RESEARCH PAPER

Reproductive biology of *Ferocactus recurvus* (Mill.) Borg subsp. *recurvus* (Cactaceae) in the Tehuacán-Cuicatlán Valley, Mexico

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ABSTRACT

- Mexico has one of the highest diversities of barrel cacti species worldwide; however, all are threatened and require conservation policies. Information on their reproductive biology is crucial, but few studies are available. *Ferocactus recurvus* subsp. *recurvus* is a barrel cactus endemic to the Tehuacán-Cuicatlán Valley. Our research aimed to characterise its floral and pollination biology. We hypothesised bee pollination, as suggested by its floral morphology and behaviour, and self-incompatibility, like most barrel cacti studied.

- Three study sites were selected in the semiarid Zapotitlán Valley, Mexico. We examined 190 flowers from 180 plants to determine: morphometry and behaviour of flowers, flower visitors and probable pollinators, and breeding system.

- Flowers showed diurnal anthesis, lasting 2–5 days, the stigma being receptive on day 2 or 3 after the start of anthesis. Flowers produced scarce/no nectar and main visitors were bees (Apidae), followed by flies (Muscidae), ants (Formicidae), thrips (Thripidae) and hummingbirds (Throchilidae); however, only native bees and occasionally wasps contacted the stigma and anthers. Pollination experiments revealed that this species is self-incompatible and xenogamous. In natural conditions, fruit set was 60% and cross-pollination fruit set was 100%. Percentage seed germination resulting from cross-pollination was higher than in the control treatment.

- Our results provide ecological information for conservation programmes to ensure a high probability of breeding and seed production in natural populations of *F. recurvus*.

INTRODUCTION

Cacti have characteristic diverse functional architecture, a variety of habits and highly specialised structures representing morphological, physiological and ecological adaptations to extreme temperatures and low water availability (Gibson & Nobel 1986; Zavala-Hurtado *et al.* 1998; Nobel & Loik 1999; Altesor & Ezcurra 2003). These plants have developed different reproductive strategies that allow them to maximise reproductive success in terms of fruit and seed (Feinsinger 1983; von Herversen & von Helversen 1999; Tschapka & Dressler 2002; Mandujano *et al.* 2010). Among strategies to attract floral visitors, the floral display can vary in size, colour, scent and rewards, attracting a diverse guild of potential pollinators (Faegri & van der Pijl 1979; Moller & Eriksson 1995; Tschapka & Dressler 2002), such as bats, birds and insects. In addition, the breeding system influences the proportion of successful fruits (fruit set), proportion of seeds produced per fruit (seed set) and thus genetic diversity (Sutherland 1986; Golubov & Mandujano 2005). For instance, breeding systems (all aspects of sexual expression that affect the relative contribution to the next generation) in Cactaceae can be self-compatible or self-incompatible, with and without inbreeding depression (Mandujano *et al.* 2010).

Studies on reproductive biology of columnar cacti (Fleming *et al.* 1996; Nassar *et al.* 1997; Valiente-Banuet *et al.* 1997; Casas *et al.* 1999; Cruz & Casas 2002; Molina-Freaner *et al.* 2004; Ibarra-Cerdeña *et al.* 2005; Méndez *et al.* 2005; Dar *et al.* 2006; Ortíz *et al.* 2010) and *Opuntia* species (Mandujano *et al.* 1996, 2010; Negrón-Ortiz 1998; Reyes-Agüero *et al.* 2006) are relatively well represented in the scientific literature, but studies on barrel cacti are scarce (del Castillo 1994; Piña 2000; McIntosh 2002, 2005; Nassar & Ramírez 2004; Jiménez-Sierra 2010), even though they represent a large portion of cactus diversity.

In inter-tropical arid regions, including the Tehuacán-Cuicatlán Valley, Mexico, there are representative species of barrel cactus, particularly species of *Echinocactus* and *Ferocactus*. In this area, Arias *et al.* (2012) reported one species of *Echinocactus* (*E. platyacanthus* Link & Otto) and five species of *Ferocactus* (*F. flavovirens* (Scheidw.) Britton & Rose, *F. robustus* (Pfeiff.) Britton & Rose, *F. haematacanthus* (Salm-Dick) Bravo, *F. macrodiscus* (Mart.) Britton & Rose and *F. recurvus* (Mill.) Borg). According to Cota-Sánchez (2008), the genus *Ferocactus*

is distributed in arid and semiarid regions of central and northern México and the southwest USA. Species of this genus exhibit co-adaptation among their flowers and pollinators, especially bees and bumblebees. Cota-Sánchez (2008) reported that the cactus flowers are often visited by other insects, *e.g.* beetles and ants that feed on their pollen and nectar but have no participation in pollination.

