

RESEARCH PAPER

How specialised is bird pollination in the Cactaceae?

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ABSTRACT

Many cactus species produce 'bird' flowers; however, the reproductive biology of the majority of these species has not been studied. Here, we report on a study of the pollination of two species from the *Cleistocactus* genus, cited as an ornithophilous genus, in the context of the different ways in which they are specialised to bird pollination. In addition, we re-evaluate the level of specialisation of previous studies of cacti with bird pollination and evaluate how common phenotypic specialisation to birds is in this family. Both *Cleistocactus* species exhibited ornithophilous floral traits. *Cleistocactus baumannii* was pollinated by hummingbirds, whereas *Cleistocactus smaragdiflorus* was pollinated by hummingbirds and bees. Pollination by birds has been recorded in 27 cactus species, many of which exhibit ornithophilous traits; however, they show generalised pollination systems with bees, bats or moths in addition to birds being their floral visitors. Of all cactus species, 27% have reddish flowers. This trait is associated with diurnal anthesis and a tubular shape. Phenotypic specialisation to bird pollination is recognised in many cactus species; however, it is not predictive of functional and ecological specialisation in this family.

INTRODUCTION

A common concept in floral biology is to understand floral traits as a result of the selective pressures of the animals that pollinate them. It is widely believed that floral traits correspond to specific pollen vectors; therefore, the large floral diversity may be arranged into different floral syndromes (Faegri & van der Pijl 1979). Even if this concept has been used to predict the main pollinators of several plant species (Stebbins 1970; Fenster *et al.* 2004), in the majority of cases this prediction is not accurate because most flowering plants actually have a generalised pollination system (Ollerton 1996; Waser *et al.* 1996; Ollerton *et al.* 2009). The majority of studies based on the concept of floral syndromes do not consider the natural dynamics beyond floral traits within the community, such as fluctuations in populations of the pollinators, their effectiveness, preferences or interactions with other species. This variability in time and space and the effects on plant reproduction would favour generalised rather than specialised pollination systems (Ollerton 1996; Waser *et al.* 1996; Gómez & Zamora 1999; Hegland & Totland 2005; Ollerton *et al.* 2007).

Ollerton *et al.* (2007) proposed that flowers show three different modes of generalisation (or specialisation): phenotypic (the adaptations exhibited by a flower), functional (the diversity of functional groups that pollinate a plant species) and ecological (the number of effective pollinators that interact with a plant species). This differentiation enables the characterisation of pollination systems using an approach that avoids the conceptual conflict that occurs when defined only as generalised or specialised (Waser 2006; Ollerton *et al.* 2007).

Flowers are a very distinctive characteristic of cactus species and show large diversity in shape, size, colour and anthesis

time (Gibson & Nobel 1986; Pimienta-Barrios & del Castillo 2002). Pollination systems in the Cactaceae are said to be specialised (Gibson & Nobel 1986; Pimienta-Barrios & del Castillo 2002; Mandujano *et al.* 2010); however, the majority of systems studied are generalist, with bats, bees, moths and birds being effective pollinators (Fleming *et al.* 2001; Bustamante *et al.* 2010; Ortega-Baes *et al.* 2011; Alonso-Pedano & Ortega-Baes 2012). At present, only one case of specialised pollination has been described in Cactaceae, the senita cactus-senita moth obligate mutualism (Fleming & Holland 1998; Fleming *et al.* 2001).

Bird flowers have been described for a large number of cactus species of different lineages, particularly in South America. In general, the taxonomic description of many of these species assumes that they have pollination systems specialised to birds, primarily hummingbirds (see Anderson 2001; Hunt 2006). In some South American cactus genera, such as *Oreocereus*, *Cleistocactus*, *Matucana* and *Denmoza*, ornithophilous flowers are a common characteristic (Anderson 2001; Hunt 2006), suggesting that these lineages have evolved toward bird specialisation. However, little is known of the relationship between birds and the cactus flowers (Díaz & Cocucci 2003; Nassar & Ramírez 2004; Colaço *et al.* 2006; Saleme Aona *et al.* 2006; Nassar *et al.* 2007; Fagua & Ackerman 2011; Larrea-Alcázar & López 2011).

Here, using the *Cleistocactus* genus (Tribe Trichocereae) as a study model to test the idea that cacti with bird flowers establish specialised relationships with birds, we determine in which mode these interactions are specialised, *sensu* Ollerton *et al.* (2007). The *Cleistocactus* genus includes 37–48 species distributed typically in tropical regions of South America, from Peru to Argentina (Anderson 2001; Hunt 2006). This genus is cited as an example of extreme phenotypic specialisation to bird pollination (Scogin 1985; Rose & Barthlott 1994; Anderson 2001;

Hunt 2006); however, no studies on its reproductive biology exist. Specifically, we studied the pollination biology of *Cleistocactus baumannii* and *C. smaragdiflorus*, two cactus species that are sympatric along part of their geographic range in northwest Argentina. We hypothesised that these two species have phenotypic and functional specialisation to hummingbirds. However, because many hummingbird species have been cited for the Dry Chaco ecoregion in Argentina (Aizen & Feinsinger 1994; Heredia 2011), where these two species are distributed, we also hypothesised that both cactus species are visited by several hummingbird species and that they therefore show ecological generalisation. In particular, we address the following questions: (i) do *C. baumannii* and *C. smaragdiflorus* flowers show phenotypic specialisation to bird-pollination; (ii) do both species depend on pollinators for fruit and seed production; (iii) do floral visitors correspond to their floral traits; and (iv) what level of specialisation *sensu* Ollerton *et al.* (2007) do they show? Additionally, we used the approach of Ollerton *et al.* (2007) to re-evaluate the modes by which previously studied cactus pollination systems that include birds as pollinators are specialised. Finally, using the available information, we analysed how common phenotypic specialisation is within the different lineages of Cactaceae. Specifically, we used different floral traits, cited as ornithophilous, for this analysis and considered colour as the most descriptive trait for this type of flower (Wilson *et al.* 2004; Curti & Ortega-Baes 2011).

MATERIAL AND METHODS

Study area

This study was performed in La Bodeguita ($25^{\circ}17'31.72''$ S, $65^{\circ}16'25.13''$ W), Salta province, Argentina (Fig. 1). The study area corresponds to the Dry Chaco ecoregion. The mean annual temperature and precipitation in this site are 18.4°C and 416 mm, respectively (Bianchi & Yañez 1992; Bianchi 1996). In addition, we searched and located other populations of both *Cleistocactus* species in the Salta province to record floral visitors. For this, we used MaxEnt software (Phillips *et al.* 2006) at select ten sites with a high probability of occurrence (≥ 0.75) of each species.

Natural history of the species

Both *Cleistocactus* species are shrubby, erect or arching cacti that are branched at their base (Fig. 2A, D). Flowers of *C. baumannii* (Lem.) Lem. are red, tubular, slightly curved and 's'-shaped (Fig. 2B). This species produces small spherical reddish fruits with white pulp and black seeds (Anderson 2001; Hunt 2006). *Cleistocactus smaragdiflorus* (F.A.C. Weber) Britton & Rose has straight tubular pink flowers, with green apical tepals (Fig. 2C); its fruits are pink or red, spherical and contain white pulp and black seeds (Anderson 2001; Hunt 2006). The seeds of both of these species do not show dormancy (Ortega-Baes *et al.* 2010) and are positively photoblastic (L. Lindow-López, G. Galíndez & P. Ortega-Baes, unpublished data).

