



## Morphological variation and reproductive biology of *Polaskia chende* (Cactaceae) under domestication in Central Mexico

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The columnar cactus *Polaskia chende*, endemic to Central Mexico, occurs in the wild and in silviculturally managed populations where artificial selection for better edible fruits operates by sparing and favouring desirable phenotypes during vegetation clearance. Wild and managed populations were compared in morphology and reproductive biology to analyse if human selection has influenced phenotypic divergence, if it has modified breeding systems and if reproductive isolation exists between them. Fruits from managed populations were larger ( $6.36 \pm 0.17 \text{ cm}^3$ ), heavier ( $19.62 \pm 1.32$ ), sweeter ( $9.07 \pm 0.25$  °Brix), and with thinner peel ( $2.09 \pm 0.13 \text{ mm}$ ) than those from wild populations ( $5.77 \pm 0.18 \text{ cm}^3$ ,  $15.73 \pm 0.85 \text{ g}$ ,  $8.23 \pm 0.31$  °Brix, and  $2.58 \pm 0.14 \text{ mm}$ , respectively). Both population types bloom synchronously, show diurnal anthesis, with bees as the most likely pollinators, and have breeding system predominantly self-incompatible. Spatial and temporal barriers to pollen exchange between wild and managed populations are unlikely, and morphological differences would be related with an ongoing process of artificial selection.

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### Introduction

Mexico is the main centre of diversity of columnar cacti in the world, with nearly 70 species of these plants occurring in its territory (Bravo-Hollis & Sánchez-Mejorada, 1991; Valiente-Banuet *et al.*, 1996). In turn, with 20 species, the Tehuacán-Cuicatlán Valley is one of the richest zones in columnar cacti of Mexico. In this region, columnar cacti have been utilized as food by indigenous cultures since the first phases of human occupation of the area (MacNeish, 1967). At present, useful products of these plants are commonly obtained from gathering in wild populations, but species such as *Myrtillocactus schenkii* (J. Purpus) Backeb., *Pachycereus hollianus* (F.A.C. Weber)

Buxbaum, *P. marginatus* (DC) Britton & Rose, *Polaskia chende*, *P. chichipe* (Gosselin) Backeb., *Stenocereus pruinosus* (Otto) F. Buxbaum, *S. stellatus* (Pfeiffer) Riccob. and *S. treleasei* (F. Vaupel) Backeb. are also cultivated in home gardens (Casas *et al.*, 1999a). In addition, Casas *et al.* (1999a) documented that indigenous peoples of the Tehuacán Valley practise silvicultural forms of management of wild populations of columnar cacti *in situ*. Such management regime consists in letting stand individuals of species such as *Escontria chiotilla* (F.A.C. Weber) Rose, *Myrtillocactus geometrizans*, *M. schenkii*, *Pachycereus hollianus*, *P. marginatus*, *Stenocereus stellatus*, *S. pruinosus* (Otto) F. Buxbaum, *Polaskia chichipe* and *P. chende* and even of giant columnar cacti such as *Pachycereus weberi* and *Neobuxbaumia* spp. during clearance of vegetation for agriculture and other purposes. Also, people promote the abundance of the spared individuals through vegetative propagation of their branches or by protecting and transplanting seedlings and young plants of the desirable plants. People not only choose useful plants to spare and promote at species level, but also particular phenotypes of those species which show outstanding attributes (in flavour, colour, texture, among other characteristics) of their useful parts. As a result of this form of management, the plant community managed may be more abundant in the species and phenotypes spared than the natural wild populations.

Management *in situ* may involve artificial selection (artificial selection *in situ*) that may have consequences in altering both phenotypic and genotypic frequencies in the managed populations. It has been documented that in species such as *Stenocereus stellatus* (Casas *et al.*, 1999b), *S. pruinosus* (Luna 1999), and *Escontria chiotilla* (Arellano, 2001), these forms of management have influenced a significant morphological and, presumably, genetic divergence between wild and managed *in situ* populations. Such divergence has been considered as domestication process since it is an expression of evolution guided by human influence (Casas & Barbera, *in press*; Casas *et al.*, 1999 b). Domestication under silvicultural management appears to be a common process in a number of plant species among Mesoamerican human cultures but it is until recently when this process has been evaluated (Colunga *et al.*, 1986, 1996; Casas & Caballero, 1996; Casas *et al.*, 1997, 1998, 1999a,b; Colunga, 1998; Zárata, 1998; Luna & Aguirre, 2001; Arellano, 2001; Rendón *et al.*, *in press*).

*Polaskia chende*, locally called 'chende', is a species endemic to the Tehuacán Valley, occurring in areas with soils derived from volcanic rocks at 1700–2100 m. According to Bravo-Hollis (1978), these plants are 2–7 m tall, have a well-defined short stem with numerous branches showing 7 or 8 ribs. Flowers, produced at the top of the branches, are 4.5–5 cm long and have a short receptacle tube 1 cm long with a nectar chamber 6 mm in length.

According to the ethnobotanical information obtained in this study, fruits of *P. chende* are greatly appreciated by people because of their good flavour, and are directly consumed or commercialized in regional markets as fresh or dried fruits or as flavoured water, ice cream, and jams. Casas *et al.* (1999a) described this species as gathered from wild and managed *in situ* populations as well as cultivated in home gardens of some villages of the region. However, in this study preliminary fieldwork allowed to observe that cultivation of *P. chende* is rare and that the main form of manipulation is under silvicultural management. The coexistence of wild and manipulated populations in the area makes, therefore, possible to study how artificial selection and domestication processes may operate under silvicultural management.