Ferocactus recurvus (Mill.) Borg subsp. *recurvus* is endemic to the Tehuacán-Cuicatlán Valley, and there is currently no information available on its reproductive biology. The aim of this study was therefore to analyse its floral biology, identify flower visitors and possible pollinators, as well as determining the breeding system for fruit and seed production. This information is relevant to understand factors that influence the reproductive biology of this species, as well as other plants of arid environments, which are characterised by a fluctuating environment and limited resources. Information on the early stages of the life cycle in this species is crucial to understand the conditions for survival and recruitment of seedlings and young plants, and to provide criteria to design strategies for conservation of the species and the biotic communities in which it occurs.

MATERIAL AND METHODS

Species studied

Individuals of *F. recurvus* are 10–50-cm high with a spiral arrangement of ribs and radial, curved red spines (Arias *et al.* 2012). Populations are distributed in the semiarid inter-tropical region of Mexico, in the states of Puebla and Oaxaca. Individuals generally grow beneath the canopy of tree and shrub species, which play a role as nurse plants; in particular beneath *Prosopis laevigata* (Humb. *et* Bonpl. ex Willd) M. C. Johnston, *Mimosa luisana* Bandegree and *Parkinsonia praecox* (Ruíz *et* Pav. ex Hook.) J. Hawkins (Valiente-Banuet *et al.* 1991; Altesor *et al.* 1992; Mandujano *et al.* 2002; Valiente-Banuet & Godínez-Álvarez 2002; García-Chávez *et al.* 2014). The blooming period starts in October and mature fruits can be found in March (Arias *et al.* 2012). The reproductive structures (*i.e.* flowers and fruits) develop around the apex of the stem, and have extrafloral nectaries that commonly attract ants (Marazzi *et al.* 2013). According to Arias *et al.* (2012), flowers of *F. recurvus* are hermaphrodite, with purple or yellow tepals 3.4–5.0 cm in length, stamens 0.3–1.4-mm long, stigma 1.6–3.0-cm long, and fruits 2.5–8.0-cm long. Seeds are dark brown, 1.2–1.6-mm long and 0.6–1.0-mm wide.

Study area

We selected three study sites within the research area of the Botanical Garden Helia Bravo Hollis, in the semiarid valley of Zapotitlán in the Tehuacán-Cuicatlán Biosphere Reserve, Mexico (Fig. 1). This is an inter-tropical zone with a summer rainy season, annual mean temperatures from 18 °C to 22 °C and average annual precipitation of nearly 400 mm. The semiarid conditions of the region are caused by the effect of orographic shade from the Sierra Madre Oriental (Zavala-Hurtado *et al.* 1998), and vegetation characteristic of the zone is a xerophytic shrubland, according to Rzedowski (1978).

Floral morphometry

In January 2014 we selected 30 individual plants, and one flower was collected from each plant at two sites in the study area. The flowers were labelled and stored in plastic bags with 70% ethanol and then in FAA solution. Using digital callipers of 0.01 mm resolution (Mitutoyo, Japan) we measured the following floral characters: (i) perianth diameter (mm), (ii) floral tube length (mm), (iii) flower length (mm), (iv) diameter of middle flower (mm), (v) diameter of basal flower (mm), (vi) distance between stigma and anthers (mm), (vii) style length (mm), (viii) stigma lobe length (mm), (ix) nectar chamber length (mm) and (x) equatorial and polar diameters of the ovary (mm) (Fig. 2). We also counted the number pollen grains, number of stamens per flower, number of stigma lobes and number of ovules per ovary.

Breeding system

We investigated the breeding system of *F. recurvus* by calculating the outcrossing index (OCI) and the pollen/ovule ratio (Cruden 1977). For calculating these indices, we used information on floral characteristics (perianth diameter, distance between anthers and stigma; herkogamy), as well as flower behaviour, *i.e.* the interval between anther dehiscence and stigma receptivity.

