RESEARCH ARTICLE

Morphological divergence between wild and cultivated chaya (*Cnidoscolus aconitifolius*) (Mill.) I.M. Johnst

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Abstract Chaya (*Cnidoscolus aconitifolius*) (Mill.) I.M. Johnst is a neglected and underutilized crop, cultivated clonally by the Maya from pre-Hispanic times to the present in their home gardens for their own consumption. Like other crops in the early stages of domestication, cultivated chaya co-occurs with its wild relatives, however, the degree of domestication of chaya is unclear. Wild and cultivated chaya exhibit some morphological differences, however, no study has assessed quantitatively the degree of divergence between these varieties. The main objective of this study was to assess the domestication degree of chaya, by measuring morphological divergence in vegetative traits (leaf number, area, toughness, trichome number, as well as plant size, number of branches and trunk width) in wild and cultivated plants. Our results show that cultivated and wild plants had only a minor degree of overlap in the multivariate morphological space. The cultivated variety had more branches and leaves, and their leaves were softer and had fewer trichomes than the wild variety. This divergence is probably reinforced by the fact that desirable traits (number of branches and leaves) are negatively correlated with undesired traits (number of trichomes and toughness). Despite significant divergences between the two varieties, a few cultivated plants fell within phenotypic range of variation for wild plants, which could be due to recent artificial selection.

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Introduction

Plant domestication is a human-driven process in which a suite of traits beneficial to growers are selected (Harlan 1975). As a result of domestication, desirable traits (and other correlated traits) are exhibited in a number of phylogenetically unrelated crops, and this trait combination is known as domestication

syndrome (Hammer 1984; Harlan 1975). Because the evolutionary trajectory of domesticated plants is driven by humans instead of natural selection, some degree of phenotypic divergence between domesticated plants and their wild relatives is expected (Ladizinsky 1998; Meyer et al. 2012). However, the time needed to observe morphological divergence between crops and their wild relatives varies among crop species depending on variables such as life history traits (i.e. perennial vs. annual; Miller and Gross 2011), propagation mode (seed vs. clonal; McKey et al. 2010) and the existence of gene flow with crop wild relatives (Ellstrand et al. 1999). A notable proportion of neglected and underutilized crop species in the Neotropics co-occurs with wild relatives in traditional agroecosystems and surroundings, and are frequently in the early stages of the domestication process; that is, little divergence between crops and their wild relatives is observed (Galluzzi and López-Noriega 2014; Galluzzi et al. 2010; Meyer et al. 2012). Co-occurrence of wild and cultivated plants may promote between-variety gene flow and this, together with relatively recent artificial selection, may explain at least partially the low degree of morphological divergence observed in underutilized crops with respect to their wild relatives (Cruz and Casas 2002; Dzul-Tejero et al. 2014). Information regarding the degree of domestication of underutilized crops is urgently needed to identify which of these crops may have the potential to contribute to the diet of a growing human population (Williams and Haq 2002).

