RESEARCH PAPER

# Pollination and reproductive system of synchronopatric species of Cactaceae (Cactoideae) subject to interspecific flow of pollen: an example of ecological adaptation in the Brazilian Chaco

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## ABSTRACT

- Three synchronopatric Cactaceae species, *Echinopsis rhodotricha*, *Harrisia balansae* and *Praecereus saxicola*, have mostly nocturnal anthesis and similar flowers, characteristics that motivated us to perform a comparative study of reproductive ecology.

- Reproductive phenology was sampled monthly from December 2014 to November 2015. We describe floral biology, breeding system *via* pollination treatments and evaluate floral visitors from focal and filming observations. Pollen grains found on moth proboscis were compared among cactus species under light microscopy. We used fluorescent dye particles to test intra- and interspecific pollen flow.

- These three species have extended flowering with greater intensity in the wet season, causing high overlap. They have white and hypocrateriformis flowers that open at twilight or nightfall and last about 15 h. *H. balansae* seems to be self-incompatible, while *E. rhodotricha* presented self-compatibility. *P. saxicola* presented self-fertility, but most of the population seems to be self-incompatible. We suggest sphingophily for the three species, but only *P. saxicola* was visited by *Manduca rustica* (Sphingidae). However, we observed pollen grains of all three species on the proboscis of moths, especially *M. rustica* and *M. sexta*. Prolonged anthesis allowed bees (herein considered as secondary pollinators) to visit flowers of *E. rhodotricha* and *P. saxicola*.

- It can be concluded that the studied species share nocturnal and diurnal pollinators, suggesting interspecific pollen flow, which, however, could not be detected with fluorescent dye particles. In view of the low frequency of primary pollinators, it appears that these three species have different reproductive strategies, ensuring the fruiting and production of viable seeds.

## INTRODUCTION

*Echinopsis sensu lato* is a polyphyletic group that includes species of *Echinopsis* and other genera, such as *Praecereus* and *Harrisia* (Schlumpberger & Renner 2012). Intergeneric hybrids are mentioned among species of *Harrisia* and *Echinopsis* (Franck 2012), but they are unknown in the wild. While hybridisation seems to be of little relevance in the evolution of this group (Schlumpberger & Renner 2012), its species show a marked convergence with respect to pollination syndromes.

Pollination syndromes, however, cannot be used to determine all groups of pollinators based on the presence of overlapping characteristics, such as those across species pollinated by moths Sphingidae (sphingophily) and bats (chiropterophily) or butterflies (psicophily) and hummingbirds (ornithophily) (Faegri & van der Pijl 1980; Proctor *et al.* 1996). In addition, generalisation may occur along with the rate of several floral visitors (Waser *et al.* 1996; Lange *et al.* 2000). Therefore, the 'isolation' provided by a set of floral features is generally incomplete, such that flowers regarded as sphingophiles or chiropterophiles, for example, may be visited by other groups of animals, such as bees, beetles and hummingbirds (*e.g.* Weiss *et al.* 1994; Rivera-Marchand & Ackerman 2006; Ortega-Baes *et al.* 2010b; Moore 2011). In light of this, syndromes may reflect primary pollinators, but they do not always exclude other floral visitors who may often act as secondary pollinators (Rosas-Guerrero *et al.* 2014). Therefore, attributes related to pollination syndromes need to be evaluated quantitatively, allowing us to examine the degree of variation between apparently convergent species. In addition, the validity of the syndrome needs to be investigated in terms of the effectiveness of pollination and the abundance of predicted visitors, especially in cases of correlated species that are sympatric (Ottra *et al.* 2016), have overlapping flowering and share the same syndrome. In these cases, the possibility of interbreeding arises since synchronicity and floral similarity may subject the plant species to pollinator sharing and interspecific pollen flow (Waser 1978; Lange *et al.* 2000; Queiroz *et al.* 2015). This situation can reduce reproductive success through competition for pollination, loss of pollen grains (if transported to

non-cospecific stigma) and/or reduction of the stigmatic area with nonspecific pollen (Nadia *et al.* 2007).

