



Reproductive biology of *Cipocereus minensis* (Cactaceae)—A columnar cactus endemic to rupestrian fields of a Neotropical savannah

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ABSTRACT

We studied the reproductive biology of *Cipocereus minensis*, an endemic columnar cactus of the Espinhaço Mountain Range, Southeastern Brazil, focusing on floral biology, breeding system, and pollination. We described floral morphology and evaluated the role of nocturnal and diurnal pollinators on the reproductive success in two populations. *C. minensis* has large, horizontal, cream-colored, chiropterophilous flowers with rigid petals that open at dusk and close on the following morning. Flowers produced a huge amount of pollen grains and nectar production was nocturnal. Controlled pollination experiments revealed that the cactus is an obligate xenogamous species. Visitor-exclusion experiments revealed that the nocturnal visitors (bats) are the prominent pollinators whereas hummingbirds and social bees, which visited the flowers early in the morning, contributed little to fruit set. We conclude that the reproductive success of this endemic columnar cactus is threatened in the absence of the effective pollinating bats.

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1. Introduction

Cactaceae is a distinctive family of plants native to the Americas with approximately 1600 species. Brazil is the third global center of cacti diversity with 200 species, of which 78% are considered endemic (Taylor and Zappi, 2004; Taylor, 1997; Zappi et al., 2010). Our understanding of the reproductive biology of these plants is severely limited compared to other aspects of their biology such as morphology, physiology, biochemistry, and ethnobotany (Nobel, 2002). This is especially true in Brazil, where, the reproductive biology of less than 10% of the species has been studied (Zappi et al., 2011). These studies have included species of *Pilosocereus* (Locatelli et al., 1997; Rocha et al., 2007), *Opuntia* (Schlindwein and Wittmann, 1997), *Parodia*, *Gymnocalycium*

(Schlindwein and Wittmann, 1995), *Tacinga palmadora* (Locatelli and Machado, 1999a), *Cereus* (Locatelli and Machado, 1999b; Silva and Sazima, 1995), *Micranthocereus* (Aona et al., 2006), *Melocactus* (Colaço et al., 2006; Gomes et al., 2014; Locatelli and Machado, 1999a), and *Cipocereus* (Rego et al., 2012).

Self-incompatibility systems in cacti are common and occur in at least 30% of the genera (Boyle, 1997; Mandujano et al., 2010; Strong and Williamson, 2007). Studies on the pollination biology of columnar cacti have shown that most species are adapted to nectar-feeding bats (Fleming et al., 2001; Locatelli et al., 1997; Munguía-Rosas et al., 2010; Nassar et al., 1997; Rocha et al., 2007; Valiente-Banuet et al., 1997a). Among columnar cacti with bat-pollination systems, the relative contribution of diurnal visitors to fruit set seems to increase with increasing latitude. Thus, it is expected that in populations of extra-tropical regions, daytime visitors significantly contribute to production of seeds (Fleming et al., 2001; Munguía-Rosas et al., 2009). Some of these species are also effectively pollinated by diurnal flower visitors such as birds and bees (Fleming et al., 2001; Munguía-Rosas et al., 2009). In addition to nectar-feeding bats, some columnar cacti with nocturnal

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flowers are pollinated by hawkmoths (Clark-Tapia and Molina-Freaner, 2004; Fleming et al., 2001; Locatelli and Machado, 1999b).

All six species of the genus *Cipocereus* are endemic to rock outcrops of the state of Minas Gerais, Brazil. We studied the reproductive biology of *C. minensis* N.P. Taylor & Zappi, a columnar cactus with nocturnal flowers, endemic to the Espinhaço Mountain Range, described floral morphology, and evaluated the reproductive system in two populations. We addressed the following questions: Is *C. minensis* pollinator-dependent for reproduction? What is the role of nocturnal and diurnal pollinators for its reproductive success?

