Flowering and pollination ecology of *Cleistocactus baumannii* (Cactaceae) in the Brazilian Chaco: pollinator dependence and floral larceny

Bruno Henrique dos Santos Ferreira¹,², Camila Silveira Souza²,³*, André Luiz Silva Fachardo¹, Aline Conceição Gomes¹ and Maria Rosângela Sigrist¹,⁴

Received: June 19, 2019
Accepted: October 22, 2019

**ABSTRACT**

*Cleistocactus baumannii* is the only ornithophilous cactus species in the Brazilian Chaco. In addition, this species of Cactaceae invests heavily in flowering in the ecoregion. Such characteristics motivated us to evaluate the temporal availability of flowers in the context of its floral visitors. The reproductive system of *C. baumannii*, the number of individuals in flowering, flower abundance and the frequency and richness of floral visitors were evaluated and quantified. Nectar robbery was a frequent phenomenon in the studied population; therefore, we compared the pollen load deposited on the stigma of damaged (robbery) and undamaged flowers. In the Brazilian Chaco, *C. baumannii* is self-incompatible and has a continuous flowering pattern, providing floral resources throughout the year for nine species of floral visitors. One hummingbird species acted as a potential pollinator, and we consider *Xylocopa splendidula* to be a nectar robber. We found stigma of flowers damaged by nectar robbers to have lower pollen loads than those of undamaged flowers. This study highlights the importance of studying reproductive traits in different populations to understand changes in the reproductive success of plant species at different scales and possible causes, such as availability of floral visitors, incidence of robbers and flowering patterns.

**Keywords:** arid environments, *Chlorostilbon lucidus*, floral damage, nectar robbery, ornitophily, *Xylocopa splendidula*

**Introduction**

Cactaceae is one of the most diverse families in the Neotropics, with 1480 recognized species (Goettsch et al. 2015). This family represents some of the most conspicuous plants in the arid and semi-arid regions (Ortega-Baes et al. 2010) and an important floristic element of Caatinga and Chaco vegetation in Brazil (Pennington et al. 2000). Within the Cactaceae, bird flowers have been described for many species of cacti from different lineages, particularly in South America. In general, the taxonomic description of many of these species assumes that they have pollination systems specialised towards birds, hummingbirds, in particular (see Anderson 2001; Gorostiague & Ortega-Baes 2016).

*Cleistocactus* is cited as an example of extreme phenotypic specialisation to bird pollination (Anderson 2001). However, this specialization has previously only been described based on floral traits that suggest ornithophily (Rowley 1980; ¹ Programa de Pós-Graduação em Biologia Vegetal, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, 79070-900, Campo Grande, MS, Brazil ² Programa de Pós-Graduação em Ecologia e Conservação, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, 79070-900, Campo Grande, MS, Brazil ³ Departamento de Botânica, Programa de Pós-Graduação em Botânica, Universidade Federal do Paraná, 81531-980, Curitiba, PR, Brazil ⁴ Laboratório de Polinização, Reprodução e Fenologia de Plantas, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, 79070-900, Campo Grande, MS, Brazil * Corresponding author: souza.camila.bio@gmail.com
flowed and extended flowering (Rojas-Nossa et al. 2016; Souza et al. 2016). While nectar robbing is linked to flowers with long corolla (Maruyama et al. 2015).

A study in the plant community in the Brazilian Chaco showed that plant species with highest abundance and longest flowering period presented the highest richness and/or frequency of floral visitors (Souza et al. 2017). Considering both number of flowers and flowering time, *C. baumannii* makes a heavy investment in flowering in the Brazilian Chaco (VGN Gomes et al. 2019). The flowering time of *C. baumannii* in the Brazilian Chaco can vary from five (Freitas et al. 2013) to eleven months (VGN Gomes et al. 2019). However, this phenophase is mainly concentrated in the rainy season (Freitas et al. 2013; VGN Gomes et al. 2019), a time with more richness and frequency of floral visitors in this area (Souza et al. 2017).