Cactaceae are among the most conspicuous plants in the arid and semi-arid regions of the Neotropics, and it is common for them to be cogeneric species, or not to occur sympatrically, and to present the same pollination syndrome. Studies mainly report the co-occurrence of melitophilous (Osborn *et al.* 1986; McIntosh 2002; Giorgis *et al.* 2015), chiropterophilous (Ruiz *et al.* 2000; Petit 2001) and ornitophilous (Colaço *et al.* 2006; Fonseca *et al.* 2008) species. In these cases, a moderate or high overlap in the flowering (and fruiting) typically occurs, especially among cogeneric species, potentially resulting in different flowering patterns associated with abiotic (precipitation and/or temperature; Browers 2002; Fonseca *et al.* 2008; Bustamante & Búrquez 2008; Rojas-Sandoval & Meléndez-Ackerman 2011; Cruz & Pavón 2013) or biotic factors (population structure, size of individuals, life form; Browers 2002; Bustamante & Búrquez 2008; Rojas-Sandoval & Meléndez-Ackerman 2011; Cruz & Pavón 2013). These species exhibit similar morphology and/or floral biology (*e.g.* Osborn *et al.* 1986; Ruiz *et al.* 2000; Petit 2001; McIntosh 2002; Colaço *et al.* 2006). In spite of this, several mechanisms of reproductive isolation, either to reduce or avoid hybridisation, can occur.

Sympatric species of Cactaceae, cogeneric or not, may show similarity in floral morphology and biology and share flower visitors and pollinators. As such, many studies have investigated these species in terms of mechanisms involved in the reduction of competition by pollinators. These mechanisms include differences in flowering peak, morphology of the flowers (size or number of elements in perianth), floral biology (beginning and end of anthesis) and nectar characteristics (volume, concentration, nectar energy, period of production; Fleming *et al.* 1996, 2001; Petit & Freeman 1997; Valiente-Banuet *et al.* 1997; Fleming 2000). However, only McFarland *et al.* (1989), working with *Opuntia* spp., have investigated the occurrence of interspecific pollen flow in Cactaceae. Only one of 40 individuals examined in this study had sufficient pollen from other species to indicate interspecific pollen flow, possibly from the low effectiveness of interspecific floral visitors as pollinators (McFarland *et al.* 1989).

Chaco is a phytogeographic province with an open vegetation of about 840,000 km². It occurs throughout the alluvial plains of central South America located in northern Argentina, western Paraguay, south-eastern Bolivia and the extreme western part of Mato Grosso do Sul, Brazil (Werneck 2011). At least ten genera and 13 species of Cactaceae comprise (Freitas *et al.* 2013; M. R. Sigrist, personal observation) a subtype of Brazilian Chaquenian vegetation (Arboreal Steppe Savannah, Woody Chaco or sense-restricted Chaco; *sensu* Veloso *et al.* 1992). Seven studied species bloom and produce fruit during the rainy season (Freitas *et al.* 2013), thus differing from Bolivian Chaco where flowering begins in the dry season. However, most species produce fruit in the rainy season (Cuéllar 2000). In this area, at least seven species of Cactaceae (*Cereus bicolor*, *Cereus phatnospermus*, *Echinopsis rhodotricha*, *Harrisia balansae*, *Harrisia tortuosa*, *Hylocereus* sp. and *Praecereus saxicola*) have flowers with mainly nocturnal anthesis and similar morphology (tubular, white), suggesting the possible sharing of pollinators and, hence, occurrence of interspecific pollen flow.

Cacti with nocturnal anthesis are usually pollinated by moths, especially Sphingidae (Silva & Sazima 1995; Fleming *et al.* 2001; Rojas-Sandoval & Meléndez-Ackerman 2009) or bats (Valiente-Banuet *et al.* 1996; Nassar *et al.* 1997; Rego *et al.* 2012). Species with mixed or generalist systems of pollination have also been observed. In these species, floral longevity allows combining pollination with nighttime and daytime agents, such as species pollinated by moths and bees (Holland & Fleming 2002), bats and bees (Alcorn *et al.* 1961), bats, bees and hummingbirds (Molina-Freaner *et al.* 2004) or bats and hummingbirds (Dar *et al.* 2006). These systems are mentioned as mechanisms of competition tolerance between synchronopatric species (Ashman 2000), including Cactaceae (Fleming *et al.* 1996).

