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Shared pollinators and sequential flowering phenologies in two sympatric cactus species

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Background and aims – The reproductive characteristics of sympatric Cactaceae are important because they help to understand interfering or facilitating mechanisms that allow their coexistence. Globose Cactaceae show melittophily flowers that may be attractive to a shared set of pollinators, and if the flowering events are not overlapping, flower resources could be present for the pollinators through time. *Ariocarpus kotschoubeyanus* and *Neolloydia conoidea* are two sympatric cactus species in the southern Chihuahuan Desert. The objective of this study is to describe and compare some reproductive characteristics of these species.

Material and methods – Individuals of *Ariocarpus kotschoubeyanus* and *Neolloydia conoidea* (n = 231 and 212, respectively) were marked and monitored during one year, recording for each species, the floral phenology, behaviour and morphology, flower visitors, breeding and mating systems (through pollination experiments).

Key results – The flowering periods of these species do not overlap. The flowers of both species are melittophily, with the same shape, colour, and similar behaviour; both are obligate xenogamous plants. The mating system of *Neolloydia conoidea* is outcrossing but mixed in *Ariocarpus kotschoubeyanus*. They share 75% of pollinators (solitary and social bees).

Conclusions – The temporal blooming separation of these species could be an important factor to facilitate coexistence within the xerophyte community since they share the same set of pollinators to achieve their reproductive success.

Keywords – Bees; breeding systems; coexistence; mating systems; reproductive phenology.

INTRODUCTION

Some aspects of reproductive biology, such as the timing of flowering, may play a critical role in plant coexistence, allowing (1) a temporal distribution of resources for pollinators, fruit consumers, and seed dispersers of the sympatric species (temporary separation of niches) (Giorgis et al. 2015) or (2) co-blooming (Moeller 2004), in which species may share (Koptur 1983; Moeller 2004)

or compete for pollinators (niche overlap) (Elzinga et al. 2007). Competition may lead to a divergent phenology – through coevolution by character displacement – or competitive exclusion (Gleeson 1981). Several authors have suggested that character displacement in flowering time is an evolutionary response facing pollinator competition (Van der Kooi et al. 2016, among others). Egli & Giorgetta (2017) proposed that sequential flowering could be an

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escape mechanism that would reduce visitor competition and decrease pollen movement among species.

The diversity of floral characters in angiosperms may be an adaptive response to selection pressures mediated by pollinators (Stebbins 1970). Among these attributes, flower characteristics as size, colour, odour, pollen, and nectar quantity must be considered, as well as the number of flowers per individual present at a given time (Augspurger 1981; Waser 1983; Conner & Rush 1996). These attributes could influence species breeding and mating systems, which could have an impact on individual and population adaptation (Eguiarte et al. 1999). Environmental variables are other important factors determining flowering patterns at population and community levels. Among the main environmental variables that influence flowering phenology are temperature, relative humidity, photoperiod, and precipitation (Fenner 1998).

The Cactaceae family comprises around 100 genera and 1500 species, mainly confined to semi-desert areas of the New World. México hosts the richest cactus diversity (Anderson 2001). The family has the third highest number of threatened species with 30% of the species in any risk category (Goettsch et al. 2015). This has led to an increased interest in studying various aspects of their biology, including their reproductive biology (Mandujano et al. 2010; Díaz-Segura et al. 2017; Jiménez-Sierra et al. 2019; among others). and some studies have considered sympatric Cactaceae (McIntosh 2002; Giorgis et al. 2015; Egli & Giorgetta 2017; Matías-Palafox et al. 2017; Ferreira et al. 2018; Guerrero et al. 2019). The study of the reproductive biology

of sympatric Cactaceae may shed light on the interference or facilitation of maintaining shared pollinators, which helps to understand the ecosystem services of pollination within xerophyte communities as well as provide arguments for the conservation of these ecosystems (Eggl & Giorgetta 2017).

The hypothesis of this study is that, if species have similar flowers and are flowering sequentially, these species might share pollinators. If so, the presence of the floral resource persists through time for the pollinators, while the Cactaceae benefit because competition for pollinators is avoided and the risk of heterospecific pollen deposition is diminished. On the other hand, if species show floral similarity and synchronized flowering, competition for pollinators occurs and the risk of interference of heterospecific pollen increases.

The objective of this study is to describe and compare the flowering phenology, flower morphology, flower visitors, and mating systems of two cactus species, *Ariocarpus kotschoubeyanus* and *Neolloydia conoidea*, which coexist in a community in the southern Chihuahuan Desert in México.

MATERIAL AND METHODS

Study area

The study site is located in the west central region of the State of Querétaro, México, which constitutes the most southern part of the Chihuahuan Desert in the surroundings of San Miguel Tolimán, municipality of Tolimán (20°52'54.1"N, 99°57'05.4"W; 1400 m a.s.l.) (fig. 1). The area belongs to the Sierra Madre Oriental region included within the

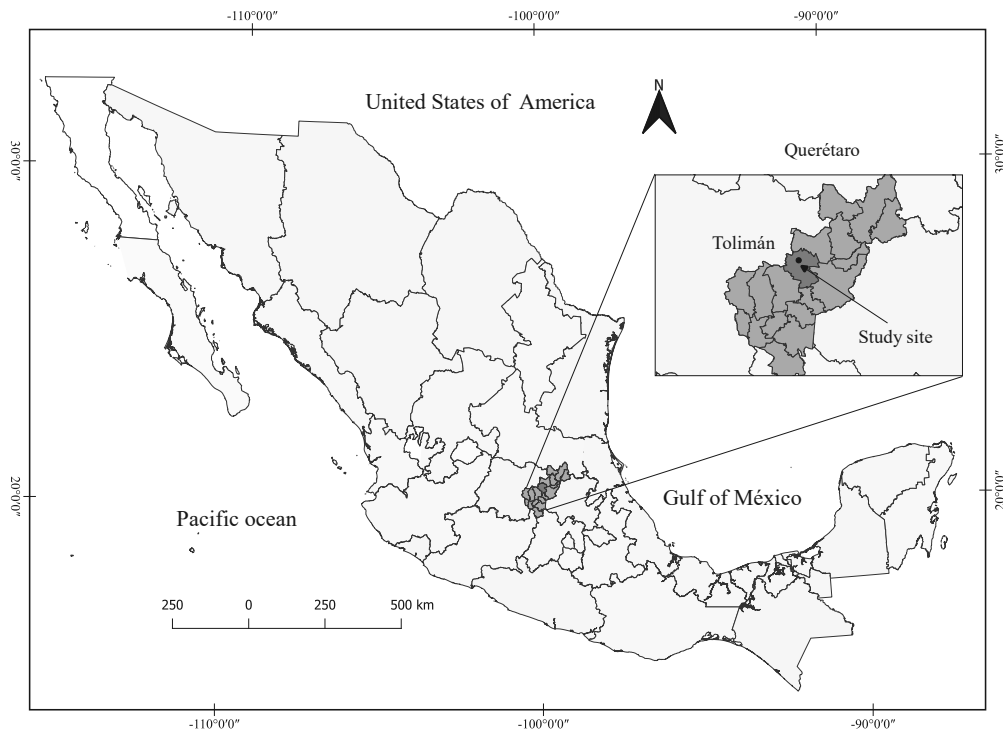


Figure 1 – Location of the study site in the municipality of Tolimán, Querétaro (México) in the southern region of the Chihuahuan Desert. Map created with QGIS (QGIS Development Team 2020).

