

RESEARCH PAPER

Pollination system of the *Pilosocereus leucocephalus* columnar cactus (tribe Cereeae) in eastern Mexico

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Keywords

Columnar cactus; nectar-feeding bats; *Pilosocereus leucocephalus*; pollination system.

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Editor

J. Arroyo

Received: 3 December 2008; Accepted: 15 July 2009

doi:10.1111/j.1438-8677.2009.00254.x

ABSTRACT

It has been suggested that there is a geographic dichotomy in the pollination systems of chiropterophilous columnar cacti: in intra-tropical areas they are pollinated almost exclusively by bats, whereas in extratropical areas they are pollinated by bats, birds and bees. However, currently the studies are clumped both taxonomically (mainly Pachycereeae species) and geographically (mainly in the Tehuacan Valley and the Sonoran Desert). This clumping limits the possibility of generalising the pattern to other regions or cactus tribes. Only four of the 36 chiropterophilous cacti in *Pilosocereus* have been studied. Despite the tropical distribution of two *Pilosocereus* species, bees account for 40–100% of their fruit set. We examined how specialised is the pollination system of *P. leucocephalus* in eastern Mexico. As we studied tropical populations, we expected a bat-specialised pollination system. However, previous studies of *Pilosocereus* suggest that a generalised pollination system is also possible. We found that this cactus is mainly bat-pollinated (bats account for 33–65% of fruit set); although to a lesser degree, diurnal visitors also caused some fruit set (7–15%). Diurnal visitors were more effective in populations containing honeybee hives. *P. leucocephalus* is partially self-compatible (14–18% of fructification) but unable to set fruit without visitors. Despite the variation in pollination system, *P. leucocephalus* shows more affinity with other columnar cacti from tropical regions than with those from extratropical regions. Although we report here that a new species of tropical *Pilosocereus* is relatively bat-specialised, this Cereeae genus is more flexible in its pollination system than the Pachycereeae genera.

INTRODUCTION

Columnar cacti are a group of New World tropical and extratropical plants characterised by their cereoid habit. These species are grouped into four tribes (Browningieae, Cereeae, Pachycereeae and Trichocereae) and all belong to the Cactoidae subfamily (Fleming & Valiente-Banuet 2002). The pollination system of columnar cacti – tribe Pachycereeae – has been the focus of several studies (Valiente-Banuet 2002; Valiente-Banuet *et al.* 2002). Most of the Mexican columnar cacti belonging to this tribe (70%) bear typical bat-pollinated flowers (Valiente-Banuet *et al.* 1996); however, a geographic dichotomy in their pollination system has been identified. Pollinator

exclusion experiments revealed that chiropterophilous columnar cacti rely almost exclusively on bats to set fruit in the tropical deserts of central Mexico (Valiente-Banuet *et al.* 2002; Ibarra-Cerdeña *et al.* 2005) and in Venezuela (Nassar *et al.* 1997) – a specialised pollination system – while both diurnal (bees and birds) and nocturnal visitors (bats) effectively pollinate flowers and set fruit in the extratropical deserts of North America – a generalised pollination system (Fleming *et al.* 1996, 2001; Valiente-Banuet *et al.* 2004). Contrasting predictability in the presence of nectar-feeding bats throughout the year has been postulated as the ultimate cause of this dichotomy (Valiente-Banuet *et al.* 1996, 1997a; b). In North America, the reliability of bat visits is lower outside the tropics,

where nectar-feeding bats are seasonal migrants. In contrast, nectar-feeding bats have resident populations in the tropics (Rojas-Martínez *et al.* 1999).

Although several pollinator-exclusion studies on approximately 21 different species support the geographic pattern described above, these studies are highly biased taxonomically and geographically. Studies on columnar cactus pollination systems are highly skewed toward species belonging to the Pachycereeae tribe (16 of 70 species in this tribe). In contrast, in only six of the approximately 100 species of the Cereae columnar cacti (*sensu* Anderson 2001) has the pollination system been studied in detail [*Cereus horrispinus* Backeb., *C. repandus* Mill. (Nassar *et al.* 1997) *Pilosocereus chrysacanthus* (Weber) Byles & Rowley (Valiente-Banuet *et al.* 1997b), *P. lanuginosus* (L.) Byles & Rowley (Nassar *et al.* 1997), *P. royenii* (L.) Byles & Rowley (Rivera-Marchand & Ackerman 2006) and *P. tuberculatus* (Werdermann) Byles & Rowley (Rocha *et al.* 2007)]. All the species of Cereae studied up to now are tropical, and most of them exhibit a bat-specialised pollination system. Of the 16 species of Pachycereeae columnar cacti whose pollination system has been studied, 12 come either from the Tehuacán-Cuicatlán Valley in central Mexico (a tropical region) or from the Sonoran Desert in northwest Mexico and from the southwest USA (an extratropical region; Valiente-Banuet *et al.* 2002). Besides these two regions, there are only a few studies addressing the pollination system of columnar cacti scattered in other regions such as Peru (Sahley 1996), Puerto Rico (Rivera-Marchand & Ackerman 2006) and Venezuela (Nassar *et al.* 1997). This taxonomic and geographic bias limits the possibility of generalising the geographic pattern identified in the Mexican columnar cactus tribe Pachycereeae by Valiente-Banuet *et al.* (1996) to other regions or taxa. The columnar cacti are a highly diverse group of approximately 170 species. The distribution of this group ranges from southern USA to southern South America (Fleming & Valiente-Banuet 2002). Increasing the taxonomic and geographic representativeness of studies on the pollination system of columnar cacti is indispensable to test whether the geographic variation in the specificity of the pollination system is consistent with that of other tribes besides Pachycereeae, and on a continental scale.