Flowering phenology (Freitas et al. 2013; VGN Gomes et al. 2019), floral morphology and pollination syndromes (Gomes & Araujo 2015), breeding system and pollination (Bianchi et al. 2000; Gorostiague & Ortega-Baes 2016; Souza et al. 2017) of *C. baumannii* have all been studied in different locations of the Gran Chaco domain. However, since the Gran Chaco is drier toward the west and more humid toward the east (e.g., Brazilian Chaco) (Lewis 1991) and floral traits and pollinators can vary between populations in different locations and climate scenarios (Schlumberger et al. 2009; Gorostiague & Ortega-Baes 2018; Rech et al. 2018), it is important to investigate new populations in different environments (Morgan 2000). Here, we aim to determine if the reproductive biology of *C. baumannii* in the Brazilian Chaco differs from that in the Argentinian Chaco. We conducted a complete study of the flowering phenology, breeding system and pollination ecology of *C. baumannii* in a remnant of Brazilian Chaco vegetation (Thorn-Forest). For this, our study to investigate the availability of flowers for pollinators over the course of one year, the morphology and floral biology of this species, the dependence of *C. baumannii* on effective pollinators for fruit set and consequent reproductive success, and the total number of floral visitors and potential pollinators.

In addition, as the incidence of nectar robbers is frequent, we also investigated the variation in pollen deposition between damaged and undamaged flowers. Finally, we will discuss possible implications on the reproductive success of this species in the Brazilian Chaco. We expected that *C. baumannii* in the Brazilian Chaco: (i) would be a self-incompatible species and hummingbird dependent for fruit set, as occur in Argentinian Chaco (e.g. Bianchi et al. 2000); (ii) will have flowers explored by many floral visitors being a key resource, where pollen deposition on stigma will be affected by nectar robbers, fact that can compromise its reproductive success. In conclusion, we expect that in periods with greater resource availability (number of flowers and individuals in flowering), the frequency and richness of floral visitors will also be higher.
Flowering and pollination ecology of *Cleistocactus baumannii* (Cactaceae) in the Brazilian Chaco: pollinator dependence and floral larceny

**Materials and methods**

**Study site**

We collected data on flowering and floral visitors’ occurrence from November/2009 to October/2010. Additional data on floral morphology and biology, breeding system, floral visitors and pollination were recorded from November/2015 to August/2016. The study was conducted in a remnant (21°42'04"S 57°53'06"W) of Chaco vegetation (Thorn-Forest) in Porto Murtinho, Mato Grosso do Sul (Carvalho & Sartori 2015). This site is characterized by a discontinuous canopy and predominance of spiny and microphyllous species (cf. Freitas et al. 2013; Souza et al. 2017). Diverse species of Cactaceae are endemic to this ecoregion, including *C. baumannii* (Ferreira et al. 2018; Gomes et al. 2018; VGN Gomes et al. 2019). Climate is hot and dry, with erratic rainfall throughout the year. The dry season is from April to September, with mean rainfall below 100 mm, and the rainy season is from November to February; March and October are considered transition months (Freitas et al. 2013). Average annual rainfall and temperature are 970.3 mm and 25 °C, respectively (Carvalho & Sartori 2015).

*Cleistocactus baumannii*

*Cleistocactus baumannii* (Lem.) Lem. is a columnar cactus (Pivatto et al. 2014), exhibiting small ascending cladodes with about 1.5 meters of branching, reaching 1.5m in height, or even more if supported by another plant (Mauseth & Plemons-Rodriguez 1998). Species has numerous flowers with bright orange-red colours (Lowry 2016). In the study area *C. baumannii* bloom more intensely in the rainy season (Freitas et al. 2013; VGN Gomes et al. 2019). Samples of *C. baumannii* were collected and deposited at the CGMS Herbarium of the Universidade Federal de Mato Grosso do Sul (CGMS 35477).

**Morphology and floral biology**

To describe the flower life events (e.g. longevity, presence of dichogamy) we marked floral buds (n = 20 flowers, two per plant) which were monitored throughout the floral anthesis. We describe the floral morphology from fresh and fixed flowers (n = 30 flowers from 14 individuals). We measured with a digital caliper the diameter and total length of the floral tube length and, anthers and stigmas length. In addition, we recorded qualitative floral traits, such as colour and presence of odour. We tested pollen viability with acetic carmine solution (Dafni 1992) from pre-anthesis floral buds fixed in 70 % FAA (n = 100, five per plant). Stigma receptivity was also assessed in situ from observations of stigmatic exudates presence across the floral anthesis of flowers marked randomly in different individuals (AC Gomes et al. 2019; VGN Gomes et al. 2019).