Therefore, the present work has undertaken a comparative study of the reproductive phenology, morphology and floral biology, as well as breeding system and pollination, of *E. rhodotricha*, *H. balansae* and *P. saxicola*, three cacti of the Brazilian Chaco, to determine if they (i) present similar and seasonal flowering and fruiting patterns; (ii) show overlapping periods of flowering and fruiting; (iii) differ in morphology and floral biology; (iv) depend on pollen vectors, considering the system of compatibility; and (v) share floral visitors and pollinators, which can result in the interspecific flow of pollen. It is hypothesised that these three species will (i) bloom and fruit in the rainy season, thus leading to interspecific overlap in reproductive phenophases; (ii) be self-incompatible, a common feature for most species of the genus studied; (iii) have differences in flower morphology and biology that may reduce the potential interspecific flow of pollen or allow pollination by various pollen vectors; and (iv) exhibit sharing of floral visitors and pollinators with greater overlap during the flowering period and/or show morphological and functional similarity of flowers.

## MATERIAL AND METHODS

### Study area

The study was conducted from October 2014 to January 2016 in two areas: Retiro Conceição Farm (21°40′57″ S, 57°46′44.46″ W) and Anahi Farm (21°41′13.05″ S, 57°44′08.02″ W). Approximately 3 km separate these two areas in Porto Murtinho City, Mato Grosso do Sul, Brazil. Both areas represent a remnant of vegetation classified as Arboreal Steppe Savannah (Veloso *et al.* 1992), which is characterised by the presence of arboreal–shrub stratum with a canopy 2–4 m in height interspersed with herbaceous strata (Alves & Sartori 2009).

The climate of the Chaco is characterised by strong seasonality, with hot summers (maximum up to 49 °C) and dry, cold winters, with frost. Rainfall declines from 1000 mm·year$^{-1}$ in the east to less than 550 mm·year$^{-1}$ in the west, with a dry season during winter and spring and a rainy season in the summer (Pennington *et al.* 2000). The soils in the Chaco region are derived from wind-driven alluvial sediment in the Quaternary. The absence of rock formations results in a compact soil that prevents drainage. The vegetation of the Chaco is subject to soil with low humidity and temporary flooding (Pennington *et al.* 2000). In the region of Porto Murtinho/MS, the rainy season occurs from November to February (rainfall ≥100 mm) and

the dry season from June to September when water deficit prevails. Transitional seasons occur in the months of March, May and October when sparse rainfall reaches 100 mm, but with no water deficit (Freitas *et al.* 2013). During the phenological study, the highest temperatures were recorded in November and January (rainy season) and higher rainfall in November and April (Figure S1).

### Studied species

*Echinopsis rhodotricha* is a short columnar cactus, while *H. balansae* and *P. saxicola* are columnar ascendant and columnar decumbent, respectively. They are distributed across Argentina, Bolivia, Brazil and Paraguay (Kiesling *et al.* 2013; Oakley & Kiesling 2013; Oakley *et al.* 2013) where they occur mainly in Chaco vegetation. *E. rhdotricha* occurs in Uruguay. They present solitary, sessile, large, hermaphrodite, hypocrateriform flowers with perigons constituted by numerous lobes arranged in several whorls, being the external calicinis and the internal petaloids. Androecium is formed from numerous stamens. Ovary is epigynous, unilocular, multicarpelar, multiovulated and encased in epidermis that forms a long floral tube (Barthlott & Hunt 1993). In all three species, the fruit is baciform, red and dehiscent through longitudinal crevices, as well as pilose (*E. rhodotricha*), glabrous (*P. saxicola*) or covered with fleshy bracts (*H. balansae*) (Kiesling 1975). The seeds are small, have black foreheads wrapped in white pulp and are produced in large quantities (Kiesling 1975). Vouchers of these species were deposited at the CGMS Herbarium, Universidade Federal de Mato Grosso do Sul (UFMS): *E. rhodotricha* (CGMS 53698), *H. balansae* (CGMS 53697) and *P. saxicola* (CGMS 48438).