The largest genus in Cereae tribe is *Pilosocereus* (36 species *sensu* Anderson 2001). Mainly distributed in eastern South America, and only three of its species [*P. leucocephalus*, *P. polygonus* (Lamarck) Byles & Rowley and *P. purpusii* (Britton & Rose) Byles & Rowley] reach extratropical regions in North America (Zappi 1994). Despite their diversity and abundance, the pollination systems of only four *Pilosocereus* species have been studied in detail: *P. chrysacanthus* (Valiente-Banuet *et al.* 1997b), *P. lanuginosus* (Nassar *et al.* 1997), *P. royenii* and *P. tuberculatus* (Rocha *et al.* 2007). Although all *Pilosocereus* species have chiropterophilous flowers, and the species studied up to now have a tropical distribution, diurnal visitors account for 40 and 100% of fruit set in *P. tuberculatus* (Rocha

et al. 2007) and *P. royenii* (Rivera-Marchand & Ackerman 2006), respectively, indicating a generalised pollination system. Thus, more research is needed to clarify whether generalised pollination systems are more frequent than expected in tropical *Pilosocereus*, or if the previous reports of generalisation in *Pilosocereus* are exceptions.

In this study we asked how specialised is the pollination system in tropical populations of *P. leucocephalus*. In addition, we describe the floral morphology, floral biology and breeding system of three populations of *P. leucocephalus* in eastern Mexico. According to the pattern of variation in pollination system among columnar cacti described above, we might expect *P. leucocephalus* to have a bat-specialised pollination system. On the other hand, the fact that a generalised pollination system is found in some populations of *Pilosocereus* species suggests this type of pollination system is also possible. Therefore, our study is more exploratory than predictive. We replicated our experimental approach in three different populations in the same region but at different altitudes to assess the consistency of the result.

MATERIAL AND METHODS

Study species and sites

Pilosocereus leucocephalus is a columnar cactus endemic to Mesoamerica, ranging from northeast Mexico to Honduras (Guzmán *et al.* 2003). This cactus exhibits pulsed blooming and fructification (two to four peaks) during spring and summer. Flowers are solitary, perfect and typically chiropterophilous (bell-shaped), pinkish to whitish, and release an unpleasant odour (Anderson 2001). Anthesis is nocturnal and flowers open only for one night.

Three populations of *P. leucocephalus* in the state of Veracruz, eastern Mexico, were selected. It is likely that these three populations belonged to a single continuous population in the past and that forest fragmentation and changes in land use separated them several decades ago. Pastures and fields dominate the matrix surrounding the three populations. The San Ignacio (19°35'26" N, 96°58'38" W, 1150 m a.s.l.) and Tenampa (19°36'25" N, 95°51'29" W; 950 m a.s.l.) populations are 2.2 km apart. The physical environment and vegetation of these two populations are similar: they develop in the ecotone between tropical dry forest and tropical montane cloud forest. The mean maximum temperature and total rainfall during the *P. leucocephalus* reproductive season were 27.19 °C and 519 mm, respectively, (mean values, data from the Xalapa climate station). In both populations, soil is scarce. Despite their similarity, the density of *P. leucocephalus* is far greater in Tenampa (600 reproductive cacti per ha) than in San Ignacio (250 reproductive cacti per ha). The third population near Actopan (19°30'21" N, 96°35'47" W, 250 m a.s.l.) is 25 km from San Ignacio and 29 km from Tenampa (Fig. 1). The cactus density in Actopan is similar to that of San Ignacio (200 reproductive cacti per ha). The vegetation is a

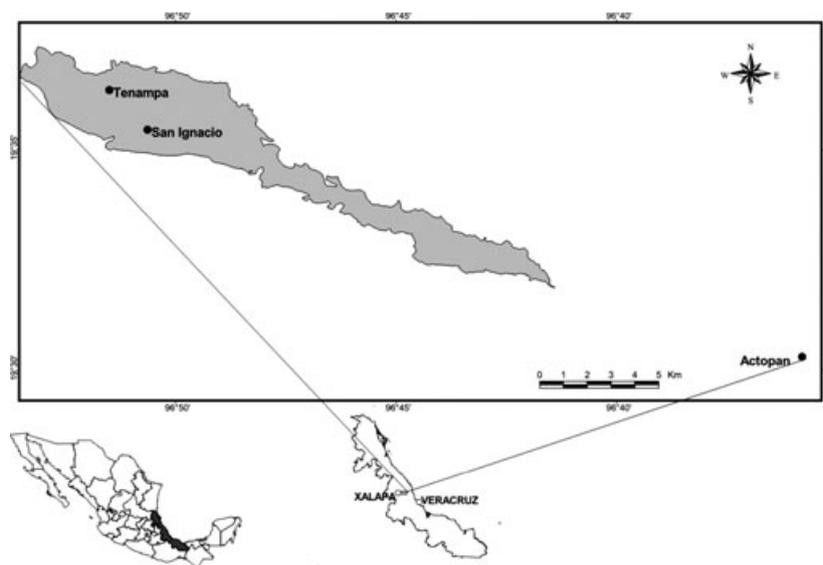


Fig. 1. Location of the three populations of *P. leucocephalus* under study: San Ignacio, Tenampa and Actopan. The grey area delimits a lava flow where the substrate is rocky and soil is very scarce. At the bottom, a map of Mexico with the state of Veracruz in black. Modified from Castillo-Campos (2003).