Quéretaro-Hidalguese semi-arid region. The vegetation is microphyllous-xerophilous scrubland on limestone substrates intercalated with mudstone outcrops (Bayona 2016). The climate is semi-dry temperate steppe with summer rains. The warmest month is May when the maximum temperature reaches 22.5–30°C and the coldest month is February with temperatures oscillating between 4.5 and 14°C. Annual precipitation is 380–470 mm, and the months with greater precipitation are May–October (CONAGUA 2010; Bayona 2016). This study was performed from March 2015 to April 2016.

Study species

Ariocarpus kotschoubeyanus (Lem.) K.Schum. (hereafter: *Ariocarpus*), locally known as “roca viva” (living rock), “pata de venado” (deer’s foot), “pezuña de venado” (deer’s cloved hoof), or “falso peyote” (false peyote), is a geophytic cactus that generally does not branch out. It has a dorsoventrally flattened stem with greyish green furrowed tubercles, whose aerial parts are approximately 7 cm in diameter, scarcely emerging from the soil surface. The funnel-shaped magenta flowers bloom from the flowering areole of the youngest tubercles (fig. 2A). The fruit is dehiscent, claviform, 5–18 mm long, with black seeds 1 mm long (Bravo-Hollis & Sánchez-Mejorada 1991). The plant is endemic to México with populations distributed in the northeast and central regions of the country (Bravo-Hollis & Sánchez-Mejorada 1991; Guzmán et al. 2003). The Mexican government has declared that this species is the subject of “Special Protection” (“Pr”) Mexican Norm (NOM-059-SEMARNAT-2010; SEMARNAT 2010). The International Union for Conservation of Nature (IUCN) considers this species as “Near Threatened” (NT) (Gómez-Hinostrosa et al. 2017), and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) includes it in Appendix I (Lüthy 2001).

Neolloydia conoidea (DC.) Britton & Rose (hereafter: *Neolloydia*) is locally known as “biznaguita” (Texas cone cactus or Chihuahuan beehive). It is an initially simple globose plant that turns into cespitose with age (Bravo-Hollis & Sánchez-Mejorada 1991). The stem is light green to greyish green, globose or cylindrical, 7–10 cm tall, and 5–7 cm in diameter with spiral series from 8–15 tubercles. The flowers are funnel-shaped and magenta-coloured (fig. 2B). The fruit is dehiscent, ovoid, light green to greyish, with black seeds 1 mm long (Bravo-Hollis & Sánchez-Mejorada 1991). The plant is widely distributed from the southern United States of America to central México (Guzmán et al. 2003). It reaches high densities and is not considered to be in any conservation risk category although its population in the State of Quéretaro has been affected by human activities and changes in land use (Scheinvar 2004).

Ariocarpus and *Neolloydia* coexist in the xerophilous scrubland at the study site, and both species have been affected by land use changes such as agricultural intensification and urban expansion, as well as by continuous illegal collection (Scheinvar 2004).

Reproductive phenology

At the study site, 231 individuals of *Ariocarpus* and 212 of *Neolloydia* were marked and censused monthly during an annual cycle (March 2015–April 2016), recording all their reproductive structures. To determine the importance of climate factors on floral bud production, climate variables – average, maximum, and minimum temperature, precipitation, and relative humidity – were recorded with the help of a Hobo Data Logger (Onset, U12-012) and a pluviometer (Onset, RG2-M), which were installed at the study site. The obtained data and floral bud emergence were analyzed through non-parametric correlations (Spearman, ρ) (Zar 2010).

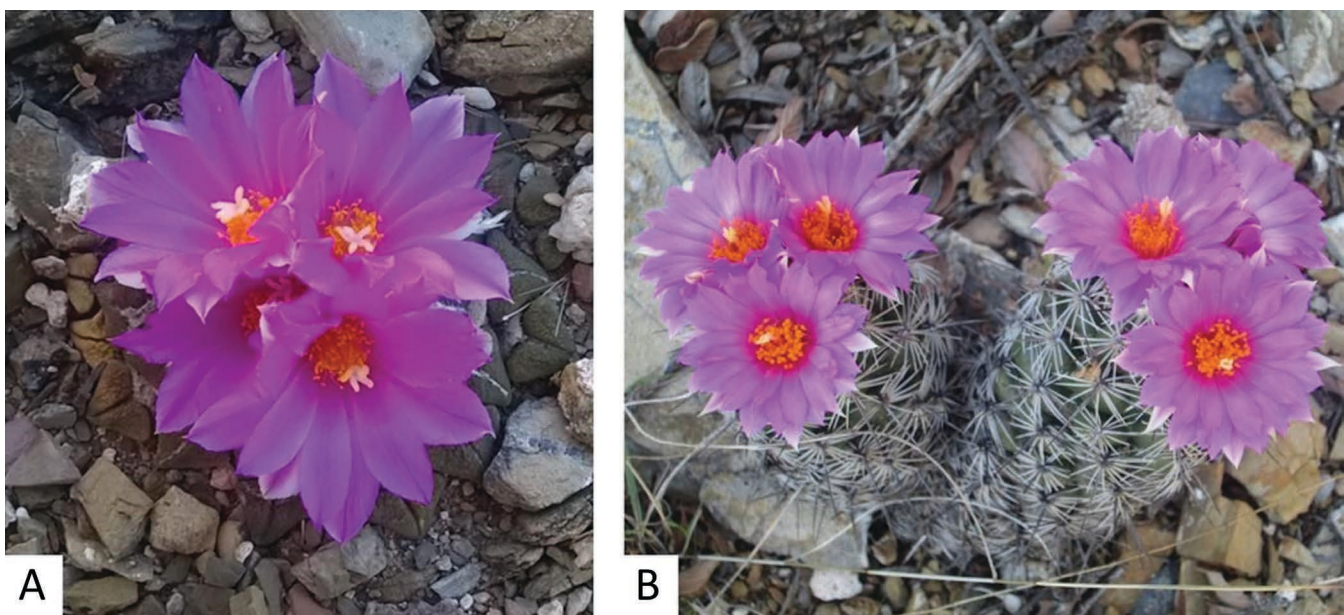


Figure 2 – Flowers of the species studied in Tolimán, Querétaro (México). A. *Ariocarpus kotschoubeyanus*. B. *Neolloydia conoidea*. Photographs by Erika Arroyo-Pérez.

Table 1 – Floral characteristics of *Ariocarpus kotschoubeyanus* and *Neolloydia conoidea* in Tolimán, México (n = 20 flowers from different individuals by species). * = significant differences.

