

## Generalized and complementary pollination system in the Andean cactus *Echinopsis schickendantzii*

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**Abstract** The considerable floral diversity present in the cactus family has often been associated with the specificity of its pollinators. However, many cactus pollination systems are generalized as their flowers are pollinated by a wide spectrum of animals. For example, cactus species with white flowers, nocturnal anthesis and extended floral cycles would present generalized pollination systems in which both nocturnal and diurnal visitors could be effective pollinators. In this article, we tested this hypothesis by studying the pollination biology of *Echinopsis schickendantzii*, an Andean cactus with sphingophilous flowers. In addition, we evaluated whether the cactus's pollination system is complementary or redundant regarding the relative contributions of nocturnal and diurnal pollinators. Specifically, we studied the floral cycle, the reproductive system and the pollination effectiveness of floral visitors. The flowers of *E. schickendantzii* are self-incompatible; they opened at crepuscule and have an extended floral cycle. Moths were frequent visitors at night, whereas bees were frequent visitors during the day; both were effective pollinators of the cactus. Our results indicated that the flowers of this species present phenotypic, functional and ecological generalization, and their fruit set is determined by the contributions of both pollinator functional groups, i.e., they have complementary pollination systems. These results support the hypothesis that cacti in the extra-tropical deserts of South America have generalized pollination systems.

**Keywords** Bee-pollination · Columnar cactus · *Echinopsis schickendantzii* · Generalization · Moth-pollination

### Pollination system in an Andean cactus

Flowering plants present a great diversity of floral types that vary in shape, size, color and pollinator reward. Although several of these floral traits have been associated with specific pollinators in the conceptual framework of pollination syndromes (Fenster et al. 2004), most extant floral plant species have generalized pollination systems (Waser et al. 1996; Ollerton et al. 2007). Ollerton et al. (2007) suggested that the generalization or specialization shown by a flower may be influenced by the different biological and ecological characteristics of the flower, and the interactions of these characteristics with floral visitors in a community context. The authors defined three ways in which a flower can be generalized or specialized: phenotypic, functional and ecological. These authors have shown that flowers with both phenotypic generalization and specialization can exhibit functional or ecological generalization, and that generalization can vary across the geographic range of each species.

Cacti are conspicuous plants native to the arid and semiarid regions of the Western hemisphere (Ortega-Baes and Godínez-Alvarez 2006; Ortega-Baes et al. 2010c). In these environments, they establish positive interactions with other plants and animals through pollination, seed dispersal and seedling establishment processes (Godínez-Alvarez et al. 2003). With regards to the pollination process, it has been suggested that the different floral types described for cacti have a close relationship with pollinating animals that visit each species as each animal may

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require different floral characters for efficient pollination (Pimienta-Barrios and del Castillo 2002). However, many cactus pollination systems are generalized because their flowers are pollinated by a wide spectrum of taxonomical and functionally different animals, including animal types that are unexpected for a particular floral phenotype (Fleming et al. 2001; Ortega-Baes et al. 2011b).

White cactus flowers with nocturnal anthesis and nocturnal nectar production have been associated primarily with bats or moths (Fleming et al. 2001; Munguía-Rosas et al. 2009; Ortega-Baes et al. 2011b). However, several of these cactus species present floral cycles that extend into the following morning, a form of phenotypic generalization. This type of cycle allows both nocturnal visitors (bats or moths) and diurnal visitors (bees and/or birds) to access the flower. Fleming et al. (2001) suggested that this floral trait is a strategy to ensure sexual reproduction in the cactus when the main pollinator (nocturnal pollinator) is unpredictable. These authors have recorded different degrees of generalization in the pollination systems of three columnar cactus species in the Sonoran desert, indicating that the systems can be complementary or redundant. A pollination system is complementary if its fruit set is determined by the contributions of both nocturnal and diurnal pollinators, while it is redundant if one pollinator group can be replaced by the other without a loss in the fruit set (Fleming et al. 2001).