In studies with *Stenocereus stellatus*, *S. pruinosus*, and *Escontria chiotilla*, Casas *et al.* (1997, 1999b), Luna (1999), and Arellano (2001), respectively, evaluated the influence of artificial selection under management *in situ* and cultivation by comparing the morphology of individuals of these types of populations with wild individuals.

These authors found that individuals of managed *in situ* and cultivated populations generally produce significantly larger fruits with sweeter pulp, thinner peel, fewer spines and more and heavier seeds than individuals of wild populations. Variation in these fruit characters is the basis for traditional systems of classification of variants of both species and for artificial selection by indigenous peoples. The divergence in these characteristics between populations is mainly caused by differential sparing and enhancing in managed environments. In this study, it was, therefore, expected that if this pattern of selection is occurring in *P. chende*, a similar morphological divergence would be found among wild and managed *in situ* populations.

In the case of *Stenocereus stellatus*, Casas *et al.* (1999c) analysed the reproductive biology of wild and manipulated populations in order to examine if sexual reproduction patterns have been modified under domestication and if such modifications could have determined barriers to pollen exchange between wild, managed *in situ* and cultivated populations of that species. These authors found that individuals of all populations studied are self-incompatible, with nocturnal anthesis, and bats as the most probable pollinators. This information indicated that human selection has not modified the reproductive biology of the species and that spatial barriers to pollen exchange are unlikely since bats may fly longer distances than those separating the different types of populations studied. All populations of *S. stellatus* also overlapped the blooming season, indicating that temporal barriers are also improbable. However, the authors found barriers due to pollen incompatibility that are limiting pollen exchange between wild, managed *in situ* and cultivated populations. In order to test if some of these mechanisms are contributing to maintain morphological divergence between wild and managed populations of *P. chende*, this study was directed to document aspects of the reproductive biology of this species.

Bravo-Hollis (1978) described flowers of *P. chende* as having diurnal anthesis but no formal studies on pollination biology of this species have been published until now. Therefore, one of the suppositions to test in this study was that anthesis, activity of flower visitors, and pollination in this species occurs during the daylight in both types of populations.

As mentioned, all species of columnar cacti of the Tehuacán Valley studied until present have been found to be self-incompatible. Therefore, if this pattern is general in columnar cacti, it would be expected that *P. chende* had also self-incompatible breeding system. However, since domestication has modified breeding system in some plant species (see Proctor *et al.*, 1996) it would alternatively be expected that self-incompatibility could have been turned to self-compatible breeding system in manipulated populations.

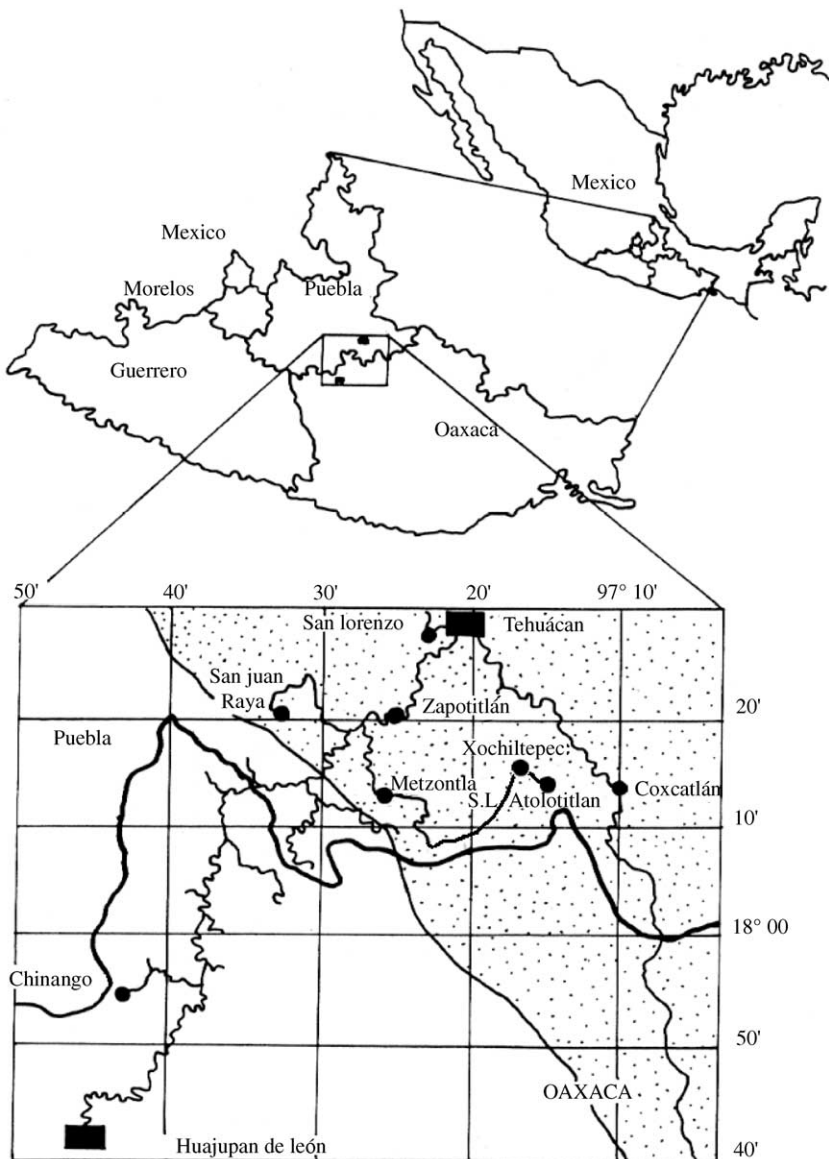
Considering the possibility that artificial selection has caused divergence between wild and managed populations, it is important to analyse the mechanisms that maintain such divergence. One mechanism that in theory could be acting is artificial selection favouring desirable phenotypes and eliminating the undesirable ones. But both spatial and/or temporal barriers to pollen or seed exchange between wild and managed populations could also be intervening. Distance or geographic barriers to pollen or seed dispersers may determine spatial isolation of populations, whereas temporal barriers may be due to differences in blooming season between populations. The main supposition for testing in this part of the study was that human management of populations of *P. chende* has modified aspects of the pollination biology and/or of the flower or fruit production behaviour determining, therefore, some kind of isolation.