	<i>A. kotschoubeyanus</i>	<i>N. conoidea</i>	p
Perianth diameter (cm)	3.15 (0.51)	3.66 (0.44)	0.007*
Length of flower (cm)	3.13 (0.67)	3.02 (0.32)	0.346
Length of style (cm)	1.42 (0.23)	1.29 (0.13)	0.035*
Number of stigma lobes	4.65 (0.87)	4.95 (0.60)	0.215
Length of stigma lobes (cm)	0.23 (0.08)	0.28 (0.06)	0.033*
Length of stamens (cm)	1.12 (0.28)	1.20 (0.16)	0.159
Number of stamens	131.85 (48.98)	146.5 (43.66)	0.324
Number of pollen grains per flower	57804 (21476)	58087 (16122)	0.583
Equatorial diameter of the ovary (cm)	0.15 (0.7)	0.19 (0.05)	0.130
Polar diameter of the ovary (cm)	0.26 (0.07)	0.28 (0.06)	0.170
Number of ovules/ovary	37.95 (7.96)	58.69 (11.25)	0.001*
Herkogamy (cm)	0.18 (0.17)	0.32 (0.12)	0.0038*

Anthesis and floral morphology

During the flowering peak of each species, 20 buds were marked on 20 different plants, separated by at least 5 m. The produced flowers were monitored during anthesis. The observations were performed at intervals of 60 min, recording the perianth diameter with a digital calliper (Standard Truper, CALDI-6MP). To determine the presence of herkogamy (spatial separation of the sexual organs), the minimum distance between the stigma lobes and the anthers was determined, and dichogamy (temporal function separation of the sexual organs) was ascertained by determining the beginning of anther dehiscence (observed with a magnifying glass) and the receptivity of the stigma (lobes opening and pollen presence) (Martínez-Peralta et al. 2014b). Additionally, the temperature was recorded when recording the anthesis variables. To determine the amount of nectar produced, 10 buds from 10 different plants (n = 10) on the point of opening were isolated. Although these flowers remained covered by organza bags during anthesis therefore preventing nectar removal by floral visitors and although the extraction of nectar was attempted with a 5 µl microcapillary, the collection of nectar was not successful. To determine if the distances recorded in the anther-stigma separation were significantly different from zero, the Student's t-test for one sample was used, and to determine if significant differences existed between the herkogamy of the first day with the second day of the anthesis, the Paired Sample Student's t-test was used.

Floral characteristics were taken of fresh undamaged flowers at anthesis (n = 20 flowers/species from different individuals) (table 1). The mean number of pollen grains/anther was determined by squashing the anthers on a slide (n = 4 anthers of 5 floral buds/species) and counting the pollen using an optical microscope (Carl Zeiss; 25; 10×), (Matías-Palafox et al. 2017). To determine the average number of pollen grains/flower/species, the average pollen grain count of the anthers was multiplied by the total number of stamens

for each species. The average number of ovules/flower was determined by counting the ovules directly in the ovaries. The average value and standard deviation were estimated for each variable per species. For interspecific comparison of these variables, Student's t-tests were applied (Zar 2010). To determine which variables contributed significantly to the interspecific variability, a principal component analysis was performed starting from a matrix correlation as well as a discriminant analysis using JMP® v.10.0.0 (SAS 2012).

Floral visitors

During the flowering peak of *Ariocarpus* (3–4 Nov. 2015) and *Neolloydia* (23–24 May 2015) 20 buds were selected from 20 different individuals (n = 20). The floral visitors were recorded in 30-min periods at an interval of one hour during anthesis. The observations were performed for two consecutive days for a total of 3 h, since the flowers of both species open in the morning and close in the afternoon, and only live for two days. The insects visiting the flowers were collected, trying to have five replicates per morphospecies. The collection was performed with test tubes with cyanide salts, and the samples were mounted on entomology pins and identified by a specialist.

The following variables were recorded for each visitor: time of arrival, visit duration, and activity performed (contact with the reproductive structures). Additionally, air temperature was recorded throughout the day (OAKTON digital thermometer). The visitation frequency was calculated as the number of visits per day and hour. Chi squared tests were used to determinate the number of flowers that received significant more visits (mean+1ED) than conspecific flowers, and to determine if there are differences in visitors of the flowers of the species studied. To compare pollinator assemblages between the two species studied, we calculated a global measure of pollinator overlap between *Ariocarpus* and *Neolloydia* using a proportional similarity test (PS; range 0–1), calculated as

$$PS = 1 - 0.5 \sum_{i=1}^n |P_{ai} - P_{bi}|$$

where P_{ai} and P_{bi} are the proportions of visitation rates by pollinator species i to flower of species a and b , respectively (Kay & Schemske 2003; Natalis & Wesselingh 2012). This index estimates the similarity between the relative frequencies of the observed bee species, with values close to 1 indicating a high resemblance between pollinator assemblages.

Breeding systems

The breeding system of the studied species (sensu Mandujano et al. 2010) was determined by Cruden's outcrossing index (OCI) and pollen/ovule (P/O) ratio (Cruden 1977). The OCI takes into account floral characteristics, such as (1) flower size, (2) dichogamy (temporal separation between sexes), and (3) herkogamy (spatial separation between female and male structures) (Cruden 1977). For pollen/ovule ratio determination, the number of pollen grains/flower were divided by the number of ovules/flower (Cruden 1977; Matias-Palafox et al. 2017).

Mating system

To determine the mating system (sensu Mandujano et al. 2010) for each species, controlled pollination experiments were performed, using the following treatments ($n = 20$ flowers per treatment of different individuals): (1) control (natural conditions), flowers marked but not manipulated; (2) artificial cross-pollination, isolated flowers and manually pollinated with pollen collected from five different flowers of different individuals of the same species found more than 20 m apart; (3) manual self-pollination, isolated flowers and manually pollinated with pollen from their own anthers; and (4) natural self-fertilization (automatic spontaneous), isolated non-manipulated flowers. At the end of anthesis, the flowers were isolated with organza bags to avoid fruit predation. Three months after flowering, mature fruits were collected, and the number of seeds was counted. Chi squared test were performed to compare the fruit set per treatment; a one-way analysis of variance (ANOVA) was performed to compare the seed set among treatments within each species (JMP® v.10.0.0; SAS 2012).

The index of self-incompatibility (ISI) (Ruiz-Zapata & Arroyo 1978) was estimated for each species dividing the average number of fruits or seeds obtained per self-pollination between the numbers of fruits or seeds obtained per manual interbreeding. ISI values above 0.2 indicate self-compatibility and autogamy (Ruiz-Zapata & Arroyo 1978).

RESULTS

Floral phenology

The flowering periods of the species did not overlap during the year of study (March 2015–April 2016) (fig. 3A). *Ariocarpus* showed an autumn flowering period that lasted three months (October–December), which coincided with

the start of the driest season, while fruit production occurred in February–March. *Neolloydia* showed a spring–summer flowering period, which is the most humid and warmest season of the year, where May is the month with the highest number of flowers (42% of the total number of annual flowers) while fruiting was observed in June–October, June being the month with the largest quantity of fruits (fig. 3B).

For *Ariocarpus*, no correlations were found between the monthly flower bud production and any environmental variable of the same or previous month. For *Neolloydia*, positive correlations were found between the number of flower buds produced monthly and the environmental variables of the same month: average monthly temperature ($r = 0.695$, $p = 0.0083$), minimum monthly temperature ($r = 0.717$, $p = 0.0057$), maximum monthly temperature ($r = 0.860$, $p = 0.0002$), monthly average relative humidity ($r = 0.740$, $p = 0.0031$), and monthly precipitation ($r = 0.776$, $p = 0.0030$).