Counting of pollen grains per anther and number of ovules per ovary was carried out in flowers collected at the beginning of anthesis and stored in FAA ($N = 30$ flowers). In total we removed 20 anthers (one per flower), which were placed in 2×2 cm Petri dishes. We dissected anthers to release pollen grains that were then counted using a stereoscopic microscope with a manual counter (Kearns & Inouye 1993). The total number of pollen grains per flower was calculated considering the total number of grains per anther × number of stamens (Kearns & Inouye 1993). We divided each ovary into four equal parts and counted the number of ovules per quarter ovary, extrapolating this to estimate the total number of ovules per ovary.

Flower behaviour and floral visitors

In December 2013, we conducted observations on flower behaviour in 30 individuals of *F. recurvus* in site 2 (Fig. 1). We selected 30 flower buds before anthesis, which were labelled and tracked through time. Time of occurrence of the following stages was recorded: (i) start and end of anthesis; (ii) maximum diameter of open flowers; (iii) percentage dehiscence of anthers; (iv) presence/absence of pollen in the flower (pollen on perianth and anthers); (v) receptivity of stigma (turgidity of stigma lobes) and (vi) floral visitors.

Observations were taken every 2 h, from 08:00 h to 18:00 h, when anthesis started and flowers closed, respectively. We observed labelled flowers every day until the final closure of flowers after 5 days, the maximum time of flower opening in this species. The fruits produced from labelled flowers were collected in paper bags, and seeds counted in the laboratory using digital photographs processed with the software Image Tool version 3.0 (Wilcox *et al.* 2002).

We conducted observations of flower visitors every 2 h, at intervals of 15 min, simultaneously to observations of flower
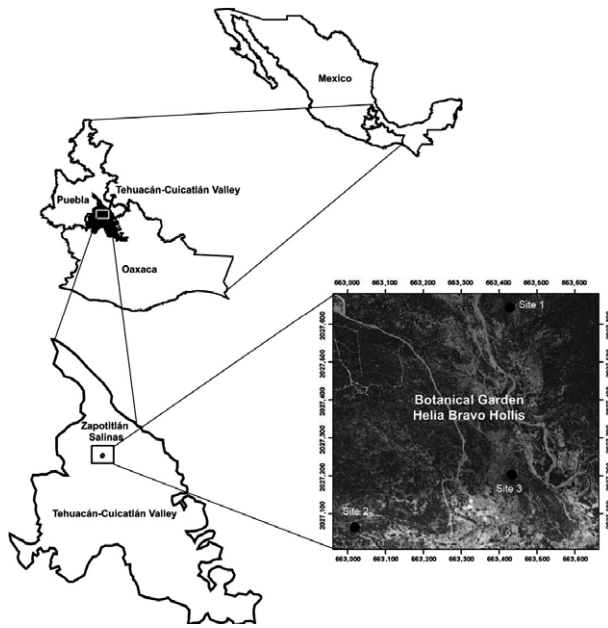
Fig. 1. The study site within the research area of the Botanical Garden Helia Bravo Hollis at Zapotitlán Salinas, Puebla, Mexico.

behaviour in the same flowers. In January 2014, we continued the observations of floral visitors and collected specimens, which were preserved in 70% ethanol for later identification.

Mating system

In order to determine the mating system of *F. recurvus,* we conducted a field experiment with four treatments of controlled pollination in the three study sites. We labelled 120 individuals and established experimental treatments controlling pollination using mosquito net bags before and after pollination was performed. From October 2013 to January 2014, we labelled 130 flower buds and randomly assigned them to the following treatments. (i) Open-pollination control (OP; n = 40) with labelled flowers exposed to natural conditions of pollination; (ii) Hand cross-pollination (HCP; n = 30) using pollen from flowers of 20 individuals located at least 15 m from the pollinated plants. We used brushes for pollen loads on stigmas (only 1 day); these were washed in ethanol and dried before being reused. The selected flower buds were labelled with strips of masking tape and covered with mosquito net bags to isolate them from any potential pollinator; (iii) Hand self-pollination (HSP; n = 30) using pollen from the same flower. In these flowers, nectar was extracted using 10 µl micropipettes (Brand) before flowers closed, between 15:00 h and 17:00 h. Selected flower buds were labelled and covered with mosquito net bags as described above; (iv) Natural self-pollination (NSP; n = 30) where flower buds were covered with mosquito net bags and monitored until abortion or fruit production; no pollen manipulation was performed in this treatment.