Our study was, therefore, aimed at comparing morphology of fruits and reproductive biology of wild and managed populations of *P. chende* in order to analyse and evaluate possible effects of artificial selection on these aspects under silvicultural management.

## Materials and methods

### *Study area*

The biosphere reserve Tehuacán-Cuicatlán is located at the southeast of the state of Puebla and the northwest of the state of Oaxaca, Mexico (Fig. 1). It is a semi-arid region 10,000 km<sup>2</sup> extent, with an annual mean temperature of 21°C and an average annual rainfall of 400 mm (Dávila *et al.*, 1993). It is one of the semi-arid regions of Mexico with highest biodiversity of nearly 3000 vascular plant species (Dávila *et al.*,



**Figure 1.** Study area. (▨) valle de Tehuacán; (▨) La Mixteca Baja; (—) 10 km. The Tehuacán Valley and San Luis Atolotitlán, Puebla, México.

1993) associated in 29 vegetation types (Valiente-Banuet *et al.*, 2000). The region is also rich in human cultures with peoples of seven indigenous ethnic groups living in the area. Predecessors of these peoples inhabited the area for nearly 10,000 years and accumulated a long experience in domestication of plants, as revealed by MacNeish (1967).

#### *Populations of P. chende studied*

The wild population is located approximately 3 km southeast of the village of San Luis Atolotitlán, (18°10'47"N, 97°26'41"W) in the Municipality of Caltepec, Puebla, growing on soils derived from volcanic rocks at 2100 m, as part of a 'chichipera' forest (Valiente-Banuet *et al.*, 2000), characterized by the abundance of *P. chichipe*. Other important species are *Pachycereus marginatus*, *Stenocereus dumortieri* (Scheidw.) F. Buxbaum, *S. pruinosus*, *Myrtillocactus schenkii*, *Opuntia pilifera* F.A.C. Weber, *Agave potatorum* Zucc., *Senecio praecox* (Cav.) DC, *Ipomoea arborescens* G. Don, *I. murucoides* Roemer and Schultes, *Acacia cochlyacantha* Humb. & Bonpl. ex Willd., *A. constricta* Benth., *Mimosa luisana* Brandegee, *Eyisenhardtia polisthachya* (Ortega), and *Ferocactus latispinus* (Haw.) Britton & Rose. In this population, *P. chende* reproduces mainly by sexual means. Vegetative propagation is possible but it was observed to be rather rare. In this population, *P. chende* has a density of 14 reproductive individuals ha<sup>-1</sup>.

The managed *in situ* population is located approximately 1 km west of the wild population, at 1970 m (18°10'53" and 97°26'44") occurring also in volcanic soils, in an area previously utilised for cultivation of maize (12 years ago) which has been recurrently cleared and abandoned. Original wild vegetation was similar to that described above as appears from the remaining plant elements (*Stenocereus stellatus*, *P. chende*, *P. chichipe*, *Myrtillocactus schenkii*, *Ipomoea arborescens*, *I. murucoides*, *Acacia cochlyacantha*, *A. constricta*, *Mimosa luisana*, among the most important). In this population, *P. chende* also reproduces mainly by sexual means. Vegetative propagation of branches both naturally and artificially is also rare, but people used to transplant young plants from place to place in order to optimize the use of the area for agricultural purposes. In this population, density of *P. chende* is 10 reproductive individuals ha<sup>-1</sup>.

#### *Morphological variation*

A total of 20 individuals were randomly sampled per population and 3–5 mature fruits and flowers were collected per individual. Fruit diameter and length were measured with a calliper in order to estimate fruit volume using the formula  $V=2(2\pi r^2/h/3)$ , where  $r$  is the radius and  $h$  the height of fruits ( $\frac{1}{2}$  of the fruit diameter and length, respectively). Peel thickness was also measured with a calliper. The total number of areoles per fruit was counted to evaluate their thorniness. Total fruit weight was measured by an analytical balance. Pulp and peel weight were measured to estimate the proportion of these parts per fruit. Total number of seeds was counted and weighed and the weight of individual seeds estimated. An indirect estimation of the amount of sugar in pulp, measured as total soluble solids, was conducted by a portable refractometer Erma model REF-103 type Brix 0–32% with a resolution of 0.2%. The maximum dimensions of the following flower structures were measured with a calliper: length and diameter of pericarpel; length and diameter of perianth; length and diameter of ovary; style length; diameter and length of the nectar chamber. The number of stigma lobes was counted. The mean value of each character was calculated per individual and compared among populations by one-way ANOVA using SYSTAT 7.0 (SYSTAT, 1997). The matrix of mean values per individual of the two populations

was utilized to perform a principal component analysis (PCA) in order to visualize the multivariate similarity among wild and managed *in situ* individuals. This analysis was performed by NTSYS (Rohlf, 1993), based on a similarity matrix calculated using the Pearson correlation coefficient between characters.

#### *Floral biology*

Records of the events occurring during anthesis were made by direct observations of a sample of nine flowers per population every hour throughout the complete process. Particular attention was concentrated on important events such as the moment of complete opening and closing of flowers, the anther dehiscence and the maximum turgidity of stigma. Photographs of flowers were taken with a fixed camera at intervals of 1 h.