Table 1. Morphological traits of flowers from three populations of the columnar cactus, *Pilosocereus leucocephalus*. The first column (n) is the number of flowers and plants measured. Data are mean (in mm) \pm 1 standard error. A MANOVA of floral traits was significant; statistics of univariate ANOVAs are shown in the bottom row.

population	n (flowers, plants)	flower length	ovary diameter	perianth width	herkogamy
San Ignacio	31, 16	60.93 \pm 1.44 ^a	20.27 \pm 0.57 ^a	55.30 \pm 0.86 ^{a,b}	17.21 \pm 1.10 ^a
Tenampa	35, 24	64.15 \pm 1.11 ^a	21.40 \pm 0.47 ^a	51.92 \pm 1.18 ^b	16.97 \pm 0.97 ^a
Actopan	35, 10	61.95 \pm 1.99 ^a	27.03 \pm 0.88 ^b	57.06 \pm 1.17 ^a	13.49 \pm 1.08 ^b
statistics		F _{2,98} = 1.1 n.s.	F _{2,94} = 28.47**	F _{2,98} = 5.84**	F _{2,97} = 3.97*

Different letters in superscript indicate statistical differences among populations.

n.s. = No significant difference.

*P < 0.05, **P < 0.01.

tropical dry forest with soil that is much more developed than that of the other two populations. The climate in Actopan is more xeric and seasonal than in San Ignacio or Tenampa: mean maximum temperature and total rainfall during the reproductive season of *P. leucocephalus* were 33.8 °C and 370.6 mm, respectively, (data from the Actopan climate station). There are some low-impact human activities in the study area: goats are occasionally allowed to wander and browse in all three sites, but *P. leucocephalus* do not appear to be damaged by these animals. Artificial honeybee hives have been introduced in Actopan and San Ignacio, but not in Tenampa. For a detailed physical description of the area and its flora see Ortega (1981) and Castillo-Campos (2003).

Flower morphology and biology

During spring and summer 2006 and 2007, total flower length, diameter at the ovary level, perianth width and distance between anthers and stigmas (herkogamy) were measured on the plant at night using Vernier callipers. Sample sizes (number of flowers and plants measured) are shown in Table 1. Since all morphological measure-

ments were made on each flower measured, the response variables are correlated. Therefore, we did a multivariate analysis of variance (MANOVA) to assess differences in flower morphology among populations. As the MANOVA detected significant differences, each morphological trait was compared among populations using univariate ANOVA (Crawley 2007).

Given that only a few flowers were accessible on any given night, nectar measurements were made only in Tenampa and Actopan during the reproductive season in 2006 and 2007. Nectar volume and concentration were estimated using two methods: with several measurements for each flower from a group (repeated nectar measurements), and only once in each flower for several groups of flowers, each group measured at a different time (cumulative nectar measurements). As well as providing an accurate estimate of nectar production, this combination of approaches reveals whether flowers can replenish nectar, whether nectar secretion is stimulated by nectar removal and whether nectar is reabsorbed. Repeated measures were performed every 2 h on six flowers of four different plants in Tenampa, and on nine flowers of six different plants in Actopan. Cumulative measurements

were made every 2 h on three or four flowers in Tenampa, and on four or five flowers in Actopan. A 3-ml syringe was used to extract and measure nectar volume. Nectar concentration was measured using a field refractometer with automatic temperature compensation (ERMA Brix/ATC 113, St. Louis, Missouri, USA). Before and between measuring times, flowers were bagged with mosquito netting. At the same time as nectar was measured, time of flower anthesis and closure, time of pollen release and stigma appearance (colour and turgidity) were recorded. These observations were also made in the San Ignacio population over two whole nights.

Pollen limitation and breeding system

To assess pollen limitation, experiments supplementing pollen were conducted over 2 years, 2006 and 2007, in the three studied populations. Using a small brush, xenogamous pollen from two different donors was placed on the stigmas of flowers from a third plant up to stigma saturation. Another group of flowers was just tagged and used as a control group (open-pollinated flowers). Sample size per treatment per population is shown in Table 2. To evaluate differences in fruit set percentage between the two pollination treatments and populations, a generalised linear model with a binomial error distribution and logit link function was fitted to the corresponding two-way contingency table. The effects of the main factors (treatment and population) as well as their interaction were the linear predictors. It was assumed that if pollen-supplemented flowers set substantially more fruit than open-pollinated flowers the system was pollen-limited. Although some authors encourage pollen supplementation at the plant level in order to avoid confusion with resource re-allocation (Knight *et al.* 2006), this is clearly not possible when working with giant columnar cacti. However, some authors have addressed pollen limitation in columnar cacti using the same approach as us and have provided considerable insight into differences in

Table 2. Fruit set (%) produced under natural conditions (Open) and from flowers supplemented with pollen (Supplementation) in *Pilosocereus leucocephalus* from three populations.

population	treatment		subtotal
	open	supplementation	
San Ignacio	51.8 (27, 11)	57.85 (7, 6)	37.5 ^a (34, 15)
Tenampa	25 (32, 22)	36.84 (19, 14)	24.28 ^b (51, 30)
Actopan	50 (38, 9)	80 (15, 10)	42.42 ^a (53, 12)
subtotal	42.26 ^a (97, 42)	57.81 ^b (41, 30)	

Numbers in parentheses are the number of flowers and plants to which each treatment was applied. Shared superscript letters in subtotals indicate no statistical differences.

response to hand-pollination for the studied cacti (*e.g.* Fleming *et al.* 2001).