After maturation, fruits were collected in paper bags, taken to the laboratory, dried and weighed using a digital balance (Sartorius M-PROVE, AY612, Göttingen, Germany). We counted the number of seeds produced per fruit from digital photographs processed with the software Image Tool version 3.0 (Wilcox *et al.* 2002).

We estimated the outcrossing rate (t_e) and autogamy (IA) index, considering fruit set (Mandujano *et al.* 2010) and seed set in the experimental treatments (Jaimes & Ramírez 1999). Outcrossing rate (t_e) was calculated using fruit set or seed set for selfing (w_s) and outcrossing (w_x) treatments: $t_e = 1 - s$; where *s* is the selfing rate: $s = w_s/(w_x + w_s)$. IA was calculated as the fruit/flower and total seeds/total ovules from pollination ratios, divided by the fruit/flower and total seeds/total ovules from hand cross-pollination ratios. Based on Ruíz-Zapata & Arroyo (1978), IA values above 0.2 indicate self-compatibility and autogamy.

We tested differences in number of seeds produced from each pollination treatment using ANOVA; the number of seeds per fruit being the response variable and the type of treatment as the factor. We used the statistical software NCSS (Hintze 2001).

Seed germination from pollination treatments

We chose 50 seeds from fruits of OP (control) and HCP treatments, which were the only experimental treatments that produced fruits. We disinfected seeds in 90% sodium hypochlorite for 15 min. The seeds were placed in Petri dishes with 1% bacteriological agar and put in a germination chamber (LAB-LINE Instruments, Melrose Park Illinois, USA) at a 25 °C constant temperature and a 12 h:12 h photoperiod, and percentage germination was recorded every day for 30 days. In order to test differences among treatments, we used Generalised Linear Models (GLM), taking as response variable the number of germinated seeds and as factor the type of treatment, using the statistical software NCSS (Hintze 2001).

RESULTS

Floral phenology

The reproductive structures of *F. recurvus* develop in the apical region of the stem, being exposed to pollinators and/or dispersers (Fig. 3). Floral buds appear in October and their production ceases by early January. The flowering peak is in December, while the fruiting period is from February to March. The fruits mature in 2–3 months and detach easily from the plant. However, in some cases, fruits remain attached to the plant for several months. Under the OP treatment, successful fruit set was 60% and an average fruit naturally produced 2051 ± 200 seeds (mean ± SE).

Floral morphometry

The flowers of *F. recurvus* are hermaphroditic and have herkogamy. There is a separation between the most distal anthers and the stigma (Table 1).

Flower behaviour and floral visitors

The flowers of *F. recurvus* had diurnal anthesis and remain open for 2–5 days. Anthesis begins in the morning, between 11:00 and 11:30 h, and maximum aperture of the perianth occurs between 13:00 h and 15:00 h. Flowers gradually close from 16:00 h and this process concludes at dusk, around 18:00 h. At the beginning of anthesis, the
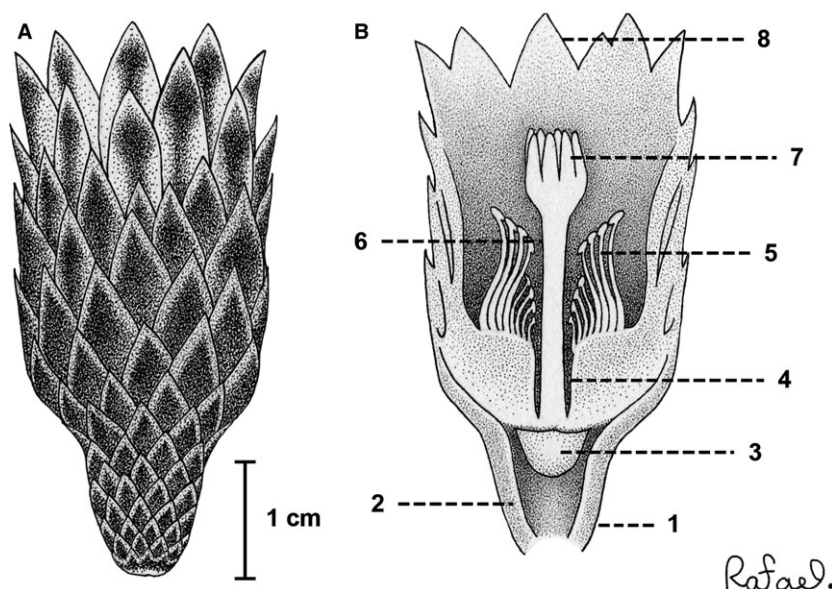
Fig. 2. Schematic representation of the floral type of *F. recurvus*. A: External view of flower; B: Longitudinal section: (1) peduncle, (2) pericarp, (3) ovary chamber, (4) nectar chamber, (5) stamens, (6) style, (7) stigma lobes, (8) external perianth segments.