Nectar production was also measured during anthesis by a refractometer. A total of 33 flower buds per population were covered with exclusion bags before opening in order to prevent the consumption of nectar by flower visitors. Amounts of nectar produced by samples of three flowers were measured every hour from the opening (0800 h) to the closing of the flowers (1800 h) by insulin syringes of five volumetric units (1 unit: 0.01 ml). Concentration of sugar was measured in °Brix in a sample of three flowers at the time of major offer of nectar (1200 h).

#### *Flower visitors*

Mist nets were used to capture birds visiting flowers of *P. chende*. A total of three nets per population (of 3 m high by 12 m long, separated by ~100 m), were set up on a random transect of *P. chende* in wild and managed populations. The species captured were photographed and identified, the hour of capture was recorded, and birds were then liberated. Insects were captured with mosquito nets when visiting flowers, and then maintained in 70% ethanol for later identification. Once identified the flower visitors, continuous observations in a sample of six flowers were made on the frequency of visits of each species throughout anthesis. Data were organized at intervals of 1 h.

#### *Breeding systems*

In order to analyse the nature of the breeding system of *P. chende* and if this system has been modified by artificial selection, the following experimental treatments (10 flowers per treatment according to the methods developed by Valiente-Banuet *et al.*, 1996) in field were conducted in the populations studied: (a) non-manipulated self-pollination, flower buds were covered with exclusion bags before anthesis and left until the maturation or abortion of the fruits. The purpose of this treatment was to analyse if self-pollination occurs without the intervention of external agents; (b) manual self-pollination, flowers previously excluded were pollinated manually with their own pollen using paintbrushes and then re-covered with bags. The purpose was to test whether self-pollination is possible with the intervention of external agents and to discard possible physical mechanisms preventing this process; (c) manual cross-pollination, a total of 20 flowers were excluded per population. Ten of them were used as donors of pollen to cross-pollinate by hand 10 other flowers. In all cases, pollen donor and receptor individuals were separated by at least 300 m. This treatment tested the viability of cross-pollination and compared its effectiveness with self-pollination; (d) natural pollination, a sample of 10 flowers was marked and followed until either abortion or successful production of fruits.

*Phenology*

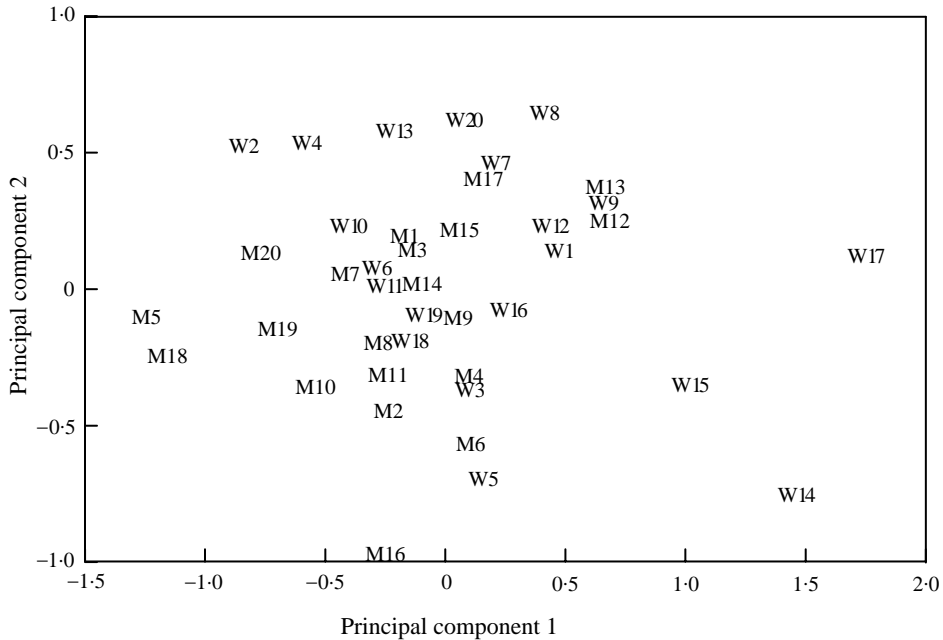
These studies were directed to compare the flowering dynamics in the wild and managed *in situ* populations and to evaluate possible temporal barriers to pollen exchange between these populations. A total of 10 individuals were randomly chosen and labelled per population. Five main ramifications were labelled per individual and the number of terminal branches per ramification was counted. Number of flower buds, flowers in anthesis, immature and mature fruits was counted per terminal branch every 30 days during the reproductive period. The proportion of each phenological stage was calculated per individual and then averaged per population on every recording date. One-way ANOVAs were performed to compare the proportions of phenological stages between populations per recording date.

**Results***Morphological variation*

Table 1 shows that fruits of the managed population were significantly larger, heavier, with a higher amount of sweeter pulp and with thinner peel than fruits of wild individuals, whereas pericarpel was larger. These results were consistent with those from principal component analysis (Fig. 2) since, although individuals were not classified in discrete groups according to their provenance, it is possible to observe that

**Table 1.** Means  $\pm$  standard errors of fruit and flower characters of individuals from wild and managed *in situ* populations of *Polaskia chende* ( $n = 20$  individuals per population)