In order to assess the breeding system, three hand-pollination treatments were carried out; an addition group of open-pollinated flowers was tagged as a control. The treatments applied were: (1) outcrossing, where pollen grains were taken and placed on the stigma of a different flower on a different plant to stigma saturation; (2) manual self-pollination, where pollen was placed on the stigma of the same flower used as pollen source to stigma saturation; and (3) autonomous self-pollination, where there was no manual pollination and flowers were just bagged. Flowers subjected to the first two treatments were also bagged before and after manipulation. Sample size is shown in Table 3. Statistical analysis and sources of variation considered were as for the supplementation experiment described above.

Hand-pollination treatments (treatments 1–3 described above) were applied between 21:00 h and 24:00 h, and pollen supplementation was performed between 01:00 h and 03:00 h. There was a difference in the time of pollen deposition because the goal of the pollen supplementation was to add more pollen to that previously placed by pollinators. Therefore, we conducted supplementation after midnight when the peak in activity of nectar-feeding bats had passed (Fenton & Kunz 1977). In contrast, for the hand-pollination treatments, we were only interested in the pollen we added, rather than the time elapsed, and were interested in maintaining a defined time interval for the manipulations. In both the supplementation and hand-pollination experiments, open-pollinated flowers were carefully selected; *i.e.* each night a similar number of flowers were selected from those close to the flowers used in the pollination treatments. Hence, the variation associated with time and space was reduced.

Pollinators and pollination system

Bats and birds were caught with three or four mist nets (9 × 2 m) from 20:00 h to 24:00 h or from 06:00 h to 10:00 h, respectively. Bats were caught during the reproductive season of *P. leucocephalus* in 2005, 2006 and 2007, and birds only in 2007. Mist nets were placed near and between flowering cacti. The total sampling effort was 176, 112 and 140 h per net in San Ignacio, Tenampa and Actopan, respectively, for bats, and 48 h per net in each population for birds. The bats and birds caught were identified and freed in the field after collecting pollen grains from their faces using jelly cubes (Beattie 1971). An optical microscope was used to identify pollen grains from *P. leucocephalus* by comparing the samples collected with reference samples. It was assumed that bats and birds caught with pollen from *P. leucocephalus* flowers on them had visited flowers of this cactus.

In order to obtain a more accurate description of the pollination system, both pollinator visitation rate and pollinator effectiveness were assessed. Pollinator visitation rate was recorded with a Sony digital camcorder equipped with

Table 3. Fruit set (%) produced under natural conditions (Open) and by hand-pollination (Treatments) on flowers of *Pilosocereus leucocephalus* in three populations to assess breeding system.

population	treatment				subtotal
	open	outcrossing	hand self-pollination	autonomous self-pollination	
San Ignacio	51.8 (27, 11)	54.5 (33, 19)	14.2 (21, 15)	6.6 (15, 13)	37.5 ^a (96, 58)
Tenampa	25 (32, 22)	60 (10, 9)	18.18 (11, 11)	5.8 (17, 15)	28.57 ^a (70, 57)
Actopan	50 (38, 9)	63.6 (11, 8)	22.2 (9, 7)	0 (8, 7)	42.42 ^a (66, 29)
subtotal	42.26 ^a (97, 42)	57.81 ^a (54, 36)	17.07 ^b (41, 33)	5.1 ^c (40, 35)	

Numbers in parentheses are the number of flowers and plants to which each treatment was applied. Shared superscript letters in subtotals indicate no statistical differences.

an infrared nightshot (Sony Corporation, Tokyo, Japan) in 2006 and 2007. The camera was set on a tripod approximately 2.5 m away from one or two focal flowers. Filming started when anthesis was completed and ended 2.5–4.5 h later. Only one or two flowers were filmed per night per site in order to gain statistical independence. In total, 10, 10 and 11 flowers were filmed in San Ignacio, Tenampa and Actopan at night, respectively. Using the same method, three, five and three different flowers were filmed in San Ignacio, Tenampa and Actopan, respectively, during the day (06:00–09:00 h). On checking the video, the number of legitimate visits (when visitors touched either or both of the reproductive organs) was counted and effective visiting rate per flower per h calculated for diurnal and nocturnal visitors. Data obtained from the film were analysed with an ANOVA; population, visitor guild (diurnal or nocturnal) and its interaction were considered as factors.