Floral morphology

Twenty *C. baumannii* and 33 *C. smaragdiflorus* open flowers from ten individuals that were chosen at random were collected

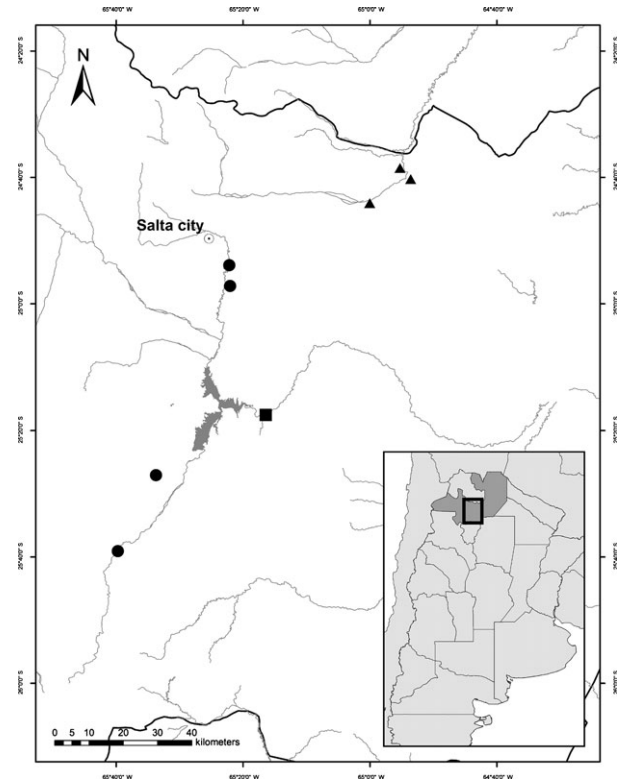


Fig. 1. Study area including the La Bodeguita site (square) and other populations of *Cleistocactus baumannii* (triangles) and *C. smaragdiflorus* (circles) in Salta, Argentina.

and preserved in 70% alcohol for morphometric analyses. The following floral traits were recorded: corolla diameter, flower length, gynoecium length, style length, stigma length and diameter, longest and shortest stamen length, ovary length and diameter, internal ovary length and diameter, nectar chamber length and diameter, stamen number, number of lobes and ovule number. We also described qualitatively the corolla colour and pollen colour from fresh open flowers.

Floral cycle

To determine the period in which flowers were available to pollinators, the phenological stages of 20 flowers from each species were recorded every 2 h, from flower budding until senescence.

Nectar production and sugar concentration

The nectar volume produced per flower was recorded for 15 flowers from each species. Flowers were covered with voile bags to prevent consumption by floral visitors. Nectar was extracted every 4 h using 80- μl capillary tubes. Sugar concentration was determined for ten flowers from each species every 4 h using a hand-held Brix refractometer.

Reproductive system

We performed an experiment during 2011 in the La Bodeguita site to determine the reproductive system of *C. baumannii* and



Fig. 2. *Cleistocactus baumannii* individuals (A) with their red, tubular-shaped flowers (B). *Cleistocactus smaragdiflorus* pink, tubular flowers with green, apical tepals (C), and a group of individuals (D). The hummingbird *Chlorostilbon lucidus* introducing its head into *Cleistocactus baumannii* flowers (E).

C. smaragdiflorus. The treatments were: (i) natural pollination: flowers not manipulated; (ii) autonomous self-pollination: flowers excluded without manipulation; (iii) hand self-pollination: flowers emasculated and hand-pollinated using pollen from the same flower and from other flowers of the same plant; and (iv) hand cross-pollination: flowers emasculated and hand-pollinated using pollen of five flowers from other individuals. Flowers from treatments (2), (3) and (4) were excluded from pollinators using voile bags following the treatments. Natural pollination was also performed in 2012 using two additional populations of *C. baumannii* and *C. smaragdiflorus*, in areas inhabited by only one of the species. Response variables were fruit set (number of fruits/number of flowers) and seed set (number of seeds/number of ovules).

Floral visitors

Floral visitor recording was conducted based on focal observations of five flowers from *C. baumannii* and *C. smaragdiflorus*, over 15 min every 2 h from 07:00 to 18:00 h. This observation was conducted over seven non-consecutive days during the flowering period in the La Bodeguita site, where these species overlap in distribution. We also videotaped four

C. baumannii flowers for 225 min and four *C. smaragdiflorus* flowers for 150 min, distributed over three non-consecutive days. An additional sampling of floral visitors was examined during the 2012 flowering period in the La Bodeguita site and in ten additional sites for each species selected based on the MaxEnt model. It was only possible to record floral visitors in three additional sites for *C. baumannii* and four additional sites for *C. smaragdiflorus* (Fig. 1) because the remaining populations did not produce flowers. In all additional sites, focal observations were performed over two non-consecutive days. Insect visitors were collected using hand nets and later determined and deposited in the Entomology section in the Museo Argentino de Ciencias Naturales Bernardino Rivadavia (Buenos Aires, Argentina). Bees were recorded as visitors only if they went into the flower. Birds were identified using binoculars with the aid of a field guide (Narosky & Yzurieta 2010; Heredia 2011). Because flowers remained open during the night, we collected 15 stigmas (on three non-consecutive days) of closed flowers from each species that were later examined in the laboratory for moth scales, to evaluate moth visits during the night. In addition, we also set a UV light trap to capture moths from 22:00 to 01:00 h during three non-consecutive nights in 2010, 2011

Table 1. Floral traits (mean, SD, min and max) of *Cleistocactus baumannii* (n = 20) and *C. smaragdiflorus* (n = 33).

	<i>Cleistocactus baumannii</i>				<i>Cleistocactus smaragdiflorus</i>			
	mean	SD	min	max	mean	SD	min	max
corolla diameter	7.19	1.68	3.39	9.32	6.97	1.89	3.18	10.15
flower length	50.2	9.53	28.85	62.19	44.71	4.09	34.01	52.47
gynoecium length	41.28	11.05	19.62	55.79	35.1	4.9	18.75	43.54
style length	38.61	10.53	18.03	52.38	32.57	4.71	15.89	40.09
stigma length	2.67	0.89	1.13	4.13	2.66	0.73	0.96	4.42
stigma diameter	3.16	0.75	2.01	4.29	3.8	1.2	1.69	6.73
longest stamen length	35.81	8.35	18.32	45.43	31.43	4.44	19.57	38.94
shortest stamen length	27.27	7.42	7.38	37.15	25.93	6.27	9.95	36.89
ovary length	8.39	1.26	6	10.59	9.58	1.4	6.69	12.95
ovary diameter	6.87	0.99	5.13	8.21	7.63	0.88	5.67	9.43
internal ovary length	5.02	1.4	1.97	7.39	5.96	1.24	3.56	9.49
internal ovary diameter	3.62	0.97	2.11	4.92	3.85	0.82	2.33	5.93
nectar chamber length	7.39	2.93	1.71	11.13	4.33	1.16	2.39	6.52
nectar chamber diameter	3.92	1.15	1.5	5.57	3.24	0.86	1.78	5.43
stamen number	254.90	56.56	151	380	230.42	32.91	143	283
number of lobes	7.35	0.59	6	8	7.55	1.23	6	11
ovule number	915.15	364.01	200	1800	1325.64	303.17	772	2027

and 2012. Captured moths were taken to the laboratory to extract pollen samples from their bodies.