stamens are arranged in a spiral around the style, with the anthers immersed in the centre. Anther dehiscence is gradual and begins around 12:30 h, *i.e.* 1.0–1.5 h after the start of anthesis. The percentage dehisced anthers increases throughout the days the flowers remain open. Generally, stigma lobes gradually open between the first and/or second day and the stigma is receptive from day 2 or 3 with all lobes completely open. In most of the observed flowers the stigma is pink when receptive and yellow when not receptive. Fruit set in the monitored sample of flowers used to determine floral behaviour (n = 30) was 53.3% (16 fruits produced). Flowers of *F. recurvus* were visited mainly by bees (Hymenoptera), followed by flies (Diptera), ants (Hymenoptera), thrips (Thysanoptera) and occasionally some hummingbirds (Trochilidae: Apodiformes). Specimens collected were mainly native bee species (oligolectic species) such as *Diadasia australis* (N = 2; Hymenoptera: Apidae), *Augochlorella* spp. (N = 2; Hymenoptera: Halictidae), *Macrotera* (*Macrotera*) *bicolor* (N = 2) and *Macrotera* (*Cockrellula*) *azteca* (N = 1; Hymenoptera: Andrenidae). It is noteworthy that only native bees made contact with the anthers and stigma, and carried large pollen loads on their hind legs. We also observed native bees landing on the stigma during visits to flowers, depositing the pollen they carried. These visits coincided with times of maximum opening of the perianth. Moreover, some ants were found protecting extrafloral nectaries, such as *Camponotus rubrithorax* (Hymenoptera: Formicidae), and occasionally visiting flowers, but not making contact with the anthers and stigma (acting as indirect mutualists). Thrips inside the flowers between stamens did not contact the stigma, and we considered them floral parasites. The collection of floral visitors was low, due to cloudy cold and windy days, when the abundance of Hymenoptera floral visitors decreased.

Another important feature was that flowers of *F. recurvus* produced little or no nectar. The maximum volume extracted from a single flower was 4.5 µl. Subsequent records of nectar extraction were practically nil in all flowers (mean ± SE, 0.13 ± 0.032 µl). Therefore, measurement of sugar concentration was not possible with a manual refractometer.

Breeding system

The floral traits of *F. recurvus* suggest that the breeding system is obligatorily xenogamous (outcrossing index OCI; Cruden 1977) because: (i) the diameter of the flower is >6 mm, (ii) the stigma lobes and anthers were separated (herkogamy; 4.19 ± 0.24 mm), and (iii) type of habitat. However, the pollen/ovule ratio (Cruden1977) suggests a xenogamous facultative system, at 1015.83:1.

Mating system

No seeds or fruits set in our selfing treatments, so $t_e = 1$ and IA = 0, indicating that *F. recurvus* is a self-incompatible species (Table 2); therefore, flowers of this species require animal vectors to ensure transfer of pollen grains. The hand self-pollination (HSP) and natural self-pollination (NSP) experiments did not lead to any fruit production. However, hand cross-pollination (HCP) led to 100% of flowers producing fruits (n = 30). The proportion of successful fruits obtained in the open-pollination (OP) treatment was 60%. The mean number of seeds produced in the OP experiment (control) was 2051 ± 200, with 1292 ± 157 seeds after HCP (ANOVA: $F = 9.11$, $P = 0.0039$; Table 3). The mean seed weights for both experiments were 0.60 ± 0.005 g and 0.49 ± 0.05 g, respectively. The relatively high number of seeds produced in the control indicated that despite having a significant percentage of floral abortion, floral visitors seem to be efficient pollinators.

