

Pollination biology of *Myrtillocactus schenckii* (Cactaceae) in wild and managed populations of the Tehuacán Valley, México

Fernando Ortíz, Kathryn E. Stoner, Edgar Pérez-Negrón, Alejandro Casas*

Centro de Investigaciones en Ecosistemas, UNAM, Campus Morelia, Apartado Postal 27-3 (Santa María de Guido), Morelia, Michoacán 58190, México

ARTICLE INFO

Article history:

Received 7 March 2009

Received in revised form

26 January 2010

Accepted 29 January 2010

Available online 24 February 2010

Keywords:

Columnar cacti

Domestication

Garambullo

Non-timber forest products

Pollination

ABSTRACT

Myrtillocactus schenckii occurs in thorn-scrub forests of the Tehuacán Valley where people gather its edible fruits. It is also silviculturally managed and cultivated, people selecting plants with higher fruit production. We compared phenology, pollination biology, and breeding systems of wild and managed populations to determine whether humans have influenced changes in these aspects. Flower production was markedly higher in cultivated populations. Flowering peak of all managed populations occurred in December whereas in the wild it occurred in February. Anthesis was diurnal, with *Plebeia mexicana* being the most frequent flower visitor in the wild, *Apis mellifera* and *Tabanus* sp. in silvicultural populations, and *Xylocopa mexicanorum* in cultivated populations. Natural pollination was more successful in cultivated than in wild and silvicultural populations (83.3%, 40%, 26.6% fruits, respectively). Out-crossing was the most successful breeding system in all populations. Self-pollination was higher in cultivated (23.3% successful fruits) than in other populations (3.3% successful fruits in wild, and 10.0% in silviculture populations). Differences in flower production influenced by artificial selection and pollinator type explain different fruit production in managed and wild populations, whereas environmental heterogeneity influences differences in reproductive patterns, limiting pollen flow and contributing to maintaining phenotypic divergence among populations of *M. schenckii*.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Archaeological studies in the Tehuacán Valley, central Mexico have revealed that interactions between people and plants in this region are more than 10,000 years old (MacNeish, 1967), and that several species of flat-padded prickly pear, spherical, barrel, and columnar cacti were among the main plant resources used by humans since prehistory (Smith, 1967). Ethnobotanical studies have documented that currently people of the region still know and use nearly 1600 plant species (Lira et al., 2009), among them 20 native species of columnar cacti mainly used for their edible fruits (Casas et al., 1999a). A total of 14 species of columnar cacti are managed through silvicultural techniques where vegetation is cleared to establish agricultural fields and individual plants are left standing and promoted; eight of these species are also under cultivation in homegardens (Casas et al., 1999a).

Both silvicultural management and cultivation may involve artificial selection in favor of morphological types desirable to humans. For instance, people generally prefer more productive

plants producing larger and sweeter fruits, with fewer spines and thinner peels, and in some species this selection has caused changes in phenotype frequencies of managed populations compared with wild ones (Casas et al., 2007). Likewise, some studies have documented differences in genetic variation, germination and reproductive biology in managed and unmanaged populations, which suggests that artificial selection is causing processes of domestication (Casas et al., 2007).

Myrtillocactus schenckii is appreciated for its edible fruits, and according to Blancas et al. (2009) it is under silvicultural management and cultivation. Artificial selection appears to operate by leaving individuals with favorable phenotypes standing and by planting branches and young plants of desirable individuals from both wild and cultivated populations into the managed stands. These authors found that in cultivated populations fruit size is significantly larger and number of fruits per branch is much higher than in wild and silvicultural managed populations, suggesting that a process of domestication could be operating. However, the question of how such differences could be maintained in coexisting populations are yet to be answered since no information on pollination biology, seed dispersal and gene flow is available. Our study investigates aspects of pollination biology in order to determine whether humans have influenced changes in reproduction of *M.*

* Corresponding author. Tel.: +52 43 22 27 38.

E-mail address: acasas@oikos.unam.mx (A. Casas).

schenckii and to identify possible reproductive barriers between wild, silviculture, and cultivated populations.

Previous studies among columnar cacti with a similar approach were conducted by Casas et al. (1999b) with *Stenocereus stellatus*, Cruz and Casas (2002) with *Polaskia chende*, Otero-Arnaiz et al. (2003) with *Polaskia chichipe*, and Oaxaca-Villa et al. (2006) with *Escontria chiotilla*, in which species phenology, pollination biology and breeding systems were compared. In all these species breeding systems are predominantly self-incompatible and the information suggests that temporal and spatial barriers to pollen flow among populations are unlikely. This consideration has been confirmed by population genetic studies (Otero-Arnaiz et al., 2005; Casas et al., 2006; Ruíz-Durán, 2007; Tinoco et al., 2005), which revealed high levels of gene flow between managed and wild populations of all of the species studied. Authors of these studies suggest that high gene flow continually counteracts the effects of artificial selection which is therefore the main force responsible for maintaining the morphological, physiological and genetic divergences identified. This argument also has been used to explain why the divergence between managed and unmanaged populations is slight and the process of domestication should be characterized as incipient (Casas et al., 2007).

However, in all these species additional studies recorded information suggesting that the problem requires a deeper understanding of reproductive biology for each species. For instance, in *S. stellatus*, Arias-Cóyotl et al. (2006) found that even when wild and managed populations overlap their blooming season, flowers of wild populations are mainly visited by the bat species *Choeronycteris mexicana*, whereas flowers of cultivated plants are mainly visited by bats of the genus *Leptonycteris*. In this case the differential affluence of species of flower visitors is related to their different ability to tolerate habitat disturbance, and these differences may influence partial barriers to pollen flow. In the cases of *P. chende* and *P. chichipe* Cruz and Casas (2002) and Otero-Arnaiz et al. (2003), respectively, reported the occurrence of self-pollination. Otero-Arnaiz et al. (2003) found that the number of individual plants of *P. chichipe* showing self-pollination was significantly higher in cultivated populations. In addition flowering peaks differed between populations suggesting that human management (because of either disturbance or artificial selection) has influenced phenological changes which add other partial barriers to pollen flow. Finally, this same study found that manipulated crosses between individuals of a population were more successful than between individuals of different populations. In sum all of these studies show that a detailed analysis of the reproductive biology of each species is crucial to understanding factors influencing gene flow between populations.