Modes of bird specialisation in previously studied cactus species

To analyse the modes of specialisation in previously published studies on cacti that presented birds as pollinators, we performed an intensive article survey using Scopus (<http://www.scopus.com>) and Google Scholar (<http://scholar.google.com>) up to December 2013. For this search, we used combinations of the following keywords: Cactaceae, cactus, pollination, bird and hummingbird. Additionally, we examined the literature cited in the articles obtained. From each studied species, we summarised information on floral shape, colour, anthesis, corolla length and width, nectar production, sugar concentration, floral syndrome and floral visitors. Scientific names used were those originally used by the author(s). In addition, we included in this analysis the two *Cleistocactus* species. With the purpose of visualising variability among these traits, a principal coordinates analysis using multidimensional scaling (MDS), as used in Wilson *et al.* (2004), was conducted for seven quantitative and qualitative variables (mean flower length, mean flower width, mean nectar production per flower, mean sugar concentration, anthesis, floral shape and colour). Variables were standardised and Euclidean distance was used for this analysis. This provides a graphic visualisation of the dissimilarity between flowers of each species based on traits associated with pollination. To use colour as a quantitative variable, species were coded numerically from 1 to 4, where one was used for completely white flowers and four was used for completely red flowers, and two or three were used for intermediate colours. For floral shape, species were coded from 1 to 3 (one for bowl-shaped, two for funnel-shaped and three for tubular-shaped). Finally, for floral cycle, two categories were considered: (i) nocturnal and (ii) diurnal flowers. Nocturnal flowers were those that showed nocturnal opening and floral

cycles that extended into the following morning, whereas diurnal flowers included those flowers that were exclusively diurnal (1-day flowers) and those that opened during the day and remained open for more than 1 day. To complete missing records for floral size and sugar concentration (only three were missing), we assigned mean values for the genus or the median value for the variable in the case of the genus with only one species.

Phenotypic specialisation to birds in the Cactaceae

To analyse how common phenotypic specialisation is to bird pollination across the cactus family lineages, we built a database of the 1430 recognised cactus species using the information available in Hunt (2006). We also took into account all recognised subspecies that showed differences among floral traits, thereby reaching 1584 records. The floral traits considered, in addition to colour, were: size (length and diameter), shape and anthesis time. We considered as reddish flowers all those that were red, pink, purple, orange and combinations thereof. Flowers that exhibited combinations of other colours in the corolla were not considered reddish.

We performed a randomisation test to evaluate whether the mean size (length and diameter) of reddish flowers was significantly different from the mean size of all cactus flowers. Because the distribution of these variables was asymmetric, median sizes were also evaluated. Additionally, we tested whether the percentage of diurnal flowers and tubular-shaped flowers among reddish flowers was significantly different from the percentage within the entire family. For each species, we indicated the corresponding subfamily and tribe and determined the percentage of reddish-flower species for each of these groups. We built a representation of the family phylogeny that included these groups, placing them based on the phylogeny proposed in Bárcenas *et al.* (2011). The independence between the proportion of reddish flowers in each group and in the entire family was evaluated using a randomisation test.

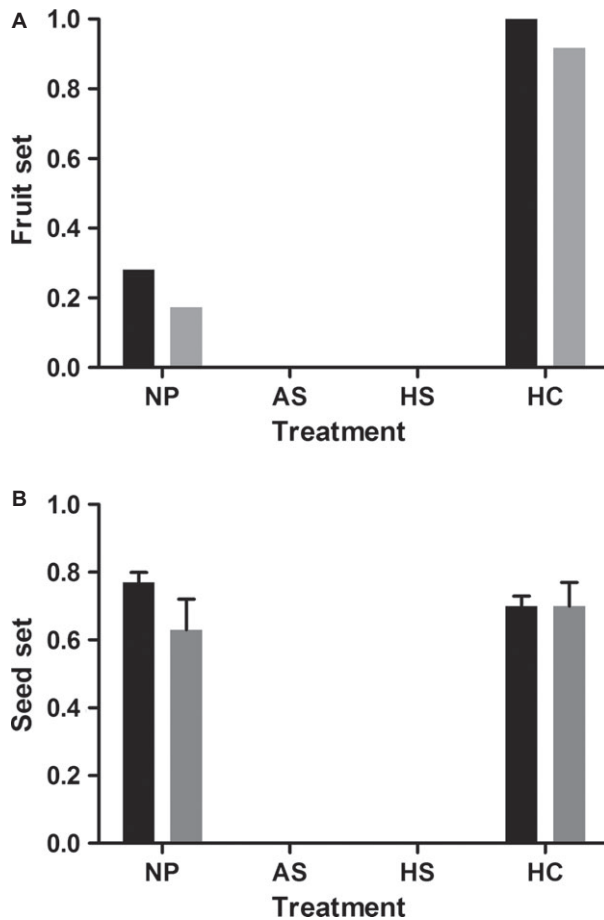


Fig. 3. Fruit set (A) and seed set (B) recorded for each treatment in the reproductive system experiment for *Cleistocactus baumannii* (black bars) and *C. smaragdiflorus* (grey bars). Treatments were: natural pollination (NP); autonomous self-pollination (AS); hand self-pollination (HS); and hand cross-pollination (HC).

For all randomisation tests, 1000 random samples without replacement were taken to obtain 95% confidence intervals.

Statistical analysis

The mean gynoecium length and mean longest stamen length were compared using a *t*-test to check stigma exertion. Nectar production and sugar concentration were compared between the *Cleistocactus* species using a *t*-test. Fruit sets between treatments from the reproductive system experiment were compared using Fisher's tests, whereas seed sets were compared using a *t*-test. All analyses were performed using Infostat software version 2009 (Di Rienzo *et al.* 2009) except for the randomisation tests, which were performed using R (R Development Core Team 2012).

RESULTS

Floral morphology

Cleistocactus baumannii flowers were tubular, slightly curved, with a red corolla and pink or red pollen (Fig. 2B). They were

50.2 ± 9.5 mm in length and 7.2 ± 1.7 mm in diameter. The flowers were hermaphroditic, with 254.9 ± 56.6 stamens and 915.2 ± 364 ovules (Table 1). *Cleistocactus smaragdiflorus* flowers were a tubular, straight in shape with a pink corolla and green apical tepals (Fig. 2D). They were 44.7 ± 4.1 mm in length and 6.9 ± 1.9 mm in diameter; their pollen was yellow or pink, and the flowers were hermaphroditic with 230.4 ± 32.9 stamens and 1325.6 ± 303.2 ovules (Table 1). A *t*-test of mean gynoecium length and mean longest stamen length indicated that stigmas were exerted in both *C. baumannii* ($t = 5.6$, $P < 0.001$) and *C. smaragdiflorus* ($t = 6.8$, $P < 0.001$).

Floral cycle

Cleistocactus baumannii and *C. smaragdiflorus* flowers began to open in the afternoon (16:00 h) and remained open until the morning of the third day, thus were open for approximately 48 h.