Nectar total volume was quantified in flowers previously bagged at the bud stage using microliter syringes of 200 μL (Hamilton, Reno, NV, USA) (n = 10 flowers of different individuals). Sugar concentration (% mass/mass of sucrose equivalents) was measured with a digital refractometer (n = 10 flowers from five individuals). To evaluate the nectar secretion pattern, we measured the volume and concentration of solutes in the accumulated nectar until 11 a.m. and again at 5 p.m.

**Breeding system**

We performed diverse reproductive treatments (Ferreira et al. 2018) (n = 12 flowers per treatment from different individuals) as follows: (1) natural pollination (control) – flowers were observed under natural conditions of pollination, without manipulation; (2) hand cross-pollination – flowers were emasculated and pollinated with exogenous pollen from other individuals; (3) “geitonogamy” – flowers were pollinated with pollen from other flowers of the same individual (Arroyo 1976); (4) spontaneous self-pollination – flowers were bagged the day before anthesis and observed until fruit set; (5) hand self-pollination – flowers were pollinated with endogenous pollen; (6) apomixis/agamospermy – floral buds were bagged the day before anthesis and then emasculated and bagged again. After approximately 40 days, we recorded the fruit set and then compared our results with those in the literature for other populations of *C. baumannii*. For a description of the breeding systems, we used such classic terminology as self-sterile and self-incompatible (Zapata & Arroyo 1978; Lloyd & Schoen 1992).

**Phenology: flowering and occurrence of floral visitors**

We carried out the flowering phenology and floral visitors sampling in 213 plants of *C. baumannii*. In relation the flowering, we quantified monthly the number of flowers (abundance) and number of flowering plants. Concomitantly, we sampled all floral visitors by means of focal observations from 6:00 a.m. to 6:00 p.m. totalling 96 hours of observation. During these observations, we sampled the frequency of each floral visitor (each contact of the animal with a flower), and these visits, when possible, were photographed.

Flowering descriptors (number of flowers (abundance) and number of flowering plants) were classified according to Newstrom et al. (1994). We used circular statistics to calculate the mean angle (or vector-μ) of each descriptor (phenophase), as well as its length (r). These analyses were performed using Oriana 2.0 software (Kovach 2004), applying Rayleigh’s test (Z) to verify the probability level (p) (Zar 2010). The mean angle (μ) represents the average...
date of phenological activity, and $r$ represents the degree of synchronization (clustering) of the phenophases around the average date (Morellato et al. 2010). Higher value of $r$ (> 0.5) correlates with greater aggregation of phenophase around the average date (possible seasonal or concentration phenophase) when $Z$ values are significant ($p$<0.05).

We next verified the pattern of occurrence of floral visitors (richness and frequency) phenology. To accomplish this, we used the same circular statistics as those used for flowering data. We performed a simple linear regression to verify the relationship between richness and frequency of floral visitors (pollinators and non-pollinators, thieves and robbers) with the abundance and number of C. baumannii flowering plants using the vegan package (Oksanen et al. 2018) in R programming (R Development Core Team 2017).

Behavior of floral visitors

We performed focal observations of floral visitors during floral anthesis. Insects were collected with an entomological net and/or bottle with ethyl acetate. Later, specimens were mounted or placed in 70% alcohol and sent to specialists for identification. Hummingbirds were pre-identified in the field with the help of an illustrated guide (Sigrist 2007), photographed, and then confirmed by specialists (see Souza et al. 2017). Insects collected were deposited in the Zoological Collection of the Universidade Federal de Mato Grosso do Sul (ZUFMS).

We classified floral visitors using the terminology developed to characterize floral larceny (Inouye 1980; Irwin et al. 2010) as follows: potential pollinators, non-pollinators, thieves, and robbers. This approach is pragmatic for revealing pollinators in a system (Jacobs et al. 2010), but requires more detailed testing to rank effectiveness (Gross & Mackay 1998; Gross et al. 2017). Visitors classified as potential pollinators were those that contacted both staminate (anthers) and pistillate (stigma) structures with pollen on the body, demonstrating the ability to transport pollen within and between flowers of different individuals. The latter is recognized as an important step in discerning pollinators from non-pollinators (Popic et al. 2013). Thieves were visitors observed collecting pollen and/or nectar without contact anthers or stigma (to nectar collect) and without damage the floral parts. Robbers were visitors observed damaging the flowers, as in chewing the corolla with the mouthparts, for example, to access the resource illegitimately without contacting the reproductive structures (primary robber). When the damage made by a primary robber was used for other floral visitor to obtain illegitimately the resource (e.g. nectar), the floral visitor was classified as secondary robber (Inouye 1980).