Compared with species like *Stenocereus pruinosus* and *S. stellatus* which are widely cultivated in the region and whose relatively fast growth allows rapid replacement and intense artificial selection, and compared also with species like *P. chende* and *E. chiotilla* which have shown slow growth, difficult management, and relatively little artificial selection, *M. schenckii* can be considered to be under an intermediate degree of management and artificial selection intensity. Therefore, we hypothesize that human management of *M. schenckii* will result in changes in phenology, pollination biology and/or breeding systems similar to those observed in species like *P. chichipe* which is also under an intermediate level of management.

The larger fruits and higher fruit production of cultivated populations of *M. schenckii* compared to wild and silviculture populations (Blancas et al., 2009) could be associated to differences in breeding systems and pollination biology, and the maintenance of differences in such features and phenology, could involve reproductive barriers operating along with artificial selection. Additionally, differences in these reproductive aspects could be

related to heterogeneity in environmental conditions, albeit natural, or associated with human disturbance. For instance, in the case of *S. stellatus*, Arias-Cóyotl et al. (2006) concluded that human disturbance likely affected the differential affluence of flower visitors observed in managed and wild populations. Similarly in *P. chichipe*, different flowering peaks in wild and managed populations were attributed to natural environmental heterogeneity and human disturbance (Otero-Arnaiz et al., 2003). The purpose of our study is to test these hypotheses by documenting and comparing phenology, flower and pollination biology, and breeding systems of wild, silvicultural managed and cultivated populations of *M. schenckii*.

2. Materials and methods

2.1. Species studied

M. schenckii is a species endemic to semiarid areas of Puebla and Oaxaca in central Mexico, growing wild in tropical deciduous and thorn-scrub forests on soils derived from volcanic rocks at elevations of 1300–1900 m. It is commonly called “garambullo” and stands 3–5 m in height, with branches dark green averaging 10 cm in diameter. Blooming season is reported to occur from November to April and fruit production from May to July (Bravo-Hollis, 1978; Blancas et al., 2009). Flowers are small (an open corolla nearly 2.5 cm diameter), white, with diurnal anthesis; pollination has not been described. Fruits are 0.8–1.5 cm long and 0.6–1.5 cm wide, spherical or ellipsoid (Bravo-Hollis, 1978) and highly consumed by birds and ants. Local people consume fruits of this species as food and its stems and branches are used as fodder and for establishing living fences (Blancas et al., 2009).

2.2. Populations studied

The study was conducted in and surrounding the town of San Luis Atolotitlán (Fig. 1), municipality of Caltepec, Puebla, Mexico. Located at an elevation of 1900 m, this region of Central Mexico has a semiarid climate with a mean annual temperature of 18 °C and a mean annual precipitation of 546 mm (García, 1981). The wild population is located approximately 5 km southeast of the village of San Luis Atolotitlán (18°10'28" N and 97°27'16" W), in a thorn-scrub forest where *M. schenckii* and *P. chichipe* are dominant species. *M. schenckii* occurs at a density of 46 individuals per hectare. Other cacti occurring in the area are *S. stellatus*, *Marginatocereus marginatus*, *Ferocactus latispinus*, *Opuntia pillifera*, and *O. tomentosa*, and trees and shrubs such as *Pittocaulon praecox*, *Agave salmiana*, *Mimosa luisana*, *Eyinsenhardia polisthachya*, *Acacia cochlyacantha*, *Ipomoea arborescens*, and *I. murucoides*, are among the more abundant.

The silvicultural managed populations are located approximately 6 km southeast of the village of San Luis Atolotitlán (18°10'41" N and 97°26'46" W), and nearly 2.5 km southwest from the wild population described above. They form part of the vegetation of fallow agroforestry fields which have not been cultivated for three to ten years. *P. chichipe*, *P. chende*, *M. marginatus*, *P. praecox*, *F. latispinus*, *O. pillifera*, *I. arborescens*, and *A. salmiana* also occur in the fallow fields because they were left standing when the fields were cleared for cultivation. Average density of *M. schenckii* in this area is 6 individuals per hectare and our study was conducted in four agroforestry fields separated by 50–700 m.

The cultivated populations are formed by individual plants cultivated within homegardens in the village of San Luis Atolotitlán (18°11'12" N and 97°27'15" W). A typical homegarden in this village consists of approximately 200 m² and frequently contains as many as 60 species of edible, medicinal and ornamental plant species.