Nectar production and sugar concentration

Total nectar production per flower was 24.9 ± 20.2 μ l in *C. baumannii* and 26.5 ± 21.3 μ l in *C. smaragdiflorus*, with no significant difference noted between the species ($t = -0.2$, $P = 0.9$). The largest proportion of nectar was available at the time of floral opening (79.2% in *C. baumannii* and 73.5% in *C. smaragdiflorus*). For both species, the flowers did not produce more nectar during the second day of the floral cycle. The mean sugar concentration was $64.5 \pm 4.6\%$ in *C. baumannii* and $62.2 \pm 3.79\%$ in *C. smaragdiflorus*, with no significant difference noted between the species ($t = 1.22$, $P = 0.2384$).

Reproductive system

For both species, fruits were produced only from natural pollination and hand cross-pollination treatments (Fig. 3A). Fruit set was significantly higher for hand cross-pollination compared with natural pollination (*C. baumannii*: $F = -0.7$, $P < 0.0001$; *C. smaragdiflorus*: $F = -0.7$, $P < 0.001$; Fig. 3A). No significant differences were found for seed set between these treatments for *C. baumannii* ($F = -1.7$, $P = 0.1$) or *C. smaragdiflorus* ($F = 0.6$, $P = 0.7$; Fig. 3B).

The fruit sets from natural pollination on the three *C. baumannii* populations (La Bodeguita: 0.28; additional populations: 0.21 and 0.33) did not differ significantly ($\chi^2 = 0.68$, $P = 0.7133$). However, in the case of *C. smaragdiflorus*, the differences were significant between the populations ($\chi^2 = 15.83$, $P = 0.0004$), with a lower fruit set in the La Bodeguita site (0.17) compared with the two other populations (0.46 and 0.64).

Floral visitors

Cleistocactus baumannii was visited exclusively by hummingbirds (Trochilidae). In the La Bodeguita site, we recorded seven visits of *Chlorostilbon lucidus* and two of *Colibri coruscans* in 2011, and one visit of each of these species in 2012. Together with the three other populations, where the species did not share habitat with *C. smaragdiflorus*, we recorded nine *C. lucidus* visits, two of which were observed *via* video recordings.

Cleistocactus smaragdiflorus was visited by *Xylocopa* sp. and by the hummingbirds *C. lucidus* and *C. coruscans*. In the La Bodeguita site, we recorded seven *Xylocopa* bees and two *C. lucidus* visits in 2011; whereas in 2012, we recorded only one *C. lucidus* visit and no visit by bees. We recorded one *C. lucidus* and two *C. coruscans* visits in only one of the four additional populations sampled. In the rest of the populations, we did not see floral visitors; videotaped flowers showed no visits.

For both cactus species, *C. lucidus* introduced its head almost completely into the flower during the visit, whereas the larger *C. coruscans* introduced its bill and only part of its head. *Xylocopa* bees attempted to access the corolla and touched the stigma and stamens with their head and abdomen. Floral visits to both species were recorded in the morning and in the afternoon. No moth scales were observed in collected stigmas, and none of the captured moths in the UV light trap showed *Cleistocactus* pollen on their bodies. Nectar thieves or signs of their activity were not seen.

Modes of bird specialisation in previously studied cactus species

Bird pollination in cactus flowers has been documented for 27 species, including the two *Cleistocactus* species (Table S1). This list includes species of subfamilies Opuntioideae (tribe Opuntieae) and Cactoideae (tribes Cereeae, Pachycereeae and Trichocereae). In all cases, species were visited by some other animals in addition to birds, with the exception of *C. baumannii*. The birds that visited cactus flowers included hummingbirds (Trochilidae) for all cases, with the exception of two *Opuntia* species from the Galápagos (visited by finches and mockingbirds) and the North American columnar cactus *Carnegiea gigantea* (visited by several bird species, but mainly doves). In addition to birds, 81% of the species were visited by bees and 52% by nocturnal animals (bats and/or moths). Ornithophilous syndrome was recognised by the author(s) in 59% of bird-pollinated cacti. Of all species studied, 63% had reddish flowers, and the majority (88%) were tubular in shape. A PCA revealed a spatial differentiation between species with red flowers and species with white or yellow flowers (Fig. 4).

Phenotypic specialisation to birds in the Cactaceae

With respect to all cactus species, 1517 records have been generated for flower colour (including all species and subspecies with different floral traits), of which 424 are reddish (27%). We recorded a high variation in floral size within species with reddish flowers, from very small flowers on the one hand (e.g. *Mammillaria* genus), to some of the biggest flowers in the family on the other hand (e.g. *Arrojadoa dinae*, *Cereus stenogonus*, *Discocactus ackermannii* and *Hylocereus stenopterus*). A randomisation test indicated that the corolla length (mean and median) of reddish flowers did not differ from that of all species together, whereas the corolla diameter (mean and median) was significantly lower for reddish flowers.

With regard to floral cycle, we obtained information on 621 taxa, 66% of which showed diurnal anthesis. In this group, reddish flower species were represented by 139 species, with a significantly higher proportion of diurnal species (93%) than all evaluated taxa together ($P < 0.001$).

The total number of species for which we had information on floral shape was 1137, and 7.92% had tubular flowers. Within reddish-flowered species, the proportion of tubular flowers (20.1%) was significantly higher when compared with all species evaluated ($P < 0.001$). A similar result was obtained when the proportion of partially tubular-shaped flowers (i.e. tubular–funnel, tubular–campanulate) in the entire family (20.1%) was compared with the proportion within reddish-flowered species (29.2%). In addition, the proportion of reddish-flowered species within the tubular-shaped group (71.1%) or partially tubular-shaped (40.6%) group was significantly higher than expected by chance ($P < 0.001$).

For all subfamilies, with the exception of Maihuenioideae, we recorded species with reddish flowers. The subfamily with the highest proportion of reddish flowers was Pereskioideae (Fig. 5). Within the Opuntioideae subfamily, the highest proportion of reddish-flowered species was found in the Austrocylindropuntieae tribe, followed by Opuntieae and Cylindropuntieae. In the subfamily Cactoideae, the highest proportion of reddish-flowered species was seen in the tribe Pachycereeae, followed by Cacteae, Cereeae and Trichocereae. Randomisation tests indicated that for the majority of subfamilies and tribes, the proportion of reddish flowers was not significantly different from that expected by chance (Fig. 5). For this, the proportion of reddish flowers was calculated for subfamilies and also for the entire family, and similar results were obtained for all cases. In summary, 65 cactus genera (52.4% of total) had at least one species with reddish flowers, and 12 genera had more than 70% of their species with reddish flowers: *Castellanosia*, *Cleistocactus*, *Denmoza*, *Disocactus*, *Geohintonia*, *Matucana*, *Melocactus*, *Neolloydia*, *Nopalea*, *Oreocereus*, *Pelecyphora* and *Schlumbergera*.

