Notes on the floral biology and pollination syndrome of *Echinopsis chiloensis* (Colla) Friedrich & G.D.Rowley (Cactaceae) in a population of semiarid Chile

Notas sobre la biología floral y síndrome de polinización de *Echinopsis chiloensis* (Colla) Friedrich & G.D.Rowley (Cactaceae) en una población de Chile semiárido

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ABSTRACT

We report some aspects of the floral biology of *Echinopsis chiloensis* ssp. *chiloensis* in a population near to the northern limit of its distribution. Anthesis is strictly diurnal and flowers remain open during 492 min on the average. *Echinopsis chiloensis* is self-incompatible. Flower longevity, nectar production along day, and floral visitors indicate that this population fits well to a diurnal insect pollination syndrome. These results contrast with a previous report indicating nocturnal anthesis and mixed mellitophylous-sphingophylous pollination syndrome in populations near to the southern limit of the distribution range. We suggest that the pollination syndrome of *E. chiloensis* is a labile rather than fixed condition that may depend on the abiotic characteristics and geographical location of the population under assessment.

Keywords: Flower longevity, nectar production, pollination syndrome, flower adaptation.

RESUMEN

Documentamos algunos aspectos de la biología floral de *Echinopsis chiloensis* ssp. *chiloensis* en una población del límite norte de su rango de distribución. La antesis es estrictamente diurna y las flores permanecen abiertas durante 492 min en promedio. La especie es auto incompatible. La longevidad floral, la marcha de néctar y los registros de polinizadores indican que la población de *E. chiloensis* se ajusta bien a un síndrome de polinización por insectos diurnos, lo cual contrasta con la antesis nocturna y el síndrome mixto melitófilo-esfingófilo descrito en poblaciones del límite sur de la distribución de la especie. Sugerimos que el síndrome de polinización de *E. chiloensis* es lábil y no fijo, siendo dependiente de la población bajo estudio y de su ubicación geográfica.

Palabras clave: Longevidad floral, producción de néctar, síndrome de polinización, adaptación floral.

INTRODUCTION

Pollination syndromes are useful categorizations to understanding floral diversification because they permit classification of pollinators according to putative similarities in the selection forces they impose on the flower phenotype (Fenster *et al*. 2004). Most columnar cacti are hermaphrodite plants that strongly depend on animals for reproduction (Gibson & Nobel 1986). While columnar cacti often rely on bats as pollinators in the tropics (e.g., Nassar *et al.* 1997, Fleming & Valiente-Banuet 2002, Ibarra-Cerdeña *et al*. 2005), more generalized and mixed pollination syndromes have been suggested to be prevalent in temperate ecosystems (Fleming *et al.* 1996, 2001, Valiente-Banuet *et al.* 2002). The few studies performed on pollination syndromes in

extra-tropical South America provide support to such generalization. For example Valiente-Banuet *et al*. (2004) reported that flowers of *Pachycereus pecten-aboriginum* (Engelm. ex S. Watson) Britton & Rose in tropical Mexican populations open at night and close early in the morning, the nectar is secreted only during the night, and pollination is restricted to bats. In northern populations, anthesis and nectar secretion occur indistinctly during the night and day, allowing the visitation and effective pollination by both nocturnal and diurnal pollinators. In Perú, the anthesis of *Weberbauerocereus weberbaueri* (K. Schum. ex Vaupel) Backeb. is nocturnal, and pollination occurs mainly by bats and hummingbirds in severe drought conditions (Sahley 1996). In Bolivia, in spite of the extensive anthesis period that covers diurnal and nocturnal, *Oreocereus celsianus* (Lem.