Chaya (*Cnidoscolus aconitifolius*) (Mill.) I.M. Johnst (Fig. 1) is considered a neglected and underutilized crop owing to limited general knowledge about it, its poor representation in ex situ collections, and the lack of conservation programs (Galluzzi and López-Noriega 2014). This plant species is a perennial, fast growing and shade-intolerant shrub (Standley and Steyermark 1949; Parra-Tabla et al. 2004). Its leaves are edible and it has been clonally propagated by peninsular Maya people in their home gardens for self-consumption for more than 500 years from pre-Hispanic times to the present (Ross-Ibarra and Molina-Cruz 2002; Ross-Ibarra 2003). Owing to the ecological affinity of wild chaya to anthropized habitats (Parra-Tabla et al. 2004), it often co-occurs with cultivated chaya in the Yucatan. However, cultivated plants rarely produce seeds (Ross-Ibarra
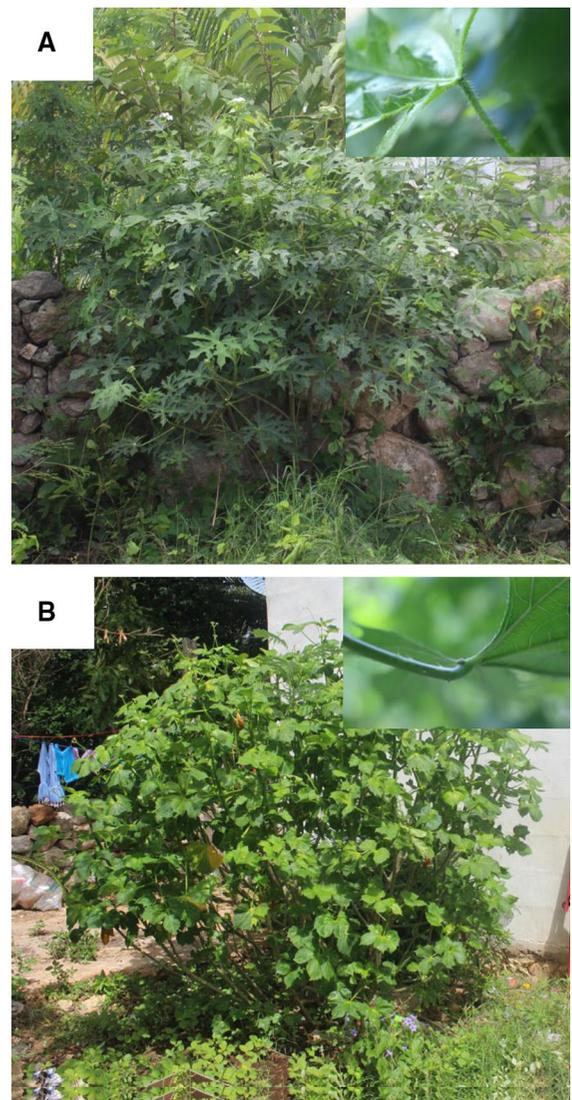

Fig. 1 Pictures of wild (**a**) and cultivated chaya (**b**) under field conditions in the Yucatan. The inset (upper right) in each picture shows differences in trichome density of the petiole

and Molina-Cruz 2002; Ross-Ibarra 2003) and therefore, inter-variety gene flow may be low despite their sympatric distribution. Even though chaya is a perennial plant, it is known that clonal propagation makes the fixation of selected traits occur more quickly in domesticated plants (McKey et al. 2010). Currently, there is some debate regarding the degree of domestication of chaya. While some authors have stated that it is undomesticated (Breckon 1975) or semi-domesticated (Aguilar-Luna et al. 2012), others suggest that it is fully domesticated (Ross-Ibarra and Molina-Cruz

2002; Ross-Ibarra 2003). Relative to wild chaya, cultivated plants apparently produce more and bigger leaves with fewer trichomes (Ross-Ibarra 2003); however, morphological divergence between wild and cultivated chaya has not been assessed quantitatively. Therefore, though some research has suggested that chaya is fully-domesticated (Ross-Ibarra 2003), for a proper evaluation of the degree of its domestication, a statistical analysis is required to identify the presence of any discontinuities in quantitative traits between wild and cultivated plants (Casas et al. 1999; Colunga-García Marín and May-Pat 1997; Parker et al. 2010).

The main objective of our study was to quantitatively assess morphological divergence (i.e. domestication degree) between wild and cultivated chaya in leaf and plant traits identified by growers as relevant to human consumption. Because chaya has been managed for centuries, we predicted discontinuous variation in its morphological individual traits, as well as a complete separation between wild and cultivated plants in the multivariate phenotypic space. In other words, we predicted that cultivated chaya is domesticated.