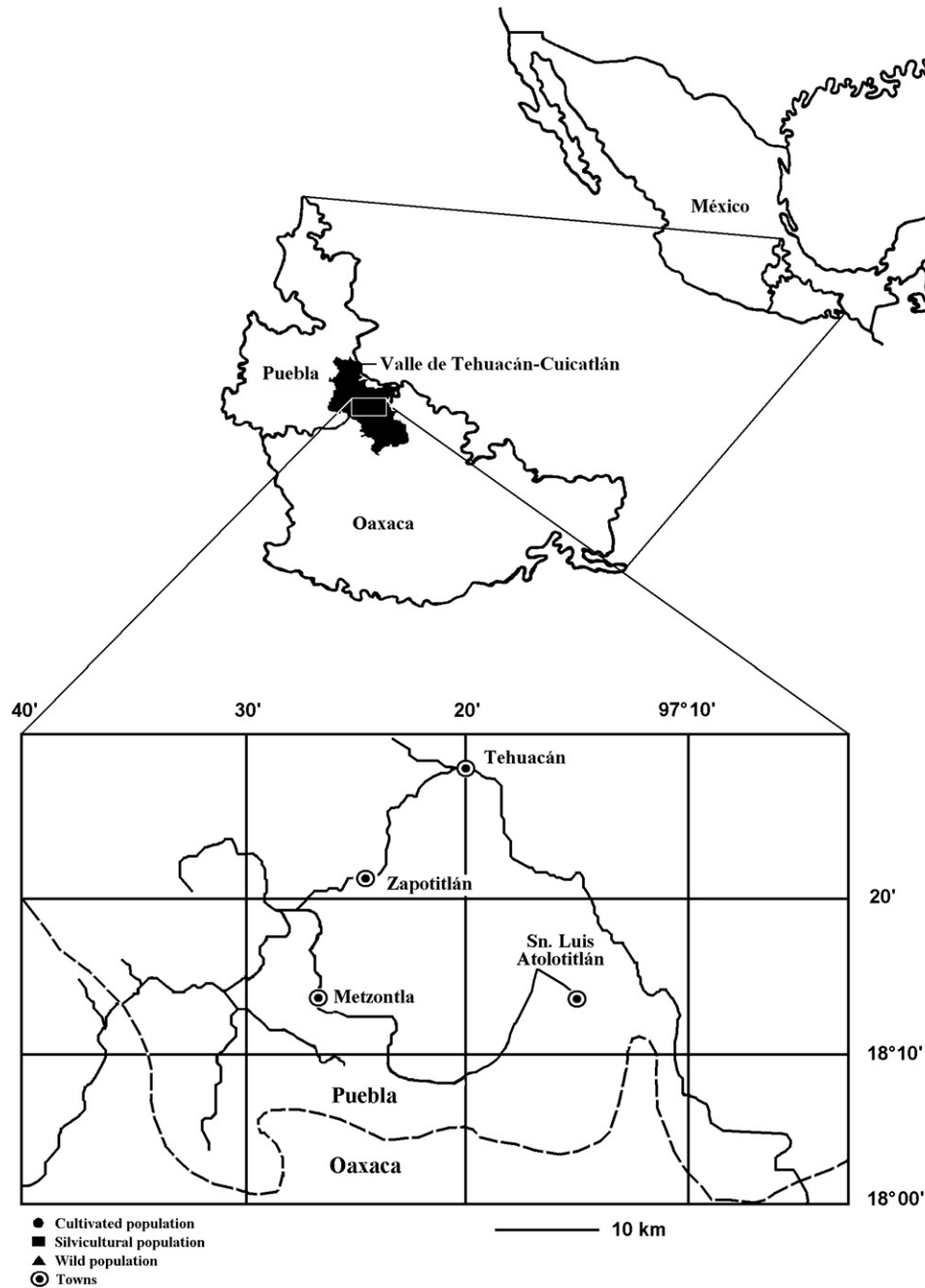


Fig. 1. Location of San Luis Atolotitlán within the Tehuacán–Cuicatlán Biosphere Reserve and the wild, silviculture, and cultivated populations of *Myrtillocactus schenckii* studied.

Other columnar cacti are cultivated in homegardens, the most common being *S. pruinosus* (11 individual plants per plot on average) and *S. stellatus* (7 individual plants per plot on average), followed by *P. chichipe* (5 individual plants per plot on average) and *M. schenckii* (3 individual plants per plot on average). Our study was conducted in seven homegardens plots.

2.3. Phenology

During one year phenological data were collected once a month in samples of 10 individual plants per population. To avoid possible effects of sun orientation affecting phenological development, 20 main branches per individual plant were evaluated—5 of them oriented to the north, 5 to the south, 5 to the east and 5 to the west.

For each individual plant, reproductive phenological stages (flower buds, open flowers, and fruits) were counted for the 20 main branches. The proportions of flower buds, flowers in anthesis, and fruits were estimated per individual plant per month and averaged for the population. Proportion data were transformed and compared with ANOVA and Tukey multiple range tests. Total number of open flowers and fruits per branch during the whole reproductive season were also averaged for each population and compared among populations with a one-way ANOVA.

2.4. Flower visitors and pollinators

In order to elaborate an inventory of flower visitors of *M. schenckii*, all insects visiting 20 flowers of five individual plants per

population throughout three days in late February were captured with entomological nets and fixed in 70% ethanol to facilitate identification. In addition, flowers and their visitors were filmed with video cameras (Sony DCR-TRV27). Six days of filming were conducted in each population with two fixed cameras filming 4–5 flowers continuously during anthesis. For all flower visitors the number and duration of visits at different hours throughout anthesis were recorded and compared with ANOVA. These detailed videos allowed us to distinguish potential effective visits (contact with reproductive structures) from ineffective visits (no contact with reproductive structures), and this parameter was also recorded.

2.5. Floral biology

Events occurring during anthesis were recorded in late December (winter) and early March (spring) through direct observations and video filming of flowers from the three population types. Direct observations were conducted at 1 h intervals during anthesis from a sample of ten flowers from three individual plants per population. Video filming was conducted continually throughout anthesis in 4–5 flowers from two individual plants per population. The time of the following events was recorded: initiation of anthesis, flowers completely open, maximum turgidity of stigma, pollen release, flowers begin to close, and flowers completely closed.