Pollinator effectiveness was assessed using pollinator exclusion experiments in the three populations in 2007. Nocturnal visitors were excluded with mosquito net bags before anthesis started and until sunrise (19:30–06:30 h). Diurnal visitors were excluded from sunrise (06:30 h) until the flowers wilted. A group of open-pollinated

flowers (selected as described in the previous section) were tagged and used as controls. Fruit set was recorded and developing fruits were protected with metallic mesh. When ripe, the fruits were collected and seeds counted. Unfortunately, ants were able to take the seeds through the mesh and only a few whole and ripe fruit were obtained. To evaluate differences in fruit set and seed number per fruit between pollination treatments and populations (see Table 4 for sample size), generalised linear models were fitted. Fruit set was the response variable in a generalised linear model with a binomial distribution and logit link function, and seed number per fruit was the response variable in a generalised linear model with a Poisson distribution and log link function. In both models, the pollinator exclusion treatments, populations, and interaction between them were the linear predictors.

All the statistical analyses were run using S-Plus software version 2000. Overdispersion in generalised linear models was corrected by re-scaling to an F test (Crawley 2007). When one factor was statistically significant, either a *post hoc* Tukey's test (for ANOVAs) or an *a posteriori* contrast analysis (for generalised linear models) was done to detect the main differences between factor levels.

Table 4. Fruit set (%) and seed number per fruit (mean \pm standard error) achieved by nocturnal visitors only (nocturnal pollination), diurnal visitors only (diurnal pollination) and the whole guild of visitors (open pollination) in three populations of *Pilosocereus leucocephalus*.

population	fruit set (flowers, plants)				seeds per fruit (fruits)			
	open pollination	nocturnal pollination	diurnal pollination	subtotal	open pollination	nocturnal pollination	diurnal pollination	subtotal
San Ignacio	62.5 (10, 10)	65 (20, 11)	11.11 (9, 6)	42.62 (39, 27)	860 \pm 222 (8)	1701 \pm 209 (5)	787 (1)	1378 \pm 257 ^b (14)
Tenampa	57.7 (26, 15)	57.9 (19, 16)	6.9 (43, 27)	32.95 (88, 58)	857 \pm 195 (7)	1081 \pm 191 (7)	542 \pm 416 (3)	894 \pm 133 ^a (17)
Actopan	20 (25, 9)	33.6 (46, 10)	15.8 (57, 16)	22.65 (128, 35)	1450 \pm 303 (4)	686 \pm 237 (5)	216 \pm 136 (2)	784 \pm 187 ^a (11)
subtotal	42.62 (61, 34)	45.88 (85, 37)	11.92 (109, 49)		1055 \pm 180 ^a (19)	1147 \pm 150 ^a (17)	474 \pm 209 ^b (6)	

Numbers in parentheses are sample sizes. Shared superscript letters in totals indicate there is no significant difference in the number of seeds per fruit. A significant interaction was found between the main factors (exclusion treatment and population) for fruit set. Subtotals for seeds per fruit are mean values for the corresponding treatment.

RESULTS

Flower morphology and floral biology

According to the MANOVA, flower morphology differed among populations ($F_{8,182} = 7.54$, Pillai-Bartlett = 0.5, $P < 0.001$). More specifically, flowers from the three populations did not differ in their length but did vary in ovary diameter, perianth width and level of herkogamy. Ovary diameter in San Ignacio and Tenampa was smaller than in flowers from Actopan. The greatest difference in perianth width was found between Tenampa and Actopan. Perianth width in flowers from San Ignacio was intermediate in size, but was not significantly different from that of the other two populations. Herkogamy was less evident in Actopan than in the other two populations (Table 1).

Flowers in the three populations started anthesis in the early evening (20:00–20:30 h) and had opened completely by 21:00 h in all three populations. At this time, the nectar chambers of the flowers in the three populations contained some nectar, the anthers had already released the pollen and the stigma exhibited receptivity (turgid and light yellow colour). The duration of nectar secretion was 2–3 h longer in Tenampa than in Actopan. Most flowers stopped, or notably reduced, nectar production around 07:00 h in Actopan, but in Tenampa nectar secretion was copious until 09:00–10:00 h. Nectar secretion peaked (approximately 1.3 ml) in these two populations around 01:00 h. Using the cumulative measurement approach, a few traces of nectar were found in flowers at 07:00 h in Actopan, while at the same time a considerable quantity of this reward was found in Tenampa. The scarcity or total absence of nectar in the early morning for flowers that had been bagged overnight in Actopan suggests the

existence of a reabsorption mechanism in this population. The total quantity of nectar produced by the flowers that had been stimulated by repeated removal in the early morning was by far higher than the cumulative quantity of nectar produced by flowers that had been bagged overnight, suggesting nectar production is stimulated by nectar removal in Tenampa and Actopan. Nectar concentration varied slightly throughout the night according to the cumulative nectar measurement, but the concentration of replenished nectar was increasingly diluted when nectar was repeatedly removed (Fig. 2). Flowers in Actopan closed approximately 3 h earlier than in San Ignacio or Tenampa; we noted that flowers remained open longer on particularly cold days.

Pollen limitation and breeding system

The results of the pollen limitation experiment are summarised in Table 2. The analysis conducted to assess pollen limitation revealed a significant effect from both the pollination treatment ($\chi^2_1 = 4.28$, $P = 0.04$) and the population ($\chi^2_2 = 10.12$, $P < 0.01$). The interaction was not significant ($\chi^2_2 = 1.04$, $P = 0.59$). On the whole, pollen supplementation increased fructification success relative to open-pollinated flowers; however, this increase was small (1.3-fold). At the population level, pollen limitation was significantly higher in Actopan than in San Ignacio. Tenampa had intermediate levels of pollen limitation with respect to the other two populations, but the difference was not statistically significant.

