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SHORT COMMUNICATION

Pollination biology of *Oreocereus celsianus* (Cactaceae), a columnar cactus inhabiting the high subtropical Andes

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Abstract The geographical dichotomy hypothesis suggests that columnar cacti in the tropics depend primarily on bats for pollination. This dependence may to be less in the outer tropics where many columnar cactus species (or their populations) show a relatively generalized pollination system with both nocturnal (moths and bats) and diurnal pollinators (bees and hummingbirds) (geographical dichotomy hypothesis). This hypothesis has been mostly tested in the northern tropics; nonetheless, our knowledge of columnar cactus species inhabiting the southern tropics is still scarce. The aim of this project was to evaluate the pollination biology of *Oreocereus celsianus*, a columnar cactus with restricted distribution in the subtropical Andes, to determine if the pollination system of this cactus tends to be more generalized than specialized because of the geographical position where it occurs. Observations of frequency of visit showed that *Patagona gigas* (Giant Hummingbird) is the main pollinator of the flowers, visiting them when they are opening (afternoon of the first day). Bees, wasps and moths were occasional visitors of the flowers. None of them seem to act as pollinator. Autogamy, geitonogamy and xenogamy treatments produced high fruit set, showing that *O. celsianus* has an unusual mixed mating system. The results suggest that this Andean columnar cactus is partially specialized on hummingbirds, with most pollination service performed by a single species, and it has the capacity of selfing ('fail-safe' pollination system). This mixed mating system (both outcrossing and selfing) may be a response to the unpredictable environment of the Prepuna in the subtropical Andes.

Keywords Andes · Hummingbirds · Nectar robbing · Semi-deserts · Self-compatible system · Southern tropics

Introduction

Angiosperms flowers offer an extraordinary range of shapes, colors and scents, reflecting high rates of evolutionary change in these traits, which can be grouped by the senses used by visitors to perceive them: visual, olfactory or auditory cues (Herrera and Pellmyr 2002). Plant pollination systems are thought to form a continuum from highly specialized systems with a single pollinator to generalized systems with many pollinator species (Johnson and Steiner 2000; Fleming et al. 1996; Fleming 2002). Several studies have demonstrated that columnar cacti in the intra-tropics depend primarily on nectar-feeding bats for pollination (Fleming 1993; Nassar et al. 1997; Valiente-Banuet et al. 1997a; Ibarra-Cerdeña et al. 2005; Munguía-Rosas et al. 2009). Their large flowers with nocturnal anthesis, large nectar quantities, wide corolla entrance and strong scent supported this interpretation (Godínez-Álvarez

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et al. 2003). Other columnar cactus species show a strong pollinator limitation in populations inhabiting extra-tropical latitudes and, thus, they can show a relatively generalized pollination system with both nocturnal (moths and bats) and diurnal pollinators (bees and hummingbirds) (Fleming et al. 1996; Molina-Freaner et al. 2004). Several studies conducted within (Mexico and Venezuela) and outside (USA) the northern tropics have confirmed this geographical pattern (even though there are some exceptions, see Rivera-Marchand and Ackerman 2006), but very few studies have considered it from a geographically wider perspective, for example, evaluating the variants that may be occurring in species inhabiting the southern subtropics (Sahley 1996).

Columnar cactus species inhabiting the subtropical Andes may be less dependent on bats because the strong elevation of the Andes may restrict the bats' altitudinal and latitudinal distribution (Koopman 1981; Aguirre 2007). Nectarivorous bats are the main pollinators of other columnar cactus species inhabiting the northern tropical desert regions (Nassar et al. 1997; Valiente-Banuet et al. 2004; Munguía-Rosas et al. 2009); however, in the southern hemisphere the altitudinal distribution of their species (e.g., genus *Glossophaga*, among others) do not surpass 2,000 m (Terán and Aguirre 2007).This suggests the relative importance that hummingbirds, bees and moths may have for outcrossing in these cacti. In fact, columnar cactus species in the Andean region have been reported as xenogamous with a generalized pollination system (de Viana et al. 2001; Badano and Schlumpberger 2005; Schlumpberger and Badano 2005). Similarly, it has been hypothesized that unpredictable pollinator activity can be the main selective factor for maintaining an unspecialized pollination system, because visitor populations may be spatially fluctuating (Valiente-Banuet et al. 1996, 1997a, b). This pattern has been supported from northern extra-tropical regions. Based on this geographical trend, equivalent patterns in response to floral characteristics of columnar cacti can be assumed to occur in the subtropical Andes, but no studies have been published to date.