2.6. Breeding systems

Breeding systems were analyzed using samples of 7–10 individual plants from wild, silvicultural managed and cultivated populations. Field experiments were conducted in early March. Except in the treatment testing efficiency of natural pollination (control), all experimental treatments were preceded by covering samples of flowers (30 flowers per treatment) with exclusion bags; after their manipulation flowers were covered again with exclusion bags. The following treatments were conducted: (1) Non-manipulated self-pollination—Flowers covered with exclusion bags were maintained in this condition until fruits matured. This experiment tested whether self-pollination occurs and whether or not its proportion is different in managed and unmanaged populations. (2) Manual cross-pollination—Pollen from 3 to 5 individual plants of a population was collected and used to pollinate flowers of other individual plants whose stamens were previously removed. This treatment was directed to test occurrence of cross-pollination and its efficiency compared to other treatments. (3) Control—Flowers were labeled and left without bags. In all treatments, the total number of successful fruits was recorded and fruit set was estimated with this data. The number of seeds per successful fruit was counted and compared with an average number of ovules of flowers per population estimated from samples of ten flowers per population. These numbers were used to calculate seed-set per treatment.

3. Results

3.1. Phenology

In all populations the production of flower buds started in early June and the blooming season started in early September and finished in mid to late March. Flowering peak occurred in December in the silvicultural managed and cultivated populations, whereas it occurred in February in the wild populations (Fig. 2a). Fruits were available throughout the year in the cultivated populations, whereas they were found between January and June in the

wild and silviculture populations (Fig. 2b). The highest availability of fruits in all populations occurred from February to April and the highest number of fruits was produced in the cultivated populations (Fig. 2b). According to our field observations fruit maturation takes 29–35 days after the flower is pollinated, fruits are indehiscent and do not fall after ripening but they are highly predated by birds and ants as soon as they ripen. During the reproductive season, the average total number of open flowers and fruits, respectively, were recorded per branch as: (1) 82.23 ± 7.62 and 52.75 ± 9.25 in the wild, (2) 93.34 ± 7.82 and 68.63 ± 8.65 in the silvicultural managed, and (3) 266.27 ± 13.27 and 209.54 ± 12.51 in the cultivated. For both flowers and fruits significant differences were found between the cultivated, and the wild and silviculture populations ($F = 58.22$, $p < 0.001$ for flowers and $F = 51.34$, $p < 0.001$ for fruits, respectively), however these last populations did not differ among themselves (Fig. 2).

3.2. Floral biology

In all the populations studied anthesis of *M. schenckii* was predominantly diurnal. In spring average duration of anthesis was 11 h, starting approximately in the interval between 0700 and 0800 h with the separation of the flower tepals. Differences in anthesis starting time appear to be related to the orientation of branches and flowers, anthesis starting first in those flowers receiving solar radiation first. Between 0830 and 0930 h flowers were completely open, and pollen release started about 1200 h and continued until 1330 h; maximum turgidity of stigmas was recorded approximately at 1300 h. Flowers started to close about 1730 h and they were completely closed at 1800 h. Winter anthesis duration was irregular with most flowers being open nearly 11 h as in spring, but a few (two of twenty individual plants observed in spring) were open as long as 29 h. In these last cases anthesis started at 0600 h to 0630 and finished at 1100–1130 h the following day; pollen shedding and stigma turgidity had a similar timing as in spring (Table 1).

3.3. Flower visitors and pollinators

Flowers of *M. schenckii* were mainly visited by carpenter bees (*Xylocopa mexicanorum*), common honey bees (*Apis mellifera*), stingless bees (*Plebeia mexicana*), and a horsefly species (*Tabanus* sp., Diptera). Occasionally flowers were visited by hummingbirds (*Amazilia violiceps*) and hawkmoths (Sphingidae), but in the few visits recorded these animals were observed obtaining nectar without making contact with the reproductive structures of flowers.

The carpenter bee was the most frequent visitor of flowers of *M. schenckii* in the cultivated plants with an average of 46.2 visits per flower per day and a duration of 1.88 ± 0.32 s ($N = 230$). Its visits started at 0900 h and continued until 1600 h with the highest activity being recorded between 1000 and 1400 h (Fig. 3). Carpenter bees posed completely on the reproductive structures of the flowers in 99% of the visits recorded ($N = 230$), allowing full contact with the stigma and anthers impregnating their bodies with pollen, especially their abdomen and feet.

The small stingless bee *P. mexicana* was the most frequent visitor of flowers of the wild population with an average of 33.3 visits per flower per day and duration of 15.31 ± 1.38 s. Its highest activity was recorded between 1100 and 1200 h (Fig. 3). During most of its visits this bee entered the nectar chamber through lateral spaces between the tepals of the flower without making contact with anthers; only in 11.5 times on average (nearly 35% of visits) it made contact with both anthers and stigma. When this bee made contact with anthers, pollen was carried on its legs and abdomen, but