2. Materials and methods

2.1. Study area

We studied two populations of *C. minensis* apart about 80 km from each other (Fig. 1) in the Espinhaço Mountain Range, Minas Gerais. One population was located on the Diamantina Plateau (18°11'48.23''S, 43°34'8.74''W), in an area of expansive rocky outcrops surrounding the town of Diamantina. The other was in Rio Preto State Park (18°07'12.9''S, 43°20'36.9''W), a nature reserve located in the municipality of São Gonçalo do Rio Preto. The climate of both areas is characterized by a well-defined rainy season from November to March, and a cooler dry season from June to September. Both populations of *C. minensis* were located in quartzitic open grasslands at altitudes between 1020 (Diamantina) and 950 m a.s.l. (Rio Preto State Park). The main vegetation within the study area is 'campo rupestre' (rupestrian fields) (Pirani and Harley, 1997), forming a mosaic with riparian forests and cerrado. The studies in both areas were carried out from May 2011 to December 2012.

2.2. Studied species

The genus *Cipocereus* differs from others in the tribe Cereeae in having blue, globose, indehiscent fruits with translucent flesh (Fig. 2A). The flowers have cream-colored petals with blue sepals (Zappi et al., 2010) (Fig. 2B). The cacti occur on rocky quartzitic outcrops and their roots usually extend into the fissures of rocks or are associated with termite mounds. Two subspecies are recognized within *C. minensis*, subspecies *leiocarpus*, the taxon studied herein, and subspecies *minensis*. The first has larger flowers and non-ribbed smooth, and blue fruit. Those of subspecies *minensis* are ribbed with a few spine-bearing areoles, and are brownish, pale green, whitish, or bluish (Taylor and Zappi, 2004).

2.3. Floral morphology and biology

For both populations we recorded color, odor and time of anthesis of the flowers. To describe floral morphology we used 25 flowers from 15 plant individuals from the Diamantina population and eight flowers from eight plant individuals from the Rio Preto State Park. The diameter of the corolla and the length of the flower, nectar chamber, and stigma + style were measured with digital calipers.

We collected 30 flowers in pre-anthesis from 16 individuals of the Diamantina population and counted the number of stamens and pollen grains per flower. We then multiplied the mean number of pollen grains per anther by the mean number of anthers per flower (Dafni et al., 2005).

To assess the rate of nectar secretion and the sugar concentration of nectar, we bagged eight flower buds of seven individuals. We then emptied all nectar from each flower in two-hour intervals from 19:00 h to 09:00 h. The flowers were re-bagged after each measurement to exclude flower visitors. We measured nectar volume using graduated microcapillary tubes and nectar sugar

concentration with a pocket refractometer (Atago® N1, Brix scale 0–32%).

2.4. Breeding system

To determine the breeding system of *C. minensis*, we performed four treatments: (1) non-manipulated self-pollination—flowers in pre-anthesis were maintained bagged without further manipulation; (2) hand self-pollination—flowers were hand-pollinated with their own pollen; (3) hand cross-pollination—flowers were emasculated and pollinated with pollen grains from at least two flowers of different individuals; and (4) natural pollination—flowers accessible to pollinators were individually marked (control).

The treatments were conducted on 126 flowers from 5 individuals of the Diamantina population and 202 flowers from 31 individuals of the Rio Preto State Park population. With the exception of the controls, flower buds were bagged with voile bags. We bagged the flowers of all treatments after senescence to protect the fruits from possible predation and determined the fruit set in each.

2.5. Floral visitors

In order to record flower visits by nocturnal animals, we set six camera traps (Tigrinus® and Bushnell®), each one in front of a cactus, during three blooming seasons of *C. minensis* in Diamantina. The trapping effort was of 528 h (April 2011), 4320 h (August to October 2011) and 3600 h (January to February 2012). Diurnal floral visitors were recorded *ad libitum*, (no systematic method; Altman, 1974) and through photographic records for Diamantina, throughout the study period.

