation (H).

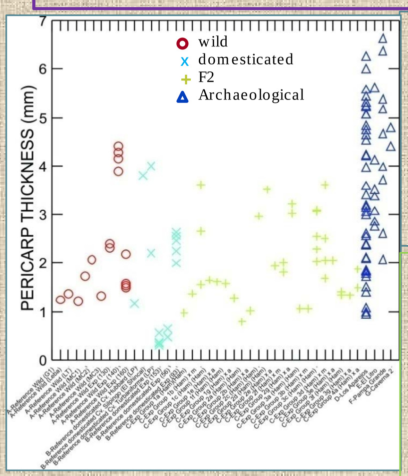
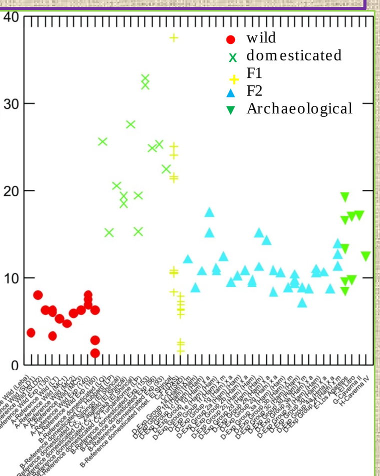


Fig. 10. Dispersion of basal diameter of peduncles in archaeological and current material (see reference names in Table 1 to 4).

Fig. 11. Dispersion of pericarp thickness in archaeological and current material (see reference names in Table 1 to 4).



5. Discussion and Conclusions

Results suggest that analyzed archaeological remains correspond to a stage posterior of the domestication of the species. The general trend after having tamed *C. maxima* subsp. *maxima* appear to have been the generation of new forms of seeds (perhaps managing different altitudinal microenvironments), but keeping in time hybrid populations (Archaic). Later, variability in shape and size reduces, the presence of hybrids forms also diminishes but still persist (Formative and Late periods).The analysis of peduncles corroborated that those archaeobotanical remains from Pampa Grande with intermediate characters were hybrid specimens. Quantitative characters of pericarp did not show a great diagnostic value in identifying ways of handling, however, the study of its anatomy allowed the confirmation in Pampa Grande of the presence of the subsp. *andreana*. From these results and others generated by our team work, we can say that, in the archaeological site of Pampa Grande, domesticated and wild/spontaneous *C. maxima* coexisted with hybrid forms resulting from introgression and hybridization processes between the two subspecies. This can be seen in modern correlates of conscious and unconscious processes of human selection which allow genetic interchange between weed, wild and domesticated forms [7]. Physiological studies allowed us to characterize dormancy demonstrating the crucial role of the testa for the restoration of seed growth. Also, lead us to propose as a hypothesis that practices tending to the maintenance of populations with differential dormancy may have been a strategy to reduce the potential risks of having homogeneous maturities while a source of diversity of selected cultivars adaptated to different environmental conditions. In sum, this conjunction of approaches, developed in the frame of a multi-disciplinary research group, let us to obtain a most comprehensive picture of *Cucurbita maxima* domestication history.

References: [1] Millán R. 1945 Variaciones del zapallito amargo *Cucurbita andreana* y el origen de *Cucurbita maxima*. *Revista Argentina de Agronomía* 12: 86-93. [2] Whitaker T.W. and Bemis W.P. 1964 Evolution in the genus *Cucurbita* Evolution 18 (4): 553-559. [3] Sanjur O., Piperno D., Andres T. and Wessel-Beaver L 2002 Phylogenetic relationships among domesticated and wild species of Cucurbita (Cucurbitaceae) inferred from a mitochondrial gene: Implications for crop plant evolution and areas of origin. *PNAS* 99 (1): 535-540. [4] Lema, V. 2011 The possible influence of post-harvest objectives on *Cucurbita maxima* ssp. *maxima* and ssp. *andreana* evolution under cultivation at the Argentinean Northwest: an archaeological example. *Journal of Anthropological and Archaeological Sciences* 3 (1):113- 139 . [5] Lema, V. 2015 Non domestication cultivation in the Andes: plant management and nurturing in the Argentinean Northwest. *Vegetation History and archaeobotany* 24:143- 150 . [6] Nee M. 1990 The domestication of *Cucurbita* (Cucurbitaceae) *Economic Botany* 44 (3) Supplement: Bretting (ed.) *New perspectives on the origin and evolution of New World domesticated plants*. [7] Montes- Hernández S. and Eguiarte L. 2002 Genetic structure and indirect estimates of gene flow in three taxa of Cucurbita (Cucurbitaceae) in Western Mexico American Journal of Botany 89(7): 1156–1163. [8] - Wilson H., Lira R. and Rodríguez I. 1994 Cop/weed gene flow: *Cucurbita argyrosperma* Hubelt and *C. fraterna* L. H. Bailey (Cucurbitaceae) *Economic Botany* 48(3) : 293-300. [9] Ellstrand N., Prentice H. and Hancock J. 1999 Gene Flow and Introgression from Domesticated Plants into Their Wild Relatives *Annual Review of Ecology and Systematics*, 30 : 539-563. [10] Jungers,, W.L., A. Falsetti & C.E. Wall - 1995- Shape, relative size and size-adjustments in morphometrics. *Yrbk Phys Anthropol* 38:137- 161. [11] Cowan C.W. and Smith B.D. 1993 New perspectives on a wild gourd in Eastern North America *Jounal of ethnobiology* 13(1):17-54