### Reproductive phenology

We carried out phenological sampling from December 2014 to November 2015 in 15 labelled adult plants of each species. The number of buds and flowers (phenophase flowering) as well as unripe and ripe fruit (in dispersion) (fruiting) was quantified monthly. We have calculated the mean angle (or vector – μ) for these phenophases, as well as the mean vector SD, mean vector length (r) and Rayleigh test (Z and P) using circular statistics supplied in the Oriana 2.0 program (Kovach Computing Services, Anglesey, UK). The mean angle represents the mean phenological activity date, and r represents the degree of synchronisation (clustering) of the phenophases for each species. Values >0.5 for r and significant Rayleigh test indicate aggregation (seasonality). The overlapping degree of the phenophases between species was calculated using the Pianka (Ojk) overlap index (Pianka 1973), with EcoSim software (Gotelli & Entsminger 2000), and the overlap could be high (>0.6), intermediate (between 0.6 and 0.4) or low (<0.4). The duration of the phenophases was classified according to Newstrom *et al.* (1994).

Multiple regressions were applied to verify the relationship between separate climate factors during the study period (total rainfall, mean temperature, photoperiod, relative air humidity) and reproductive phenophases (see Souza *et al.* 2016). The Akaike information criterion (AIC; Johnson & Omland 2004) was used to select the simplest and most parsimonious model, using the vegan package (Oksanen *et al.* 2010) in the R environment (R Development Core Team 2012). The photoperiod

was calculated using the Online Photoperiod Calculator (Lammi 1996–2015). Climatological variables of the phenological study period were obtained from the Centro de Monitoramento do Tempo, do Clima e Recursos Hídricos de Mato Grosso do Sul (Cemtec- Mato Grosso do Sul, Brazil).

### Morphology and floral biology

We recorded the number of open flowers per plant per day and the arrangement of these flowers in the plants. We studied the floral morphology from fresh material and/or material fixed in 70% alcohol and from photographic records. For each species, we measured the flowers with the aid of digital calipers (n = 15 flowers, one per plant). Pollen viability was tested with acetic carmine solution (Dafni 1992) from pre-anthesis buttons fixed in 70% FAA (n = 20, one per plant). This dye allowed us to count 200 pollen grains by scanning semi-permanent sheets. We qualitatively determined stigmatic receptivity based on turgor, aspect (moist or 'glossy') and removal of stigmatic lobes. We calculated the pollen/ovule (P/O) ratio (*sensu* Cruden 1977) using five pre-anthesis buds per species, one per plant.

We measured the volume of accumulated nectar until 05:00 h, depending on the hours between sunset and sunrise, and the concentration of solutes of the floral nectar from flowers bagged during pre-anthesis (n = 8 flowers, one per plant; Dafni 1992) with the aid of a graduated microsyringe and digital refractometer, respectively. We estimated the caloric content of nectar based on the equation of Scogin (1985) for Cactaceae species: Energy/flower = concentration (% of solutes) × volume (ml) × 39, where 39 is the volume conversion unit that reflects calories per gram sucrose. We verified other floral attributes, such as colour, presence of odour, dichogamy, anthesis time, floral longevity and period of availability of nectar and pollen, using direct observations in the field of marked pre-anthesis flowers until the time of wilt/closure (n = 20 flowers, two flowers per plant).

We compared the difference of characters between species through the Kruskal–Wallis test (non-parametric ANOVA) with Dunn post-test (Zar 2010). For both tests, we considered a level of significance of 5%. The floral dimensions (total flower length, perianth diameter, floral throat diameter, length and diameter of the floral tube) of the three species were grouped in a two-dimensional dispersion diagram with 95% confidence ellipses by principal components analysis (PCA) from a variance-covariance matrix between the groups (Legendre & Legendre 1998).

### Reproductive systems

In the field, we performed treatments to verify the occurrence of spontaneous self-pollination, hand self-pollination and cross-pollination using pollen from flowers of the same plant (geitonogamy) Arroyo (1976) or different plant (xenogamy) bagged in pre-anthesis, either emasculated (cross-pollination) or not (self-pollination). All manual pollination treatments (self and cross) were performed at night from 01:00 to 05:00 h. Twenty flowers were used as samples for each treatment, but varied from one to 20 flowers, depending on their availability during field campaigns.