Floral anthesis and morphology

The annual production of flowers/individual in *Ariocarpus* varied from one to four, while *Neolloydia* produced 1–39 flowers. The flowers usually open for two days, closing at night. They are dichogamous, partially protandrous since on the first day, anther dehiscence began in the first hours of anthesis, while the stigma lobes were not receptive until the last hours of the day. The flower anthesis of both species started at 09:00–10:00 and ended at 14:00–15:00. The maximum opening of the perianth coincided with the maximum environmental temperature (25°C for *Ariocarpus* and 30°C for *Neolloydia*).

The flowers of both species are herkogamous for the two anthesis days, maintaining the stigma lobes above the most distal anthers ($t_{\text{day1}} = 6.38$; $p < 0.001$; $t_{\text{day2}} = 3.45$; $p < 0.01$; d.f. = 19 for *Ariocarpus*; and $t_{\text{day1}} = 3.69$; $p < 0.01$; $t_{\text{day2}} = 4.58$; $p < 0.001$; d.f. = 19 for *Neolloydia*). Significant differences were found for both species in herkogamy (H) on the first and second day of anthesis ($H_{\text{day1}} = 0.17 \pm 0.12$ cm; $H_{\text{day2}} = 0.09 \pm 0.1$; $t = 3.19$; $p < 0.002$; d.f. = 19 for *Ariocarpus*; and $H_{\text{day1}} = 0.35 \pm 0.42$; $H_{\text{day2}} = 0.32 \pm 0.31$; $t = 5.42$; $p < 0.001$; d.f. = 19 for *Neolloydia*). In both species, the stigma lobes curved outward at the end of the second day, in such a way that the lobes and anthers made contact although at that moment the amount of pollen in the anthers was low.

Table 1 shows the floral characteristics of each species. The principal component analysis indicated that the first three components explained 68.18% of the interspecific floral morphological variance. The discriminant multiple analysis indicated that only the perianth diameter, style length, and stamen length were the variables with a significant contribution to the morphometric differences between these species (λ of Wilks = 0.21; $F = 12.20$; $p = 0.0001$).

Floral visitors

The total number of visitors recorded in *Ariocarpus* flowers was 578 (4.81 visits/flower/hour) and 694 (6.94 visits/flower/hour) for *Neolloydia*. The highest number

of visitors corresponded to bees and ants for both species. The bees that visited the flowers of both species were *Apis mellifera* (Apidae), *Augochlora* sp1, and *Lasioglossum* sp1 (Halictidae). *Agapostemon* sp1 only visited *Ariocarpus*, while beetles (Nitidulidae) and wasps (Vespidae) were exclusive of *Neolloydia* (table 2).

The percentage of total visitor species shared was 60%. The number of floral visitors/species varies among the cacti studied. For example, while *Augochlora* is the main visitor of *Ariocarpus* (37.8% of total visits), it only constitutes 14.7% of the visits to *Neolloydia* in which *Apis mellifera* is the main visitor (34.10% of total visits) vs 8.3% to *Ariocarpus*. These differences reduce the global proportional similarity of insects considered as pollinators (see below) between the two species of cactus (PS = 0.48).

Total visits number/day/hour, as visits number/flower/hour for both species is shown in fig. 4A–B. In both species, the majority of the visits occurred when environmental temperature was around 25°C. Even though no significant differences in environmental temperatures occurred between

the first and second day of anthesis, differences in visit frequencies between these two days were observed for both species, which received more visitors the first day of anthesis (for *Ariocarpus*, $\chi^2 = 22.72$, d.f. = 5, $p < 0.001$; and for *Neolloydia* $\chi^2 = 19.19$, d.f. = 5, $p < 0.001$). The frequency of visitors varied throughout the day (fig. 4C–D). For example, *Apis mellifera* showed the greatest frequency in the flowers of *Ariocarpus* in the afternoon, while its highest frequency was observed around 11:00 in *Neolloydia* flowers. On the other hand, the number of visits per flower within each species was heterogenous since 20% of the *Ariocarpus* flowers and 25% of *Neolloydia* received significant more visits than the estimated mean (+ 1 sd) of conspecific flowers ($\chi^2 = 98.24$, d.f. = 19, $p < 0.001$; and $\chi^2 = 121.04$, d.f. = 19, $p < 0.001$, respectively).

With respect to the behaviour of the floral visitors to both species, only bees were considered as pollinators since they were the only insects that made contact with the sexual organs upon arrival or leaving the flowers. Beetles (Nitidulidae) and ants remained in the bottom of the flower

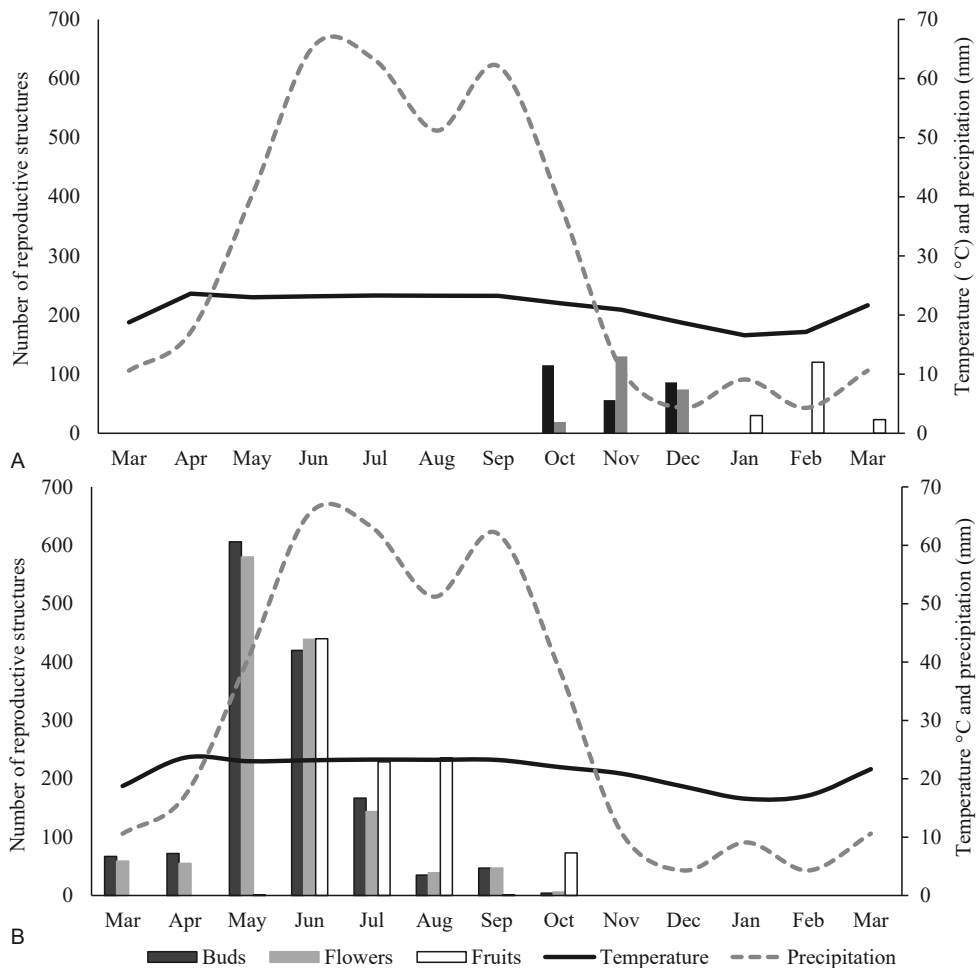


Figure 3 – Reproductive phenology (number of buds, flowers, and fruit/month), mean temperature, and monthly precipitation for 2015 and 2016 at Tolimán, Querétaro (México). **A.** *Ariocarpus kotschoubeyanus* (n = 231 individuals). **B.** *Neolloydia conoidea* (n = 212 individuals).