2.6. Visitor-exclusion experiments

To evaluate the effectiveness of diurnal and nocturnal pollinators, we conducted exclusion experiments during four consecutive days/nights for Rio Preto and six days/nights for Diamantina. In order to exclude diurnal visitors, flowers were bagged at around 5:30 h in the morning until the end of anthesis (5 individuals and 33 flowers – Diamantina; 20 individuals and 51 flowers – Rio Preto). To exclude nocturnal visitors, flowers were bagged from 17:30 h to 5:30 h the next morning (5 individuals and 33 flowers – Diamantina; 20 individuals and 56 flowers – Rio Preto).

2.7. Statistical analysis

Generalized linear models (GLM) using quasi-poisson error distribution with log link function (after residual analyses) were constructed to compare fruit sets in the visitor-exclusion experiments and among the pollination treatments used to determine the breeding system of the plant. The response variable was fruit set and the explanatory variables were treatment and plant individual (used as a random factor). Contrast analyses were performed after the construction of the models. A multivariate analysis of variance (MANOVA) was performed to assess differences in flower morphology between the two populations. Analyses were performed in Statistica 8.0 (MANOVA) and R (GLM).

3. Results

3.1. Floral morphology and biology

C. minensis showed large, robust, cream colored flowers, with a large number of stamens (303 ± 42) and pollen grains (417.865 ± 3.345). They have a wide nectar chamber, which is approximately one third of the total length of the flower. Flower

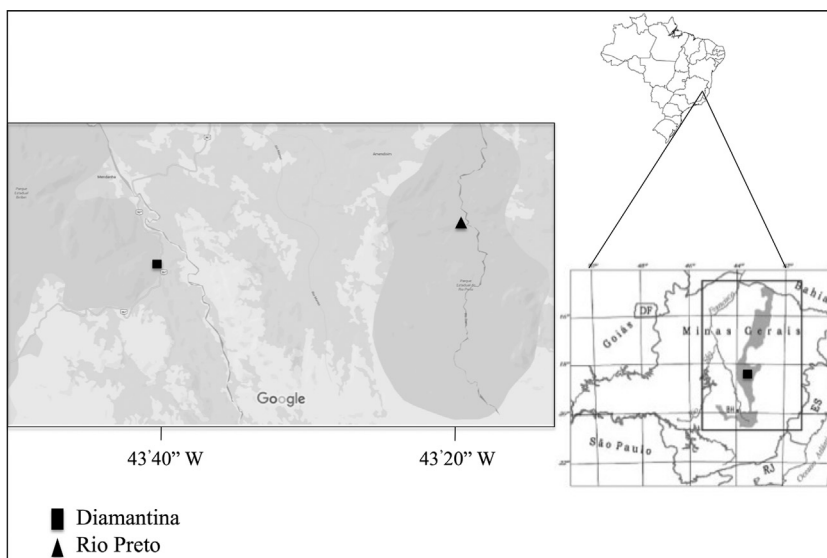


Fig. 1. Location of the two studied populations of *Cipocereus minensis*: Square represents Diamantina Plateau and Black triangle State Park of Rio Preto. Modified from Meyer and Franceschinelli (2011).