Seed germination from experimental pollinations
The percentage germination of open-pollinated flowers was 63.75%, and rose to 83.33% in the HCP treatment. The total
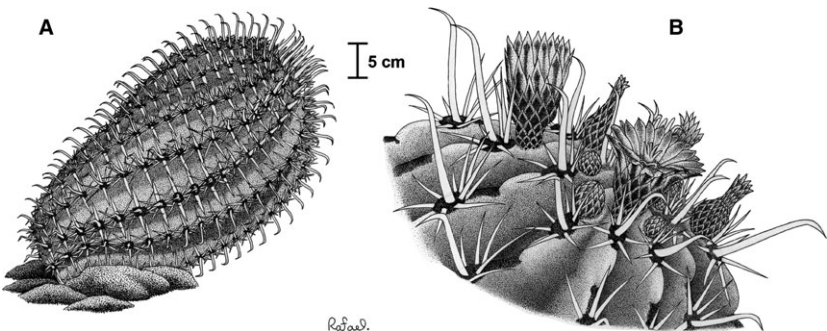
Fig. 3. *Ferocactus recurvus* subsp. *recurvus*: A: Stem, B: Reproductive structures in the apical region.

Table 1. Floral traits (mean ± SE, min and max) of *F. recurvus* in the Tehuacán-Cuicatlán Valley, Mexico.

floral traits	N	Mean	SE	min	max
Perianth diameter (mm)	30	21.77	1.34	19.03	24.52
Floral tube length (mm)	30	13.9	0.36	13.16	14.64
Flower length (mm)	30	41.13	0.56	39.96	42.29
Distance between stigma and anthers (mm)	30	4.19	0.24	3.69	4.68
Style length (mm)	30	25.58	0.31	24.94	26.23
Stigma lobe length (mm)	30	6.08	0.27	5.51	6.65
Number of stigma lobes	30	16.46	0.53	15.36	17.56
Nectar chamber length (mm)	30	6.53	0.21	6.08	6.98
Equatorial diameter of ovary (mm)	30	5.57	0.12	5.3	5.83
Polar diameter of ovary (mm)	30	4.66	0.19	4.25	5.06
Ovule number/ovary	30	1476	39.89	1394	1557
Number of stamens/flower	30	1626	33.32	1557	1694
Number of pollen grains	20	1,499,376.24	30,725.53	1,147,030	1,825,659
Diameter of middle flower (mm)	30	19.7	0.4	18.87	20.54
Diameter of basal flower (mm)	30	10.9	0.27	10.35	11.46

number of germinated seeds in OP was 765, and 1200 in HCP. There were significant differences in the number of germinated seeds from open-pollination (control) and the hand cross-pollination. The GLM showed significant differences between treatments (GLM: $F = 8.99$, $P = 0.004$; Table 4). HCP produced most successful in percentage seed germination over time (30 days; Fig. 4).

DISCUSSION

The results of this study provide important information on the reproductive biology of this group of plants growing in arid and semiarid environments. *F. recurvus* has diurnal anthesis, flowers with large corollas, large amounts of pollen and negligible nectar production. These traits suggest that this cactus is pollinated by insects, particularly bees (Faegri & van der Pijl 1979), and that floral morphology is closely correlated with the vectors of pollination (Galen 1999; Castro 2009). Our observations confirmed this assumption: flowers of *F. recurvus* have a wide range of floral visitors: bees (Apidae), flies (Muscidae), ants (Formicidae) and thrips (Thripidae). However, only native bees and occasionally wasps made contact when they landed on the stigma and anthers and carried pollen loads on their extremities, suggesting that pollen is the major floral reward in flowers of *F. recurvus*. Despite not

testing if a given visitor was indeed contributing to successful pollen exchange (*e.g.* single visit effectiveness), there was no contact with the stigma and anthers of the other occasional floral visitors, flies, ants (indirect mutualists) and thrips (floral parasites). In this sense, McIntosh (2005) found that visits by flies to flowers of *F. wislizeni* and *F. cylindraceus* did not produce fruits. Also, LeVan *et al.* (2014) reported that floral visitation by the Argentine ant reduced pollinator visitation and seed set in the coast barrel cactus, *F. viridescens*. These results highlight the well-documented observation that not all visitors are pollinators.