Table 2 – Floral visitors observed in the flowers of *Ariocarpus kotschoubeyanus* and *Neolloydia conoidea* in Tolimán (n = 20 flowers). Bold letters indicate pollinators.

Order and family		<i>A. kotschoubeyanus</i> (%)	n	Number of visits/ flower/hour	<i>N. conoidea</i> (%)	n	Number of visits/ flower/hour
Hymenoptera							
Halictidae	<i>Agapostemon</i> sp1	33.39	193	1.61	0	0	0
Halictidae	<i>Augochlora</i> sp1	37.80	218	1.82	14.7	102	1.02
Apidae	<i>Apis mellifera</i>	8.30	48	0.4	34.10	237	2.37
Halictidae	<i>Lasioglossum</i> sp1	1.36	8	0.93	7.63	53	0.53
Vespidae	Wasps	0	0	0	1.4	10	0.1
Formicidae	Ants	19.15	111	0.07	23.64	164	1.64
Coleoptera							
Nitidulidae	Beetles	0	0	0	18.44	128	1.28
Total		100	578	4.81	100	694	6.94

tube around the filament bases or between the anthers during anthesis. The wasps had only contact with the perianth and stigma without touching the stamens.

Breeding system

According to the OCI, both species are obligate xenogams, the diameter of their flowers are greater than 6 mm, and they are herkogamous and dichogamous. The pollen/ovule ratio for both species also agrees with an obligate xenogamy system (*Ariocarpus* = 1523:1 and *Neolloydia* = 989:1).

Mating system

Ariocarpus produced fruits in all the pollination treatments, which suggested a mixed mating system, with a tendency to outcrossing. Additionally, the ISI value was 0.15 for fruit set and 0.05 for seed set, which suggests that the species tends to be self-incompatible. Although the fruit set and seed set of artificial cross-pollination does not differ statistically from the control, they tend to be higher (table 3). *Neolloydia* only produced fruits in the control and artificial cross-pollination treatments, which indicates that it is an obligate outcrosser. No significant differences were found between the fruit set

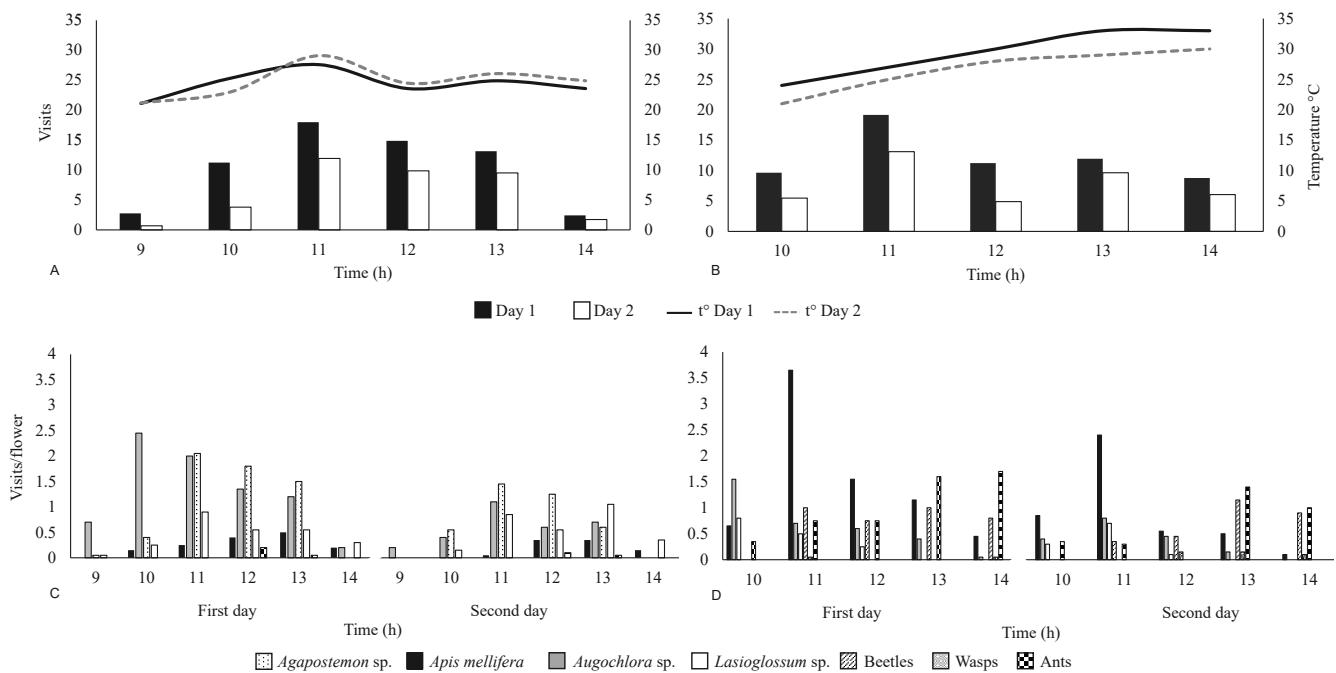


Figure 4 – A–B. Number of visits/day during the two days of anthesis. A. *Ariocarpus kotschoubeyanus*. B. *Neolloydia conoidea*. C–D. Number of visitor species/flower/hour. C. *Ariocarpus kotschoubeyanus*. D. *Neolloydia conoidea*.

Table 3 – Results of pollination experiments by species. Different letters per column indicate significant differences per treatment and species (n = 20 flowers/treatment).

<i>A. kotschoubeyanus</i>		
Treatments	Fruit set	Seed set
Control	0.85 ^a	0.88 (± 0.29) ^a
Artificial cross-pollination	1 ^a	1 (± 0.28) ^a
Natural self-pollination	0.05 ^b	0.02 ^b
Hand self-pollination	0.15 ^b	0.05 (± 0.04) ^b
Statistical test	$\chi^2 = 55.08$; p < 0.0001; d.f. = 3	F = 13.15; p < 0.001
<i>N. conoidea</i>		
Control	0.90 ^a	0.73(± 0.19) ^a
Artificial cross-pollination	0.95 ^a	0.96 (± 0.30) ^b
Natural self-pollination	0	0
Hand self-pollination	0	0
Statistical test	$\chi^2 = 0.35$; p > 0.05; d.f. = 1	F = 6.38; p < 0.001

of these treatments (0.90 ± 0.07 vs 0.95 ± 0.05 , respectively). However, significant differences were observed in the seed set of these treatments (0.73 ± 0.19 vs 0.96 ± 0.30 ; control vs artificial cross-pollination, respectively) (table 3).