The results of the experiment conducted to assess breeding system are summarised in Table 3. The effect of pollination treatment on fruit set was highly significant

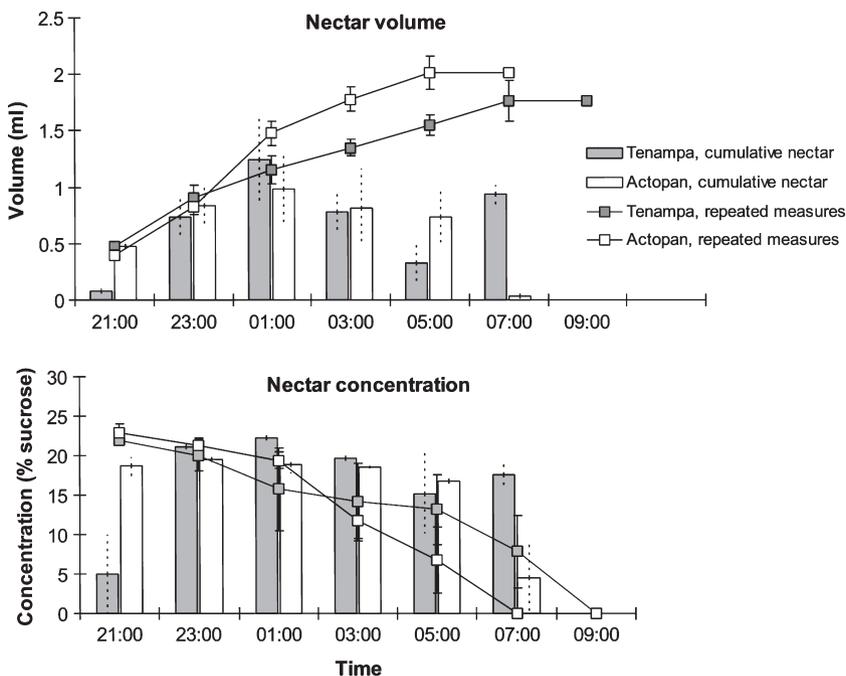


Fig. 2. Mean nectar volume in millilitre (above) and mean concentration in percentage sucrose (below) for flowers from three populations of *Pilosocereus leucocephalus*. Nectar volume and concentration were measured by two methods: several measurements of the same group of flower (repeated measures, lines) and each measurement on different groups of flowers and at different times (cumulative, bars). Cumulative nectar volume, measured repeatedly, is given (graph above) in order to compare the total nectar production with (lines) and without (bars) the stimulus of nectar removal. Both bars and square symbols on lines indicate the mean ± 1 standard error.

($\chi^2_3 = 38.56$, $P < 0.001$). Neither the effect of population ($\chi^2_2 = 5.56$, $P = 0.06$) nor that of the interaction between pollination treatment and population were significant ($\chi^2_6 = 4.47$, $P = 0.61$). On the whole, the fruit set of out-crossed and open-pollinated plants was very similar, and higher than that recorded for the self-pollination treatments. However, up to 22% of manual self-pollinated flowers developed fruit.

Pollinators and pollination system

The relative abundance of nectar-feeding bats with pollen on their faces was almost the same in the three populations: 0.079, 0.098 and 0.1 bats per net per h in San Ignacio, Tenampa and Actopan, respectively. The nectarivorous bat species that had pollen on their faces and were caught most frequently in the three populations were *Choeroniscus godmani* Thomas (11, six and seven bats in San Ignacio, Tenampa and Actopan, respectively) and *Glossophaga soricina* Pallas (two, five and three bats in San Ignacio, Tenampa and Actopan, respectively). *Leptonycteris* bats (*L. curasoae yerbabuena* Miller and *L. nivalis* Saussure) were commonly captured in Actopan (five), rarely in Tenampa (one) and were absent from San Ignacio. The nectar-feeding birds most frequently caught were hummingbirds (*Amazilia tzacatl* De la Llave and *A. yucatanensis* Cabot); however, no pollen grains of *P. leucocephalus* were found on their faces. Pollen from *P. leucocephalus* was detected on six species of bird (the number of individuals captured per species is given in parenthesis): *Empidonax* sp. Cabanis (two), *Campylorhynchus rufinucha* Lesson (one), *Vireo belli* Audubon (one), *Columbina passerina* L. (one) and *Icterus gularis* Wagler (one).

According to nocturnal filming, 87% of the legitimate visits to flowers were by bats and 13% by small moths. During the day, 97% of the legitimate visits were by bees and 3% by large birds. Large hawkmoths and hummingbirds visited the flowers but did not touch the reproductive organs. The legitimate visitation rate (legitimate visits per flower per h) of the diurnal visitors was not different from that of nocturnal visitors ($F_{1,36} = 0.34$, $P = 0.56$). The population factor was highly significant ($F_{2,36} = 0.34$, $P < 0.01$). Visiting rate was clearly different between Actopan (5.69 ± 1.11) and Tenampa (1.61 ± 0.52), however there was no difference between these two populations and San Ignacio (3.9 ± 0.95). The interaction between the main factors, population and visitor guild, was not statistically significant ($F_{2,36} = 2.07$, $P = 0.14$).