Character	Wild population	Managed population	<i>F</i>	<i>p</i>
Number of areoles per fruit	10.18 $\pm$ 0.50	10.81 $\pm$ 0.39	0.997	0.335
Fruit size (cm <sup>3</sup> )	5.77 $\pm$ 0.18	6.36 $\pm$ 0.17	5.734	0.022*
Fruit weight (g)	15.73 $\pm$ 0.85	19.62 $\pm$ 1.32	6.151	0.018*
Peel thickness (mm)	2.58 $\pm$ 0.14	2.09 $\pm$ 0.13	6.700	0.014*
Amount of pulp (g)	8.63 $\pm$ 0.97	11.53 $\pm$ 0.53	6.890	0.012*
Pulp/peel weight	1.20 $\pm$ 0.10	1.42 $\pm$ 0.10	2.790	0.103
Number of seeds	602.65 $\pm$ 45.29	657.53 $\pm$ 28.38	1.054	0.311
Individual seed weight (mg)	0.694 $\pm$ 0.020	0.657 $\pm$ 0.012	0.498	0.122
°Brix in pulp	8.23 $\pm$ 0.31	9.07 $\pm$ 0.25	4.523	0.040*
Pericarpel diameter (cm)	1.29 $\pm$ 0.04	1.53 $\pm$ 0.06	10.65	0.012*
Pericarpel length (cm)	2.26 $\pm$ 0.10	2.92 $\pm$ 0.17	10.6	0.012*
Perianth diameter (cm)	6.57 $\pm$ 0.11	6.29 $\pm$ 0.07	4.83	0.060
Perianth length (cm)	3.34 $\pm$ 0.12	3.18 $\pm$ 0.21	0.74	0.430
Ovary length (cm)	0.46 $\pm$ 0.03	0.53 $\pm$ 0.12	0.32	0.600
Ovary diameter (cm)	0.50 $\pm$ 0.04	0.49 $\pm$ 0.03	0.02	0.880
Nectar chamber diameter (cm)	0.38 $\pm$ 0.03	0.34 $\pm$ 0.01	2.24	0.170
Nectar chamber length (cm)	0.79 $\pm$ 0.02	0.73 $\pm$ 0.04	2.09	0.190
Stile length (cm)	2.64 $\pm$ 0.06	2.87 $\pm$ 0.04	9.68	0.010*
Number of stigma lobes	12.20 $\pm$ 0.37	12.80 $\pm$ 0.66	0.62	0.460



**Figure 2.** Projection of individuals of *Polaskia chende* in the space of the first and second principal components; (W), wild individuals; (M) individuals from managed *in situ* population.

most of the wild individuals occupy the left and lower side of the plot, whereas most of the managed *in situ* individuals occupy the right and upper part of the plot. However, it is notorious that some wild individuals were classified along with the main group of managed individuals and vice versa. The most meaningful characters for grouping individuals were fruit size, amount of pulp, peel thickness, the relation between pulp and peel weight and total soluble solids in pulp in the principal component 1, whereas seed weight is the most meaningful in the principal component 2 (Table 2).

#### *Flower biology*

Our study confirmed that anthesis of *P. chende* is diurnal, occurring between 0800 and 1800 h. A marked asynchrony of 3 h in the starting of the flowers opening and closing was observed. Flowers started anthesis at different times between 0800 and 1100 h, and this interval may be present in flowers from an individual. Flowers starting

**Table 2.** Eigenvectors for principal component analysis of morphological variation between wild and managed *in situ* populations of *Polaskia chende*

Character	PC1	PC2	PC3
Number of areoles	0.564	0.411	0.353
Fruit size	-0.813	-0.148	-0.176
Peel thickness	0.734	-0.042	-0.291
Fruit weight	-0.187	-0.535	0.147
Pulp weight	-0.821	0.288	0.046
Pulp/peel weight	-0.876	0.233	-0.050
Seed weight	-0.103	0.853	0.038
Number of seeds	0.022	-0.120	0.933
°Brix	-0.718	-0.152	0.172



anthesis at 0800 h showed a turgid perianth with tepals starting to separate between 0800 and 0900 h, and this process continued until 1200 h, when flowers were completely open. Stigma lobes started to be turgid between 1000 and 1100 h, and at 1200 h most of them were completely separated and receptive (as shown by our successful pollination experiments discussed below conducted at this hour).

Stamens and anthers were generally beneath the stigma with an average distance of  $0.79 \text{ mm} \pm 0.01 \text{ mm}$  ( $X \pm S, n=15$ ) between anthers and stigma. There was an internal group of stamens, generally compacted around the stile, which determined that small flower visitors, such as the Meliponinae (Apidae) bees *Plabeia mexicana* Ayala and *Augochlora smaragdina* Friese, invariably made contact with anthers when introduced into flowers. The external group of stamens was more separated around the stile, but larger flower visitors such as *Apis mellifera* L. (Apinae, Apidae), bumble bees such as *Bombus pennsylvanicus pennsylvanicus* DeGeer, (Bombinae, Apidae) and *Xylocopa mexicana* Cockerell (Xylocopinae, Anthophoridae), as well as hummingbirds invariably touched both anthers and stigma during visits to flowers.

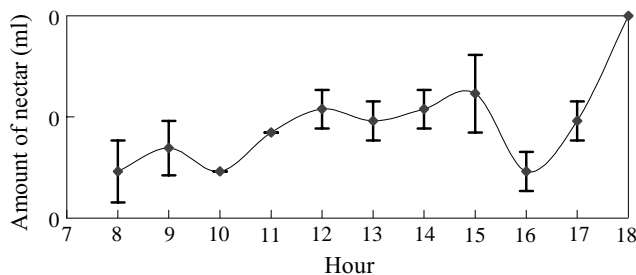
Approximately at 1200 h flower structures showed a granular aspect indicating anther dehiscence. Turgidity of stigma and anther dehiscence occur approximately at the same time. However, considering the asynchrony of the starting of anthesis, these events can be observed occurring between 1200 and 1500 h in different flowers. Approximately at 1400 h, the process of flower closing initiates and it is completed at 1500 h for flowers opening at 0800 h and at 1800 h for flowers opening at 1100 h. Each flower is open approximately 7 h, but in a population, flowers allow the entrance to flower visitors between 0800 and 1800 h, and this behaviour is similar in both wild and managed populations.