Many Andean cactus species of the genus *Echinopsis* Zuccarini (Tribe Trichocereae) present white flowers with nocturnal anthesis that are classified as sphingophilous flowers. Some *Echinopsis* species have extended their floral cycles into the following day (up to 48 h), allowing pollination by both nocturnal and diurnal animals (de Viana et al. 2001; Schlumpberger and Badano 2005; Schlumpberger et al. 2009; Walter 2010; Ortega-Baes et al. 2011b; but see Ossa and Medel 2011). Ortega-Baes et al. (2011b) have demonstrated that *Echinopsis terscheckii* (Parmentier) H. Friedrich and G.D. Rowley, an Argentine columnar cactus with these floral traits, has a generalized pollination system in which moths (Sphingidae and Noctuidae) are the main pollinators. The authors proposed that several columnar cacti native to the extra-tropical desert of Andean South America, which have nocturnal flowers, would also have generalized pollination systems and would be effectively pollinated by both nocturnal (moths) and diurnal visitors (bees and birds). Previous studies suggest that both groups of animal visitors contribute to the total fruit set (Walter 2010; Ortega-Baes et al. 2011b).

To examine this proposal, we studied the reproductive biology of *Echinopsis schickendantzii* F.A.C. Weber, an Andean cactus with nocturnal flowers. We tested the

hypothesis that the pollination systems of nocturnal-flowing *Echinopsis* species of the Andean extra-tropical region are generalist and complementary. Consequently, we expected that (1) *E. schickendantzii* has extended floral cycles, (2) both nocturnal and diurnal visitors are effective pollinators, and (3) the total fruit set and seed set are determined by the relative contributions both of nocturnal and diurnal visitors. To test our hypothesis, it was also essential to clarify the breeding system of this species to determine its dependence (or lack thereof) on pollinators to produce fruit and seeds.

## Materials and methods

### Study area

The study was conducted in the Quebrada El Sunchal (25° 10'S; 65° 49'W; Salta province, Argentina), located in the Central Andean Puna ecoregion (Olson and Dinerstein 2002). In this study area, the climate is semiarid, with a mean annual rainfall of 667 mm (Bianchi and Yañez 1992) and a mean temperature of 10.2 °C (14.6 °C in summer and 4.4 °C in winter; Bianchi 1996). *E. schickendantzii* is found in a community with several other cacti, such as *E. walteri* (R. Kiesling) H. Friedrich and Glaetzle, *Austrocylindropuntia vershafeltii* (Cels ex F.A.C. Weber) Backeberg and *Tunilla corrugata* (Salm-Dyck) D.R. Hunt and Iliff.

### Natural history of the species

*Echinopsis schickendantzii* is a long-lived, short, columnar cactus with a clumped growth form, distributed in Argentina and Bolivia (Fig. 1). The studied population was previously considered a different species (*Echinopsis smrziana* Backeberg; Kiesling 1978; Hunt 2006). The flowers of *E. schickendantzii* are funnel-shaped, white–pink (Fig. 1), and have an external length of  $11.88 \pm 0.23$  cm and an internal length of  $9.19 \pm 0.08$  cm. The internal diameter varies between 3.1 and 4.7 cm, while the external diameter varies between 10.5 and 14 cm. The flowers are hermaphroditic, with  $739.6 \pm 23.16$  stamens arranged in two series. The stigmas are exerted during anthesis. The number of ovules per flower is  $8,644.8 \pm 599.7$ , and the number of pollen grains per flower is  $2,379 \times 10^3 \pm 195 \times 10^3$ . The flowers do not produce nectar (Alonso-Pedano unpublished data). The fleshy fruits have black seeds that are positive photoblastic and non-dormant (Ortega-Baes et al. 2010a, b), and they germinate at a wide range of both alternating and constant temperatures (Ortega-Baes et al. 2011a).