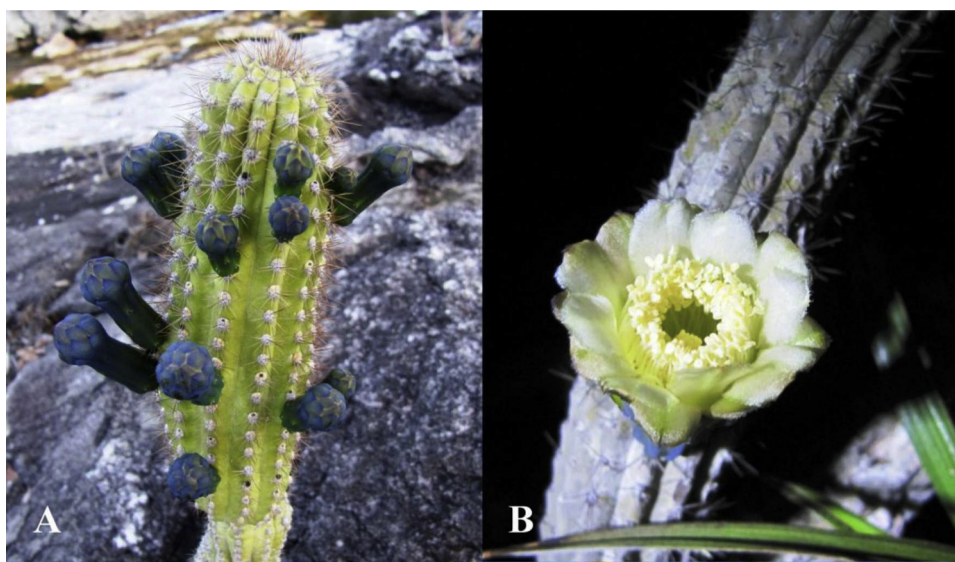


Fig. 2. *Cipocereus minensis*. (A) Flower buds and (B) flower.

Table 1
Floral morphology of *Cipocereus minensis* (mean \pm SD).

Size (cm)	Diamantina (n = 15)	Range	Rio Preto (n = 8)	Range
Diameter of the corolla	3.28 \pm 0.37 ^a	2.69–3.97	3.06 \pm 0.26 ^a	2.69–3.74
Flower length	4.61 \pm 0.66 ^a	3.54–6.34	4.90 \pm 0.44 ^a	4.46–5.47
Length of the nectar chamber	1.30 \pm 0.22 ^a	0.91–1.89	1.16 \pm 0.09 ^b	1.04–1.26
Length of stigma + style	2.74 \pm 0.47 ^a	2.34–3.33	3.31 \pm 0.34 ^b	2.73–3.68

Statistical differences are represented by different letters.

Table 2
Fruit set after different treatments of flowers of *Cipocereus minensis* of the Serra do Espinhaço, Minas Gerais, Brazil.

Treatment	Plants	Diamantina			Plants	Rio Preto		
		Flowers	Fruits	%		Flowers	Fruits	%
Spontaneous self pollination	5	33	0	–	19	84	0	–
Manual self pollination	5	20	0	–	12	28	0	–
Manual cross pollination	5	23	19	83	7	16	16	100
Natural pollination (control)	5	50	40	80	21	74	46	62
Night pollination	5	33	11	33	14	51	27	53
Day pollination	5	33	4	12	15	56	9	16

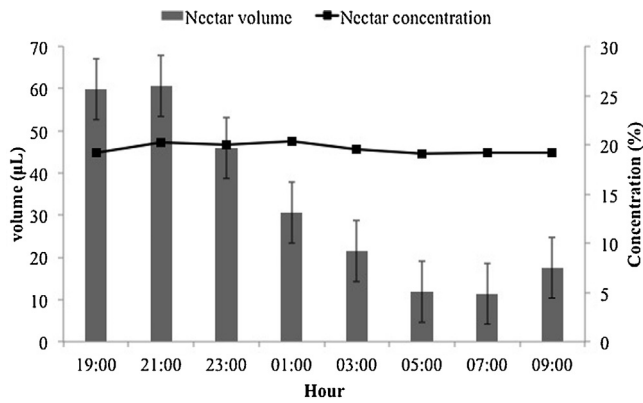


Fig. 3. Average production and concentration (given as production per two hours) of nectar from *Cipocereus minensis* (eight flowers from seven individuals) throughout the anthesis. Whiskers indicate mean \pm standard error.

morphology only differs between populations by the nectar chamber ($F_{3,22} = -3.67$, $p < 0.001$) and stigma + style length ($F_{3,22} = -3.05$, $p < 0.01$) (Table 1).