#### Nectar production

Nectar production oscillated throughout anthesis (Fig. 3), which is probably due to the asynchrony in the beginning of anthesis in the flowers sampled. Before anthesis there was already a small amount of nectar, and it increased notably at 1100 h. Nectar production was continuous and the maximum production occurred between 1200 and 1500 h. The average sugar concentration was  $25.86 \pm 2.3$  °Brix ( $X \pm S, n=3$ ).

#### Flower visitors

The most frequent visitors of flowers of *P. chende* were *Apis mellifera*, *Bombus pennsylvanicus* and *Xylocopa mexicana* (Table 3). Other visitors recorded are Meliponinae bees, flies, wasps, beetles, mosquitoes and hummingbirds, but, as shown in Table 3, their visits take place before or after 1200–1500 h, when maximum stigma turgidity and anther dehiscence occur. Ants are also common visitors but they interact with flowers as predators, consuming entirely the flower structures. Foraging pattern



**Figure 3.** Mean nectar production throughout anthesis in flowers of *P. chende*. Vertical bars indicate standard errors.

**Table 3.** Flower visitors of *Polaskia chende* and visit frequency throughout the day ( $n=6$  flowers)

Species/Hour	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16
<b>Hymenoptera</b>								
<i>Apis mellifera</i> L.	3	12	18	41	38	15	2	0
<i>Bombus</i> <i>pensylvanicus</i> - <i>pensylvanicus</i> DeGeer	0	1	5	11	10	4	1	0
<i>Xylocopa mexicanorum</i> Cockerell	0	0	0	7	0	1	1	0
<i>Plabeia mexicana</i> Ayala	0	0	1	0	2	3	0	2
<i>Augochlora smaragdina</i> Friese	0	0	0	0	2	3	0	2
<i>Polistes mexicanus</i> Bequaerf	0	0	1	0	0	0	0	0
<i>Brachygastra mellifica</i> Say	0	0	0	0	0	0	0	1
<i>Atta cephalota</i>	0	0	5	0	0	6	3	0
<b>Coleoptera</b>								
Beetle sp. 1	0	2	3	0	0	2	3	2
Beetle sp. 2	0	0	0	0	0	2	0	0
<b>Diptera</b>								
<i>Antrax</i> sp.	0	0	0	0	0	1	1	0
<i>Exoprosopa</i> sp.	0	1	1	0	1	1	0	4
Mosquito sp.1	0	0	0	0	3	4	0	1
Mosquito sp.2	0	0	0	0	0	0	0	1
<b>Avian (Trochilidae)</b>								
<i>Cynanthus sordidus</i>	0	0	1	0	0	0	0	0
<i>Amazilia violiceps</i>	2	1	0	0	0	1	0	0

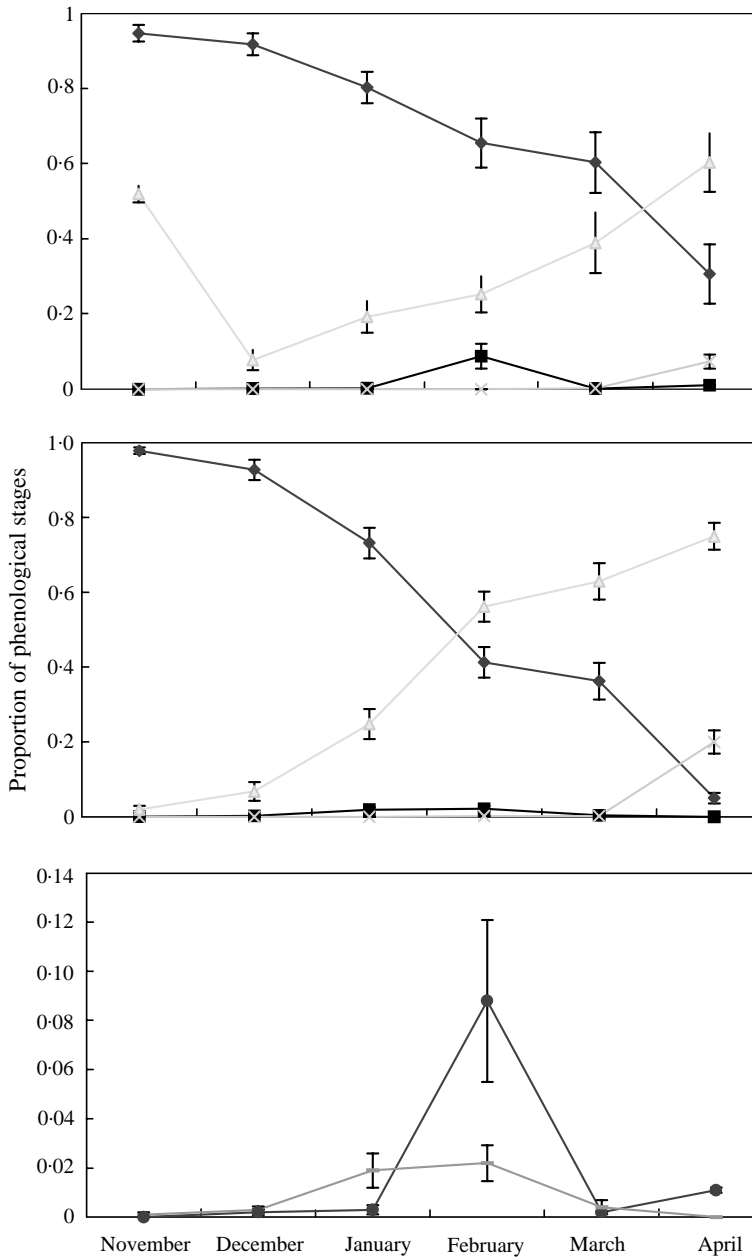
of flower visitors is generally of short flights, visiting flowers of the same individual or other close neighbours, although hummingbirds may fly longer distances.