Here, we evaluated the pollination biology of *Oreocereus celsianus*, a columnar cactus inhabiting the Prepuna biogeographical region in the subtropical Andes. The two aims of this study were: (1) to characterize the pollination biology of this cactus species, and (2) to determine the degree of effectiveness and relative importance of hummingbirds, bees and moths as pollination agents. We hypothesized that if abundance and unpredictability of flower visitors (hummingbirds, moths and bees) has been an important force in the evolution of the pollination system in *O. celsianus*, then we would expect a relatively generalized pollination system in this Andean columnar cactus.

Materials and methods

Study site

Field work was carried out in the Prepuna, which is one of South America's main biogeographical provinces (Cabrera and Willink 1973). It is located in the central Andes from around 20° to 26–27° southern latitude, between 2,000 and 3,300 m, approximately (López 2000; López and Beck 2000). Mean temperatures range from around 12 to 19°C, depending on altitude. In winter very cold temperatures have been recorded (lower than −10°C). A north-south gradient of decreasing precipitation exists, from an annual 300 mm in the Bolivian Prepuna to the less than 100 mm in parts of the Argentinean Prepuna. Vegetation is characterized by *Prosopis ferox*, *Cercidium andicola*, *Acacia feddeana*, *Senna crassiramea* (all legumes), *Oreocereus* and *Trichocereus* spp. (columnar cacti); many Cactaceae with different growth forms, *Bougainvillea spinosa* (Nyctaginaceae) and *Larrea divaricata* (Zygophyllaceae); different species of Compositae (*Aphyllocladus spartioides*, *Flourensia fiebrigii*, *Gochnatia* spp.), among others (López et al. 2007). The study was conducted in a locality within the Bolivian Prepuna located close to Cotagaita town, at an altitude of 3,140 m (20°45.052 S and 65°37.857 W). The study site contains one large population of *O. celsianus* compared to other sites within the Prepuna, making it appropriate for conducting this study. The project was implemented during two flowering periods in the summer seasons (December 2007 and February 2008) of the cactus species from 2007 to 2008.

The cactus species

Oreocereus celsianus (Lem. ex Salm-Dyck) Riccob (Tribe Trichocereeae) is a columnar cactus that grows in the high Andes of Bolivia, Perú and northern Argentina (Hunt et al. 2006). It is a branched columnar cactus (1–36 branches, min–max) that reaches a height of 6 m and grows as a codominant with *Trichocereus tacaquirensis* (tribe Trichocereeae); however, *O. celsianus* seems to be relatively more abundant in the plain sites compared to hills with medium to high slopes. Mature individuals branch basally with several erect columnar stems covered by white hairs and spines. The lateral stems grow up to 2 m in height. The ribs (from 10 to 25) are rounded and tuberculate. Areoles are large, white and densely spiny, with wool up to 5–6 cm long. Spines are yellowish to reddish brown. Central spines (1–4 stout) can grow up to 8 cm long. Radial spines (7–9) can grow up to 2 cm long. Flowers are hermaphroditic; they are pale purplish pink and are located laterally near the stem tips. Fruits are globose and indehiscent with white pulp, and their pericarp changes from green to yellow when ripe (Hunt et al. 2006). Flowering and fruiting of *O. celsianus* occurs between December and March. Fruit mature ca. 40–45 days after flowers close. Reproductive maturation seems to occur when plants are 1.4 m tall (none of the censused plants below this height produced flowers). Their mature individuals produce about 25–30 fruits/cactus (1–83; min–max; n=18), and their fruits contain about 850 seeds/fruit (170–1,221; $n = 9$). Size of seeds fluctuates from 1.0 to 1.6 mm in length. In the northern Prepuna it reaches 6 m in height, and in many localities forms, together with *Trichocereus tacaquirensis* and *T. werdermannianus,* dense and mixed cactus stands.