At the beginning of anthesis, at dusk, the flowers already emitted a mild sweet odor. In the Diamantina population, flowers started opening from 17:30 h to 18:30 h and were fully open between 19:00 h and 21:00 h. In the Rio Preto population, the beginning of anthesis was one to two hours delayed. The flowers remained open until late morning and closed definitively around 11:00 h in both populations.

The mean total production of nectar per flower was 260 μ L and the mean sugar concentration $20 \pm 0.5\%$ ($n = 8$). Nectar production per flower was high in the first hours of anthesis, between 19:00 h and 21:00 h (60 μ L/2 h), and diminished continuously until dawn (10 μ L/2 h). Sugar concentration of nectar remained stable throughout anthesis (Fig. 3).

3.2. Breeding system

C. minensis was self-incompatible and fruit set occurred only after hand cross-pollination and natural pollination (control) at a rate of 83% and 80%, respectively, for the Diamantina population and 100% and 62.2%, respectively, for the Rio Preto population (Table 2). Fruit set was different among the treatments both in Diamantina ($F_{3,17} = 22.97$, $p < 0.001$) and Rio Preto ($F_{3,51} = 5.218$, $p < 0.004$). Fruit set between individuals was not different, both in Rio Preto ($F_{25} = 0.794$, $p = 0.710$) and Diamantina ($F_4 = 0.932$, $p = 0.230$). Hand cross-pollination and natural pollination did not differ in the Diamantina population ($F_{1,9} = 0.234$, $p > 0.05$), whereas in the Rio Preto population hand cross-pollinated flowers set significantly more fruits than those accessible to pollinators (control) ($F_{1,24} = 13.909$, $p < 0.005$) (Table 2).

3.3. Visitor-exclusion experiment

Flowers available exclusively to nocturnal visitors set significantly more fruits than those available exclusively to diurnal visitors in both populations (Diamantina— $F_{1,8} = 5.69$, $p = 0.04$ /Rio Preto— $F_{1,27} = 9.64$, $p < 0.001$). Fruit set by nocturnal visitors was 33% and 53% in Diamantina and Rio Preto populations, respectively. Fruit set in flowers exclusively accessible to diurnal visitors was only 12% and 16.7%, respectively (Table 2).

3.4. Floral visitors

In the Diamantina population, ten nocturnal visits by bats of *Anoura* sp. (Phyllostomidae) and six diurnal visits by hummingbirds

of *Phaethornis pretrei* and *Eupetomena macroura* (Trochilidae) were recorded in the *C. minensis* flowers using camera traps. Worker bees of *Apis mellifera* and *Trigona* sp. (Apidae), as well as small beetles (Nitidulidae) were observed visiting flowers early in the morning.

4. Discussion

Our study shows that the endemic columnar cactus *C. minensis* is a self-incompatible species, pollinated almost exclusively by nocturnal flower visitors in both studied populations. The diurnal generalist visitors, highly social honeybees and stingless bees, and nectar seeking hummingbirds, provided only a minor contribution to fruit set. Floral traits such as large, robust and fleshy cream-colored flowers without nectar guides, a short flower tube with numerous stamens with a huge amount of pollen, and the high rates of nectar production during the night decreasing until dawn, are all typical adaptations to bat pollination (chiropterophily) (Faegri and Van Der Pijl, 1979; Vogel, 1968).

The extended period of anthesis and the continuous, but low, nectar secretion during the following morning, also permits visits to the flowers by generalist eusocial bees looking for pollen, and sporadic visits by hummingbirds which take-up the remaining nectar. However, considering the results of the exclusion experiment, bats, but not bees and hummingbirds, are the best pollinators of *C. minensis*, in spite of their low frequency of flower visits. Furthermore, floral biology and morphology as well as nocturnal anthesis are consistent with chiropterophily, reinforcing the statement that only part of the pollinator species or functional groups of them (bats in our case) exert strong selective pressures on floral traits (Fenster et al., 2004; Reynolds et al., 2009).