DISCUSSION

Reproductive biology of *Cleistocactus*

Both *Cleistocactus* species studied here presented red tubular flowers with exerted stigma and coloured pollen. Among the floral traits indicated as typical of bird flowers, a red colour is considered to define ornithophilous flowers most accurately (Thomson *et al.* 2000; Wilson *et al.* 2004; Curti & Ortega-Baes 2011). In particular, it is a recurrent trait within cactus species with birds as pollinators. Moreover, a tubular shape is also cited as an ornithophilous trait (Faegri & van der Pijl 1979) that may operate as a physical barrier to some other pollinators (Brown & Kodric-Brown 1979). In the case of the species studied here, the narrow tubular shape of the flowers could restrict access to the nectar to other animals beyond hummingbirds. In the Cactaceae, reddish tubular flowers have been cited for various bird-pollinated species (Locatelli & Machado 1999; Scobell & Scott 2002; Nassar & Ramírez 2004; Colaço *et al.* 2006; Nassar *et al.* 2007; Fagua & Ackerman 2011). These traits are also present within other cacti of the tribe Trichocereae, such as *Oreocereus celsianus*, whose main pollinators are hummingbirds (Larrea-Alcázar & López 2011). The pollen colour of the studied species supports Rose & Barthlott (1994) idea that red pollen is an ornithophilous trait in cacti; however, some bird-pollinated species have yellow pollen, as reported here for some *C. smaragdiflorus* individuals.

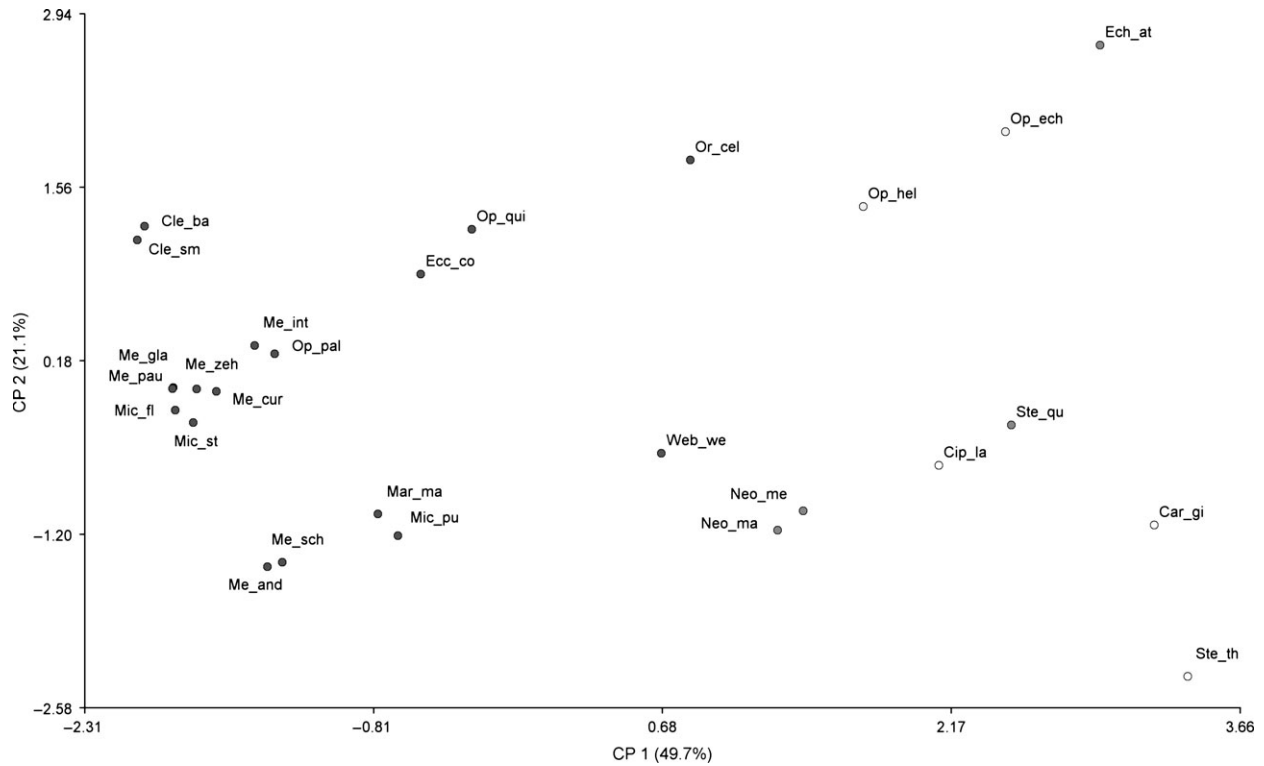


Fig. 4. Multi-dimensional scaling PCA for floral traits associated with pollination including all species studied in which bird pollination has been recorded.

The amount of nectar produced per flower was similar for both of the studied species, and was intermediate in value when compared with other bird-pollinated cacti. However, the sugar concentration in *Cleistocactus baumannii* and *C. smaragdiflorus* was the highest among cactus species pollinated by birds. This contrasts with the dilute concentrations that have been found for some bird flowers (Pyke & Waser 1981; Scogin 1985; Stiles

& Freeman 1993; Nicolson & Fleming 2003; Fenster *et al.* 2006; Curti & Ortega-Baes 2011); however, these findings are not the only recorded exception (Parra *et al.* 1993; Nassar & Ramírez 2004). Nectar production was lower and sugar concentration higher (more than double) than those found by Scogin (1985) for the same species; however, our values were recorded in natural populations. This difference is most likely a consequence of the climate conditions in the Dry Chaco ecoregion during the reproductive period.

Floral lifespan extended for more than a day in both species, an uncommon trait in the Cactaceae because the majority of species show 1-day flowers (Fleming *et al.* 1996, 2001; Ortega-Baes *et al.* 2011; Alonso-Pedano & Ortega-Baes 2012; Ortega-Baes & Gorostiague 2013). An extended floral cycle may be considered a strategy to reduce competition for pollinators (Nassar *et al.* 2007) or increase the likelihood of receiving visits from pollen vectors to mitigate their scarcity or low frequency (Primack 1985; Torres-Díaz *et al.* 2011).

The species studied were self-incompatible; they therefore depended exclusively on their pollinators for fruit and seed production. This feature is common in cactus species of the Trichocereae tribe (Ortega-Baes *et al.* 2011; Alonso-Pedano & Ortega-Baes 2012; Ortega-Baes & Gorostiague 2013); however, self-compatible species have also been found for this tribe (Sahley 1996; Larrea-Alcázar & López 2011). Despite this dependence on pollinators, our results revealed a low visitation rate for both species. *Cleistocactus baumannii* flowers were visited by two hummingbird species, whereas *C. smaragdiflorus* flowers were visited by two hummingbird species and also by bees. Nocturnal visits by moths have been recorded for other ornithophilous cactus species with extended floral lifespans (e.g. *Oreocereus celcianus*; Larrea-Alcázar & López 2011); however, there

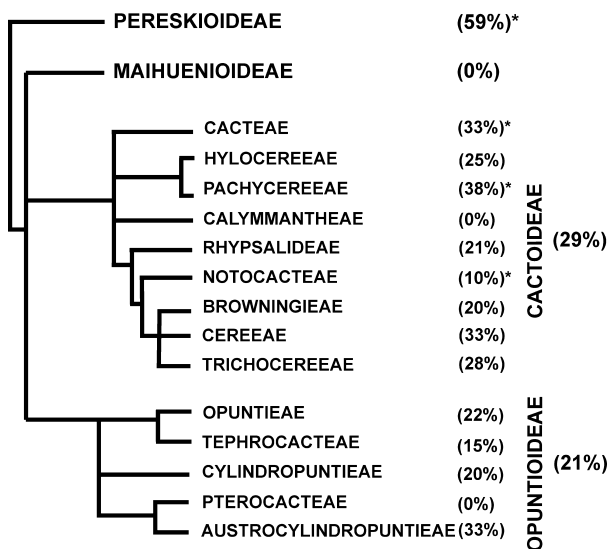


Fig. 5. Proportion of species with reddish flowers in each subfamily and tribe in the Cactaceae. Groups were organised based on the phylogeny of Bárcenas *et al.* (2011). *Indicates that the proportion of reddish-flowered species is significantly different from that expected by chance ($P < 0.005$).

was no evidence of nocturnal visits to the species studied here, even though the flowers remained open at night.