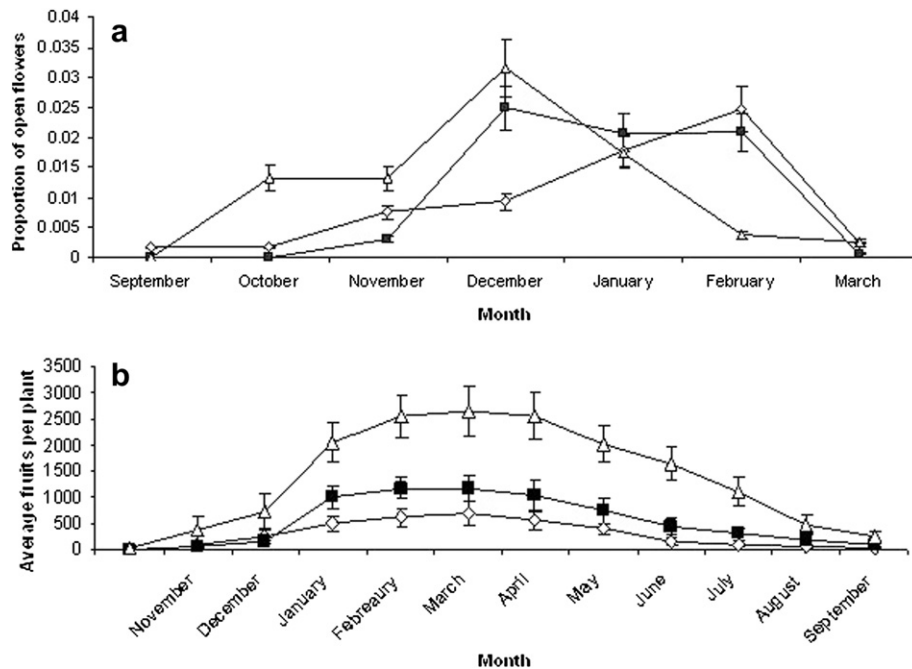


Fig. 2. Blooming and fruit production of the populations of *M. schenckii* studied over time. (a) Proportion of open flowers in relation to the total number of flower buds and fruits per plant, (b) average number of fruits per plant in the populations studied. ◇ Wild population, ■ Silviculture population, Δ cultivated population. Bars are confidence intervals estimated through ANOVA and Tukey multiple range tests.

because of its body size only a small amount of pollen was actually removed. The video films clearly showed that pollen loads of *P. mexicana* were markedly lower than those of carpenter bees. Because of little contact with stigmas and the relatively lower pollen load we consider this species is less effective as a pollinator of *M. schenckii* than the carpenter bee.

A. mellifera was observed visiting flowers of *M. schenckii* in the three population types, but the highest frequency of visits occurred in flowers of the silvicultural managed population (16.8 visits per flower per day) followed by flowers of the cultivated population (8.6 visits per flower per day). The duration of visits per flower per day was 17.16 ± 2.67 s ($N = 84$) in the silvicultural managed population and 5.49 ± 0.75 s ($N = 43$) in the cultivated population. In the silvicultural managed population activity of *A. mellifera* started at 0800 h and their maximum activity occurred at 1100 h (Fig. 4). Honey bees were observed making contact with anthers and stigma in nearly 60% of their visits, whereas in nearly 40% of their visits they only contacted the anthers (Fig. 4).

Tabanus sp. and *A. mellifera* were the most frequent species observed visiting flowers in the silvicultural managed population of *M. schenckii* and were observed between 0800 and 1600 h. Average horsefly visits were 17 per day with duration of 25.28 ± 2.73 s per flower (Fig. 5). During all their visits horseflies posed on both anthers and stigma impregnating their bodies with pollen.

3.4. Breeding systems

The experimental crosses showed that *M. schenckii* is predominantly out-crossing (Table 2). The cross pollination treatment resulted in 80% fruit set with 79 ± 12.9 seeds per fruit in the wild plants, 53% fruit set with 77.8 ± 11.6 seeds per fruit in the silvicultural managed plants, and 50% fruit set with 90.6 ± 13.4 seeds per fruit in the cultivated plants. No significant differences in seed production were identified through a one-way ANOVA ($F = 0.31$, $p = 0.21$). The cultivated plants showed the greatest success for the treatment of non-manipulated self-pollination with 23.3% fruit set

with 53 ± 13.1 seeds per fruit. Nevertheless, this treatment resulted in 3% and 10% fruit set for wild and silvicultural managed populations, respectively, with an average of less than 3 seeds per fruit (differences in seed production through one way ANOVA were significant, $F = 5.32$, $p < 0.001$).

Similar to the cross-pollination treatment, the control treatment was generally successful and varied among populations. Fruit set was 83% in the cultivated population with 87.2 ± 7.8 seeds per fruit, 40% in the wild population with 68.6 ± 13.9 seeds per fruit, and 27% with 65.7 ± 16.5 seeds per fruit in the silvicultural managed population. Number of seeds produced in the cultivated population was significantly higher than in the other populations ($F = 0.15$, $p = 0.04$).

4. Discussion

Blooming season generally overlapped among the wild, cultivated and silvicultural managed populations. In particular January was the month in which estimated flower production was most similar among populations. It is probably during this month when pollen interchange among populations is most likely. Flowering peak, which represents the highest offer of pollen per population, nevertheless, occurred at different times (December in cultivated and silvicultural managed populations, and February in the wild population) increasing the probability that crosses at this moment occur more commonly within a population. This result is similar to that reported for the sympatric species *P. chichipe* (Otero-Arnaiz et al., 2003). The similar behavior exhibited by *M. schenckii* and *P. chichipe*, and the fact that ethnobotanical information by Blancas et al. (2009) does not identify a particular preference or selection in favor of a specific interval of blooming time of *M. schenckii*, supports the hypothesis by Otero-Arnaiz et al. (2003) that phenological differences are associated with natural environmental heterogeneity and these would only be magnified by human disturbance. Environmental heterogeneity, whether a result of human-induced or natural disturbance, or a combination of these