Materials and methods

Study system

Cnidoscolus aconitifolius (Mill) I.M. Johnst (Euphorbiaceae) (Fig. 1) is a shrub that grows up to 4.5 m in height (Parra-Tabla et al. 2004). This plant species produces male and female flowers on the same plant, flowers are short-lived (24 h) and insect-pollinated (Carbajal 1998). In the study area, the flowering season extends from May to November with a flowering peak in summer (June–September). The fruit are dry capsules with up to three seeds dispersed ballistically (Standley and Steyermark 1949). Although fruit production is abundant in wild plants, it is infrequent in cultivated plants (Ross-Ibarra 2003). Four cultivated varieties (i.e. plant groupings within a single taxon defined by the reproducibility of its distinguishing characteristics; FAO 2009) have been reported, however, only the wild (tzin-tzin chay) and one cultivated variety (chay) occur and are named by locals in the Yucatan (Ross-Ibarra and Molina-Cruz 2002). The existence of only two clearly distinguishable varieties in the Yucatan is clearly advantageous for our objectives because their identification is simply, relative to other sites where more than two morphologically similar varieties co-occur. Cultivation is usually done with stem cuttings and this is also possible for the wild variety. This species has an extensive distribution, mainly in the tropics, growing wild from southern Texas along the coast of the Gulf of Mexico through the Yucatan Peninsula, and from Chiapas to the northern part of South America (Ross-Ibarra and Molina-Cruz 2002). However, its cultivation is far more limited geographically, as it is a more popular vegetable on the Yucatan Peninsula and in Guatemala (Ross-Ibarra 2003). The Yucatan is the only site were this plant species has been named in an indigenous language. Additionally, the Yucatec Maya have broader knowledge and make more extensive use of this plant than any other culture (Ross-Ibarra and Molina-Cruz 2002). Based on this ethnobotanical information, some authors have suggested that this species was domesticated in the Yucatan (Ross-Ibarra and Molina-Cruz 2002). Chaya has highly urticant trichomes on its stems, leaves and fruit (Parra-Tabla et al. 2004), apparently, cultivated plants have fewer and a more restricted distribution of trichomes (Ross-Ibarra 2003), but this has not been tested statistically. Some of these traits (leaf size and shape, as well as trichome density) exhibit phenotypic plasticity (Abdala-Roberts and Parra-Tabla 2005; Parra-Tabla et al. 2004).

Owing to its nutritional quality, ease of cultivation, productivity and the lack of intensive plantations, some authors have suggested that this is an underutilized crop, but one that has a high potential to become an intensively cultivated crop (Aye 2012; Victor et al. 2016).

Sampling and experimental design

In the summer of 2017, the stems of cultivated and wild chaya were collected from the young, secondary branches of adult plants at the equatorial zone of the canopy in 40 different locations scattered all over the Yucatan peninsula (See Appendix S1). Cultivated plants were those planted in home gardens (confirmed by the owner) and wild plants were those growing in secondary forest, apparently having established naturally. Also, these two plant categories are easily differentiated because the stems of wild plants are far

thornier than cultivated plants even to the naked eye (Fig. 1). Two to four stems per locality were collected, and in most cases two stems per variety: wild versus cultivated were collected per locality. However, in some sites only one variety was found and when this occurred, the other variety was collected in a different site. In total 160 accessions were collected.

Although the wild variety produces several seeds, we decided to use stems for standardization because, as mentioned above, the cultivated variety produces only a few or no seeds at all. The stems were grown in a plant nursery located within the natural range of distribution of chaya in Merida, in central Yucatan (21° 00′ 32.97″N, 89° 38′ 40.49″W). Stems were planted in 6 L pots with a mixture of gravel and soil (70:30) and watered as needed. Only 60% of the plants successfully rooted after 4 months and of these, we selected a subsample of 68 apparently healthy plants that were similar in height to be planted in a common garden at the same nursery (50 × 35 m). Selected plants were planted in random positions, 1–1.5 m apart and tagged. Plants were grown in a common garden to control the effect of the environment on morphological variation and because our main interest was genetically-based morphological variation. The plants included in the experiment, the variety to which they belong and the site of origin for each are listed in Appendix S 1.

To identify the morphological traits that growers consider important, we asked the owners of home gardens about the traits they prefer a plant to have and how the mother plants (i.e. plants from which a stem is cut for propagation purposes) are selected. All of them agreed that the number of leaves, the lack or a reduced number of urticant trichomes, and the general appearance of the leaves were the most important traits. Most informants mentioned that big plants with many leaves are preferred as mother plants and from them, succulent stems are selected for propagation because they have greater chance of rooting. Therefore, in May 2018, we counted the total number of leaves and secondary branches per plant. Leaf area (cm^2) and leaf toughness (N/mm^2) were measured with a portable leaf area meter (CID-Biosciences, model CI-202) and a self-made penetrometer in four randomly chosen and completely expanded leaves per plant. For the same leaves, the number of trichomes on the main rib and on the edge were counted. Chaya trichomes are translucent, whitish structures from 2 to 6 mm long, and though they are visible to the naked eye, we used a stereoscopic microscope (2X-10X) to reduce bias. We counted all the trichomes visible in the field with the aid of a hand-held counter and once all the trichomes had been counted, we advanced to a new portion of the leaf and repeated the procedure. The number of trichomes on both sides of the main rib and on the edge were counted.