Effects of nectar robbing by counting pollen grains

Since C. baumannii received a high frequency of nectar robbers, we marked approximately 50 flowers for visitation throughout anthesis. On the next morning (~12h after end of the anthesis), we randomly collected 30 of these flowers, 15 flowers with signs of nectar robbing and 15 flowers with no signs. The collected flowers were stored in individual bottles containing 70% FAA fixative and transported to the laboratory. In the laboratory, the stigmas of these flowers were mounted on slides and covered with lamina for analysis under optical microscopy. All C. baumannii pollen grains (checked with control material obtained from buds) that adhered to stigmatic tissue were quantified by scanning the slides, always moving it in the same direction. We performed a t-test (normal distribution) to compare differences in the number of pollen grains deposited on damaged and undamaged flowers using the vegan package (Oksanen et al. 2018) in R programming (R Development Core Team 2017).

Results

Morphology and floral biology

Flowers are hermaphrodite, diurnal, tubular, slightly curved and reddish (Fig. 1A), with floral tube extremities orange. In the human sense, C. baumannii flowers are showy, but not odoriferous. Floral tube measures an average of 48.19 ($\pm$ 1.71) mm in length and 9.25 ($\pm$ 0.61) mm in diameter. Androecium is polystemonous and heterodynamous with approximately 129 stamens, which form a staminal column with 49.76 ($\pm$ 3.65) mm of length. Anthers have wine colour and are basifixid, rimose, and produce white pollen with high viability (89.06 $\pm$ 17.19%). Gynoecium is syncarpous with a single style and multilocular stigma, which has 50.25 ($\pm$ 3.68) mm in height. Stigma has with up to nine lobes but in the most flowers there is six (30.8 %). Ovary is inferior and presents around 505 ($\pm$ 147) ovules. Nectar is produced in nectariferous tissue that lines the floral tube and is stored in the lower third of this tube.

Floral longevity of C. baumannii is approximately 48 hours. In pre-anthesis, it is possible to perceive a yellowish tone in the extremities of floral buds. This indicates that the perianth elements have already begun to move away. In the next morning (6:00 a.m.), the corolla and anthers are already open, but the stigmatic lobes are still leaning and not yet receptive. Thus, the flowers are functionally staminate at the beginning of anthesis. From 8:00 a.m. there is pollen in the anthers, and the stigmatic lobes are partially distended and receptive. By around 10:00 a.m., there is little pollen in the anthers, starting the "pistillate phase" of the flower until the next day. In the afternoon of the second day of anthesis, the stigmatic lobes begin to wilt, and the next morning, they are completely closed. Until end of the first morning (11:00 a.m.) of floral life, flowers produce and accumulate, on average, 59.8 ($\pm$ 64.25) $\mu$L of nectar with 20.24% ($\pm$ 4.9) of solute concentration. We did not find new accumulation of nectar at 5:00 p.m.
Flowering and pollination ecology of *Cleistocactus baumannii* (Cactaceae) in the Brazilian Chaco: pollinator dependence and floral larceny

Breeding system: does *Cleistocactus baumannii* need pollinators?

In all studied populations, *C. baumannii* fruit set only by hand cross- and natural pollinations (Tab. 1). In Brazilian population, fruit set by natural pollination was greater than cross-pollination. However, cross-pollination fruit set in Brazil was lower compared to Argentinean populations, but similar for natural pollination (Tab. 1). In all populations, *C. baumannii* is self-sterile and self-incompatible and therefore, totally pollinator dependent.