ex Salm-Dyck) Riccob. is pollinated almost exclusively by hummingbirds (Larrea-Alcázar & López 2011). In Argentina, *Echinopsis atacamensis* (Phil.) Friedrich & G.D.Rowley ssp. *pasacana* (F.A.C.Weber) G. Navarro is visited by diurnal and nocturnal animals, including bees, moths and birds (de Viana *et al.* 2001, Schlumpberger & Badano 2005), and *Echinopsis terscheckii* (Parm. ex Pfeiff.) Friedrich & G.D. Rowley, a species with nocturnal and diurnal flowers that open at dusk and close the next day, is visited by moths, bees, and birds, being moths the most efficient pollinators (Ortega-Baes *et al.* 2011). Unlike the species indicated above, *Echinopsis ancistrophora* Speg. presents diurnal or nocturnal anthesis depending on the population, and their flowers are pollinated by bees and hawkmoths (Schlumpberger *et al.* 2009). Recently, Walter (2010) described that, contrary to previous reports indicating diurnal anthesis (Hoffmann 1989, Hoffmann & Walter 2004), flowers of *Echinopsis chiloensis* (Colla) Friedrich & G.D.Rowley open at dusk and have a long longevity (17 - 42 h), which permits a mixed pollinator (bees and hawkmoths) syndrome in populations near to the southern distribution limit of the species. In this note we report some aspects of the floral biology of *Echinopsis chiloensis* ssp. *chiloensis* that confirm the existence of strict diurnal anthesis in a population near to the northern limit of the distribution range of the species. By doing so, we provide data on the breeding system, flower longevity, and flower visitors of *E. chiloensis* in a different environmental setting than that provided by Walter (2010) and discuss the consequences of this finding.

MATERIALS AND METHODS

This study was conducted during November 2006 at the National Reserve Las Chinchillas (31º30' S, 71º06' W), located 300 km northeast of Santiago. The climate is of a semiarid Mediterranean type with most rainfall concentrated in the winter season. Mean annual precipitation is 167 mm (data from 1974 to 1999), with ample inter-annual variation and long-lasting droughts alternated with unusual years of high precipitation that seemingly co-occur with ENSO events (Jaksic 2001). The reproductive season of *E. chiloensis* extends from early September through mid-November. Vegetation in the study site is thorny, with *Flourensia thurifera* DC. (Asteraceae), *Bahia ambrosioides* Lag. (Asteraceae), and *Porlieria chilensis* I.M.Johnst. (Zygophyllaceae) as the most common shrub species. In the study site, *E. chiloensis* is parasitized extensively by the leafless mistletoe *Tristerix aphyllus* (Miers ex DC.) Barlow & Wiens (Medel *et al.* 2004). Additional cactus species are represented by the columnar *Eulychnia acida* Phil., and the spheric-shaped *Cumulopuntia sphaerica* (Foerster) E.F. Anderson and *Eriosyce aurata* (Pfeiff.) Backeb.

A total of 43 flowers were tagged and monitored on an hourly basis during 4 consecutive days to determine flower longevity. Temperature was recorded with a thermocouple adhered to the cactus surface and oriented in the NW exposition. Nectar volume was measured on 20 previously bagged flower buds in an independent set of plants. Nectar was collected using 1 ml-microcapillars, every hour after flower opening until withering. To record potential pollinators, we performed focal observations of 30 min in 26 flowers from independent plants during 5 sunny days. Insects that entered the tubular hypanthium and contacted the reproductive organs of the flower were considered as flower visitors. All observations were performed between 8:30 - 16:00 h. Samples of flower visitors were collected at the end of the 5-day observation period for subsequent identification in the laboratory.

To determine the breeding system, we tagged flower buds from different cacti, and assigned them to one of the following treatments: (1) natural pollination (N = 40 flowers): flowers entirely available to visitors; (2) autonomous self-pollination (N = 84 flowers): pollinators excluded from flowers with white tulle bags, flowers without manipulation; (3) manual self-pollination (N = 47 flowers): pollinators excluded from flowers, stigmas fertilized with pollen of the same flower; (4) manual cross-pollination (N = 55 flowers): pollinators excluded from flowers, flowers emasculated, and stigmas fertilized with pollen of plants distant at least 5 m from the focal plant. Experiments were performed in the morning to ensure that stigmas were receptive and pollen was viable. We evaluated the outcome of treatments on: (a) fruit production or abortion (1/0), and (b) seed production of fruits. Treatment effects on dependent variables were examined in GLM using a binomial distribution for residuals and a logit-link function for independent and dependent variables. Coefficients were estimated using maximum likelihood. We calculate the index of self-incompatibility (ISI) proposed by Ruiz-Zapata and Arroyo (1978), which indicates the physiological capacity to carry out the self-fecundation. The index was calculated by dividing the number of seeds produced by manual self-pollination (treatment 3) and the number of seeds produced by manual cross-pollination (treatment 4). The index indicates self-incompatibility in the range 0 - 0.2, partial or incomplete self-compatibility when $0.2 <$ ISI value < 1, and complete self-compatibility when ISI = 1.