Flowering intensity and flower availability

Flowering intensity and flower availability were estimated by counting for 5 consecutive days the number of buds, open flowers and buds and flowers aborted in 94 mature individuals of *O. celsianus*. We marked 20 mature cacti with aluminium wire in order to examine the flower characteristic (of flower development). Flowering intensity was defined as the proportion of flowering individuals and number of flowers per mature cacti.

Floral biology

Twenty-nine flowers from 20 individual plants were used to describe floral morphology. Measurements included flower length (i.e., total length, distance to the top of the nectar chamber from corolla opening), width (i.e., diameter of corolla and corolla opening) and reproductive traits (i.e., number of stamens, stigma area, external and internal stamen height, and number of ovules per flower). The stigmatic area ($A = \pi r^2$) was calculated measuring the total diameter ($2r$) of the stigma with a stereoscopic microscope. Three hundred buds from 19 plants were marked and followed through an entire flowering cycle to determine opening and closing times. The condition of each bud was recorded every 24 h over the duration of the flowering cycle, about 96 h (4 days). Nectar volume secreted by flowers was measured every 2 h during one 24-h period (from 1800 hours of first day to 1800 hours of the second day of the anthesis) in a sample of 31 bagged flowers from adult cacti. Nectar was extracted using graduated 0.5-ml insulin syringes and microcapillaries (0.5 ml). Sugar concentration was determined using a hand-held refractometer (Kelilong Electron Co., Ltd., China; Brix value 0–90%). Likewise, 13 flowers from 10 plants were measured for daily nectar accumulation between floral opening and closure over 24-h periods (3 days in total). All flowers used were covered with fine wedding veil. The base of the flowers was dusted with a commercial formicide (MAPEX, cypermetrine 0.3%) to deter leaf-cutting ants.

Breeding system

We conducted a pollination experiment to determine if flowers of *O. celsianus* are self-compatible, as well as the effectiveness of nocturnal and diurnal pollinators. The field experiment was carried out between December 2008 and February 2009 (3 months). One hundred forty-one flower buds from different adult plants were marked and/or bagged with fine wedding veil. Because of the low number of flower buds available, each flower shoot was considered as a unit of replication. We assigned from 14 to 25 flowers to each of the following treatments: (1) Autonomous (or nonmanipulated) self-pollination: 19 flower buds were bagged and monitored until they aborted or set fruit. (2) Nocturnal pollination: 14 flower buds were bagged before opening; when these flowers opened up, they were exposed to nocturnal floral visitors by removal of the bag from 1930 to 0500 hours (before sunrise) and rebagged. This procedure was repeated for 3 days. The observations were undertaken until abortion or fruit production. (3) Diurnal pollination: 21 flower buds were bagged before opening; when the flowers opened up, they were exposed to diurnal floral visitors by removal of the bag from 0500 to 1930 hours (before night) and rebagged. This procedure was repeated for 3 days. The observations were undertaken until abortion or fruit production. (4) Cross-pollination: 24 flower buds were bagged; when the flowers opened up, they were hand-pollinated using fresh pollen obtained from different adult plants located between 50 and 120 m from each other. (5) Self-pollination: 22 flower buds were bagged; when the flowers opened up, they were hand-pollinated using fresh pollen from the same flower. (6) Unmanipulated open-pollinated flowers: 22 flowers were only marked and monitored until abortion or fruit production (natural pollination). (7) Agamospermy or apomixis: 25 flower buds were bagged; when the flowers opened up, they were emasculated (i.e., hand suppression of anthers) and monitored until abortion or fruit production. In all cases, the base of the flowers was dusted with a commercial formicide (MAPEX, cypermetrine 0.3%). The number of mature seeds per each fruit formed was counted. Differences in the number of viable seeds per fruit were analyzed using separate one-way ANOVAs for unbalanced samples, which were implemented with GLM (General Linear Modeling). The dependent variable was the number of seeds per fruit formed. The independent variable was the pollination treatment. Because the fate of one flower bud on one plant can be influenced by the fate of another bud on the same plant, the lack of independence of units of measure was assumed in the interpretation of the results found.