#### Breeding system

Table 4 shows the results of experiments testing the nature of breeding system. In the two populations sampled, the treatments for non-manipulated self-pollination

**Table 4.** Fruit set percentage in treatments for testing breeding system in wild and managed populations of *Polaskia chende*

Treatment	Flower buds number	Fruit set percentage	
		Wild population	Managed population
Non-manipulated self-pollination	10	0	0
Manual self-pollination	10	10	0
Manual cross-pollination	10	70	80
Natural pollination	10	50	100



**Figure 4.** Phenology of reproductive stages in populations of *P. chende*: (a) Wild population; (b) managed *in situ* population. (◆) flower buds; (■) flowers in anthesis, (▲) immature fruits, (×) mature fruits. (c) Detailed view of flower production in populations of *P. chende* studied. (●) Wild population; (■) managed *in situ* population. Vertical bars indicate standard errors.

determined the abortion of 100% of fruits. This result was similar in the treatments of manual self-pollination, although 10% of the treatments determined successful production of fruits in the wild population. In contrast, treatments for manual cross-pollination determined 70% of successful production of fruits in the wild population

**Table 5.** Mean  $\pm$  standard deviation of the number of flower buds (B), flowers in anthesis (Fl) and fruits (Fr) per branch among wild and managed in situ populations of *Polaskia chende* (asterisk denotes 0.95% significant differences between populations)

Population	Nov			Dec			Jan		
	B	Fl	Fr*	B	Fl	Fr	B	Fl*	Fr
Wild	13.81 $\pm$ 11.40	0 $\pm$ 0	5.29 $\pm$ 9.38	14.53 $\pm$ 11.42	0.02 $\pm$ 0.10	1.34 $\pm$ 2.73	12.98 $\pm$ 7.98	0.01 $\pm$ 0.03	3.61 $\pm$ 5.32
Managed	15.48 $\pm$ 11.64	0.26 $\pm$ 1.83	7.18 $\pm$ 13.54	15.61 $\pm$ 11.41	0.01 $\pm$ 0.03	2.20 $\pm$ 5.42	12.15 $\pm$ 9.35	0.19 $\pm$ 0.38	4.66 $\pm$ 4.85

Population	Feb			Mar			Apr		
	B*	Fl	Fr*	B	Fl	Fr*	B*	Fl	Fr*
Wild	8.55 $\pm$ 4.53	4.21 $\pm$ 7.69	3.77 $\pm$ 4.11	4.69 $\pm$ 3.20	0.002 $\pm$ 0.014	4.04 $\pm$ 5.92	1.01 $\pm$ 0.85	0.008 $\pm$ 0.043	%
Managed	6.22 $\pm$ 6.95	0.18 $\pm$ 0.35	7.47 $\pm$ 6.83	4.76 $\pm$ 6.40	0.01 $\pm$ 0.05	8.32 $\pm$ 8.32	0.37 $\pm$ 0.46	0 $\pm$ 0	2.69 $\pm$ 0.42 3.93 $\pm$ 0.63

and 80% in the managed population. Treatments of natural pollination determined 50 and 100% of successful production of fruits in the wild and managed populations, respectively, but the low production of fruits in the natural pollination treatments of the wild population was due to predation of flowers by ants.

### *Phenology*

The temporal production of flower buds, flowers in anthesis, immature and mature fruits in both populations are presented in Fig. 4. Figure 4(c) shows details of flower production in the two populations. It is notorious that proportion of flowers in anthesis is relatively small per day, compared with other phenological stages. Also notorious is the overlap of flower production, with the flowering peak, although shorter and more pronounced in the wild population, occurring during the second and third week of February in both populations. Mature fruits are available from the third week of March, and the reproductive season finishes by the end of May. Production of buds and flowers per branch are similar in both populations, although the number of fruits is significantly higher in the managed *in situ* population (Table 5).

### **Discussion and conclusions**

Our studies on morphological variation show that there are significant differences in fruits from wild and managed *in situ* individuals. Fruits from managed individuals are in average larger, heavier with more and sweeter pulp and thinner peel. This information is similar to the data found in *Stenocereus stellatus* by Casas *et al.* (1999b,c), *S. pruinosus* by Luna (1999), *Escontria chiotilla* by Arellano (2001) and in several species of *Opuntia* by Colunga *et al.* (1986). These characteristics, along with fewer spines in peel, are the features preferred by people for utilizing fruits of cacti, and for favouring individuals through artificial selection. Favouring these characteristics appears to be, therefore, guiding the evolution under domestication of several species of *Opuntia* and columnar cacti among them, seemingly, *P. chende*. As in all the cases referred to above, the morphological differences found in *P. chichipe* may be, in part, a determined by genetic variation modelled by artificial selection; but also, they may be, in part, a consequence of differences in the environmental conditions where the populations studied occur, especially differences in soils since the managed population is located in an area where agriculture has recurrently been practiced, the last time 12 years ago. To evaluate the contribution of environment and artificial selection in explaining the variation observed, further research would be necessary utilizing a randomized statistical design including more of the scarce populations, as well as quantitative genetics experiments in order to estimate the variance due to both genetic and environmental factors. However, from the multi-variate analysis, it is notorious that individuals from both wild and managed populations overlap in morphological similarity indicating that similar phenotypes can be found in different populations. Also, it is clear that different phenotypes coexist within the same population. This observation suggests that environmental factors alone are not the morphological differences. The main difference at population level consists in that phenotypes with outstanding attributes are more frequent in the managed population, and this would be related with a process of artificial selection.