**Fig. 1** **a** Quebrada El Sunchal site where the pollination biology of *Echinopsis schickendantzii* was studied. **b** Individuals, **c** buds, **d** flowers and **e** flower visitor of *Echinopsis schickendantzii*

### Floral cycle

The floral cycle was studied to determine whether the flowers were available to nocturnal and diurnal visitors. Thirty buds from ten individuals were tagged at noon, and the phenological stage of each flower was recorded every 3 h. The phenological stages considered were: (1) flower bud, (2) flower partially open, (3) flower completely open, (4) flower partially closed and (5) flower completely closed. We recorded the availability of pollen grains at the time of anthesis and the receptivity of the stigma throughout the floral cycle. Pollen availability was qualitatively determined

by touching the anthers with a piece of black paper to observe whether pollen grains adhered to it. Stigmatic receptivity was experimentally determined from 20 flowers (from 20 different individuals). During this experiment, pollinators were excluded using voile bags. Ten flowers were assigned to each of the following treatments: (1) nocturnal hand cross-pollination, in which emasculated flowers were hand-pollinated with pollen from other individuals during the night (00:00 h); (2) diurnal hand cross-pollination, in which emasculated flowers were hand-pollinated with pollen from other individuals during the day (12:00 h). The response variables were fruit set and seed set.

## Breeding system

We conducted an experiment during November 2005 to determine the breeding system of *E. schickendantzii*. Fifteen bagged buds were used in each of the following treatments: (1) natural pollination, in which flowers were not manipulated; (2) automatic self-pollination, in which flowers were bagged without manipulation; (3) hand self-pollination, in which flowers were hand pollinated using pollen from the same flower and pollen from other flowers of the same individual; (4) hand cross-pollination, in which emasculated flowers were hand pollinated using pollen from three flowers from other individuals. Pollinators were excluded from flowers in treatments (2), (3) and (4) using voile bags. Each flower used in this experiment was selected from a different individual. The response variables were fruit set and seed set.

## Floral visitors

Focal observations were performed on three flowers every 2 h for 30-min periods, during the night and during the day, over 3 nonconsecutive days (in November 2005 and 2006). The nighttime observations were conducted from 21:00 to 01:00 h using soft light, while diurnal visitors were observed from 08:00 to 16:00 h. As no visits were registered at night, we captured moths using a UV light-trap that was opened from 22:00 to 01:00 h over 3 nonconsecutive nights (in November 2005 and 2006). Pollen samples were taken from the bodies of captured animals using agar and analyzed in the laboratory. Moth visits to flowers were also evaluated by determining the proportion of stigmas with moth scales. Ten stigmas were collected before dawn during 3 consecutive nights in 2005, 2006 and 2007. Bees that visited the flowers were identified and housed in the Bernardino Rivadavia Museum (Argentina).

## Effectiveness of nocturnal and diurnal visitors

The effectiveness of nocturnal and diurnal visitors was evaluated with an experiment conducted in December 2005, 2006 and 2007. Forty-five buds (from 45 different individuals) were marked and bagged, and 15 were assigned to each of the following treatments: (1) natural pollination, in which emasculated flowers were not manipulated with pollen; (2) nocturnal natural pollination, in which emasculated flowers were available only to nocturnal pollinators from 20:00 to 05:00 h and diurnal pollinators were excluded using voile bags; (3) diurnal natural pollination, in which emasculated flowers were available only to diurnal pollinators from 07:00 h until flower closing and nocturnal pollinators were excluded using voile bags. The response variables were fruit set and seed set.

## Statistical analysis

Seed sets were compared between the different treatments of the stigmatic receptivity and breeding system experiments using *t* tests (Zar 1984) because only two of the treatments produced fruits in both experiments. Fruit sets for the different treatments of the effectiveness experiment were compared using a Cochran-Mantel-Haenzel test, taking the year into account as the stratification criteria (Agresti 1990). To compare seed sets of this experiment, we used a model that controlled variance heterogeneity. Fisher's test was used to detect which treatments were different. All analyses were performed using Infostat software (Di Rienzo et al. 2010).

## Results

### Floral cycle

The floral cycle lasts approximately 24 h. The flowers started opening at 18:00 h and were completely opened by 24:00 h. At 13:00 h of the following day, some flowers began to close, and 70 % of the flowers were closed at 18:00 h (Fig. 2). All flowers presented pollen at the beginning of anthesis. The fruit set was 0.9 for the nocturnal receptivity treatment (NR), and it was 1 for the diurnal receptivity treatment (DR). No significant difference was observed in the seed set between these treatments (NR:  $0.91 \pm 0.04$ , DR:  $0.92 \pm 0.02$ ;  $t = 0.30$ ,  $P = 0.77$ ).