The results of the pollinator-exclusion experiment are summarised in Table 4. Variation in fruit set was significantly explained by the interaction of the two factors: exclusion treatment and population ($\chi^2_4 = 9.98$, $P = 0.04$). Nocturnal visitors caused higher fructification success than diurnal visitors in the three populations. However, the effectiveness of diurnal visitors is spatially variable: diurnal visitors had a poor effect on fruit set in Tenampa, a remarkable effect in Actopan and a moderate

effect in San Ignacio. Seed number per fruit differed statistically among exclusion treatments ($F_{2,23} = 2.58$, $P = 0.04$) and populations ($F_{2,23} = 3.98$, $P = 0.03$), however the interaction was not significant ($F_{2,23} = 0.83$, $P = 0.48$). *A posteriori* contrasts indicated that the seed number accounted for by diurnal pollinators was lower than that from nocturnal visitors, or the entire guild. At the same time, the seed yield from nocturnal pollinators and the whole pollinator guild was comparable. Seed number per fruit was higher in San Ignacio than in the other two populations. The interaction between population and pollinator treatment was not statistically significant (Table 4).

DISCUSSION

The results of the pollinator exclusion experiments and filming show that *P. leucocephalus* is mainly pollinated by bats. However, this cactus species is not as dependent on nectar-feeding bats as reported for other tropical columnar cacti in the Pachycereeae tribe [e.g. *Neobuxbaumia tetetzo* (F.A.C.Weber ex K.Schum.) Backeb. (Valiente-Banuet *et al.* 1996), *Pachycereus pecten-aboriginum* Britton & Rose (Valiente-Banuet *et al.* 2004), *Pachycereus weberi* (J.M. Coult.) Backeb. (Valiente-Banuet *et al.* 1997b), *Stenocereus griseus* (Haw.) Buxb. (Nassar *et al.* 1997) and *Stenocereus stellatus* (Pfeiff.) Riccob. (Casas *et al.* 1999)]. In the study area, diurnal visitors, in addition to nocturnal visitors, contribute to the fruit set of *P. leucocephalus* in the three populations, but to variable degrees. Therefore, the pollination system of *P. leucocephalus*, although essentially bat-specialised, exhibits certain deviations toward generalisation, especially in Actopan and San Ignacio. Surprisingly, diurnal visitors did not lead to higher fruit set in the two populations where flower longevity was increased (Tenampa and San Ignacio). Instead, the maximum effectiveness of diurnal visitors was recorded in the population where there were honeybee hives (Actopan and San Ignacio). It is likely that the variation identified in *P. leucocephalus* pollination system is the result of bringing honeybees into these areas. An important role of carpenter bees (*Xylocopa*) in fruit set of tropical *Pilosocereus* has been reported; bees account for up to 40% of *P. tuberculatus* (Rocha *et al.* 2007) fruit set and almost all of the fruit set observed in *P. royenii* (Rivera-Merchand & Ackerman 2006). In contrast, diurnal visitors yielded no fruit in *P. chrysacanthus* in central Mexico (Valiente-Banuet *et al.* 1997b). Although our results indicate that the pollination system of *P. leucocephalus* in eastern Mexico is primarily bat-specialised, thus answering our research question, further research is needed on extra-tropical populations of this species to determine whether its pollination system varies with latitude.

Filming data revealed that there is no difference in visitation rate between diurnal and nocturnal visitors. Although no difference was detected in visitation rate, visitor guilds differed in their effectiveness (fruit set and seeds per fruit). Differences between diurnal and

nocturnal visitors in terms of the size of the pollen load carried, in addition to the loss of receptivity in the stigma during the day, could explain the contrast in effectiveness between diurnal and nocturnal visitors. Also, we have to recognise that the small sample size (especially in diurnal filming) may have influenced the statistical power of the test. However, the pollinator exclusion experiments provide unbiased evidence of pollinator effectiveness and, in turn, of the pollination system. The sample size per population reached in the pollinator exclusion experiment is similar to those reported in previous studies (e.g. Valiente-Banuet *et al.* 1996, 1997a,b; Méndez *et al.* 2005). In fact, the sample size shown in subtotals (Table 4, bottom row) is robust; therefore, our characterisation of the pollination system of *P. leucocephalus* is reliable. Although visiting rate was not effective in predicting effectiveness of visitors, the data obtained by filming are highly valuable in documenting pollinator visiting behaviour. It was this technique that allowed us to discount hummingbirds and large hawkmoths as effective pollinators (they did not touch the reproductive organs while visiting the flowers), and also explains the absence of pollen from *P. leucocephalus* on hummingbirds.