Flower visitors

Through direct observations on several flowers, floral visitors of *O. celsianus* were recorded in 30-min periods (3,630 min, 60.5 h in total) between 0600 and 1900 hours (diurnal visitors, 2,610 min, 43.5 h) and 1900 and 0600 hours (nocturnal visitors, 1,020 min, 17 h) for 15 days. In each 30-min period all flower-visiting vertebrates (hummingbirds) and invertebrates (butterflies, flies, bees and moths) were counted, and their behavior was observed and described. Nocturnal visitors (moths) were caught and identified up to family level. The identification up to genus or species level was not possible because of the lack of entomological taxonomic keys for the region. We considered as legitimate visitors those species that contacted the stigma during their visit, while those species that did not touch the stigma were considered as illegitimate visitors. Because of the relative low amount of available flowers, the observations were done without distinguishing the two types of flowers [i.e., flowers located in the erect columnar stem tips (up to 6 m in height) and flowers located in the lateral stem tips (up to 2 m in height)]. Likewise, each individual flower was considered as a unit of replication. The relationship between nectar production and visitor frequency was evaluated using classical linear regression analysis.

Results

Floral biology

Oreocereus celsianus is an hermaphroditic cactus species. Flowers lasted about 3 days, opening at sunset (1600–1800 hours, local time) of the first day and closing at midday (1500–1600 hours) of the fourth day (ca. 72 h or 3 days of anthesis). The flowers are located laterally close to the stem tips. Small (1.2 m tall), and taller (4.9 m tall) branches may contain flowers at their tips, causing the flowers to be located at two levels. The upper and lower levels may be separated by 2 m. The mean proportion of reproductive branches (branches with at least one reproductive trait: buds, flowers or fruits) was 43% (min–max 0–100%, SD = 0.39, $n = 94$). The mean number of buds and open flowers per cactus was 2.5 (min–max 0–29, SD = 5.03) and 2 (min–max 0–26, SD = 4.11), respectively. The mean number of abortions (mostly flowers) was 28.1 (min–max 0–461, SD = 59.73) (Fig. 1).

External flower length was 78.1 mm (min–max 62–96, SD = 10.6, $n = 29$), while internal length was 60 mm (min–max 44–78, SD = 8.6). Perianth mean width was 60 mm when open (min–max 21–38, SD = 8.6). The anthers are grouped in two sets that differ in height and location (external group: top anthers; internal group: base anthers). External anther height was 24 mm (min–max 14–37, SD = 4.8), while internal anther height was 50.9 mm (min–max 38–65, SD = 8.6). Stigma height was 75 mm (min–max 60–90, SD = 9.0). Top and base anther height does not exceed stigma height; therefore, *O. celsianus* can be considered as a markedly herkogamous species.

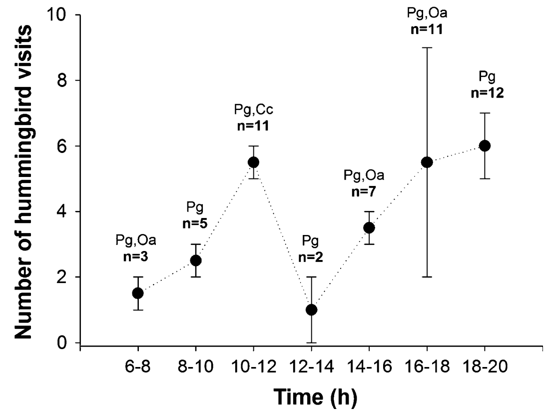

Fig. 1 Number of floral visits (mean ± 1 SE) by hummingbirds in *Oreocereus celsianus* (Cactaceae) in the Prepuna of the Bolivian Andes. Values shown were calculated from the total number of visits recorded at 30-min intervals. The total number of records (*n*) for each 2-h period are shown. Pg, *Patagona gigas*; Oa, *Oreotrochilus adela*; Cc, *Colibri coruscans*

The flowers are highly funnel-shaped, with thick walls of fleshy to leathery tissue surrounding the sexual organs. They also produce a weak odor. The dominant color of the petaloid segments or perianth was purplish pink and can be considered visually attractive to diurnal visitors. The pistil has a gaudy green color and a single long central style with a multilobed stigma (6–8 lobes) surrounded by two rings of numerous stamens (mean: 257 total stamens/flower, min–max 155–333). The number of ovules produced per flower was relatively high with a mean value of 1,580 (min–max 1,131–2,104, SD = 232.2).