But the multivariate analysis also indicates that the morphological distinction between wild and managed individuals of *P. chende* is not as strong as in other species (see for a comparison the case of *Stenocereus stellatus* reported by Casas *et al.*, 1999b). Although *P. chende* is very much appreciated by people as a plant resource, its populations and individuals within populations are scarce, and this appears to be

determining that during management *in situ*, artificial selection does not operate as in other species. For instance, the elimination of undesirable individuals is not taking place, and the enhancing of numbers of outstanding phenotypes through vegetative propagation is not as strong as in other species since this form of propagation is not always successful, and it is rather rare in natural habitats. Our data show that the process of morphological divergence between wild and managed populations is incipient. It is important to mention that in the managed population fruit production was higher than in the wild (Table 5) suggesting that although artificial selection could not have been strong, the effect of management of populations is favourable to fruit production. It is still to be clarified if such higher fruit yield is a result of environmental differences or of artificial selection.

Our studies on floral biology confirm that anthesis in *P. chende* is completely diurnal. The maximum production of nectar occurs between 1200 and 1500 h, when anther dehiscence also begins and stigma are receptive. Although individual flowers are open 7 h on average, at population level, considering the asynchrony of anthesis, open flowers are available to visitors nearly 10 h day<sup>-1</sup>. The activity of flower visitors is more intense between 1100 and 1400 h overlapping with the time when events of anthesis crucial for pollination occur.

The most important flower visitors seem to be *Apis mellifera*, the bumble bee *Bombus pensylvanicus*, *Xylocopa mexicanorum*, the Meliponinae bees *Plabeia mexicana* and *Augochlora smaragdina*, as well as the humming birds *Cynanthus sordidus* and *Amazilia violiceps*, since their activity, in terms of visit frequency to flowers, is higher than that of other visitors. The maximum activity of *Apis mellifera*, the bumble bee, and *Xylocopa mexicanorum* coincides with the time the stigmata are receptive and anther dehiscence takes place. As mentioned, these visitors carry out short journeys, visiting in this way several flowers of a single individual. This behaviour favours that pollen of an individual continually arrives to the stigma of other flowers of the same individual. However, the asynchrony of anthesis and the small amount of flowers in anthesis per day apparently favour that flower visitors are forced to visit flowers of different individuals in a given moment allowing continuous pollen exchange between different individuals. Although the behaviour of bees visiting flowers of *P. chende* has not been studied in detail, to have an idea of the distances that these insects would be able to travel the information reported by Janzen (1971) is useful which says that some species of bees of the tribe Euglossinae are able to move within a radius of 23 km. Loading of *P. chende* pollen by bees has not been quantified, but it was observed that *Apis mellifera*, *Bombus pensylvanicus*, and *Xylocopa mexicanorum* load significantly more pollen than other visitors such as flies, Meliponinae bees, mosquitoes and beetles whose role as effective pollinators of *P. chende* is presumably limited. Role of ants as pollinators can be discarded because of their destructive activity in flowers. Summarizing, considering the frequency of visits, the moment in which the visits take place and the amount of pollen loading, *Apis mellifera*, bumble bees, and *Xylocopa mexicanorum* appear to be the most probable effective pollinators of *P. chende*.

Both morphology and behaviour of flowers, as well as floral visitors are similar in wild and managed populations. Therefore, considering that the distance between these populations is ~1 km and that visitors are able to travel this distance in their activity radius, spatial barriers to pollen exchange are unlikely between wild and managed populations.

In both the studied populations, self-pollination is mostly ineffective. Only in one flower of the wild population it was effective. On the other hand, cross-pollination is the predominant breeding system. However, it is important to remark that since cross-pollination has been the only breeding system reported in all studies with columnar cacti of the Tehuacán Valley (Valiente-Banuet *et al.*, 1996; 1997a,b; Casas *et al.*, 1999b,c), this is, therefore, the first report of self-pollination in columnar cacti of this area, and reveals that, although at low frequency, alleles for self-compatibility are

present in wild populations. Morphologic features of flowers, such as the considerable separation of the stigma lobes from the anthers could favour cross-pollination, even in self-compatible individuals. For this reason, self-pollination was recorded only in manipulated treatments.

The results of the experiments showed the occurrence of an out-breeding system in *P. chende* in both wild and managed populations. Human manipulation, therefore, apparently has not modified the characteristic breeding system of wild populations. The information from this study also indicates that temporary barriers for pollen exchange between both populations are unlikely since flowering season occurs simultaneously.

Summarizing, our study indicates the occurrence of artificial selection under *in situ* management determining incipient morphological divergence between wild and managed *in situ* populations. There are neither spatial nor temporal apparent barriers to pollen exchange between these populations, and divergence appears to be in relation with artificial selection.

There are still many aspects to understand about the biology and ecology of this cactus species and their mutualists. To analyse general aspects on fruit yield, it would be important to determine more precisely the amount of fruits that are naturally unsuccessful and the relation of this phenomenon with the population structure and dynamics of floral visitors. For assessing this, it would be necessary to increase the sample size of the treatment of natural pollination and to investigate on population biology of bees. Also, it is necessary to make exclusion experiments to determine the effectiveness of the different bee species as pollinators of *P. chende* in comparison with other species.

According to the people of San Luis Atolotitlán, it is common to find in the area individuals of *P. chende* 'grafted' with *P. chichipe*, *Myrtillocactus schenkii* and *Escontria chiotilla*. These 'grafted' cacti are apparently inter-specific hybrids involving all these species as a complex. It would be important to demonstrate the hybrid nature of such 'grafted' cacti and to test experimentally the viability of crosses between these species. The study of these processes would add new light to the understanding of natural evolution of these plants and domestication of these species considered as a complex.

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