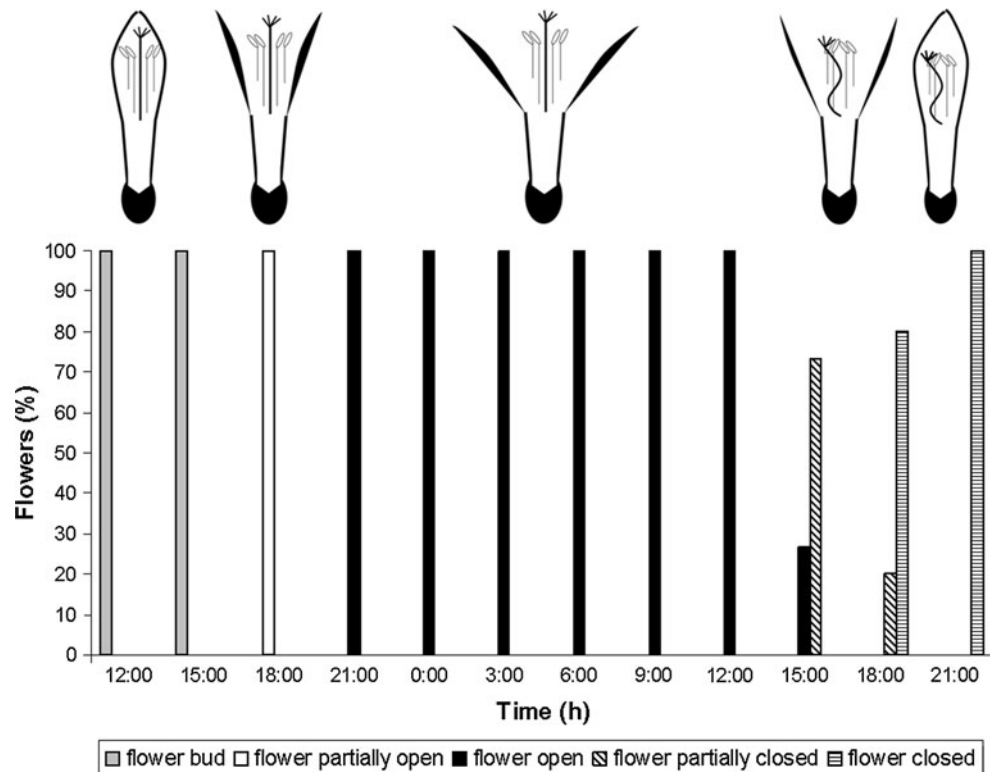
### Breeding system

Fruits were only produced in the natural and hand cross-pollination treatments, with similar levels of fruit set (0.8 and 0.87, respectively). The seed set was significantly different between treatments ( $t = 3.03$ ,  $P = 0.0084$ ) and was higher for the cross pollination treatment ( $0.91 \pm 0.03$ ).

### Floral visitors

Moths were not observed visiting the flowers of *E. schickendantzii*. In the UV-light traps, however, 15 morpho-species of moths were captured. Three moth species belonged to the Sphingidae family (*Hyles euphorbium*, *Sphinx maura* and *Manduca stuarti*) and one to the Saturniidae family. The remaining small moths were not identified. Pollen grains of *E. schickendantzii* were not registered on any of the moths. The proportion of stigmas with moth scales was similar among years (2005: 52.5 %; 2006: 61 %; 2007: 58 %). *Apis mellifera*, *Bombus atratus*, *Brachygllossula communis* and *Megachile mitcheli* were recorded among the diurnal visitors.

**Fig. 2** Proportions of *Echinopsis schickendantzii* flowers in different stages of the floral cycle



#### Effectiveness of nocturnal and diurnal visitors

The fruit set depended on the treatment, regardless of the year (Cochran-Mantel-Haenzel = 12.13,  $gl = 2$ ,  $P = 0.0023$ ; Fig. 3). The fruit set was greatest for the natural treatment (82 %), followed by the diurnal treatment (62 %). The highest deviations from what was expected under independence were observed in the nocturnal and natural treatments (minor and major differences from the expected values, respectively).