Nectar production starts during the first day with high production and concentration (Table 1). Three peaks of nectar production were found. The former (and more accentuated compared to others) occurred between 1800 and 2000 hours of the first day of the anthesis. The following peak nectar production was recorded between midnight and 0200 hours of the second day. The latter peak occurred between 0400 and 0600 hours of the same day (Table 1). The average volume of floral nectar during this period was 0.16 ml (min–max 0.02–0.51, SD = 0.14). Moreover, the nectar concentration in the flowers was higher at the beginning of the anthesis (1800–2000 hours of

Table 1 Nectar concentration and nectar produced per 2-h periods in *Oreocereus celsianus* (Cactaceae) in the Prepuna of the Bolivian Andes

Time (h)	Nectar concentration (% Brix)		Nectar volume (ml)	
	Mean (SD)	Min–max	Mean (SD)	Min–max
1800–2000	32.3 (16.2)	31–33	0.51 (0.31)	0.21–0.83
2000–2200	31.3 (1.2)	30–32	0.27 (0.33)	0.04–0.64
2200–0000	30.3 (2.8)	28–32	0.12 (0.14)	0.01–0.28
0000–0200	30.5 (0.7)	30–31	0.26 (0.08)	0.20–0.31
0200–0400	33.3 (4.0)	29–37	0.08 (0.10)	0.01–0.20
0400–0600	28.7 (1.1)	28–29.5	0.31 (0.28)	0.10–0.64
0600–0800	24.9 (5.8)	5–30.6	0.19 (0.14)	0.01–0.49
0800–1000	23.9 (5.6)	12–30.6	0.09 (0.12)	0.01–0.39
1000–1200	22.5 (8.9)	7–35.6	0.06 (0.06)	<0.01–0.25
1200–1400	23.7 (5.4)	14–33.4	0.07 (0.09)	<0.01–0.37
1400–1600	21.9 (9.0)	11–33.2	0.06 (0.11)	<0.01–0.41
1600–1800	20.4 (7.4)	6–27.8	0.06 (0.06)	<0.01–0.14
1800–2000	16.1 (7.2)	4–24	0.02 (0.01)	<0.01–0.04

the first day) and diminished gradually until the second day (1800–2000 hours of the second day, Table 1). The average nectar concentration during this period was 26.1% (min–max 16.1–33.3, SD = 5.3).

The mean nectar concentration and nectar volume accumulated during the first day of the anthesis (from opening until 1800 of the second day) were 25.5% (Brix, sucrose equivalent units) (min–max 17–32%, SD = 5.6; $n = 11$) and 2.24 ml (min–max 0.06–0.74, SD = 0.24; $n = 11$), respectively. The second day (until 1800 of the third day) they were 24% (min–max 18–24%, SD = 2.5; $n = 11$) and 0.19 ml (min–max 0.01–0.78, SD = 0.27; $n = 11$), respectively. The mean of nectar concentration and nectar volume accumulated during the third day (until 18:00 of the fourth day) were <0.1% and <0.1 ml, respectively. A positive and significant correlation between nectar concentration and nectar production was found ($r_s = 0.79$, $P < 0.05$, $n = 94$).

Breeding system

Autogamy (nonmanipulated), geitonogamy (hand self-pollination) and xenogamy (cross-pollination) treatments produced fruits (fruit: flower ratios from 0.32 to 0.62, Table 2), suggesting that this species has a mixed breeding system (autogamy + xenogamy). The diurnal fruit set (nocturnal visitors excluded) was also high, whereas the nocturnal fruit set (diurnal visitors excluded) was comparatively low, with only three fruits formed. Agamospermy treatment did not produce fruits, showing that the species does not generate fruits and seeds in the absence of pollen. Likewise, the fruit set via natural pollination was high as well (see Table 2).

Differences between the six treatments with formed fruits were marginally significant [$F_{(5,82)} = 2.25$, $P = 0.07$]. The number of seeds formed in the nocturnal treatment was significantly lower than that in the cross-pollination treatment (Tukey test = 255.3, $P < 0.05$). No difference was found among other experiments (Tukey tests, $P > 0.05$). Fruit: flower ratios in cross- and natural pollination were equivalent, indicating that fruit set in *O. celsianus* is not pollen limited (Table 2).