Phenology: more flowers and flowering plants lead to greater richness and frequency of floral visitors in *Cleistocactus baumannii*

In the Brazilian Chaco, *C. baumannii* flowered for 12 months with mean dates in March (number of plants in flowering) and April (number of flowers) in the transitional season (from rainy to dry season) (Tab. 2, Fig. 2). Thus, *C. baumannii* flowering pattern is continuous (sensu Newstrom et al. 1994) and seasonal ($r > 0.5$; $p < 0.0001$). The same was recorded for floral visitors (pollinators, non-pollinators) ($r > 0.6$; $p < 0.0001$), except for richness of the pollinators (non-seasonal) ($r = 0.3$, $p = 0.2$) and the pattern of non-pollinators (extended, nine months), which were not sampled in part of the dry season (June-August). Moreover, all descriptors of the floral visitors (richness, frequency) presented mean dates in December. (Tab. 2, Fig. 2). Both richness ($r^2 = 0.87$, $p < 0.0001$) and frequency ($r^2 = 0.93$, $p < 0.0001$) of non-pollinators and richness ($r^2 = 0.47$, $p < 0.05$) 0.0001) and frequency of pollinators ($r^2 = 0.54$, $p < 0.05$) were highly correlated with the number of flowers in *C. baumannii*. The same was found for the number of flowering plants in that the richness ($r^2 = 0.54$, $p < 0.05$) and frequency ($r^2 = 0.35$, $p < 0.05$) of pollinators and richness ($r^2 = 0.68$, $p < 0.0001$) and frequency ($r^2 = 0.80$; $p < 0.0001$) of non-pollinators were also highly correlated with the number of *C. baumannii* individuals in bloom.

Are the floral visitors of *Cleistocactus baumannii* all potential pollinators? Effects of damage by nectar robbers on stigma pollen deposition

*Cleistocactus baumannii* flowers were visited by bees (n = 5 spp.), ants (n = 2 spp.), butterfly (*Pyrisitia* sp.) and hummingbird (*Chlorostilbon lucidus*) (Fig. 1C) (Tab. 3), which collected nectar (seven spp.) and/or pollen (bees: *Apis mellifera*, *Megachile* sp., *Xylocopa splendula*). *Xylocopa splendula* (Fig. 1B), *C. lucidus* and *A. mellifera* were the most frequent floral visitors and occurred in most sampling months (Tab. 3). To collect nectar, *C. lucidus* hovers in front of the flower, introduces the beak into the floral tube and collects nectar legitimately, contacting anthers and stigma.

---

Figure 1. A. *Cleistocactus baumannii*: plants and flowers in Brazilian Chaco. B. Bee *Xylocopa splendida* (nectar robber) and C. The hummingbird *Chlorostilbon lucidus* (potential pollinator) collecting nectar in *C. baumannii* flowers.
with the upper portion of the beak and front of the head (Fig. 1C). Its visits last about two seconds, and then it flies to another plant, visiting usually only one flower per plant. Therefore, *C. lucidus* is a potential pollinator of *C. baumannii*.

*Apis mellifera*, *Megachile* sp. and *X. splendidula* to collect pollen land on the anthers and gather pollen with the first and second pairs of legs, then transferring it to the third pair, contacting the anthers and stigma with the ventral portion of the body and legs, but acting mainly as pollen thieves (see discussion). Besides, in all visits, *X. splendidula* collected nectar as follow: the bee lands on the perianth and walks to the base of the floral tube where it pierced the tube to rob nectar (see Video S1 in supplementary material). After a few seconds the bee goes to another flower usually from the same plant where it performs similar behaviour, being considered as primary nectar robber. The hole left by *X. splendida* was used by the bees *Ceratina* (*Rhysoceratina*) *prox.* *volintans* and *Tetragonisca angustula* and ants to access the floral nectar, acting as secondary nectar robbers. *Pyrisitia* sp. was considered as nectar thief because it does not contact anthers and stigma to collect nectar.

By the end of floral anthesis, we recorded less pollen grains adhered to the stigmatic surface of flowers damaged by primary nectar robbing (*X. splendidula*) than non-damaged flowers. We found higher pollen deposition on stigmas of undamaged flower (825 ± 215; *t* = 3.45; df = 28; *p* < 0.0001) in relation to damaged flowers by nectar robbing (*X. splendidula*) (542 ± 236; Fig. 3).

### Table 1. Breeding system of different natural populations of *Cleistocactus baumannii* in Gran Chaco (South America).