Although the reproductive traits (flowers, fruit or seed) were not relevant to growers, the production of these structures is clearly different between varieties, so we also counted reproductive structures to explore allocation to reproductive functions and their relationship to vegetative growth. To this end, two times a month for 6 months (May–October 2018), we counted the total number of leaves, measured plant height (cm) with a measuring tape, and measured the diameter of the main trunk at the base (mm) with a digital calliper. Simultaneously, we counted the number of inflorescences on all the plants, as well as the number of flowers in the most apical inflorescence.

All measurements were conducted in the common garden and started 7 months after transplant (May 2018), when all the plants had reached sexual maturity (plants produce flowers and/or fruit) and a height > 55 cm. Our main interest in this study was between-variety variation of genetically-based traits; therefore, interannual variation was not relevant for our study because temporal variation in morphological traits is a plastic response.

Statistical analyses

To assess multivariate morphological divergence, we first conducted a principal components analysis (PCA) with the eight standardized vegetative morphological variables: toughness, number of leaves, number of branches, number of trichomes on the main rib and edge, leaf area, plant height and the diameter of the main trunk, and we visually assessed whether the two varieties of chaya separated on the axes of the two first principal components (PC1 & PC2). Then, a multivariate analysis of variance was run to assess statistical differences between the two varieties for the eight morphological variables together. To improve the normality of the data, the number of leaves, branches and trichomes (on the main rib and edge) were log transformed.

To assess the divergence of individual traits we compared each trait between the two varieties using mixed linear models (8 models in total) with the morphological trait as the response variable and the variety and the site of origin as the fixed and random factors, respectively. To improve the normality of the data, the number of leaves, branches and trichomes (on the main rib and edge) were log transformed before running the analyses. We corrected the P value for multiple tests with the false rate discovery method. For statistically significant variables, we also calculated the percentage of change (from wild to cultivated variety) as: $(V_c - V_w)/|V_c|) \times 100$, where V_c is the value of the trait in the cultivated variety and V_w is the value of the same trait in the wild variety. Because domestication not only affects mean trait values but also trait variance (Parker et al. 2010), differences between varieties in terms of trait variance were assessed with Fisher's tests.

To assess phenotypic correlation among vegetative traits, we calculated Pearson's correlation between all possible combinations of vegetative trait pairs. We also used linear mixed effect models to assess if allocation to flower production can be explained by allocation to vegetative traits. Thus, in the model, the number of flowers produced over 6 months was the response variable, the growth of the main trunk (change in the volume of the main trunk over 6 months), leaf production during the same period, the variety, and second order interactions were included as predictors. The site of origin was included in the random part of the model. Because the error distribution was approximately normal, no data transformation was needed.

To assess whether sample size was sufficient to detect differences between the two plant varieties, we conducted power analyses for each variable. Statistical power (β) was in general sufficient (0.86 ± 0.07, hereafter mean values $\pm$ SE). Only for leaf area, statistical power was relatively low ($\beta = 0.47$). Previous research on the topic with perennial plants used a similar sample size (e.g. Cruz and Casas 2002; Thul et al. 2009). Although it has been suggested that the domestication of long-lived perennials may take longer than annuals (Miller and Gross 2011), it also known that the fixation of desired traits in crops is faster in clonally propagated crops than sexually propagated crops (McKey et al. 2010). Considering

this information, the sample sized used in this study was appropriate for our stated purpose.

All statistical analyses were run in R 3.5.1 (R Development Core Team 2018).