RESULTS

The flowers of *E. chiloensis* are funnel-shaped and displayed in lateral or apical position on the columns. Like most flowers in the genus, the flowers are tubular, external tepals are green and the internal tepals are white. Flowers are hermaphroditic with a lobed stigma that most of the

time exceeds the height of anthers (mean style length = 90.4 mm, mean height of the highest stamen = 74.7 mm). In general, flowers tend to be smaller than reported in southern populations by Walter (2010) (range flower length: 107 - 153 mm and 140 - 160 mm, respectively). The flowers began to open between 07:00 - 08:00 h when the temperature reaches 10ºC (Fig. 1a). The ratio of open flowers increased quickly from 0.29 at 07:00 - 08:00 to 0.97 at 09:00 - 10:00 h, when the air temperature is over 15ºC. After that, the ratio remains relatively stable until 14:00 h (temperature above 33ºC) when most flowers begin to close. No flower was open later than 17:00 h. Overall, individual flowers do not live for more than 492 min on the average (SD = 101), and do not re-open after closing. The daily pattern of nectar production showed two well-defined picks in the intervals 07:00 - 11:00 h and one additional pick at 15:00 - 16:00 h (Fig. 1b). The lowest

nectar production occurred in the interval 17:00 - 18:00 h after flowers experienced the maximum temperature (35ºC) (Fig. 1a, Fig. 1b). Flowers produced 54.1 µL of nectar along day on the average (SD = 49.5, range = 0 - 173 µL, N = 20).

The chance of fruit production differed among the four reproductive treatments (N = 226, df = 3, χ^2 = 181.4, P < 0.001) (see Table I). The flowers subject to autonomous self-pollination test produced less fruits than control flowers 1.2% vs. 90.0%, respectively (df = 1, χ^2 = 114.3, P < 0.001), indicating that pollen vectors are necessary for successful fertilization. The flowers subjected to manual cross-pollination had a higher fruit production than flowers subject to manual self-pollination tests (76.4% vs 4.2%, respectively, df = 1, χ^2 = 101.0, P < 0.001). This result is consistent with the low self-incompatibility index (0.043), suggesting a physiologic self-incompatibility in *E. chiloensis*.
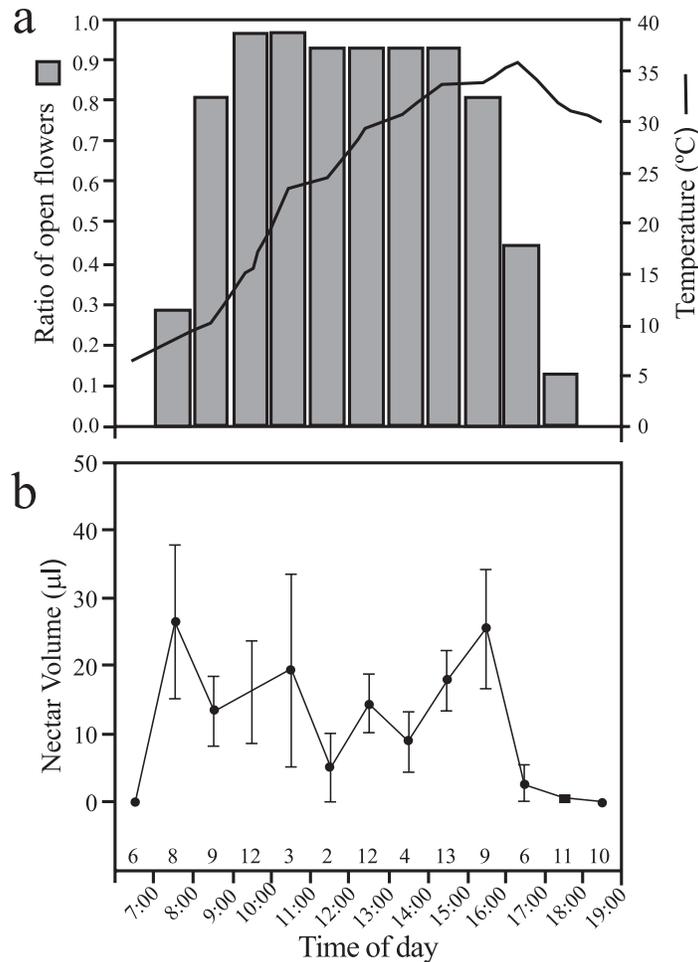