<table>
<thead>
<tr>
<th>Country</th>
<th>Location</th>
<th>Vegetation Type</th>
<th>Treatments</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>Porto Murtinho, Mato Grosso do Sul</td>
<td>Chaco Thorn-Forest</td>
<td>Spontaneous self-pollination</td>
<td>“Geitonogamy”</td>
</tr>
<tr>
<td>Argentina</td>
<td>La Bodeguita, Salta</td>
<td>Dry Chaco</td>
<td>Spontaneous self-pollination</td>
<td>“Geitonogamy”</td>
</tr>
<tr>
<td>Argentina</td>
<td>Las Gamas, Santa Fé</td>
<td>Chaco Woodland</td>
<td>Spontaneous self-pollination</td>
<td>“Geitonogamy”</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>Spontaneous self-pollination</td>
<td>“Geitonogamy”</td>
</tr>
</tbody>
</table>

**HYMENOPTERA**

- **Bees**
  - *Apis mellifera* (Linnaeus, 1758) 105 Pollen thief 8 (Nov-May/Oct)
  - *Xylocopa splendidula* Lepeletier, 1841 269 Primary nectar robber, Pollen thief 8 (Nov-May/Oct)
  - *Ceratina* (*Rhysoceratina*) *prox.* *Volintans* (Schrottley, 1907) 22 Secondary nectar robber 7 (Nov-Mar/Sep-Oct)
  - *Tetragonisca angustula* (Schwarz, 1938) 6 Secondary nectar robber 3 (Dec/Sep-Oct)
  - *Megachile* sp. 6 Pollen thief 3 (Nov-Jan)

**Ants**

- *Camponotus cf.* *senex* (Smith 1858) 7 Secondary nectar robber 4 (Nov-Jan/Oct)
- *Crematogaster* sp. 3 Secondary nectar robber 2 (Nov/Jan)

**LEPIDOPTERA** (butterfly)

- *Pyrisitia* sp. 4 Nectar thief 2 (Nov/Jan)

**TROCHILIDAE** (hummingbird)

- *Chlorostilbon lucidus* (Shaw, 1812) 251 Potential Pollinator 12 (all months)

Table 1. Breeding system of different natural populations of *Cleistocactus baumannii* in Gran Chaco (South America).
Discussion

In the studied population, *C. baumannii* has continuous flowering pattern (all year), differing slightly to reported for Cactaceae in some arid or semi-arid ecosystems, such as deserts (McIntosh 2002), savannas (Fonseca et al. 2008) and thorn-forests (Ortíz et al. 2010), including in Brazilian Chaco (Gomes et al. 2016; Ferreira et al. 2018; VGN Gomes et al. 2019), which the predominant pattern was extended (sensu Newstrom et al. 1994). According to VGN Gomes et al. (2019) this fact demonstrates the importance of cacti as resources for animals throughout the year, mainly in dry environments.

*Cleistocactus baumannii* flowers are source of pollen and nectar for anthophilous fauna. In the Brazilian Chaco, *C. baumannii* nectar is more voluminous (average = 59.8 μl) than that recorded in Argentina (average = 24.9 μl), but it is more diluted (average = 20.24%) in relation Dry Chaco population (average = 64.5%) (Gorostiague & Ortega-Baes 2016). According to Tamm & Gass (1986), it is important to investigate the combined effects of nectar volume and concentration on the rate and preference of energy intake of possible pollinators. This was done

**Figure 2.** Circular histograms of flowering and floral visitors of *Cleistocactus baumannii* across the year in the Brazilian Chaco. Letters around the circle indicate the months and numbers inside the histograms indicate the number of species/individuals in the respective month. Vectors indicating the length and direction of the mean are in red.

**Figure 3.** Pollen deposition on stigma of damaged and undamaged flowers of *Cleistocactus baumannii* in the Brazilian Chaco.
Indeed, diverse floral traits of *C. baumannii* suggest pollination by hummingbirds (ornithophilous) such as diurnal anthesis, “long” tubular flower, reddish color and copious nectar production. Although the predictive validity of pollination syndromes remains controversial, some studies provide evidence that floral traits may be linked to the main pollinator group (Armbruster *et al.* 2011; Danieli-Silva *et al.* 2012; Rosas-Guerrero *et al.* 2014). In fact, *C. baumannii* is ornithophilous, as found here (see below) and in several previous studies (Scogin 1985; Gomes & Araujo 2015; Gorostiague & Ortega-Baes 2016). This means that hummingbirds are the main functional group exerting selective pressure on the floral traits of these species, even though these traits do not exclude other floral visitors and potentially fewer effective pollinators. According to Fenster *et al.* (2004), associate effectiveness and functional groups of pollinators with floral design clearly contributes to the understanding of the relevance of pollination syndromes.