A low visitation rate of pollinators resulted in low fruit set for the natural pollination treatment: <30% in *C. baumannii* and 20% in *C. smaragdiflorus* in the La Bodeguita site. This value was similar to other evaluated populations of *C. baumannii*. However, in the remaining *C. smaragdiflorus* populations, natural pollination increased the fruit set significantly. This latter finding may be a consequence of several factors that were not evaluated in this study, including variation in pollinator abundance or plant demography (e.g. individual size, flower production). Fruit set results for natural pollination contrasted with those obtained from hand cross-pollination, suggesting that fruit production is pollen (pollinator)-limited in both species. In addition to low visitation, another cause of the low fruit production may relate to the hummingbird behaviour and cactus self-incompatibility. Hummingbirds typically visited several flowers on the same plant, depositing self-pollen in the stigma. Additionally, because both species show clonal reproduction, it is also possible that pollen received from flowers of neighbouring individuals was self-pollen. This finding has been demonstrated recently for *Echinopsis thelegona*, a clonal cactus species that lives in the same study area and in which sexual reproduction is extremely rare (Ortega-Baes & Gorostiague 2013).

Following the approach of Ollerton *et al.* (2007), both *Cleistocactus* species showed phenotypic specialisation because their flowers showed typical ornithophilous traits. However, only *C. baumannii* seems to be functionally specialised (although a longer study over a larger area might reveal other pollinators). Conversely, *C. smaragdiflorus* presented functional generalisation because its flowers were visited by more than one 'functional group'. Moreover, the pollination system of both species was ecologically generalised. This study constitutes the first report on the reproductive ecology of species of the *Cleistocactus* genus. Remarkably, it does not support the previously proposed idea that species in this genus present specialised pollination systems (Anderson 2001; Hunt 2006); at least, not for the multiple modes in which a species may be considered specialised (Ollerton *et al.* 2007).

Modes of bird specialisation in previously studied cactus species

In the Cactaceae, pollination by birds has been recorded in 27 species that are distributed through the Western hemisphere. These species include representatives of the subfamilies Opuntioideae and Cactoideae. Although a high proportion of these species are concentrated in tropical environments, we cannot state that this pollination mode is associated with a geographic component due to the sampling bias generated by the richness of these species in the tropics (Ollerton & Cranmer 2002). This plant–animal interaction appears to occur throughout the geographic range of the family and is limited primarily by the pollinator's geographic distribution rather than by the cactus species itself. Flowers with a reddish corolla were present in 63% of species studied that were visited by birds. Based on this trait, we may also differentiate species phenotypically specialised to birds based on the PCA.

Sugar concentration in the nectar produced by bird-pollinated cacti was quite uniform and corresponded with that indicated for bird flowers. Nevertheless, the amount of nectar

produced per flower was highly variable. Larger flowers produced much more nectar than smaller ones. This suggests that, at least for cacti, nectar production correlates with flower size rather than phenotypic specialisation. This finding was also found in other bird-pollinated systems (Geerts & Pauw 2009).

Only one cactus species (*Cleistocactus baumannii*) was visited exclusively by birds; *i.e.* was functionally specialised. The remaining species were also visited by other groups of animals, including bees, bats and moths. Despite presenting ornithophilous flowers, the pollination systems studied were generalist, although they were lightly classified as specialised to bird pollination by the original author(s). This pattern suggests that, in general, ornithophilous traits do not restrict other pollinators (in addition to birds) that could use the resources offered by the flower.

Phenotypic specialisation to birds in the Cactaceae

In the Cactaceae, phenotypic specialisation to birds, taking only the reddish colour trait into account, was present in 386 species. This number represents approximately 27% of the family and includes representatives of almost every subfamily and tribe. In addition, 12 cactus genera may be considered phenotypically specialised to birds, given that the majority of their species produce reddish flowers: *Castellanosia*, *Cleistocactus*, *Denmoza*, *Disocactus*, *Geohintonia*, *Matucana*, *Melocactus*, *Neolloydia*, *Nopalea*, *Oreocereus*, *Pelecypora* and *Schlumbergera*. Many have already been cited as examples of bird specialisation in the taxonomic literature (Rose & Barthlott 1994; Anderson 2001; Pimienta-Barrios & del Castillo 2002; Hunt 2006). Nonetheless, results may overestimate the number of species with actual phenotypic specialisation to bird pollination because some would be pollinated mainly by bees. This may be the case in species from the genera *Geohintonia*, *Mammillaria*, *Neolloydia*, *Pelecypora* and *Pereskia*, among others.

Our results, based on the available information for the entire family, revealed that in cacti, reddish flowers were associated with diurnal anthesis and a tubular shape. Additionally, even though there was large diversity in floral size among reddish-flowered cactus species, our results showed that floral diameter was smaller in those species compared with the rest of the family. This trend also supports the idea that a tubular shape is more common among cactus species with reddish flowers. The absence of a relationship between the size (length) of flowers and a reddish colour may indicate that there are no restrictions on the size of bird-pollinated flowers. Cactus species visited by birds included very small flowers (e.g. *Melocactus* and *Micranthocereus*) and very large flowers (e.g. *Echinopsis atacamenensis*). In turn, corolla width may restrict access to nectar for animals that cannot enter narrow flowers, as described for *Cleistocactus* species. For other plant groups, it has been shown that floral dimensions and hummingbird bills are not as strongly associated as expected; this finding may be attributed to other factors related to nectar features or interactions with other species (Temeles *et al.* 2002; but see Geerts & Pauw 2009).

Finally, we may state that phenotypic specialisation to bird pollination would have evolved several times in the Cactaceae, given that reddish flowers are a recurrent trait in its phylogeny. This specialisation has only been recorded at a genus level (e.g. *Cleistocactus*, *Melocactus*, *Oreocereus*) but not for higher taxa. It

is possible that, for some cases, this trait evolved from ancestors with bee-pollinated flowers (Pimienta-Barrios & del Castillo 2002), as has been suggested for other plant families (Alcantara & Lohmann 2010).