FIGURE 1. a) Ratio of open flowers of *Echinopsis chiloensis* as a function of the time of day. Continuous line indicates the mean temperature observed at each time interval. b) Mean nectar volume (± 1 SE) along day. Data obtained from 20 bagged flowers from independent plants. Values on the x-axis indicate the number of flowers measured at each time interval.

FIGURA 1. a) Razón de flores abiertas de *Echinopsis chiloensis* en función de la hora del día. La línea continua indica el promedio de temperatura observado en cada intervalo de tiempo. b) Promedio (± 1 EE) del volumen de néctar en 20 flores de plantas independientes a lo largo del día. Valores sobre la abscisa indican el número de flores medidas en cada intervalo horario.

Regarding flower visitors, all the species recorded were insects, mainly hymenopterans (8 species) and dipterans (7 species), followed by coleopterans (3 species) and lepidopterans (1 species) (Table II). The flowers of *E. chiloensis* were more frequently visited by hymenopterans and dipterans than coleopterans and lepidopterans (Table II). Hymenopterans were more frequent visitors than dipterans (GLM estimates, mean ± SE; hymenopterans: 2.81 ± 0.28; dipterans: 0.89 ± 0.29, $P < 0.001$). Within hymenopterans, there was a high heterogeneity in the visitation rate shown by species (LL = 520.4, χ^2 = 1040.8, df = 7, $P < 0.001$), with

Caenohalictus and *Megachile* as the most important genera. The visitation rate of the exotic honeybee *A. mellifera* albeit lower than most hymenopterans, did not differ from the native *C. nigerrima* (χ^2 = 1.21, df = 1, $P = 0.270$) and *Trichoturgus* sp 1 (χ^2 = 1.67, df = 1, $P = 0.196$) (Table II). The overall pattern of activity of insects was unimodal, reaching its highest values between 11:00 - 13:00 h (Fig. 2). Unlike hymenopterans, dipterans presented two peaks of activity along day with an almost nil activity between 12:00 - 16:00 h (Fig. 2).

TABLE I. Summary of results for reproductive tests in *Echinopsis chiloensis*.

TABLA I. Resumen de los resultados de las pruebas reproductivas en *Echinopsis chiloensis*.

TEST	FRUITS/FLOWERS	SEEDS PER FRUIT (SE)	SEEDS PER FLOWER (SE)
Autonomous self-pollination	1/84	0[†]	0[†]
Manual self-pollination	2/47	3408.0 (922)	145.0 (105.2)
Manual cross-pollination	42/55	9449.7 (279.3)	3380.3 (410.6)
Natural pollination	36/40	3040.1 (286.8)	2702. 7 (301.4)