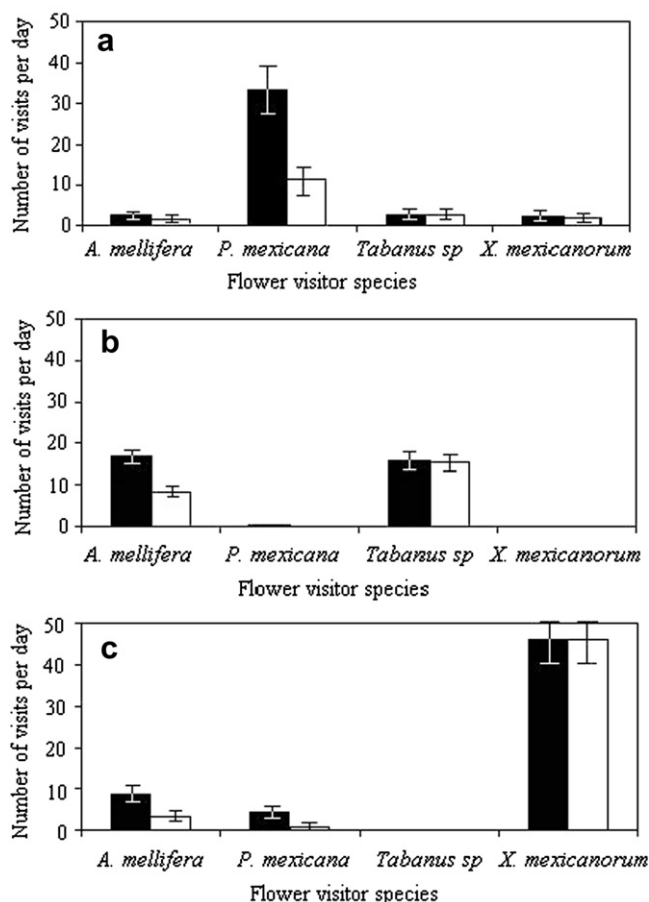


Fig. 3. Average number of visits in the wild (a), silviculture managed (b) and cultivated (c) populations studied. Bars represent confidence intervals estimated through ANOVA and Tukey multiple range tests. ■ Total number of visits. In wild population $F = 65.01$, $DF = 3$, $p < 0.001$; in silviculture population $F = 41.99$, $DF = 3$, $p < 0.001$; in cultivated population $F = 68.43$, $DF = 3$, $p < 0.001$. □ Visits in which there were contact with anthers and stigma (effective visits). In the wild $F = 14.54$, $DF = 3$, $p < 0.001$; in silviculture population $F = 44.46$, $DF = 3$, $p < 0.001$; in cultivated population $F = 91.50$, $DF = 3$, $p < 0.001$.

factors, is a major factor affecting the differences in flowering peaks we observed in *M. schenckii* and likely function as partial barriers to pollen flow among populations.

Anthesis occurred similarly in all populations and approximately at the same time, with variations within and between populations being determined mainly by the incidence of solar radiation. Pollen release and the highest stigma receptivity occurred between 1200 and 1500 h, representing the most crucial time for pollination of *M. schenckii*. This period coincides with the time of highest activity of *X. mexicanorum*, and also partially with

Table 1

Timing of flower anthesis of *M. schenckii* in spring and winter ($N = 28$ flowers spring, 6 flowers winter).

Event	Winter (h)	Spring (h)
Anthesis begins	06:00–06:30	07:00–08:00
Flowers completely open	09:00–10:00	08:30–09:30
Pollen release	12:00–13:30	12:00–13:30
Maximum turgidity of stigma	13:00–14:30	13:00–14:30
Starting of flower closing	10:00–10:30 ^a	17:30–18:00
Flowers completely closed	11:00–11:30 ^a	18:00–18:30
Total duration of anthesis	29	11

^a Day after anthesis initiates.

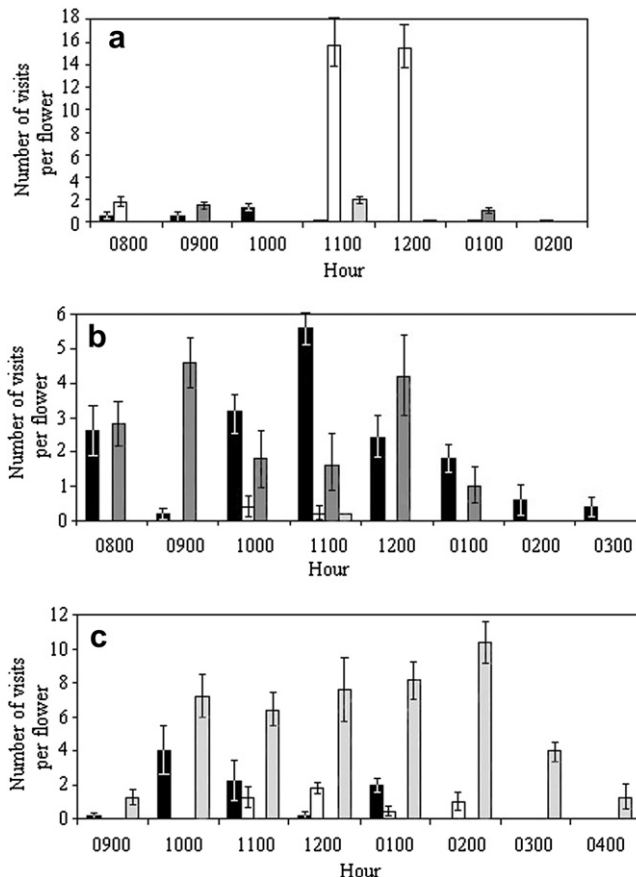


Fig. 4. Frequency of insect visits over time to flowers of (a) silviculture managed (b) and cultivated (c) populations of *M. schenckii*. ■ Common bee (*Apis mellifera*), □ Melliponinae bee (*Plebeia mexicana*), ■ Horsefly (*Tabanus sp.*), □ Carpenter bee (*Xylocopa mexicanorum*). Bars represent confidence intervals estimated through ANOVA and Tukey tests.

activity of *P. mexicana* and *Tabanus sp.*, but not with activity of *A. mellifera* which is more active earlier.