Effective pollinators are extremely important for *C. baumannii* in relation to the reproductive system of the species. Although anthers and stigma are arranged at about the same level (no herkogamy), we did not register fruit set after spontaneous self-pollination. In addition, certain temporal separation of reproductive structures occurs in flowers, as pollen is released before the stigma receptivity (partial protandry). This probably helps to reduce self-pollination and clogging of stigma with “inadequate” pollen, as the species has self-incompatibility (Ross 1981; Bianchi *et al.* 2000; Gorostiague & Ortega-Baes 2016). *Cleistocactus baumannii* does not fruit by “geitonogamy”, a process genetically similar to self-pollination (Arroyo 1976). Thus, the population studied maintains self-incompatibility, an important mechanism to prevent inbreeding (Godoy *et al.* 2018). However, the fruit set of *C. baumannii* in the Brazilian Chaco by natural conditions and mainly by hand cross-pollination was very low compared to other populations in Argentina (Bianchi *et al.* 2000; Gorostiague & Ortega-Baes 2016).

We registered only 25% fruit set under natural conditions. This low value may be related to the frequency of illegitimate visitors (Inouye 1980), especially nectar robbers (Irwin *et al.* 2001; Bergamo & Sazima 2018). Among the nine species of floral visitors that we observed, only one species - the hummingbird *C. lucidus* - act as potential pollinator; the other species (mainly bees) acted as non-pollinators, especially as nectar robber (see below), probably due to the long tube corolla, which restricts legitimate access to nectar. According to Gorostiague & Ortega-Baes (2016), ornithophilous characteristics do not restrict other pollinators (other than birds) that could use the resources offered by the flower. We show that *C. baumannii* and anthophilous fauna are related based on richness and frequency of floral visitors, pollinators or not, positive related with the flowering of this species. Therefore, the diversity of the anthophilous fauna in the studied chaquenian vegetation probably are sensitive to, or dependent on, the availability of floral resources of species as *C. baumannii* (Souza *et al.* 2017).

Bees visit the flowers of *C. baumannii* to collect pollen and nectar. The most frequent floral visitor, *X. splendidula*, while collecting pollen, contacts *C. baumannii*’s reproductive structures. However, after collecting pollen, *X. splendidula* collects nectar illegitimately as a robber. In this case, this bee species could be both pollinator and robber of flowers of the same species (Navarro 2000). However, this bee species would not be as effective pollinator considering the reproductive system of *C. baumannii* and its visiting pattern and behaviour. *Xylocopa splendidula* visited all flowers of each individual, potentially promoting mainly self-pollination and “geitonogamy” (Video S1 in supplementary material). We found that flowers with apparent signs of nectar robbery (damage) has lower deposition of pollen on their stigmas. Nectar robbers, such as *X. splendidula*, can decrease the reproductive success of *C. baumannii* in two ways: by reduce the number of flowers visited by effective pollinators (hummingbirds), and/or by clogging the stigma with pollen from the same individual (see Goulson *et al.* 1998).

**Conclusions**

We concluded that hummingbirds appear to be more efficient at promoting cross-pollination in *C. baumannii*, since they only visited one flower per plant. The increased deposition of pollen on stigma of undamaged flowers evidences the efficiency of *C. lucidus* in the pollination service, while other visitors would be less effective, decreased the reproductive success of *C. baumannii*. In fact, the occasional contribution of nectar robbers to pollination of *C. baumannii* did not show any positive effects in our study. The production of nectar, a resource collected by hummingbirds, occurs only during the onset of anthesis. This indicates that legitimate visitors with a few quick visits can deposit more pollen on the stigma of *C. baumannii* than visitors that are pollen collectors and nectar robbers. Finally, this study highlights the importance of studying reproductive traits in different populations to understand changes in the reproductive success of plant species at different scales and possible causes, such as availability of floral visitors, incidence of robbers and flowering patterns.

**Acknowledgements**

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance Code 001 and financial support by Scogin (1985) who then defended *C. baumannii* is ornithophilous.
Flowering and pollination ecology of *Cleistocactus baumannii* (Cactaceae) in the Brazilian Chaco: pollinator dependence and floral larceny

References