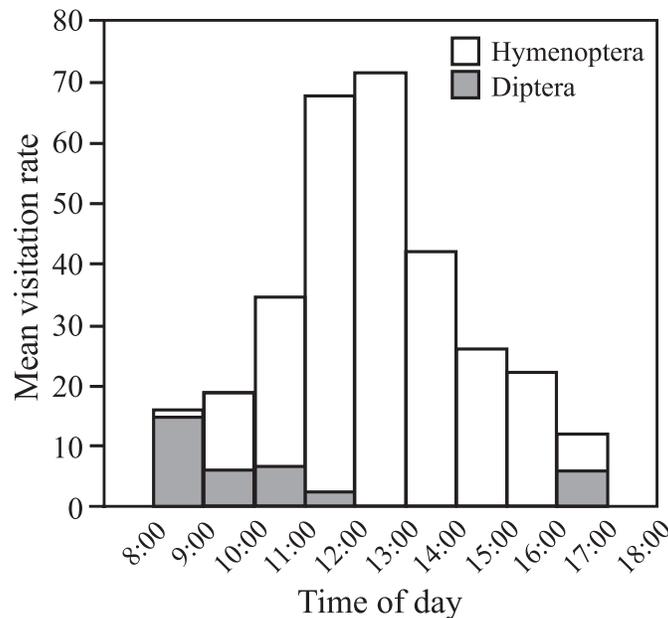† Seeds not measured in the only fruit recovered./ Semillas no medidas en el único fruto recuperado.

FIGURE 2. Mean visitation rate (visits flower[-1] h^{-1}) of hymenopterans and dipterans on individual flowers of *Echinopsis chiloensis* along day. Data on coleopteran and lepidopteran visits are not shown for simplicity (see Table II). Recordings based upon 30-min timed observations per flower.

FIGURA 2. Tasa promedio de visitas (visitas flor[-1] h^{-1}) de himenópteros y dípteros sobre flores individuales de *Echinopsis chiloensis* a lo largo del día. Los datos de visita de coleópteros y lepidópteros no se presentan en la figura para mayor simplicidad (ver Tabla II). Los registros se basaron en observaciones focales de 30 min por flor.

TABLE II. List of flower visitors in *Echinopsis chiloensis*. Visitation rate is expressed as number of visits flower[-1] h^{-1}.

TABLA II. Lista de visitantes florales en *Echinopsis chiloensis*. La tasa de visitas es expresada como el número de visitas flor[-1] h^{-1}.

ORDER/FAMILY/SPECIES	VISITATION RATE (SE)
Hymenoptera	
Apidae	
Apis mellifera	0.23 (0.09)
Antophoridae	
Centris nigerrima	0.10 (0.08)
Halictidae	
Caenohalictus sp 1	7.28 (1.54)
Caenohalictus sp 2	14.73 (5.01)
Lasioglossum sp	3.38 (1.61)
Megachilidae	
Megachile saulcyi	7.41 (2.40)
Trichoturgus sp 1	0.44 (0.27)
Trichoturgus sp 2	2.59 (0.68)
Diptera	
Mycetophilidae	
sp 1	0.26 (0.16)
sp 2	0.58 (0.28)
sp 3	2.88 (0.98)
Syrphidae	
Copestylum concinna	0.14 (0.09)
sp 1	0.08 (0.08)
sp 2	0.61 (0.36)
Sarcophagidae	
sp 1	0.08 (0.08)
Coleoptera	
Scarabeidae	
Arctodium vulpinus	0.04 (0.04)
Lichnia gallardoi	0.03 (0.08)
sp 1	0.08 (0.08)
Lepidoptera	
sp 1	0.04 (0.04)

DISCUSSION

Echinopsis chiloensis has been frequently described as an endemic columnar with white diurnal flowers (Hoffmann & Walter 2004). However, recently Walter (2010) described a mixed pollination syndrome that, in addition to diurnal insects, included dusk pollination attributable to hawkmoth species, hence suggesting the interesting possibility of a new pollination syndrome for this species (see also Ortega-Baes *et al.* 2011 for *Echinopsis terscheckii,* and Schlumpberger & Badano 2005 for *Echinopsis atacamensis*). Our results on anthesis time and flower longevity differ from data reported by Walter (2010), indicating that southern and northern populations of *E. chiloensis* differ substantially in floral traits. For example, while flowers in the southern populations open at dusk, and remain open by 17 - 42 h, flowers in the northern population are strictly diurnal and last only 8 h on the average, which exclude hawkmoths as potential pollinators.