Results

Multivariate morphological divergence

According to the results of the PCA, PC1 explained 37% and PC2 17% of the variance (cumulative explained variance = 54%). On these two axes (PC1 & PC2), there was only minor overlap between the distributions of wild and cultivated plants (Fig. 2). The first principal component (PC1) was positively influenced mainly by trichomes on the edges and on the main rib (loadings = 0.43 & 0.40), and by leaf toughness (loading = 0.39) and was negatively affected by the number of leaves (loading = − 0.48) and branches (loading = − 0.46). For principal component 2 (PC2), the diameter of the main truck (loading = 0.67), leaf area (loading = 0.58), trichomes on the edge (loading = 0.28) and on the main rib (loading = − 0.23) had the most influence. A full loadings matrix is given in Appendix S 2.
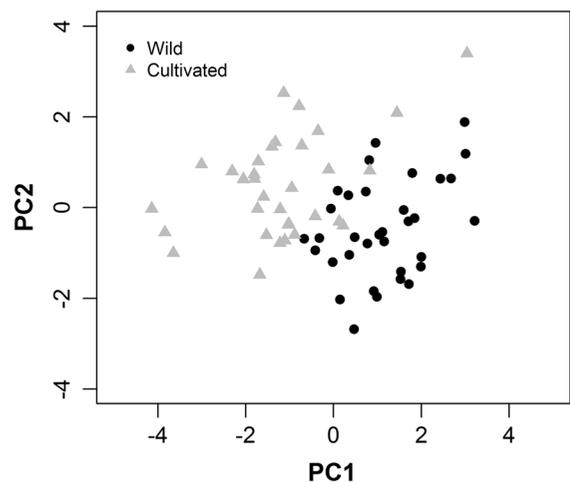

Fig. 2 Scatter plot showing the distribution of wild (black circles) and cultivated (grey triangles) plants of *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst along two principal components (PC1 & PC2) obtained from eight morphological vegetative traits relevant to artificial selection from the perspective of growers

A statistically significant difference between the cultivated and the wild variety was found when the eight morphological variables were analysed together in the MANOVA (Pillai = 0.74, $F_{8,58} = 20.89$, $P < 0.01$).

Morphological divergence of individual traits

The mean number of branches, mean diameter of the main trunk and mean number of leaves were all significantly greater in the cultivated variety, while mean leaf toughness and mean number of trichomes (on the main rib and borders) were significantly greater in the wild variety (Table 1). On average, the cultivated variety produced 244% more branches, 103% more leaves and the main trunk was 12% wider than those of the wild variety. Also, leaves of the cultivated variety were 33% softer and had 93% and 49% fewer trichomes on the main rib and on the edge than the wild variety did. Mean leaf area and mean

plant height were not statistically different between the cultivated and the wild variety (Table 1).

Regarding trait variability, the cultivated variety exhibited a statistically lower variance in leaf toughness, trichomes on the main rib and plant height than the wild variety did (Table 1). In contrast, the cultivated variety varied more than the wild variety in terms of number of branches and leaves (Table 1). Variance in the number of trichomes on the main rib, leaf toughness and plant height were 91%, 66% and 99% lower in the cultivated than in the wild variety, whereas variance in the number of branches and leaves was 4797% and 383% greater in the cultivated than in the wild variety. No statistically significant differences between the cultivated and the wild variety in terms of variance were found in the number of trichomes on the leaf edge, leaf area or the diameter of the main trunk (Table 1).

Table 1 Mean ($\pm$ 1 SE) and variance (metrics) for eight vegetative morphological traits (Trait) relevant to artificial selection in wild and cultivated *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst in a common garden

Metric	Trait	Variety		Statistic	P value adjusted
		Wild	Cultivated		
Mean	**Toughness (N/mm^2)**	**0.07 ± 0.003***	**0.05 ± 0.002**	**12.67**	**< 0.01**
	Branches (n)	**3 ± 0.13**	**10.33 ± 0.94***	**62.12**	**< 0.01**
	Trichomes on the main rib (n)	**11.79 ± 1.2***	**0.85 ± 0.35**	**76.12**	**< 0.01**
	Trichomes on leaf edge (n)	**1303.03 ± 95.7***	**658.33 ± 98.19**	**9.63**	**< 0.01**
	Leaf area (cm^2)	127.36 ± 5.17	152.27 ± 6.97	3.78	0.07
	Height (cm)	137.71 ± 27.85	115.88 ± 2.68	0.30	0.61
	Trunk diameter (mm)	**17.86 ± 0.31**	**20.05 ± 0.32***	**11.85**	**< 0.01**
	Leaves (n)	**36.29 ± 1.61**	**73.51 ± 3.53***	**55.55**	**< 0.01**
Variance	**Toughness**	**7.1 × 10^{-4}***	**2.4 × 10^{-4}**	**0.34**	**< 0.01**
	Branches	**1.21**	**59.35***	**48.97**	**< 0.01**
	Trichomes on the main rib	**96.35***	**8.57**	**0.09**	**< 0.01**
	Trichomes on leaf edge	61.3 × 10^4	64.6 × 10^4	1.052	0.91
	Leaf area	1789.83	3252.34	1.82	0.12
	Height	**51.9 × 10^3***	**0.48 × 10^3**	**0.01**	**< 0.01**
	Trunk diameter	6.28	7.19	1.14	0.80
	Leaves	**172.82**	**834.82***	**4.83**	**< 0.01**