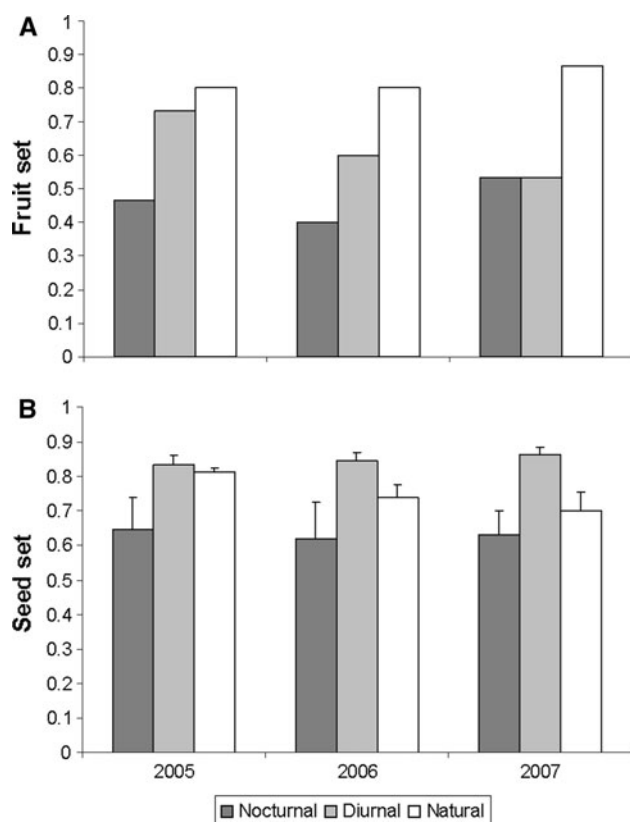
In 2005, the seed set did not significantly differ among the treatments ( $F = 1.75$ ,  $P = 0.193$ ; Fig. 3). In 2006 and 2007, differences were registered among the treatments ( $F = 4.00$ ,  $P = 0.0032$  and  $F = 8.50$ ,  $P = 0.0015$ , respectively; Fig. 3). For the last 2 years, according to Fisher's test, the seed set was greatest in the diurnal treatment (0.84 and 0.87, respectively; Fig. 3).

#### Discussion

Like many species of the subfamily Cactoideae (Pimienta-Barrios and del Castillo 2002; Mandujano et al. 2010), *E. schickendantzii* is a self-incompatible species; therefore, it depends on pollinators for seed production. The flowers of this species have nocturnal anthesis and an extended floral cycle, which lasts approximately 24 h. This floral trait appears to be common in nocturnal-flowering *Echinopsis*

species distributed across the arid regions of the South American extra-tropics (Walter 2010; Ortega-Baes et al. 2011b; but see Ossa and Medel 2011), and it has also been found in cactus species of other genera in North America and South America (Sahley 1996; Fleming et al. 2001; Bustamante et al. 2010; Larrea-Alcázar and López 2011). This trait would allow both diurnal and nocturnal visitors to access and potentially pollinate the flowers of the cactus.

According to our results, both bees and moths visited *E. schickendantzii* flowers. Although no moths were recorded visiting the flowers, these animals were apparently constant floral visitors during the night, because we found moth scales in the cactus stigmas during all 3 years of the study. Conversely, bees (native and exotic) were constant floral visitors during the day. These results are similar to those observed in other cactus species (Sahley 1996; Fleming et al. 2001; Clark-Tapia and Molina-Freaner 2004; Walter 2010; Ortega-Baes et al. 2011b). Both functional groups behaved as effective pollinators of *E. schickendantzii* flowers. This observation coincides with the results obtained by Walter (2010) for *E. chiloensis* (Colla) H. Friedrich and G.D. Rowley, but it differs from the findings for *E. terscheckii*, a columnar cactus in which bees are less effective pollinators than moths (Ortega-Baes et al. 2011b). Our results support the idea, proposed by Fleming et al. (2001), that the extended floral cycle of columnar cactus species with nocturnal flowers is a strategy to ensure sexual reproduction in the presence of unpredictable nocturnal pollinators.