For some columnar cacti, a nocturnal floral cycle that extends into the following day has been suggested to be a strategy to ensure sexual reproduction when there is spatial and temporal variation in the frequency of nocturnal pollinators (Fleming et al., 2001). Pollination studies with columnar cacti in Mexico suggest that specialized pollination systems like that of chiropterophily prevail in tropical regions, whereas in extra-tropical regions, insects and hummingbirds would frequently be complementary pollinators in bat pollinated species (Fleming et al., 2001; Valiente-Banuet et al., 1996, 1997a,b).

In only six of the approximately 100 species of Cereaceae columnar cacti (sensu Zappi et al., 2010) has the pollination system been studied in detail [*Cereus horrispinus* Backeb., *Cereus repandus* Mill. (Nassar et al., 1997) *Pilosocereus chrysacanthus* (Weber) Byles & Rowley (Valiente-Banuet et al., 1997b), *Pilosocereus lanuginosus* (L.) Byles & Rowley (Nassar et al., 1997), *Pilosocereus royenii* (L.) Byles & Rowley (Rivera-Marchand and Ackerman, 2006) and *Pilosocereus tuberculatus* (Werdermann) Byles & Rowley (Rocha et al., 2007)]. All of them are tropical and exhibit a bat-specialized pollination system, even if diurnal visitors account for a minor contribution to fruit set. However, information on reproductive biology of the Southern hemisphere cacti is scarce, preventing conclusions about the occurrence of this pattern for this region (Munguía-Rosas et al., 2009).

Fruit set exclusively from cross-pollination is widespread in cacti species (see review Mandujano et al., 2010). Fruit set exclusively from cross-pollination in *C. minensis* as well as demonstrated for co-generic species (*Cipocereus laniflorus*—Rego et al., 2011, *Cipocereus crassisepalus*—Martins et al. unpublished) and other representatives of Cereaceae (Clark-Tapia and Molina-Freaner, 2004; Ibarra-Cerdeña et al., 2005; Strong and Williamson, 2007; Valiente-Banuet et al., 1997).

Our results indicate the occurrence of pollinator limitation on fruit set in the Rio Preto population but not in Diamantina, whereas the Diamantina but not the Rio Preto population seems to be

resource limited. Low visitation frequency, variation in pollination efficiency, and pollinators sharing has been identified as possible causes of pollinator limitation (Ashman et al., 2004; Hegland and Totland, 2007; Pinto and Schlindwein, 2015). In the Rio Preto Park (and not in Diamantina), *C. minensis* occurs with further two columnar cacti, *Pilosocereus aurisetus* (Werderm.) Byles & G.D. Rowley and *Cipocereus crassisepalus* (Buining and Brederoo) Zappi & N.P. Taylor. Both species are chiropterophilous and their blooming periods overlap largely (*P. aurisetus*) or partially (*C. crassisepalus*) with that of *C. minensis* (Martins et al., pers. obs.).

Populations of *C. minensis* occur in rocky habitats, from c. 750 m to 1500 m of altitude (Taylor and Zappi, 2004), which is a constrained habitat with a huge thermic amplitude, low water and resource availability, and sporadic fire (Taylor and Zappi, 2004). Although both populations occur in such a habitat, the fruit set in manual cross pollination treatments was only less than 100% in Diamantina indicating that in this but not in the Rio Preto population there are insufficient resources to produce fruits. Furthermore, inbreeding depression might constrain fruit set in the small-sized population of Diamantina.

The flowers of the two studied populations of *C. minensis* differ only in the size of the nectar chamber and in the stigma length. Because both populations depend on bat pollination, such differences do not result in a shift from nocturnal to diurnal pollinators. An important implication for conservation of endemic and endangered species, such as *C. minensis*, is the loss of main pollinators. The entire Cactaceae family is listed as endangered in the Convention on International Trade in Endangered Species (CITES). For columnar cacti, studies on possible population decreases of the bat pollinators and their viability are required to better understand the cacti's reproductive constraints.

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