DISCUSSION

The separation of the flowering period of *Ariocarpus kotschoubeyanus* and *Neolloydia conoidea* could be an important factor in floral resource maintenance through time and avoid interspecific pollination (Elzinga et al. 2007; Giorgis et al. 2015). It is interesting to point out that during the months in which the studied species did not produce flowers, another globose cactus, *Mammillaria parkinsonii* Ehrenb., also present in the study site, flowered, therefore supplementing the presence of similar floral resources during the annual cycle. It could be an important factor to facilitate the coexistence of these cactus species (Silvertown 2004), which could require a shared set of pollinators. Despite the fact that no overlap was observed between flowering of both species, it may occasionally occur, as it was observed in 2014 when the *Neolloydia conoidea* flowering period extended until October, which overlaps with the start of the *Ariocarpus kotschoubeyanus* flowering period (personal observation). The flowers of both species are similar in colour, shape, behaviour, and rewards, which could promote competition for pollinators, if these are in low number and there is temporal overlap.

Biotic interactions are not the only factors that may determine important aspects of floral phenology, since environmental factors also exert a strong influence (Fenner 1998). Crimmins et al. (2010) mentioned that species responses could vary according to their physiological characteristics. For *Ariocarpus kotschoubeyanus*, no correlations were found between the environmental variables studied and floral bud production, which suggest that there are other environment variables that act as triggers (Fenner 1998; Crimmins et al. 2010). Nonetheless, for *Neolloydia*

conoidea, direct correlations were found between the studied environmental variables and floral bud production.

With respect to floral visitors, in both species, the main visitors were native solitary bees (*Augochlora* sp1, *Agapostemon* sp1, and *Lasioglossum* sp1) and the introduced honey bee *Apis mellifera*. *Agapostemon* sp1 was exclusive to the *Ariocarpus kotschoubeyanus* flowers. The record of these species agrees with that reported in melittophilous flowers of other globose cactus species (Martínez-Peralta & Mandujano 2012; Valverde et al. 2015; Matías-Palafox et al. 2017). Furthermore, the visitors of *Ariocarpus kotschoubeyanus* flowers reported in this study agreed with that reported for other species in the genus *Ariocarpus* (Martínez-Peralta & Mandujano 2012). The solitary bees, different from social bees as *Apis mellifera*, feed on a reduced group of plant species, which is why they are called oligolectic (Michener 2007; Martínez-Peralta & Mandujano 2012). Snelling & Danforth (1992) reported that the reproduction season of some of these species coincides with the flowering time of some cacti, thus very likely, native bees are a crucial element for the pollination process of the study species. On the other hand, the presence of *Apis mellifera* may also play an important role since it has been reported as pollinator of some cacti (Martínez-Peralta & Mandujano 2012; Valverde et al. 2015; Matías-Palafox et al. 2017). The effectiveness of *Apis mellifera* has been questioned because of its foraging strategy (Westerkamp 1991; Valido et al. 2014), since although compared with native pollinators, it is very efficient in pollen collection, it shows very low rates of deposition of pollen on stigmas (Sun et al. 2013; Valido et al. 2014). This could explain the pollen limitation found in pollination experiments with the flowers of *Neolloydia conoidea* whose main visitor was *Apis mellifera*. However, the effect of this bee on the pollination of both species is still largely unknown since *Apis mellifera* has been reported during the whole year and can extract great quantities of pollen and nectar, making it highly competitive compared to the native bee species (Paini 2004; Domínguez-Álvarez 2009). On the

other hand, its presence could be compensating the lack of native pollinators, given the current global pollinator crisis (Martínez-Peralta & Mandujano 2012; Ollerton et al. 2012).

The studied cacti species shared 75% of the pollinator species, which reinforces the idea that sequential flowering may allow the use of the same pollinator set among sympatric species (Giorgis et al. 2015; Egli & Giorgetta 2017). This would allow to minimise the risk of heterospecific pollen deposition (Kudo & Kasagi 2005) in species with very similar floral characteristics, which is the case for *Ariocarpus kotschoubeyanus* and *Neolloydia conoidea*.

The differences in visiting frequency may be due to the fact that fluctuations in insect populations may occur throughout the year or to annual or daily fluctuations in environmental variables (Kingsolver 1983) because insects – as exothermic organisms – are very susceptible to environmental changes (Tauber & Tauber 1981). Additionally, the floral resources provided by both species are present in different environmental conditions since *Ariocarpus kotschoubeyanus* produces flowers in the dry and less warm season compared to *Neolloydia conoidea* that produces flowers in the most humid and hot season. The generalist species *Apis mellifera* has low visitation rates to *Ariocarpus* flowers, which are present when the floral resource density in the community is scarce (personal observation). Two possible explanations exist: (1) *Apis mellifera* found other plant species that produced greater quantities of pollen or nectar, since it is capable of locating and communicating the location of optimum feeding sites even when found at great distances from its beehive (Winfree et al. 2009; Campos-Navarrete et al. 2013), and (2) *Ariocarpus kotschoubeyanus* flowers were not sufficiently attractive to *Apis mellifera* due to its low number of flowers per individual and their geophytic habit where the floral resource is closer to the ground compared to other plant species.

Nectar absence in flowers of the study species may be related with the melittophily pollination syndrome since the nectar reward is substituted by a high pollen production, which is a resource of great importance for bees (Valverde et al. 2015). However, it is possible that the technique used here was not adequate, as the presence of nectar has been reported in other populations of this species (Martínez-Peralta & Mandujano 2012).

With respect to the breeding system, both species showed an obligate xenogamous system, as it has been reported for the majority of the Cactaceae (Mandujano et al. 2010).

The similarities found in the reproduction systems of the cactus species studied could be influenced among others by pollinating bees, which may be acting as important selective factors both in the evolution of the mating system (Goodwillie et al. 2005; Guerrero et al. 2019) and the breeding systems (Martínez-Ramos et al. 2017), since both pollinators and seed dispersers affect the reproductive characteristics of the species with which they interact (Bodbyl Roels & Kelly 2011). In both species, the dichogamy is partial and the herkogamy varies between the first and second day of anthesis, which could promote pollen self-deposition in the last day, as reported in *Melocactus curvispinus* Pfeiff. (Nassar & Ramírez 2004). Pollen limitation has been

documented for a population of *Ariocarpus kotschoubeyanus* in northern México (Martínez-Peralta & Mandujano 2016). According to Martínez-Peralta et al. (2014b), the presence of partial self-incompatibility could be favouring the selection of self-compatible genotypes to guarantee reproductive success in the population they studied. Therefore, it would be interesting to perform more precise studies on the self-incompatibility systems in the population of Tolimán. *Neolloydia conoidea* is an obligate outcrosser, while *Ariocarpus kotschoubeyanus* has a mixed mating system that can represent a strategy to maximize its reproductive success (Martínez-Peralta et al. 2014a). Visitation rates were very high, so the higher values of fruit set and seed set obtained in artificial crosses compared to natural pollinations are probably the result of pollen deposition from flowers of the same individual (geitonogamy).

Going back to the two hypotheses set out, the results confirmed the first one since a great similarity existed between the flowers of both species. Additionally, their flowering phenology is sequential and they share 75% of the bee species that pollinate them, which is advantageous for both the cacti and the pollinators. Both cactus species benefit because they avoid direct competition for pollinators and interference by heterospecific pollen, and the flower resource is persistent through time as suggested by Egli & Giorgetta (2017). These results offer important information on the reproductive biology of two cactus species that coexist in the Chihuahuan Desert and pave the way for more precise studies on the complex interactions that take place within the xerophyte communities.