In spring, anthesis occurred during 11 h whereas in winter it took as long as 29 h, particularly on cloudy and cold days. Since flowers initiate anthesis asynchronously according to variations in their orientation and the time they start to receive solar radiation, it is possible to hypothesize that differences in anthesis duration between spring and winter have a similar principle (probably differences in light and temperature), but this hypothesis is yet to be tested. A similar pattern is found in *P. chichi* which opens its flowers 13 h in spring and 28 h in winter (Otero-Arnaiz et al., 2003). In *P. chichi* this pattern allows the possibility of effective

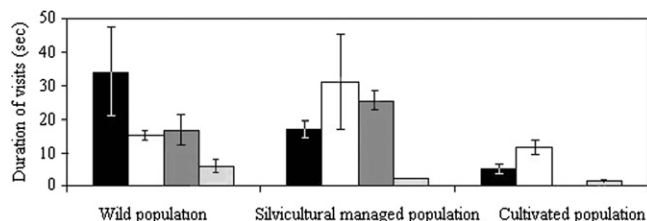


Fig. 5. Duration of insect visits recorded in the populations studied. ■ *Apis mellifera*, □ *Plebeia mexicana*, ■ *Tabanus sp.*, □ *Xylocopa mexicanorum*. Bars represent confidence intervals estimated through ANOVA and Tukey multiple range tests. In wild population $F = 5.46$, $DF = 3$, $p = 0.001$; in silvicultural population $F = 1.92$, $DF = 3$, $p = 0.127$; in cultivated population $F = 31.28$, $DF = 3$, $p < 0.000$.

Table 2

Fruit set (%) and seed production (average number \pm S.E.) in experimental treatments of pollination among wild, silviculture and cultivated populations of *M. schenckii*. $N = 30$ for each treatment within each type of population.

Treatment	Successful fruits (percentage) (seeds produced \pm S.E.)		
	Population		
	Wild	Silviculture	Cultivated
Control	40% (68.6 \pm 13.9)	26.7% (65.7 \pm 16.5)	83.3% (87.2 \pm 7.8)
Cross-pollination	80% (79 \pm 12.9)	53.3% (77.8 \pm 11.6)	50% (90.6 \pm 13.4)
Non-manipulated self-pollination	3.3% (1 \pm 0)	10% (4.6 \pm 2.7)	23.3% (53 \pm 13.1)

nocturnal pollination. The possibility of nocturnal pollination in *M. schenckii* cannot be entirely ruled out until further research is conducted.

The study of breeding systems revealed that cross pollination was the more effective system in terms of both fruit set and seed set for all three populations. Additionally, it is interesting that cultivated individual plants had greater fruit set and produced more seeds by self-pollination than the other populations. The relatively higher success of self-pollination in cultivated plants constitutes an advantage in the production of fruits and seeds even in periods of scarcity of pollinators, and probably this has been a character favored by people through artificial selection under cultivation. A similar situation has been reported for *P. chichi*pe (Otero-Arnaiz et al., 2003). In the case of *M. schenckii* it is important to point out that the morphometric study by Blancas et al. (2009) found that cultivated populations produce significantly more fruits per branch than wild and silvicultural managed populations. This information was confirmed in our study, revealing that people have favored more productive individual plants and, at least in part, this characteristic could be associated with self-pollination and for this reason these individual plants are more abundant under cultivation. But our study also found that cultivated plants produce markedly more flowers per branch during the whole reproductive season, which supports the information that cultivated plants are significantly more productive.

Natural pollination (control treatment) was significantly more successful in the cultivated individual plants (83.3% fruit set and nearly 90 seeds per fruit). This high success is likely the result of a higher number of visits of *X. mexicanorum* in this plant population. In the wild population both fruit and seed production were significantly lower than in cultivated population (Table 2) and production was even lower in the silvicultural managed population. In the wild population visits of *P. mexicana* were the most frequent, whereas in the silvicultural managed population the most frequent visitors were the horsefly and *A. mellifera*. This information suggests that *P. mexicana* is a more effective pollinator than *Tabanus* sp. and *A. mellifera*. Although our observations and video filming indicate that *P. mexicana* is less efficient since they do not touch reproductive structures in many of their visits, their significantly higher number of visits could explain their higher effectiveness.

It appears clear that *X. mexicanorum* (apparently the most effective pollinator among the flower visitors recorded in this study) preferably visits the cultivated population with almost no visits to the other populations. This is likely the main cause explaining the different reproductive success between the populations studied, since although cultivated plants produce more flowers, their high fruit production is possible because flowers are visited by *X. mexicanorum*. The number of visits of *X. mexicanorum* is lower in the wild population and even lower in the silvicultural managed population. The density of flowers in the cultivated population is markedly higher than in the wild population and in this population the density of flowers is higher than in the

silvicultural managed population. This pattern suggests the hypothesis that *X. mexicanorum* prefers to visit areas with greater resource availability, but this supposition is yet to be tested. However, the relevant aspect to this study is that such differential affluence of flower visitor types among populations could provide partial barriers to pollen flow which, along with self-pollination could influence together important barriers to pollen flow between wild and cultivated populations.

Although this study identifies some factors affecting pollen flow among the populations of *M. schenckii* studied, preliminary data on population genetics has identified high levels of gene flow between these populations (César Maldonado and Alejandro Casas unpublished data). It is still necessary to determine what other factors are affecting genetic structure among these populations and to evaluate the importance of seed dispersal and the movement of vegetative propagules by humans in maintaining connectivity.

Larger fruit size and higher fruit production per branch are main phenotypical features favored by people in cultivated populations. How much these characters are determined by environmental factors or by genetic information is still an unanswered question. According to Blancas et al. (2009), the coexistence of individual plants expressing different fruit size and productivity within a single homegarden suggests that environmental conditions are not the only determinant of plant reproductive success. According to Blancas et al. (2009) people select more productive individual plants to be planted in homegardens, and according to our data these plants produce significantly more flowers that become fruits due to a combination of self-pollination and the more frequent visits of *X. mexicanorum*. The artificial selection practiced by people is crucial in explaining the phenotypical divergence characterizing the incipient domestication process in *M. schenckii*. But in addition, the partial reproductive barriers identified in our study, may contribute to explain processes limiting genetic interactions among the managed and the unmanaged populations. The balance between artificial selection, gene flow, and reproductive barriers is crucial to analyze how domestication processes operate in areas like Mesoamerica where wild and managed population of hundreds of plant species coexist. This study aspires to contribute to a better understanding of this delicate balance.