Our results suggest that native bees, such as the metallic green-bee *Augochlorella* sp. (Halictidae), and solitary bees such as *Diadasia australis* (Apidae), *Macrotera* (*Cockerellula*) *azteca* and *Macrotera* (*Macrotera*) *bicolor* (Andrenidae), are actual or at least potential pollinators of *F. recurvus*. This confers a huge advantage compared to other flower visitors, since the probability of fertilising these flowers is very high; moreover these insects visit a large number of flowers during the day (Ollerton 1999; Castro 2009). Furthermore, the fact that flowers of this barrel cactus are relatively long-lived (2–5 days) increases the probability of visits because pollen is available for several days, increasing the transfer to other flowers. This pattern of floral longevity has also been observed in barrel cacti of the Sonoran Desert, such as *F. wislizeni* and *F. cylindraceus*

Table 2. Fruit set results of pollination treatments conducted on *F. recurvus* at Tehuacán-Cuicatlán Valley, Mexico.

treatment	number of flowers	fruit: flower ratio	number of seeds	ovule: seed ratio
Natural self-pollination (NSP)	30	0	0	0
Hand self-pollination (HSP)	30	0	0	0
Hand cross-pollination (HCP)	30	1	1292 ($\pm$157)	0.029
Open-pollination (Control)	40	0.6	2051 ($\pm$200)	0.046

Table 3. Results of one-way ANOVA, with number of seeds per fruit as response variable and type of treatment factor in *F. recurvus*.

treatment	N (number of fruits)	mean number of seeds	SE	F	P
Open-pollination (control)	24	2051	187.35	9.11	0.0039
Hand cross-pollination (HCP)	30	1292	167.57		

Table 4. Results of analysis variance of GLM, with response variable number of germinated seeds and explanatory variable type of treatment in *F. recurvus*.

treatment	N (fruit set)	mean of germinated seeds	SE	F	P
Open-pollination (control)	24	31.87	2.433	8.99	0.004
Hand cross-pollination (HCP)	30	41.66	2.176		

Fig. 4. Number of germinated seeds over 30 days between pollination treatments: hand cross-pollination (HCP) (black diamond) and open-pollination (control) (grey square) of *F. recurvus*.
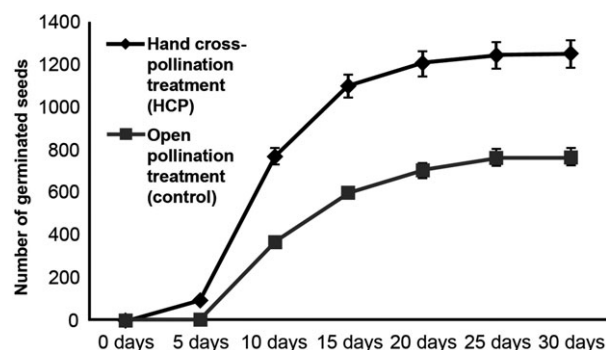
(McIntosh 2005). In contrast, the floral longevity of *E. platyacanthus* is only 2–3 days (Jiménez-Sierra 2010). Ashman (2004) and Castro (2009) found that floral longevity is a trait that can directly or indirectly influence reproductive success, probably affecting the amount of nectar and pollen transferred or received, and thus making the plants attractive to pollinators. Other authors describe the floral longevity as the result of a trade-off between male and female roles and the energy cost of maintaining open flowers or producing new structures (Ashman & Schoen 1994; Castro 2009). However, the low rate of visits observed and the poor collection of specimens on flowers of *F. recurvus* was probably due to weather conditions at the time of sampling, as the wind was very cold, making it difficult for insects to visit flowers for floral rewards. Therefore, in situations of weather unpredictability or lack of pollinators, floral longevity may be beneficial in terms of likelihood of fertilisation (Castro 2009).

In this respect, our results suggest a possible relationship between floral longevity and outcross pollination. A relatively long flowering period (2–5 days) allows longer exposure of flowers to effective pollinators, ensuring the transfer and receipt of pollen grains. Various authors reported that reproduction by animal-pollinated plants commonly involves plastic traits, because outcrossing depends of pollinators whose abundance varies within and between reproductive seasons. This variation could promote different pollination environments for individual plants (Schemske & Horvitz 1989; Harder & Johnson 2005), which favours different suites of traits to promote outcrossing success (Harder & Wilson 1994; Harder & Johnson 2005). In this sense, floral longevity and display size could be important plastic traits in attraction of abundant pollinators to flowers of *F. recurvus*.