Based on the Index of Self-Incompatibility (ISI) (Bullock 1985), *O. celsianus* is partially self-compatible (ISI = 0.43). Nonetheless, the fruit:flower ratio via cross-pollination was almost twice as high as the fruit:flower ratio via nonmanipulated self-pollination. Likewise, the high values of seeds produced per flower under natural pollination compared with hand self-pollination and cross-pollination treatments (both hand-manipulated experiments) suggest a high effectiveness in the deposition of pollen grains on the floral stigmas.

Results of the diurnal exclusion test (nocturnal pollination) showed that the proportion of flowers producing fruit decreased. Differences between fruit:flower ratios of natural, diurnal- and cross-pollination treatments compared to nocturnal treatments (Table 2) support the hypothesis that diurnal visitors may be acting as the main pollinator agents of this columnar cactus.

Flower visitors

Hummingbirds were the most important floral visitors of *O. celsianus*. During the day (0600–1900 hours), three species of hummingbirds were observed visiting the flowers, *Patagona gigas* (Giant Hummingbird), *Oreotrochilus adela* (Wedge-tailed Hillstar) and *Colibri coruscans* (Sparkling Violetear), all of which may be considered relatively common species in the Bolivian Prepuna. Individuals of *P. gigas* were the most important visitors of the flowers in

Table 2 Fruit:flower ratio from each pollination treatment and diurnal and nocturnal exclusion experiment in *Oreocereus celsianus* (Cactaceae)

Treatment	No. of flowers	Fruit:flower ratio	No. of seeds produced
Nonmanipulated self-pollination (autogamy)	19	0.32	723 (261–2,103)
Hand self-pollination (geitonogamy)	22	0.82	957 (248–1,418)
Cross-pollination (xenogamy)	24	0.75	904 (248–3,521)
Diurnal pollination	21	0.71	976 (184–1,709)
Nocturnal pollination	14	0.21	255 (130–353)
Agamospermy (apomixis)	25	0.00	–
Natural pollination (control)	22	0.73	970 (170–1,571)

Mean number of seeds produced (min–max) in each case is also shown

this period (40 records, 86.9% of the total counts) in relation to *O. adela* and *C. coruscans* individuals (6 records in total for both species, 13.1% of the total). Likewise, the frequency of visits of *P. gigas* was higher (mean ± SD 3.01 ind/h ± 2.8) compared to other hummingbird species (*O. adela*: 0.23 ind/h ± 0.43, *C. coruscans*: 0.23 ind/h ± 0.59).

The *P. gigas* visits were relatively long (mean ± SD 7.3 s ± 3.4, min–max 2–16 s, $n = 37$). The grasping (hanging in front of the corolla and supporting hummingbird's mass on spines or the base of the flower) was the most important kind of visitation behavior identified in this species. The visits by *O. adela* and *C. coruscans* were shorter (min–max; 3–5 s in both cases). *P. gigas* visited the flowers in two well-defined periods. The first period was from 0900 to 1200 hours with 12 records (26.1% of the total), while the second period occurred from 1600 to close to 2000 hours with 20 records (47.8% of the total).

Bees (*Apis mellifera* and *Augochlora* sp.) and wasps (*Polistes buyssoni*) were occasionally recorded visiting the flowers. Among them, individuals of *A. mellifera* were recorded in higher frequency (6.5 ind/h ± 4.2, mean ± SD) and were observed visiting the flowers from 0800 to 1700 hours. No contact between their bodies and the flower stigma was recorded, suggesting that they operate mostly as pollen thieves. This same pattern was observed in *Augochlora* sp. Moreover, *P. gigas*, *O. adela* and *C. coruscans* visited the flowers frontally. *P. gigas* seems to visit the flowers located at the top of the taller branches (upper level, see floral biology results) with higher frequency, while *O. adela* and *C. coruscans* visited mainly the flowers located in small and medium branches (lower level). Nectar robbing by ants (species of genus *Camponotus* and *Crematogaster*) was detected. Ants used the holes caused by *P. buyssoni* near the base of the open flowers. Hummingbirds (*O. adela* and *C. coruscans*) were occasionally recorded sucking the nectar from the holes. Flower predators were also detected (the leaf-cutting ant *Acromyrmex hispidus*).