Differences among varieties (wild vs. cultivated) were assessed with linear mixed models and *F* tests for mean trait value and trait variance, respectively. The statistics for mean values are *F* values with 1 and 32 degrees of freedom and for variances, the statistics are *F* values with 32 and 33 degrees of freedom. *P* values were adjusted for multiple tests with the false rate discovery method. Statistically significant test results are in bold, an asterisk (*) indicates which variety that had the greater value

Phenotypic correlations

From all possible combinations of morphological traits, 50% (14 of 28) were significantly correlated. However, relatively strong correlations ($r > 0.35$) were detected only for nine pairs of variables (32%): number of branches vs. number of leaves ($r = 0.81$), trichome on the main rib vs. trichomes on the leaf edge ($r = 0.51$), leaf toughness vs. trichome on leaf edge ($r = 0.49$), leaf toughness vs. trichome on the main rib ($r = 0.40$), trichome on the edge vs. number of leaves ($r = -0.39$), leaf toughness vs. number of leaves ($r = -0.37$), leaf toughness vs. number of branches ($r = -0.36$), number of branches vs. number of trichomes on the main rib ($r = -0.36$) and on the edge ($r = -0.36$) (Table 2).

Leaf production was significantly and negatively correlated (coefficient = -0.102 ± 0.02) with flower production (Table 3). Main trunk growth was not a reliable predictor of flower production (Table 3). Although there were statistically significant differences between varieties in flower production (wild = 226.03 ± 38.76, cultivated = 17.36 ± 17.36), the interaction between variety and leaf production was not significant, which was also the case for all of the other interactions included in the model (Table 3).

Discussion

Plants in the early stages of domestication usually co-occur with their wild relatives and show relatively low morphological divergence, and this seems to be the case for some neglected and underutilized crops in the Neotropics. Chaya is one such crop, cultivated in traditional agroecosystems by the Maya from pre-Hispanic times to the present, for which the degree of domestication was unclear. Our findings suggest that there is only minor overlap in the distribution of cultivated and wild plants in the multivariate phenotypic space. The cultivated variety had more branches with more edible leaves and fewer structures/properties that reduce leaf palatability (trichomes and toughness). This divergence is probably reinforced by the fact that desirable traits (number of branches and leaves) are strongly negatively correlated with undesired traits (number of trichomes and toughness). Plants with greater allocation to leaves produced fewer flowers; thus, even though flower production is not of interest to growers, it is likely that unconsciously this trait was selected against when growers selected plants that produce more leaves. Although there is a remarkable morphological divergence between cultivated and wild plants, a few cultivated plants fell within the range of variation of the wild variety in terms of morphology. The existence of cultivated plants that are morphologically similar to wild plants may be due to relatively recent artificial selection, as well as gene flow from wild to fertile cultivated plants.

We found that the number of leaves, branches and trunk diameter was greater in cultivated chaya, while leaf toughness and the number of trichomes were greater in the wild variety. This is probably because the leaves are the edible organ and therefore, plants that produce more and more palatable leaves are being selected by growers. Trunk diameter was probably

Table 2 Correlation matrix of vegetative morphological traits of *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst in a common garden

	Toughness	Branches	T. rib	T. edge	Area	Height	Diameter
Branches	**− 0.36****						
T. rib	**0.40****	**− 0.36****					
T. edge	**0.49****	**− 0.36****	**0.51****				
Area	0.13	− 0.10	0.02	0.22			
Height	− 0.02	− 0.09	− 0.01	0.32**	0.04		
Diameter	− 0.03	0.12	− 0.35**	0.04	0.25*	0.00	
Leaves	**− 0.37****	**0.81****	− 0.34**	**− 0.39****	− 0.11	− 0.12	0.25*

Values are Pearson's correlation coefficients (r), significant values are identified with one ($P < 0.05$) or two ($P < 0.01$) asterisks. Values with an $r > |0.35|$ are in bold. Branches = Number of secondary branches, T. rib = Trichomes on the main rib, T. edge = Trichomes on the leaf edges, Area = Leaf area, Height = Total plant height, Diameter = Basal diameter of the main trunk