The observation that not all floral traits of *E. chiloensis* match the standard hawkmoth pollination syndrome (Walter 2010) can be explained, in part, by the fact that northern populations did not receive visits by hawkmoths during our observation period. In consequence, sphingids cannot be invoked as important selective agents for northern populations of *E. chiloensis*. Other diurnal but unfrequent flower visitors, however, could have been undetected in our study. For example, the Chilean mockingbid *Mimus thenca* has been observed introducing its head into flowers of *E. chiloensis* in the study site (Medel, pers. obs.). Similarly, although hummingbirds are present at the study site (*Patagonas gigas* and *Sephanoides sephaniodes*), they have not been detected visiting flowers of *E. chiloensis* during more than 10 years of research in the site.

Because the physiological characteristics of flowers are generally thought to be adaptations to local environmental conditions in ways that ensure fertilization, it is likely that the discrepancy of our results with that of Walter (2010)

comes from variation in abiotic factors between sites. For instance, while southern populations receive an annual precipitation of 450 - 500 mm (Walter 2010), the population from Las Chinchillas receives an mean annual precipitation of 167 mm on the average. Similarly, the maximum daily temperature at Las Chinchillas is 5ºC higher than the southern populations. It is well known that temperature is one of the main factors determining the duration of anthesis (Primack 1985). It is likely that the high temperature and low water availability at Las Chinchillas impose physiological costs associated with flower development and anthesis to maternal plants, therefore promoting short-lived flowers as a way to circumvent abiotic constraints (see models in Ashman & Schoen 1994, 1996). In consequence, it is likely that the variable floral traits observed in *E. chiloensis* results from variation in flower longevity among populations.

An alternative explanation relies on the observation that in arid Chilean areas, pollination by moths may be low or even absent in comparison to humid and high elevation localities (Pérez *et al*. 2006). In the absence of nocturnal pollinators, probably northern populations of *E. chiloensis* shifted towards exclusively diurnal flowers. This phenomenon may not be only restricted to *E. chiloensis*. For instance, Valiente-Banuet *et al*. (2004) showed that variation in the time of anthesis across the geographic range of the columnar species *Pachycereus pecten-aboriginum*, can be explained by variation in the pollinator availability among populations. Similarly, Schlumpberger *et al*. (2009) described that depending of the population; *Echinopsis ancsistrophora* may show diurnal, short, and bee-pollinated flowers, or alternatively, nocturnal, long, and hawkmoth-pollinated flowers. Our results on *E. chiloensis* show a similar phenomenon. While flowers of southern populations are nocturnal, long, and bee- and hawkmoth-pollinated, flowers in the northern population are diurnal, short, and mostly probably bee-pollinated. More work is needed to fully appreciate the ecological and evolutionary consequences of among-population variation in the floral biology of *E. chiloensis*. The extent to which abiotic and biotic factors, or a combination of them, influence the geographic variation in anthesis, flower longevity, and pollination syndromes in *E. chiloensis* need to be assessed in future studies.

LITERATURE CITED

Ashman, T.L. & D.J. Schoen. 1994. How long should flowers live? Nature 371: 788-791.

Ashman, T.L & D.J. Schoen. 1996. Floral longevity: fitness consequences and resource costs. In: D.G. Lloyd & S.C.H. Barrett (eds.), Floral biology. pp 112-139. Chapman and Hall, New York.

De Viana, M.L., P. Ortega-Baes, M. Saravia, E.I. Badano & B. Schlumpberger. 2001. Biología floral y polinizadores de *Trichocereus pasacana* (Cactaceae). Journal of Tropical Biology 49: 277-283

Fenster, C.B., W.S. Armbruster, P. Wilson, M.R. Dudash & J.D. Thomson. 2004. Pollination syndromes and floral specialization. Annual Review of Ecology, Evolution and Systematics 35: 375-403.

Fleming, T.H., & A. Valiente-Banuet (eds). 2002. Columnar cacti and their mutualists: evolution, ecology, and conservation. University of Arizona Press, Tucson. 371 pp.

Fleming, T.H., M.D. Tuttle & M.A. Horner. 1996. Pollination biology and the relative importance of nocturnal and diurnal pollinators in three species of Sonoran Desert columnar cacti. Southwestern Naturalist 41: 257-269.