**Fig. 3** **a** Fruit set (proportion) and **b** seed set (mean  $\pm$  SE) in different treatments of a pollinator effectiveness experiment conducted during 3 consecutive years in the population of *Echinopsis schickendantzii*

In the study system, nocturnal and diurnal pollinator effectiveness on fruit set varied independently of the year. The major contribution to fruit set was due to diurnal pollinators, though it was smaller than that obtained by natural pollination. The latter demonstrates that fruit set is determined by the contributions of both pollinator functional groups. Therefore, the pollination system of *E. schickendantzii* is complementary, as it is in the columnar cacti of the Sonoran Desert (Fleming et al. 2001). On the other hand, seed set was always greater in the diurnal treatment, even greater than that obtained by natural pollination, contrary to the findings of other studies (Jennersten and Morse 1991; Guitian et al. 1993; Miyake and Yahara 1998; Wolff et al. 2003). Our results support the hypothesis that the relative contribution of diurnal visitors to fruit set and seed set increases with increasing latitude, with greater dependence on diurnal visitors for seed production in extra-tropical cactus populations (Munguía-Rosas et al. 2009; but see Bustamante et al. 2010).

The extension of the floral cycle and the period of stigma receptivity in the nocturnal sphingophilous flowers of *E. schickendantzii* allows taxonomically different and diverse nocturnal and diurnal animals to visit (and

potentially pollinate) these flowers. Therefore, the flowers of this cactus species present phenotypic, functional and ecological generalization (sensu Ollerton et al. 2007), and their fruit set is determined by the contributions of both functional groups of pollinators. Future studies should evaluate whether the high cactus diversity of the extra-tropical arid regions of South America (Ortega-Baes et al. 2010c), which apparently contain a great variety of floral phenotypic specialization, actually present specialized pollination systems. This research is essential to understand how cactus pollination systems have diversified in American deserts in relation to different lineages and growth forms.

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## References

- Agresti A (1990) Categorical data analysis. Wiley, New York
- Bianchi AR (1996) Temperaturas medias estimadas para la región noroeste de Argentina. INTA, Salta
- Bianchi AR, Yañez CE (1992) Las precipitaciones en el noroeste argentino. INTA, Salta
- Bustamante E, Casas A, Búrquez A (2010) Geographic variation in reproductive success of *Stenocereus thurberi* (Cactaceae): effects of pollination timing and pollinator guild. *Am J Bot* 97:2020–2030
- Clark-Tapia R, Molina-Freaner F (2004) Reproductive ecology of the rare clonal cactus *Stenocereus eruca* in the Sonoran Desert. *Plant Syst Evol* 247:155–164
- de Viana ML, Ortega-Baes P, Saravia M, Badano E, Schlumpberger BO (2001) Biología floral y polinizadores de *Trichocereus pasacana* (Cactaceae) en el Parque Nacional Los Cardones, Argentina. *Rev Biol Trop* 49:279–285
- Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2010) InfoStat versión 2010. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination syndromes and floral specialization. *Ann Rev Ecol Syst* 35:375–403
- Fleming TH, Shaley CT, Holland JN, Nason JD, Hamrick JL (2001) Sonoran desert columnar cacti and the evolution of generalized pollination systems. *Ecol Monogr* 71:511–530
- Godínez-Alvarez HO, Valverde T, Ortega-Baes P (2003) Demographic trends in the Cactaceae. *Bot Rev* 69:173–203
- Guitian P, Guitian J, Navarro L (1993) Pollen transfer and diurnal versus nocturnal pollination in *Lonicera etrusca*. *Acta Oecol* 14:219–227
- Hunt D (2006) The new cactus lexicon. Remous Ltd, Milborne Port
- Jennersten O, Morse DH (1991) The quality of pollination by diurnal and nocturnal insects visiting common milkweed, *Asclepias syriaca*. *Am Midl Nat* 125:18–28
- Kiesling R (1978) El género *Trichocereus* (Cactaceae): I. Las especies de la República Argentina. *Darwiniana* 21:263–330