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REFERENCES

- Anderson E.F. 2001. *The cactus family*. Timber Press, Inc, Portland, Oregon.
- Augspurger C.K. 1981. Reproductive synchrony of tropical plants: experimental effects of pollinators and seed predators on

- Hybanthus prunifolius* (Violaceae). *Ecology* 62(3): 775–788. <https://doi.org/10.2307/1937745>
- Bayona C.A. 2016. Los suelos en el estado de Querétaro. In: Jones R.W. & Serrano C.V (eds) Historia Natural de Querétaro: 87–89. Universidad Autónoma de Querétaro Press, México.
- Bravo-Hollis H. & Sánchez-Mejorada H. 1991. *Las cactáceas de México*. Vol. II. Universidad Autónoma de México Press, Mexico.
- Bodbyl Roels S.A. & Kelly J.K. 2011. Rapid evolution caused by pollinator loss in *Mimulus guttatus*. *Evolution* 65(9): 2541–2552. <https://doi.org/10.1111/j.1558-5646.2011.01326.x>
- Campos-Navarrete M.J., Parra-Tabla V., Ramos-Zapata J., Díaz-Castelazo C. & Reyes-Novelo E. 2013. Structure of plant–hymenoptera networks in two coastal shrub sites in Mexico. *Arthropod-Plant Interactions* 7(6): 607–617. <https://doi.org/10.1007/s11829-013-9280-1>
- Comisión Nacional del Agua (CONAGUA) 2010. Información climatológica por estado. Available from <https://smn.conagua.gob.mx/es/informacion-climatologica-por-estado?estado=qro> [accessed 25 Sep. 2017].
- Conner J.K. & Rush S. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia* 105: 509–516. <https://doi.org/10.1007/BF00330014>
- Crimmins T.B., Crimmins M.A. & Bertelsen C.D. 2010. Complex responses to climate drivers in Honest of spring flowering across a semi-arid elevation gradient. *Journal of Ecology* 98(5): 1042–1051. <https://doi.org/10.1111/j.1365-2745.2010.01696.x>
- Cruden R.W. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31(1): 32–46. <https://doi.org/10.2307/2407542>
- Díaz-Segura O., Jiménez-Sierra C.L. & Matías-Palafox M.L. 2017. Algunas características de la biología reproductiva del peyote queretano *Lophophora diffusa* (Croizat) Bravo, (Cactaceae). *Cactáceas y Suculentas Mexicanas* 62(4): 116–127.
- Domínguez-Álvarez L.A. 2009. Fenología de las abejas de la Reserva Ecológica del Pedregal de San Ángel y su relación con la fenología floral. MD thesis, Universidad Nacional Autónoma de México, México.
- Eggl U. & Giorgetta M. 2017. Flowering phenology and observations on the pollination biology of South American cacti. 3. Temporally robust sequential “Big Bang” flowering of two unrelated sympatric globular cacti in northern Argentina. *Haseltonia* 23: 97–109. <https://doi.org/10.2985/026.023.0113>
- Eguiarte L.E., Nuñez-Farfan J., Domínguez C. & Cordero C. 1999. Biología evolutiva de la reproducción de las plantas. In: Nuñez-Farfan J. & Eguiarte L.E. (eds) La evolución biológica: 69–86. Ciencias revista de difusión de la Facultad de Ciencias, Instituto de Ecología, UNAM, CONABIO, México.
- Elzinga J.A., Atlan A., Biere A., Gigord L., Weis A.E. & Bernasconi G. 2007. Time after time: flowering phenology and biotic interactions. *Trends in Ecology & Evolution* 22(8): 432–439. <https://doi.org/10.1016/j.tree.2007.05.006>
- Fenner M. 1998. The phenology of growth and reproduction in plants. *Perspectives in Plant Ecology, Evolution and Systematics* 1(1): 78–91. <https://doi.org/10.1078/1433-8319-00053>
- Ferreira B.H.S., Gomes A.C., Souza C.S., Fabri J.R. & Sigríst M.R. 2018. Pollination and reproductive system of synchronopatric species of Cactaceae (Cactoideae) subject to interspecific flow of pollen: an example of ecological adaptation in the Brazilian Chaco. *Plant Biology* 20(1): 101–112. <https://doi.org/10.1111/plb.12641>
- Giorgis M.A., Cingolani A.M. & Gurvich D.E. 2015. Flowering phenology, fruit set and seed mass and number of five coexisting *Gymnocalycium* (Cactaceae) species from Cordoba Mountain, Argentina. *Journal of the Torrey Botanical Society* 142(3): 220–230. <https://doi.org/10.3159/TORREY-D-14-00017.1>
- Gleeson S.K. 1981. Character displacement in flowering phenologies. *Oecologia* 51: 294–295. <https://doi.org/10.1007/BF00540618>
- Goettsch B., Hilton-Taylor C., Cruz-Piñón G., et al. 2015. High proportion of cactus species threatened with extinction. *Nature Plants* 1: 15142. <https://doi.org/10.1038/nplants.2015.142>
- Gómez-Hinostrosa C., Sotomayor M., Hernández H.M. & Smith M. 2017. *Ariocarpus kotschoubeyanus* (amended version of 2013 assessment). The IUCN Red List of Threatened Species. <https://doi.org/10.2305/IUCN.UK.2013-1.RLTS.T41217A2951984.en>
- Goodwillie C., Kalisz S. & Eckert C.G. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics* 36: 47–79. <https://doi.org/10.1146/annurev.ecolsys.36.091704.175539>
- Guerrero P.C., Antinao C.A., Vergara-Meriño B., Villagra C.A. & Carvallo G.O. 2019. Bees may drive the reproduction of four sympatric cacti in a vanishing coastal mediterranean-type ecosystem. *PeerJ* 7: e7865. <https://doi.org/10.7717/peerj.7865>
- Guzmán U., Arias S. & Dávila P. 2003. Catálogo de cactáceas mexicanas. UNAM, CONABIO, México.
- Jiménez-Sierra C.L., Segura-Venegas D., Rendón-Aguilar B., Valverde Valdés T. & Ballesteros-Barrera C. 2019. Fenología floral de *Stenocactus obvallatus* (DC) Hill (Cactaceae) en Guanajuato (México). *Cactáceas y Suculentas Mexicanas* 64(2): 47–57.
- Kay K.M. & Schemske D.W. 2003. Pollinator assemblages and visitation rates for 11 species of Neotropical *Costus* (Costaceae). *Biotropica* 35: 198–207. <https://doi.org/10.1646/02159>
- Kingsolver J.G. 1983. Thermoregulation and flight in *Colias* butterflies: elevational patterns and mechanistic limitations. *Ecology* 64(3): 534–545. <https://doi.org/10.2307/1939973>
- Koptur S. 1983. Flowering phenology and floral biology of *Inga* (Fabaceae: Mimosoideae). *Systematic Botany* 8(4): 354–368. <https://doi.org/10.2307/2418355>
- Kudo G. & Kasagi T. 2005. Microscale variations in the mating system and heterospecific incompatibility mediated by pollination competition in alpine snowbed plants. *Plant Species Biology* 20(2): 93–103. <https://doi.org/10.1111/j.1442-1984.2005.00129.x>
- Lüthy J.M. 2001. The Cacti of CITES Appendix I. CITES identification manual. CITES, Federal Veterinary Office Switzerland, Botanical Garden of the University of Berne, IOS, & Sukulent-Sammlung, Zürich.
- Mandujano M.C., Carrillo-Angeles I., Martínez-Peralta C. & Golubov J. 2010. Reproductive biology of Cactaceae. In: Ramawat K.G. (ed.) Desert plants: biology and biotechnology: 197–230. Springer, Berlin & Heidelberg. https://doi.org/10.1007/978-3-642-02550-1_10
- Martínez-Peralta C. & Mandujano M.C. 2012. Biología de la polinización y fenología reproductiva del género *Ariocarpus* Scheidweiler (Cactaceae). *Cactáceas y Suculentas Mexicanas* 54(4): 114–127.
- Martínez-Peralta C. & Mandujano M.C. 2016. Pollen limitation in a rare cactus with synchronous mass flowering. *Haseltonia* 22(22): 2–8. <https://doi.org/10.2985/026.022.0102>