Table 3 Results of mixed linear models to assess the effect of leaf production and trunk growth (increment in main trunk volume), variety (wild vs. cultivated) and second order interactions on flower production in *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst in a common garden. Statisticallly significant variables are in bolds

Response	Source of variation	Statistic	P value
Production of flowers	**Leaf production**	$F_{1,33} = 7.29$	**0.01**
	Trunk growth	$F_{1,33} = 1.79$	0.19
	Variety	$F_{1,33} = 29.81$	**< 0.01**
	Leaf production × Trunk growth	$F_{1,33} = 0.12$	0.71
	Leaf production × Variety	$F_{1,33} = 0.09$	0.76
	Trunk growth × Variety	$F_{1,33} = 0.89$	0.35

selected for clonal propagation. Those interviewed mentioned that they selected plants with bigger and more succulent stems for clonal propagation and previous studies have also related stem morphology to clonal propagation (McKey et al. 2010; Ross-Ibarra 2003). Previous work has shown that toughness and trichomes reduce leaf palatability (Fordyce and Agrawal 2002; Howard 1988), therefore, these traits could have been selected against by growers. The negative phenotypic correlation detected between leaf trichomes and desired traits such as the number of leaves/branches may have also reinforced selection against trichome number in leaves. While previous research suggests that leaf growth and trichome number are plastic traits in chaya (Abdala-Roberts and Parra-Tabla 2005; Parra-Tabla et al. 2004), the differences in these traits between the varieties observed in this study cannot be the result of phenotypic plasticity because both varieties were grown in a common garden and the traits were measured in the two varieties at the same time.

According to the PCA, the number of leaves and branches, the diameter of the main trunk as well as the number of trichomes and toughness had the greatest influence on the first two principal components. For these axes, a few (10%) cultivated plants fell within the range of variation of wild plants. The existence of intermediate phenotypes may be due to relatively recent artificial selection. Some degree of morphological overlap between cultivated and wild relatives has been reported in other crops that occur sympatrically with their wild relatives due to between-variety gene flow facilitated by spatial proximity (Cruz and Casas 2002; Parker et al. 2010; Aguirre-Dugua et al. 2012; Casas et al. 1999; Dzul-Tejero et al. 2014). Although

not properly tested, gene flow between wild and cultivated chaya cannot be completely ruled out. Even though most cultivated chaya plants did not produce pollen or seeds (Ross-Ibarra 2003), in our sample, two cultivated plants (6%) produced apparently viable seeds but no pollen at all. This leads us to think that some pollen flow from wild to cultivated plants is likely. In fact, in an ongoing study, we have seen that the two varieties share their main pollinators and some pollen from wild plants germinates on the stigmas of cultivated plants. Therefore, recent human-mediated selection in addition to gene flow may at least partially explain the existence of intermediate phenotypes in chaya.

Due to the selection bottleneck, in addition to changing mean trait values, we expect trait variation to decline in domesticated plants (Parker et al. 2010). While this holds true for leaf toughness and trichomes in chaya, the opposite occurred for the number of branches and leaves. This may also suggest weak or recent selection by humans on these traits (Parker et al. 2010). Plants are integrated phenotypes where allocation to one function may compromise allocation to another, and this is true for several crop species (Milla et al. 2015; Santos del Blanco et al. 2015). Our results show a negative relationship between leaf and flower production. Therefore, although flower production is not of interest to growers, this trait was probably selected against unconsciously when growers selected plants that produce more leaves.

In conclusion, wild and cultivated chaya show important morphological divergence. Cultivated chaya produced significantly more and softer leaves as well as fewer trichomes, and we suggest that these traits define the domestication syndrome of this crop

species as well as that of others crops with edible leaves (e.g. lettuce; de Vries 1997). The negative phenotypic correlation between desired and undesired traits reinforces human-mediated selective trends. Interestingly, a few cultivated plants fell within the range of variation of wild plants, which is probably due to recent selection or some gene flow between wild plants and fertile cultivated plants. The existence of some cultivated plants that are morphologically similar to wild plants may suggest that chaya is not yet fully domesticated. The fact that most plants exhibit desired traits, ease of cultivation, and nutritional properties suggests that this currently neglected and underutilized crop has a lot of potential to become a large-scale crop and so contribute to food security in the study area and other regions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. Informants gave verbal consent for the information they provided to be used for academic purposes.

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