- Larrea-Alcázar DM, López RP (2011) Pollination biology of *Oreocereus celsianus* (Cactaceae), a columnar cactus inhabiting the high subtropical Andes. *Plant Syst Evol* 295:129–137
- Mandujano MC, Carrillo-Angeles I, Martínez-Peralta C, Golubov J (2010) Reproductive biology of Cactaceae. In: Ramawat KG (ed) *Desert plants: biology and biotechnology*. Springer, Berlin, pp 157–173
- Miyake T, Yahara T (1998) Why does the flower of *Lonicera japonica* open at dusk? *Can J Bot* 76:1806–1811
- Munguía-Rosas MA, Sosa VJ, Ojeda MM, De-Nova JA (2009) Specialization clines in the pollination systems of agaves (Agavaceae) and columnar cacti (Cactaceae): a phylogenetically controlled meta-analysis. *Am J Bot* 96:1887–1895
- 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* 56:717–720
- Olson DM, Dinerstein E (2002) The Global 200: priority ecoregions for global conservation. *Ann Mo Bot Gard* 89:199–224
- Ortega-Baes P, Godínez-Alvarez H (2006) Global diversity and conservation priorities in the Cactaceae. *Biodivers Conserv* 15:817–827
- Ortega-Baes P, Aparicio M, Galíndez G (2010a) Vivipary in the cactus family: an evaluation of 25 species from northwestern Argentina. *J Arid Environ* 74:1359–1361
- Ortega-Baes P, Aparicio-González M, Galíndez G, del Fueyo P, Sühling S, Rojas-Aréchiga M (2010b) Are cactus growth forms related to germination responses to light? A test using *Echinopsis* species. *Acta Oncol* 36:339–342
- Ortega-Baes P, Sühling S, Sajama J, Sotola E, Alonso-Pedano M, Bravo S, Godínez-Alvarez H (2010c) Chapter 8: diversity and conservation in the cactus family. In: Ramawat KG (ed) *Desert plants: biology and biotechnology*. Springer, Heidelberg, pp 157–173
- Ortega-Baes P, Galíndez G, Sühling S, Rojas-Aréchiga M, Daws MI, Pritchard HW (2011a) Seed germination of *Echinopsis schickendantzii* (Cactaceae): the effects of constant and alternating temperatures. *Seed Sci Technol* 39:219–224
- Ortega-Baes P, Saravia M, Sühling S, Godínez-Alvarez H, Zamar M (2011b) Reproductive biology of *Echinopsis terscheckii* (Cactaceae): the role of nocturnal and diurnal pollinators. *Plant Biol* 13:33–40
- Ossa CG, Medel R (2011) Notes on the floral biology and pollination syndrome of *Echinopsis chiloensis* (Colla) Friedrich & G.D. Rowley (Cactaceae) in a population of semiarid Chile. *Gayana Bot* 68(2):213–219
- Pimienta-Barrios E, del Castillo RF (2002) Reproductive biology. In: Nobel PS (ed) *Cacti. Biology and Uses*. University of California Press, Los Angeles, pp 163–183
- Sahley CT (1996) Bat and hummingbird pollination of an autotetraploid columnar cactus, *Weberbauerocereus weberbaueri* (Cactaceae). *Am J Bot* 83:329–336
- Schlumpberger BO, Badano E (2005) Diversity of floral visitors to *Echinopsis atacamensis* ssp. *pasacana* (Cactaceae). *Haseltonia* 11:18–26
- Schlumpberger BO, Cocucci AA, Moré M, Sérsic AN, Raguso RA (2009) Extreme variation in floral characters and its consequences for pollinator attraction among populations of an Andean cactus. *Ann Bot* 103:1489–1500
- Walter HE (2010) Floral biology of *Echinopsis chiloensis* ssp. *chiloensis* (Cactaceae): evidence for a mixed pollination syndrome. *Flora* 205:757–763
- Waser NM, Chittka L, Price MV, Williams NM, Ollerton J (1996) Generalization in pollination systems and why it matters. *Ecology* 1977:1043–1060
- Wolff D, Braun M, Liede S (2003) Nocturnal versus diurnal pollination success in *Isertia iaevis* (Rubiaceae): a sphingophilous plant visited by hummingbirds. *Plant Biol* 5:71–78
- Zar JH (1984) *Biostatistical analysis*. Prentice Hall, Inc., pp 122–159