Fleming, T.H., C.T. Sahley, J.N. Holland, J.D. Nason & J.L. Hamrick. 2001. Sonoran desert columnar cacti and the evolution of generalized pollination systems. Ecological Monographs 71: 511-530.

Gibson, A.C. & P.S. Nobel. 1986. The cactus primer. Harvard University Press, Cambridge. 286 pp.

Hoffmann, A.E. 1989. Cactáceas en la flora silvestre de Chile (Primera Edición). Ediciones Fundación Claudio Gay, Santiago, Chile. 272 pp.

Hoffmann, A.E. & H.E. Walter. 2004. Cactáceas en la flora silvestre de Chile (2da Ed.). Ediciones Fundación Claudio Gay, Santiago. 307 pp.

Ibarra-Cerdeña, C.N., L.I. Iñiguez-Dávalos & V. Sánchez-Cordero. 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.

Jaksic, F.M. 2001. Ecological effects of El Niño in terrestial ecosystems of western South America. Ecography 24: 241-250.

Larrea-Alcázar, D.M. & R.P. López. 2011. Pollination biology of Oreocereus celsianus (Cactaceae), a columnar cactus inhabiting the high subtropical Andes. Plant Systematics and Evolution 295: 129-137.

Medel, R., E. Vergara, A. Silva & M. Kalin-Arroyo. 2004. Effects of vector behavior and host resistance on mistletoe aggregation. Ecology 85: 120-126.

Nassar, J.M., N. Ramírez & O. Linares. 1997. Comparative pollination biology of Venezuelan columnar cacto and the role of nectar-feeding bats in their sexual reproduction. American Journal of Botany 84: 918-927.

Ortega-Baes, P., M. Saravia, S. Suhring, H. Godínez-Alvarez & M. Zamar. 2011. Reproductive biology of *Echinopsis terscheckii* (Cactaceae): the role of nocturnal and diurnal pollinators. Plant Biology 13: 33-40.

Pérez, F., M.T.K. Arroyo, R. Medel & M.A. Hershkovitz. 2006. Ancestral reconstruction of flower morphology and

pollination systems in *Schizanthus* (Solanaceae). American Journal of Botany 93: 1029-1038.

Primack, R.B. 1985. Longevity of individual flowers. Annual Review of Ecology and Systematics 16: 15-37.

Ruiz-Zapata, T. & M.T.K. Arroyo. 1978. Plant reproductive biology of a secondary deciduous forest in Venezuela. Biotropica 10: 221-230.

Sahley, C.T. 1996. Bat and hummingbird pollination of an autotetraploid columnar cactus, *Weberbauerocereus weberbaueri* (Cactaceae). American Journal of Botany 83: 1329-1336.

Schlumpberger, B.O. & E.I. Badano. 2005. Diversity of floral visitors to *Echinopsis atacamensis* subsp. *pasacana* (Cactaceae). Haseltonia 11: 18-26.

Schlumpberger, B.O., A.A. Cocucci, M. Moré, A.N. Sérsic & R.A. Raguso. 2009. Extreme variation in floral characters and its consequences for pollinator attraction among populations of an Andean cactus. Annals of Botany 103: 1489-1500.

Valiente-Banuet, A., M.C. Arizmendi, A. Rojas- Martínez, H. Godínez-Alvarez & P. Dávila-Aranda. 2002. Biotic interactions and population dynamics of columnar cacti. In: T.H. Fleming & A. Valiente-Banuet (eds.), Columnar cacti and their mutualists: evolution, ecology, and conservation. Pp 225-240. University of Arizona Press, Tucson.

Valiente-Banuet, A. F. Molina-Freaner, A. Torres, M.C. Arizmendi & A. Casas. 2004. Geographic differentiation in the pollination system of the columnar cactus *Pachycereus pecten-aboriginum*. American Journal of Botany 91: 850-855.

Walter, H.E. 2010. Floral biology of *Echinopsis chiloensis* ssp. *chiloensis* (Cactaceae): evidence for a mixed pollination syndrome. Flora 205: 757-763.