Acknowledgements

The authors acknowledge support received from the Consejo Nacional de Ciencia y Tecnología, México (CONACYT projects 2002-C1-0544 and 103551), as well as the Dirección General de Asuntos de Personal Académico, Universidad Nacional Autónoma de México (DGAPA, UNAM projects IN220005 and IN219608). We thank Dr. Alfonso Valiente-Banuet for his criticism to an earlier version of the manuscript, and the people of San Luis Atolotitlán for their support and permission to conduct research on their land. Bees were identified by Dr. Ricardo Ayala from the Instituto de Biología at the Universidad Nacional Autónoma de México. We are grateful for technical support provided by H. Ferreira, and A. Valencia García. A UC MEXUS Visiting Scholar Fellowship provided financial support to KES during the preparation of this manuscript.

References

- Arias-Cóyotl, E., Stoner, K.E., Casas, A., 2006. Effectiveness of bats as pollinators of *Stenocereus stellatus* (Cactaceae) in wild, managed *in situ*, and cultivated populations in La Mixteca Baja, central Mexico. *American Journal of Botany* 93, 1675–1683.
- Blancas, J., Casas, A., Lira, R., Caballero, J., 2009. Traditional management and morphological patterns of *Myrtillocactus schenckii* (Cactaceae) in the Tehuacán Valley, central Mexico. *Economic Botany* 63, 375–387.

- Bravo-Hollis, H., 1978. Las cactáceas de México, vol. 1. Universidad Nacional Autónoma de México, México.
- Casas, A., Caballero, J., Valiente-Banuet, A., 1999a. Use, management and domestication of columnar cacti in south central México: a historical perspective. *Journal of Ethnobiology* 19, 71–95.
- Casas, A., Caballero, J., Valiente-Banuet, A., Rojas-Martinez, A., Dávila, P., 1999b. Reproductive biology and the process of domestication of the columnar cactus *Stenocereus stellatus* in Central Mexico. *American Journal of Botany* 86, 534–542.
- Casas, A., Cruse, J., Morales, E., Otero-Arnaiz, A., Valiente-Banuet, A., 2006. Maintenance of phenotypic and genotypic diversity of *Stenocereus stellatus* (Cactaceae) by indigenous peoples in Central Mexico. *Biodiversity and Conservation* 15, 879–898.
- Casas, A., Otero-Arnaiz, A., Pérez-Negrón, E., Valiente-Banuet, A., 2007. *In situ* management and domestication of plants in Mesoamerica. *Annals of Botany* 100, 1101–1115.
- Cruz, M., Casas, A., 2002. Morphological variation and reproductive biology of *Polaskia chende* (Cactaceae) under domestication in Central Mexico. *Journal of Arid Environments* 51, 561–576.
- García, E., 1981. Modificaciones al sistema de clasificación climática de Köpen para adaptarlo a las condiciones de la República Mexicana. Instituto de Geografía, Universidad Nacional Autónoma de México, México.
- Lira, R., Casas, A., Rosas, R., Paredes, M., Pérez-Negrón, E., Rangel-Landa, S., Solís, L., Torres, I., Dávila, P., 2009. Traditional knowledge and useful plant richness in the Tehuacán-Cuicatlán Valley, Mexico. *Economic Botany* 63, 271–287.
- MacNeish, R.S., 1967. A summary of subsistence. In: Byers, D.S. (Ed.), *The prehistory of the Tehuacan Valley. Environment and Subsistence*, vol. 1. Universidad de Texas Press, Austin, Texas, pp. 290–309.
- Oaxaca-Villa, B., Casas, A., Valiente-Banuet, A., 2006. Reproductive biology in wild and silvicultural management populations of *Escontria chiotilla* (Cactaceae) in the Tehuacán Valley, Central México. *Genetic Resources and Crop Evolution* 53, 277–287.
- Otero-Arnaiz, A., Casas, A., Bartolo, C., Pérez-Negrón, E., Valiente-Banuet, A., 2003. Evolution of *Polaskia chichi* (Cactaceae) under domestication in the Tehuacan Valley, Central México: reproductive biology. *American Journal of Botany* 90, 593–602.
- Otero-Arnaiz, A., Casas, A., Hamrick, J.L., Cruse, J., 2005. Genetic variation and evolution of *Polaskia chichi* (Cactaceae) under domestication in the Tehuacán Valley, Central Mexico analyzed by microsatellite polymorphism. *Molecular Ecology* 14, 1603–1611.
- Ruiz-Durán, M.E., 2007. Patrones de diversidad genética y proceso de domesticación de *Polaskia chende* (Cactaceae) (Glosselin) Backeb., en el Valle de Tehuacán, Puebla, México. BSc Thesis. Universidad Michoacana de San Nicolás de Hidalgo, Michoacán, Mexico.
- Smith, C.E., 1967. Plant remains. In: Byers, D.S. (Ed.), *The Prehistory of the Tehuacán Valley. Environment and Subsistence*, vol. 1. University of Texas Press, Austin, Texas, pp. 220–225.
- Tinoco, A., Casas, A., Luna, R., Oyama, K., 2005. Population genetics of wild and silvicultural managed populations of *Escontria chiotilla* in the Tehuacán Valley, Central Mexico. *Genetic Resources and Crop Evolution* 52, 525–538.