As the results of the hand-pollination experiment suggest, fructification success in *P. leucocephalus* is widely favoured by outcrossing. However, this cactus is able to set fruit by selfing. In other columnar cacti from tribe Pachycereeae self-incompatibility appears to be the rule (Bustamante & Búrquez 2005). However, the few studies that have been done on *Pilosocereus* species show that self-incompatibility is not a generality in this genus. While *P. chrysacanthus* set no fruit by self-pollination (Valiente-Banuet *et al.* 1997b), *P. lanuginosus* (Nassar *et al.* 1997) and *P. royenii* (Rivera-Merchand & Ackerman 2006) set a substantial percentage of fruit by self-pollination (22% and 100%, respectively). It is not currently known whether there is any late self-incompatibility mechanism in *Pilosocereus* cacti such as, for example, seed from self-pollinated flowers failing to germinate. Moreover, although some self-compatibility was verified, the opportunity to set fruits in the absence of pollinators is rare in *P. leucocephalus* (fruit set by autonomous self-pollination was almost null). Hence, the presence of pollinators for either outcrossing or self-pollinating the flowers is clearly needed in all the populations (Table 3). The increase in fructification success after pollen supplementation relative to fruit set in open-pollinated flowers was very low (1.3-fold higher); thus, we should not take this result as strong evidence of pollen limitation. Certainly, other authors have assessed pollen limitation by comparing fruit set in open-pollinated flowers and bagged flowers pollinated with xenogamous pollen. In those studies, a substantial increase in the fruit set of hand-pollinated flowers relative to open-pollinated flowers suggests pollen limitation (Fleming *et al.* 2001). Even though it is less likely that pollen limitation will be detected with this approach, these authors found that hand cross-pollination increases fruit set threefold and twofold in *Stenocereus thurberi*

(Engelm.) Buxb. and *Pachycereus pringlei* Britton & Rose, respectively. Using the approach of Fleming *et al.* (2001) we also did not find any differences in these treatments (Table 3), thus reinforcing our suggestion that *P. leucocephalus* is not under strong pollen limitation.

Interpopulation plasticity in floral morphology and physiology was identified in *P. leucocephalus* in the study area. Flowers from the Actopan population have larger perianth and ovary than flowers from Tenampa or San Ignacio. We suspect that differences in resource availability could have constrained biomass allocation to flowers in San Ignacio and Tenampa. Cacti in San Ignacio and Tenampa grow on poorly developed soils and under conditions of resource limitation, as manifest in cactus size: plants in these populations are shorter than cacti from Actopan, where the soil is well developed. The lower degree of herkogamy found in Actopan relative to the other two populations did not increase self-autonomous pollination. In Actopan, flowers are shorter lived and stopped producing nectar earlier than in the other two populations. Also, flowers from Actopan are able to reabsorb nectar while flowers in San Ignacio and Tenampa could not. This is not surprising because environmental conditions are more xeric in Actopan than in the other two locations. Under xeric conditions it is harder to maintain flower turgidity and nectar supply. It has been demonstrated that nectar production is positively influenced by water availability in other plants (Petanidou *et al.* 1999), and flower longevity is positively affected by temperature in *Pachycereus schottii* (Engelm.) D. R. Hunt (Holland & Fleming 2002). Nectar removal stimulated nectar replenishment in *P. leucocephalus* although this imposes a cost on nectar concentration, as suggested for other plants (Ordano & Ornelas 2004). Experimental verification is needed in order to establish the cause of intrapopulation variation in floral traits.

Considering all of the evidence (*i.e.* results from pollinator exclusion experiments, filming, floral biology and hand-pollination), we can conclude that the *P. leucocephalus* pollination system in eastern Mexico has a greater affinity with that of columnar cacti from the tropical regions of Mexico and Venezuela than with extratropical cacti from the Sonoran Desert. Although diurnal visitors set some fruit in *P. leucocephalus*, the role of these visitors is much less important than it is in extratropical columnar cacti, where diurnal visitors can account for more than half of the total fruit set (Fleming *et al.* 1996). Diurnal visitors are able to set some fruit in other tropical columnar cacti, e.g. up to 40% in *Stenocereus queretaroensis* (F.A.C. Weber) Buxb. (Ibarra-Cerdeña *et al.* 2005) and 35% in *Weberbauerocereus weberbaueri* (K. Schum.) Backeb. (Sahley 1996). For the *Pilosocereus* genus, considering the evidence provided in this and previous studies, we cannot yet determine whether this genus follows the same pattern of variation as that of species of the Pachycereeae tribe. Evidently more studies are needed, including research on *Pilosocereus* species with an extratropical distribution.

Also, it is interesting to reflect on the factors that are producing deviations from exclusively bat-pollinated cacti to moderately generalised pollination systems even in the tropics. Using meta-analytical tools, we found that the pollination systems of all columnar cacti studied to date show a clinal variation, rather than the previously suggested dichotomy. The analysis also suggests that the pattern described in the northern hemisphere might also occur in the southern hemisphere. Unfortunately, statistical support is weak because there are several locations along the gradient for which there are no data and several species that have not been studied, especially in South America where the biggest diversity of Cereaceae species is found (Munguía-Rosas *et al.* in press). The detection of a generalised geographic pattern in columnar cacti and on a continental scale remains a big challenge for researchers studying pollination in columnar cacti. We can see no other way to face this challenge than by increasing the number of studies on both species and regions.

ACKNOWLEDGEMENTS

We are very grateful to JF Ornelas and F Molina-Freaner for their comments on the first draft. Suggestions made by three anonymous reviewers significantly improved the quality of this manuscript. Norma E. Corona helped us with map shown in Fig.1. This project was funded by a BCI scholarship, IDEA WILD and a grant from the División de Posgrado, INECOL to the first author. B. Torales, A. Miranda, A. Flores, E. Testa, JM Pech, ML Quiroz-Cerón and E Ruiz, helped with fieldwork. B Delfosse revised the English.

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