Concluding remarks

This study is the first to provide a better understanding of bird specialisation in the cactus family. As with other angiosperm families, the ornithophilous floral syndrome conceived as phenotypic specialisation is also recognised in the Cactaceae. However, we cannot state that a correspondence exists between this phenotypic specialisation and the actual pollinators that visit and effectively pollinate bird flowers. The attribution of pollination service to an exclusive pollinator or pollinator guild has not always been accurate because field observations may show different interactions with other species. In addition, phenotypic specialisation does not necessarily provide advantages for pollination because an opportunistic pollinator may prove as effective as the theoretically specialised pollinator (Nassar & Ramírez 2004; Ollerton *et al.* 2007; Frick *et al.* 2013). Furthermore, cases of functional specialisation in pollination systems are scarce, and the variation in pollinators between populations of the same species is remarkable. This finding supports the idea that specialisation is not exclusively determined by the plant's own traits, but also by ecological circumstances (Ollerton *et al.* 2007). Current evidence supports the fact that functional specialisation to bird pollination in the cactus family is rare, whereas ecological specialisation appears even rarer,

given that no cases have thus far been recorded. As our results indicate that qualification of a given pollination system as specialised should be done cautiously when the mode in which it is specialised (or generalised; *sensu* Ollerton *et al.* 2007) is not clearly defined. Cacti are commonly defined as specialised to certain pollinator types; however, this determination is typically based on floral traits (floral syndrome concept) or the most effective pollinator principle (*sensu* Stebbins 1970). Due to the great diversity of this family, many species without available information on their ecological interactions remain; thus, further studies are essential to understand the interactions between cacti and their mutualists.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Floral traits and pollinators of 27 cactus species for which bird pollination has been recorded.

REFERENCES

- Aizen M.A., Feinsinger P. (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology*, **75**, 330–351.
- Alcantara S., Lohmann L.G. (2010) Evolution of floral morphology and pollination system in *Bignoniaceae* (Bignoniaceae). *American Journal of Botany*, **97**, 782–796.
- Alonso-Pedano M., Ortega-Baes P. (2012) Generalized and complementary pollination system in the Andean cactus *Echinopsis schickendantzii*. *Plant Systematics and Evolution*, **298**, 1671–1677.
- Anderson E.F. (2001) *The cactus family*. Timber Press, Portland, OR, USA, pp 776.
- Bárceñas R.T., Yesson C., Hawkins J.A. (2011) Molecular systematics of the Cactaceae. *Cladistics*, **27**, 470–489.
- Bianchi A.R. (1996) *Temperaturas medias estimadas para la región noroeste de Argentina*. INTA, Salta, Argentina.
- Bianchi A.R., Yañez C.E. (1992). *Las precipitaciones en el noroeste argentino*. INTA, Salta, Argentina.
- Brown J.H., Kodric-Brown A. (1979) Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. *Ecology*, **60**, 1022–1035.
- Bustamante E., Casas A., Búrquez A. (2010) Geographic variation in reproductive success of *Stenocereus thurberi* (Cactaceae): effects of pollination timing and pollinator guild. *American Journal of Botany*, **97**, 2020–2030.
- Colação M.A.S., Fonseca R.B.S., Lambert S.M., Costa C.B.N., Machado C.G., Borba E.L. (2006) Biología reproductiva de *Melocactus glaucescens* Buining & Brederoo e *M. paucispinus* G. Heimen & R. Paul (Cactaceae), na Chapada Diamantina, Nordeste do Brasil. *Revista Brasileira de Botânica*, **29**, 239–249.
- Curti R., Ortega-Baes P. (2011) Relationship between floral traits and floral visitors in two coexisting *Tecoma* species (Bignoniaceae). *Plant Systematics and Evolution*, **293**, 207–211.
- Dar S., Arizmendi M., Valiente-Banuet A. (2006) Diurnal and Nocturnal Pollination of *Marginatocereus marginatus* (Pachycereeae: Cactaceae) in Central Mexico. *Annals of Botany*, **97**, 423–427.
- Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. (2009) *InfoStat versión 2009*. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.
- Díaz L., Cocucci A.A. (2003) Functional gynodioecy in *Opuntia quimilo* (Cactaceae), a tree cactus pollinated by bees and hummingbirds. *Plant Biology*, **5**, 531–539.
- Faegri K., van der Pijl L. (1979) *The principles of pollination ecology*. Pergamon Press, London, UK, pp 248.
- Fagua J.C., Ackerman J.D. (2011) Consequences of floral visits by ants and invasive honeybees to the hummingbird-pollinated, Caribbean cactus *Melocactus intortus*. *Plant Species Biology*, **26**, 193–204.
- Fenster C.B., Armbruster W.S., Dudash M.R., Wilson P., Thomson J.D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology and Systematics*, **35**, 375–103.
- Fenster C.B., Cheely G., Dudash M.R., Reynolds R.J. (2006) Nectar reward and advertisement in hummingbird-pollinated *Silene virginica* (Caryophyllaceae). *American Journal of Botany*, **93**, 1800–1807.
- Fleming T.H., Holland J.N. (1998) The evolution of obligate pollination mutualisms: senita cactus and senita moth. *Oecologia*, **114**, 368–375.
- Fleming T.H., Tuttle M.D., Horner M.A. (1996) Pollination biology and the relative importance of nocturnal and diurnal pollinators in three species of Sonoran desert columnar cacti. *The Southwestern Naturalist*, **41**, 257–269.
- Fleming T.H., Shaley C.T., Holland J.N., Nason J.D., Hamrick J.L. (2001) Sonoran desert columnar cacti and the evolution of generalized pollination systems. *Ecological Monographs*, **71**, 511–530.
- Frick W.R., Price R.D., Heady P.A. III, Kay K.M. (2013) Insectivorous bat pollinates columnar cactus more effectively per visit than specialized nectar bat. *The American Naturalist*, **181**, 137–144.
- Geerts S., Pauw A. (2009) Hyper-specialization for long-billed bird pollination in a guild of South African plants: the Malachite Sunbird pollination syndrome. *South African Journal of Botany*, **75**, 699–706.
- Gibson A.C., Nobel P.S. (1986) *The cactus primer*. Harvard University Press, Cambridge, MA, USA, pp 286.
- Gómez J.M., Zamora R. (1999) Generalization vs. specialization in the pollination system of *Hormatophylla spinosa* (cruciferae). *Ecology*, **80**, 796–805.
- Grant B.R., Grant P.R. (1981) Exploitation of *Opuntia* cactus by birds on the Galápagos. *Oecologia*, **49**, 179–187.
- Hegland S.J., Totland O. (2005) Relationships between species floral traits and pollinator visitation in a temperate grassland. *Oecologia*, **145**, 586–594.