During the night (1900–0600 hours), hawk moths and owlet moths (Sphingidae and Noctuidae, respectively) were recorded (three records between 0300 and 0600 hours). These moths were occasional visitors of the flowers. None of them arrived frontally to the flowers monitored. Individuals of *P. gigas* were also detected visiting the flowers at the beginning of the night when the light is already weak (three records from 1930 to 1945 hours). These unusual visits may be an explanation for the fruits formed in the nocturnal pollination (see results about breeding system). By including the nocturnal records of *P. gigas*, the total number of counts for this species was 45 (88% of the total), and the frequency of visits for this species was 3.21 ind/h ± 2.75.

Discussion

Because of the lack of independence of the measurement units used (buds and flowers rather than individual plants), the results presented here should be considered as preliminary. However, they do suggest that flower visitation by hummingbirds can be key for the sexual reproductive success of *O. celsianus*. Because of the floral traits (attractive external color, tubular flowers and herkogamy), spatial and temporal availability of flowers (flowers located close to the stem tips), anthesis beginning during the day and patterns of floral rewards (production of moderate amounts of nectar with sugar concentration in the range preferred by birds), Baker and Baker 1975; Fægri and van der Pijl 1979 show that the flowers of *O. celsianus* present a typical hummingbird pollination syndrome (Fenster et al. 2004). Even though the anthesis covers diurnal and nocturnal periods of about 72 h (spanning 4 days), the results indicate that the effect of the hummingbirds during the first 4 h of the anthesis (from 1600 to 2000 hours) may be essential for flower pollination; however, nocturnal visitors (moths) contributed to the fruit set. Therefore, based on floral morphology, rewards and floral visitors only, this species seem to have an unusual xenogamous reproductive strategy.

The pollination system of *O. celsianus*, although slightly bird-specialized, exhibits certain deviations toward generalization. This pattern was already detected in other Andean columnar cacti [*Trichocereus pasacana*, (Badano and Schlumpberger 2005) and *Echinopsis atacamensis* subsp. *pasacana* (Schlumpberger and Badano 2005)]. On the other hand, although *O. celsianus* have a partially nocturnal anthesis and nectar production similar to other species inhabiting in the northern tropical regions, nectarivorous bats do not form part of this "transition" from a lightly bird-specialized system to a generalized breeding system. In addition, this columnar cactus species is self-compatible, likely showing an evolutionary response to the lack of these ancestral pollinators.

The main pollinator of the flowers seems to be *P. gigas*, which visits the flowers when they are opening (afternoon of the first day) or all along the morning of the next day. The temporal coupling among peaks of floral visitation, nectar production and sugar concentration during the first hours of the anthesis indicates that reward presentation is the main factor shaping the pattern of visitation displayed by hummingbirds (mostly *P. gigas*) in relation to this columnar cactus. Aggressive encounters between *P. gigas* and the others hummingbird species visiting *O. celsianus* flowers [*O. adela*, a endemic species for the central Andes, Stattersfield et al. (1998) and *C. coruscans*, a common visitor species of several Andean plant species, Martínez et al. (2010)] were recorded. This can explain partially the short visits and the nectar robbing behavior detected in the latter. Likewise, its small body size (in both cases $\leq$8.5 g, Wester and Claβen-Bockhoff 2006) and short-sized beak (both $\leq$30 mm, Wester and Claβen-Bockhoff 2006) suggest that both hummingbird species are mainly nectar-robbing of this species. On the other hand, the visiting period to the flowers by *P. gigas* is longer (from sunrise to at nightfall, 0600–1945 hours), which explains the fruits formed in the diurnal exclusion test (nocturnal pollination). However, the fruits built during the night could well result from selfing. On the other hand, the data also support the hypothesis that *P. gigas* is a regular, rather than an occasional, floral visitor of Andean columnar cacti (sensu Schlumpberger and Badano 2005). In fact, *P. gigas* is a hummingbird species common in the Bolivian Prepuna and dry Andes in general, and it can be considered a predictable visitor of most columnar cactus species (*Oreocereus* and *Trichocereus*) in the subtropical Andes. This is a seasonal migrant broadly distributed in the Neotropics and resident of the Prepuna from November to April. Other studies also support the relative importance of *P. gigas* for pollinating other CAM plants in the southern extra-tropical regions (33°S, Bromeliaceae, Gónzalez-Gómez and Valdivia 2005). The expected occurrence of *P. gigas* may sustain partially the suggestion that the tendency of presenting a more specialized pollination system is related to predictability of pollinators (Arizmendi et al. 1996; Fleming 2002; Dar et al. 2006); what seems clear is that *O. celsianus* is a key nectar source for hummingbirds inhabiting the region.