In addition, flowers of *F. recurvus* produce practically no nectar. This could be for several reasons, since production of

this floral reward represents significant energy cost (Pleasants & Chaplin 1983; Ollerton 1999; Jiménez-Sierra 2010). Some authors have documented absence of nectar in some cacti that have floral characteristics associated with pollination by bees (Scogin 1985; Piña 2000; Matías-Palafox 2006; Jiménez-Sierra 2010). Moreover, it is noteworthy that individuals of *F. recurvus* have extrafloral nectaries, which represent an additional cost in terms of allocation of resources (Ness *et al.* 2006), although higher fruit set offsets this cost. This may suggest a trade-off in producing one of these two types of nectar and somehow counteracting the relatively long period of anthesis (2–5 days). In contrast, flowers of *F. recurvus* produce large amounts of pollen grains per flower. Scogin (1985) notes that plants that do not produce nectar (*i.e.* no detectable nectar) produce large amounts of pollen as the primary reward for foraging bees. Therefore, in *F. recurvus* our observations suggest that pollen acts as the main attractant for effective pollinators such as bees. *F. recurvus* is xenogamous like other species of barrel cactus, such as *F. cylindraceus*, *F. wislizenii* (McIntosh 2002) and *F. histrix* (del Castillo 1994). According to our results, this species requires pollinator vectors (particularly bees) to ensure production of fruits and seeds. In contrast, *Echinocactus platyacanthus* (Jiménez-Sierra 2010), *Melocactus schatzlii* and *M. andinus* present a mixed mating system, *i.e.* even though self-pollination occurs, higher reproductive success is obtained from outcrossing (Nassar *et al.* 2007).

The results suggest a clear self-incompatibility system for *F. recurvus*. Additionally, data obtained from floral morphometry indicate herkogamy, a trait associated with xenogamous species. The hand cross-pollination treatment was the most successful, as was the control (open-pollination), whereas no

seed or fruit set followed selfing treatments, so *F. recurvus* is an obligate outcrosser. Although these results suggest that under natural conditions about 40% of flowers on plants fail to produce fruit (aborted flowers), flower visitors seem to be very efficient pollinators, as even a few fruits produced many seeds. However, several biotic and abiotic factors could explain the observed abortion. For instance, a decrease in temperature during the flowering peak in December could have decreased the visit rate and abundance of potential pollinators, reducing considerably the transfer of pollen to the stigma. In addition, allocation of resources to breeding may play a decisive role in this regard (Moller & Eriksson 1995; Ollerton 1999; Tschapka & Dressler 2002; McIntosh 2005; Makino 2008). Seed germination in the hand cross-pollination treatment was more successful than the control treatment. From day 5, seed germination in both treatments increased. These results suggest that most seeds produced are viable and germinate relatively quickly under controlled conditions.

In conclusion, this study is particularly relevant because, at present, knowledge of the reproductive biology of barrel cacti in arid and semiarid regions of Mexico is scarce. *F. recurvus* is endemic to the Tehuacán-Cuicatlán Valley, therefore its importance is greater due to the lack of reproductive information on a key species for the region. Particularly important is recognition that the main floral visitors that contact reproductive structures are native bees. This group is highly relevant for barrel cactus and numerous other species of cacti in the study area, since thanks to these bees the flowers are pollinated. Hence, studies on reproductive biology allow the description of basic aspects of pollination, in addition to establishing a basis for implementing management and conservation plans for threatened species (Martínez-Peralta & Mandujano 2012; Larios-Ulloa *et al.* 2015). Therefore, the protection of native bees as a regional policy is of primary importance for the conservation of *F. recurvus* as well as other species. Similarly, identification of a self-incompatibility system indicates that the reproductive success of this species could be guaranteed with measures to preserve genetic diversity of populations that are facing threats of looting of individuals and habitat fragmentation. These threats affect not only *F. recurvus* but many species and must be explicitly incorporated into conservation policies in the Tehuacán-Cuicatlán Biosphere Reserve, Mexico.

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