- Heredia J. (2011) *Picaflores de Argentina*. L.O.L.A, Buenos Aires, Argentina, pp 104.
- Hunt D. (2006) *The new cactus lexicon*. DH Books, Milborne Port, UK, 373, pp 526.
- Ibarra-Cerdeña C., Iníiguez-Dávalos L., Sánchez-Cordero V. (2005) Pollination ecology of *Stenocereus queretaroensis* (Cactaceae) a chiropterophilous columnar cactus, in a tropical dry forest of Mexico. *American Journal of Botany*, **92**, 503–509.
- Larrea-Alcázar D.M., López R.P. (2011) Pollination biology of *Oreocereus celsianus* (Cactaceae), a columnar cactus inhabiting the high subtropical Andes. *Plant Systematics and Evolution*, **295**, 129–137.
- Locatelli E., Machado I.C. (1999) Comparative study of the floral biology in two ornithophilous species of Cactaceae: *Melocactus zehntneri* and *Opuntia palmadora*. *Bradleya*, **17**, 75–85.
- Mandujano M.C., Carrillo-Angeles I., Martínez-Peralta C., Golubov J. (2010) Reproductive biology of Cactaceae. In: Ramawat K.J. (Ed), *Desert Plants*. Springer, Berlin, Germany, pp 157–171.
- Mc Gregor S.E., Alcorn S.M., Olin G. (1962) Pollination and pollinating agents of the saguaro. *Ecology*, **43**, 259–267.
- Narosky T., Yzurieta D. (2010) *Guía para la identificación de las aves de Argentina y Uruguay*. Vazquez Mazzini Editores, Buenos Aires, Argentina, pp 427.
- Nassar J.M., Ramírez N. (2004) Reproductive biology of the melon cactus, *Melocactus curvispinus* (Cactaceae). *Plant Systematics and Evolution*, **248**, 31–44.
- Nassar J.M., Ramírez N., Lampo M., González J.A., Casado R., Nava F. (2007) Reproductive biology and mating system estimates of two Andean melocacti, *Melocactus schatzlii* and *M. andinus* (Cactaceae). *Annals of Botany*, **99**, 29–38.
- Nicolson S.W., Fleming P.A. (2003) Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. *Plant Systematics and Evolution*, **238**, 139–153.
- Ollerton J. (1996) Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant–pollinator systems. *Journal of Ecology*, **84**, 767–769.
- Ollerton J., Cranmer L. (2002) Latitudinal trends in plant–pollinator interactions: are tropical plants more specialized? *Oikos*, **98**, 340–350.
- Ollerton J., Killick A., Lamborn E., Watts S., Whiston M. (2007) Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon*, **53**, 717–728.
- Ollerton J., Alarcón R., Waser N.M., Price M.V., Watts S., Cranmer L., Hingston A., Peter C.I., Rotenberg J. (2009) A global test of the pollination syndrome hypothesis. *Annals of Botany*, **103**, 1471–1480.
- Ordoñez Rego J., Franceschinelli E.V., Zappi D.C. (2012) Reproductive biology of a highly endemic species: *Cipocereus laniflorus* N.P. Taylor & Zappi (Cactaceae). *Acta Botanica Brasílica*, **26**, 243–250.
- Ortega-Baes P., Gorostiague P. (2013) Extremely reduced sexual reproduction in the clonal cactus *Echinopsis thelegona*. *Plant Systematics and Evolution*, **299**, 785–791.
- Ortega-Baes P., Aparicio-González M., Galíndez G. (2010) Vivipary in the cactus family: an evaluation of 25 species from northwestern Argentina. *Journal of Arid Environments*, **74**, 1359–1361.
- Ortega-Baes P., Saravia M., Suhring S., Godínez-Álvarez H., Zamar M. (2011) Reproductive biology of *Echinopsis terscheckii* (Cactaceae): the role of nocturnal and diurnal pollinators. *Plant Biology*, **13**, 33–40.
- Parra V., Vargas C.F., Eguarte L.E. (1993) Reproductive biology, pollen and seed dispersal, and neighborhood size in the hummingbird-pollinated *Echeveria gibbiflora* (Crassulaceae). *American Journal of Botany*, **80**, 153–159.
- Phillips S.J., Anderson R., Schapire R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.
- Pimienta-Barrios E., del Castillo R.F. (2002) Reproductive biology. In: Nobel P.S. (Ed), *Cacti: biology and uses*. University of California Press, Los Angeles, CA, USA, pp 163–183.
- Primack R.B. (1985) Longevity of individual flowers. *Annual Review of Ecology and Systematics*, **16**, 15–37.
- Pyke G.H., Waser N.M. (1981) The production of dilute nectar by hummingbird and honeyeater flowers. *Biotropica*, **13**, 260–270.
- R Development Core Team. 2012. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria: URL <http://www.R-project.org/>
- Rose M.J., Barthlott W. (1994) Coloured pollen in Cactaceae: a mimetic adaptation to hummingbird-pollination? *Botanica Acta*, **107**, 402–406.
- Sahley C.T. (1996) Bat and hummingbird pollination of an autotetraploid columnar cactus, *Weberbauerocereus weberbaueri* (Cactaceae). *American Journal of Botany*, **83**, 1329–1336.
- Saleme Aona L.Y., Machado M., Pansarin E.R., Cardoso de Castro C., Zappi D., do Amaral M.C. (2006) Pollination biology of three Brazilian species of *Micranthocereus* Backeb. (Cereaceae, Cactoideae) endemic to the “campos rupestres”. *Bradleya*, **24**, 39–52.
- Schlumpberger B.O., Badano E. (2005) Diversity of floral visitors to *Echinopsis atacamenensis* subsp. *pasacana* (Cactaceae). *Haseltonia*, **11**, 18–26.
- Scobell S.A., Scott P.E. (2002) Visitors and floral traits of a hummingbird-adapted cactus (*Echinocereus coccineus*) show only minor variation along an elevational gradient. *American Midland Naturalist*, **147**, 1–15.
- Scogin R. (1985) Nectar constituents of the Cactaceae. *The Southwestern Naturalist*, **30**, 77–82.
- Stebbins G.L. (1970) Adaptive radiation of reproductive characteristics in angiosperms I: pollination mechanisms. *Annual Review of Ecology and Systematics*, **1**, 307–326.
- Stiles F.G., Freeman C.E. (1993) Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. *Biotropica*, **25**, 191–205.
- Temeles E.J., Linhart Y.B., Masonjones M., Masonjones H.D. (2002) The role of flower width in hummingbird bill length–flower length relationships. *Biotropica*, **34**, 68–80.
- Thomson J.D., Wilson P., Valenzuela M. (2000) Pollen presentation and pollination syndromes, with special reference to *Penstemon*. *Plant Species Biology*, **15**, 11–29.
- Torres-Díaz C.S., Gómez-González S., Stotz G.C., Torres-Morales P., Paredes B., Pérez-Millaqueo M., Gianoli E. (2011) Extremely long-lived stigmas allow extended cross-pollination opportunities in a high Andean plant. *PLoS One*, **6**, e19497.
- Valiente-Banuet A., Rojas-Martínez A., Arizmendi M.C., Da’ Vila P. (1997) Pollination Biology of Two Columnar Cacti (*Neobuxbaumia mezcalaensis* and *Neobuxbaumia macrocephala*) in the Tehuacan Valley, Central Mexico. *American Journal of Botany*, **84**, 452–455.
- de Viana M.L., Ortega-Baes P., Saravia M., Badano E., Schlumpberger B. (2001) Biología floral y polinizadores de *Trichocereus pasacana* (Cactaceae) en el Parque Nacional Los Cardones, Argentina. *Revista de Biología Tropical*, **49**, 279–285.
- Waser N.M. (2006) Specialization and generalization in plant–pollinator interactions: a historical perspective. In: Waser N.M., Ollerton J. (Eds), *Plant–pollinator interactions: from specialization to generalization*. University of Chicago Press, Chicago, IL, USA, pp 445.
- Waser N.M., Chittka L., Price M.V., Williams N.M., Ollerton J. (1996) Generalization in pollination systems and why it matters. *Ecology*, **77**, 1043–1060.
- Wilson P., Castellanos M.C., Houge J.N., Thomson J.D., Armbruster S.W. (2004) A multivariate search for pollination syndromes among penstemons. *Oikos*, **104**, 346–361.