The number of seeds produced in a self-compatible way was comparable to those produced by cross- and diurnal pollination, which suggests that the need for an external pollinating agent is not great in this summer-blooming columnar cactus. In fact, *O. celsianus* produced seeds in the absence of pollinators. This result is dissimilar to those obtained from intra-tropical columnar cacti in Mexico (Valiente-Banuet et al. 1996, 1997; Dar et al. 2006) and Venezuela (Nassar et al. 1997), and it shows that even though the flowers showed traits associated with pollination by hummingbirds, they have the genetic capacity for allowing autogamy and seed set by selfing. This suggests that *O. celsianus* has in autogamy an alternative strategy to mixed pollination to ensure reproduction in a potentially unpredictable environment. However, because *P. gigas* is mainly responsible for outcrossing and gene flow in this species, it is plausible to suppose that the main input for its natural regeneration belongs to sexual individuals produced by xenogamy. Successful seed germination tests, in which seeds produced through autogamy and xenogamy are contrasted, may demonstrate the potential of this suggestion.

Our results also show that autogamy may be closer to nocturnal pollination. It seems that in autogamous flowers a low fruit set can be achieved, but the number of seeds produced had a lot of variance. In contrast, in the nocturnal treatment the fruit set was also low, but the number of seeds was lower and less variable. This can be due to lack of pollinators and a failure in the self-compatible mechanism, due maybe to pollen loss (*sensu* Fleming et al. 2001), or can be only an indication of the lower number of flowers used in this treatment, which would reduce total variation.

A similar fruit set was detected in the nocturnal exclusion test (diurnal pollination) and control treatment, suggesting that there is no pollinator limitation. Likewise, the fruit set in the hand self-pollination treatment was similar to the diurnal and natural pollination. This pattern highlights again the notion that this Andean cactus has the capacity for encouraging a 'fail-safe' pollination system (Fleming 2002; Dar et al. 2006), which could combine autogamy and xenogamy pollination. Thus, the geographical dichotomy hypothesis of pollination systems may also include the mixture between sexual and non-sexual systems instead of only the types of sexual pollination systems (diurnal and nocturnal pollination or its variants), which has been regularly detected in columnar cactus species from the northern extra-tropical latitudes. The mixed mating breeding system has been reported for globose cactus species in northern tropics (genus *Melocactus*, Nassar and Ramírez 2004; Nassar et al. 2007). This is the first study reporting a mixed mating system in a columnar cactus species from the subtropical Andes and suggests the importance of future research focused on evaluating the genetic variability within and across populations of this species (e.g., Figueredo-Urbina et al. 2010). Likewise, the results support the hypothesis that flowers with apparently specialized pollination systems have the ability to self-pollinate, contradicting the suggestion that pollination specialization reveals selective pressures to ensure high maternal outcrossing rates (sensu Fenster and Martén-Rodríguez 2007). However, we still do not know the effect that annual variation can have on the flowering season and, therefore, in the evolution of this breeding system.

In summary, based on the levels of pollination effectiveness in the *O. celsianus*, the results strongly indicate that the giant hummingbird, *P. gigas*, is the main reliable agent that this Andean columnar cactus has for their sexual reproduction. These pollination relationships may be the cause of the diversity of breeding systems detected in this cactus species and partially explain the composition of the hummingbird assemblage recorded in this study, which may strongly depend on columnar cacti to obtain nectar. Likewise, even though the results suggest the existence of a primarily bird-specialized pollination system in this species, further research is need on other populations of *O. celsianus* to determine whether its pollination system can vary with latitude and altitude.

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