- Martínez-Peralta C., Márquez-Guzmán J. & Mandujano M.C. 2014a. How common is self-incompatibility across species of the herkogamous genus *Ariocarpus*. *American Journal of Botany* 10(3): 530–538. <https://doi.org/10.3732/ajb.1400022>
- Martínez-Peralta C., Molina-Freaner F., Gulubov J., Vázquez-Lobo A. & Mandujano M.C. 2014b. Comparative study of the reproductive traits and floral morphology of a genus of geophytic cacti. *International Journal of Plant Sciences* 175(6): 663–680. <https://doi.org/10.1086/676302>
- Martínez-Ramos L.M., Mejía R.M., Rojas-Aréchiga M. & Mandujano M.C. 2017. La hercogamia como indicador del sistema reproductivo de *Thelocactus leucacanthus* spp. *schmollii*. *Cactáceas y suculentas mexicanas* 62: 13–22.
- Matías-Palafox M.L., Jiménez-Sierra C.L., Golubov J. & Mandujano M.C. 2017. Reproductive ecology of the threatened “star cactus” *Astrophytum ornatum* (Cactaceae): a strategy of continuous reproduction with low success. *Botanical Sciences* 95(2): 245–258. <https://doi.org/10.17129/botsci.780>
- McIntosh M.E. 2002. Flowering phenology and reproductive output in two sister species of *Ferocactus* (Cactaceae). *Plant Ecology* 159: 1–13. <https://doi.org/10.1023/A:1015589002987>
- Michener C.D. 2007. The bees of the world. Second edition. Johns Hopkins University Press, Baltimore.
- Moeller D.A. 2004. Facilitative interactions among plants via shared pollinators. *Ecology* 85(12): 3289–3301. <https://doi.org/10.1890/03-0810>
- Nassar J.M. & Ramírez N. 2004. Reproductive biology of the melon cactus, *Melocactus curvispinus* (Cactaceae). *Plant Systematics and Evolution* 248(1): 31–44. <https://doi.org/10.1007/s00606-004-0193-4>
- Natalis L.C. & Wesselingh R.A. 2012. Shared pollinators and pollen transfer dynamics in two hybridizing species, *Rhinanthus minor* and *R. angustifolius*. *Oecologia* 170: 709–721. <https://doi.org/10.1007/s00442-012-2346-4>
- Ollerton J., Price V., Armbruster W.S., et al. 2012. Overplaying the role of honey bees as pollinators: a comment on Aebi and Neumann (2011). *Trends in Ecology & Evolution* 27(3): 141–142. <https://doi.org/10.1016/j.tree.2011.12.001>
- Paini D.R. 2004. Impact of the introduced honey bee: a review. *Austral Ecology* 29(4): 399–407. <https://doi.org/10.1111/j.1442-9993.2004.01376.x>
- QGIS Development Team 2020. QGIS Geographic Information System. Version 3.16. Open Source Geospatial Foundation. Available from <https://qgis.org> [accessed 3 Jun. 2020].
- Ruiz-Zapata T. & Arroyo M.T.K. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica* 10(3): 221–230. <https://doi.org/10.2307/2387907>
- Scheinvar L. 2004. Flora cactológica del estado de Querétaro. Diversidad y riqueza. Fondo de Cultura Económica, México.
- SEMARNAT 2010. NORMA Oficial Mexicana NOM-059-SEMARNAT-2010, Protección ambiental-Especies nativas de México de la flora y fauna silvestre-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Diario oficial de la Federación, 30 de diciembre de 2010.
- Silvertown J. 2004. Plant coexistence and the niche. *Trends in Ecology & Evolution* 19(11): 605–611. <https://doi.org/10.1016/j.tree.2004.09.003>
- Snelling R. & Danforth B. 1992. A review of *Perdita* subgenus *Macrotera* (Hymenoptera: Andrenidae). *Contributions in Science* 436: 1–12.
- Stebbins G.L. 1970. Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. *Annual Review of Ecology and Systematics* 1: 307–326. <https://doi.org/10.1146/annurev.es.01.110170.001515>
- Sun S., Huang S. & Guo Y. 2013. Pollinator shift to managed honey bees enhances reproductive output in a bumblebee-pollinated plant. *Plant Systematics and Evolution* 299: 139–150. <https://doi.org/10.1007/s00606-012-0711-8>
- Tauber C.A. & Tauber M.J. 1981. Insect cycles: genetics and evolution. *Annual Review of Ecology and Systematics* 12: 281–303. <https://doi.org/10.1146/annurev.es.12.110181.001433>
- Valido A., Rodríguez-Rodríguez M.C. & Jordano P. 2014. Impacto de la introducción de la abeja doméstica (*Apis mellifera*, Apidae) en el Parque Nacional del Teide (Tenerife, Islas Canarias). *Ecosistemas* 23: 58–66. <https://doi.org/10.7818/ECOS.2014.23-3.08>
- Valverde P.L., Jiménez-Sierra C., López-Ortega G., et al. 2015. Floral morphology, anthesis, and pollination success of *Mammillaria pectinifera* (Cactaceae), a rare and threatened endemic species of Central Mexico. *Journal of Arid Environments* 116: 29–32. <https://doi.org/10.1016/j.jaridenv.2015.01.016>
- Van Der Kooij C.J., Pen I., Staal M., Stavenga D.G. & Elzenga J.T.M. 2016. Competition for pollinators and intra-communal spectral dissimilarity of flowers. *Plant Biology* 18(1): 56–62. <https://doi.org/10.1111/plb.12328>
- Waser N.M. 1983. Competition for pollination and floral character differences among sympatric plant species: a review of evidence. In: Jones C.E. & Little R.J. (eds) Handbook of experimental pollination biology: 277–293. Van Nostrand Reinhold, New York.
- Westerkamp C. 1991. Honeybees are poor pollinators – why? *Plant Systematics and Evolution* 177: 71–75. <https://doi.org/10.1007/BF00937827>
- Winfree R., Aguilar R., Vázquez D.P., LeBuhn G. & Aizen M.A. 2009. A meta-analysis of bees’ responses to anthropogenic disturbance. *Ecology* 90(8): 2068–2076. <https://doi.org/10.1890/08-1245.1>
- Zar J.H. 2010. Biostatistical analysis. Fifth edition. Pearson, New Jersey.
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