After 40–45 days, giving sufficient time for fruit maturation, we recorded the percentage of fruits obtained from each treatment, and these were collected to test viability of the seeds. We quantified the seeds under a magnifying glass by direct counting during fruit removal. After removing a small portion of the forehead and immersing it in 0.6% tetrazolium solution (modified from Assis *et al.* 2015) for 12 h (overnight), a 'viability reading' was taken. We chose to use tetrazolium instead of seed germination since dormancy has been observed in the seeds of *Echinopsis*, for example, and we needed a rapid response (Ortega-Baes *et al.* 2010a).

For all species and all treatments, fecundity was calculated from the ratio between the number of seeds and the mean number of ovules (Wolowski & Freitas 2010). The percentage of fruiting between treatments was compared using Fisher's exact test, while the number of seeds per fruit, fecundity and seed viability were compared using Kruskal–Wallis and one-way ANOVA.

### Floral visitors

We performed focal observations of floral visitors during anthesis, totalling 40 h for *E. rhodotricha*, 30 h for *H. balansae* and 60 h for *P. saxicola* on non-consecutive days, according to the availability of flowers in the study areas. *P. saxicola* is the species most commonly found in the flowering state (B.H.S. Ferreira, personal observation). Therefore, we were able to film 40 h of this species in anthesis with a camcorder coupled to infrared light on non-consecutive nights, totalling 100 h of observation. For all species, we recorded visiting time, number of flowers visited per visitation, frequency of visits (number of visits/total hours of observation for the species), length of visit and resource collected. When possible, we used a collecting net to pinch, tag and package insects in entomological boxes. We constructed a network of Kamada-Kawai-type interactions using the Pajek 2.02 program (Batagelj & Mrvar 1988) to visually verify or present the sharing of floral visitors. Additionally, we collected nocturnal Lepidoptera using a light trap, which consisted of two lamps of mixed light arranged 3 m apart on a white tissue during one night under the new moon (n = 10 h). After collection, the insects were dormant in a lethal chamber containing ethyl acetate and packed in properly labelled paper envelopes. Using stereomicroscopy, we conducted analysis in the laboratory to verify the presence and the place of deposition of pollen on the body of the insect. When present, the pollen was removed with fuchsin gelatin and prepared on slides, which were examined under light microscopy. This examination basically consisted of morphological comparison of the pollen grains found with a previously prepared micrographic reference of the pollen grains of the studied Cactaceae species. All insects collected will be deposited in the Zoological Collection of UFMS (ZUFMS).

### Pollen flow

We tested the occurrence of interspecific pollen flow among plants close to the studied species, marking the flower anthers (*E. rhodotricha* n = 6 flowers, *H. balansae* n = 2, *P. saxicola* n = 19) with fluorescent dye particles of different colours (adapted from Fenster *et al.* 1996) on non-consecutive days. For each 'marked' flower, we left at least one more flower per plant unmarked in order to also test intraspecific pollen flow. Toward the end of anthesis, we inspected the flowers with a black light lantern to verify the presence, colour and location of fluorescent dye particle deposition on the flowers. Based on the colour of the fluorescent dye particles, we could determine their origin, *i.e.* either from the same or from another species, plant and/or flower.

## RESULTS

### Reproductive phenology

In all species, phenophases presented an extended (duration ≥5 months; Newstrom *et al.* 1994) and seasonal (r > 0.5) pattern, with flowering and fruiting for an mean of 8 and 6 months and mean dates during the rainy season in December and January, respectively (Table 1, Fig. 1). We found overlap in the flowering period between species ($O_{jk}$ *E. rhodotricha*–*H. balansae* = 0.75; $O_{jk}$ *E. rhodotricha*–*P. saxicola* = 0.84; $O_{jk}$ *H. balansae*–*P. saxicola* = 0.71). Similarly, we observed a high overlap in fruit production between *P. saxicola* and other species ($O_{jk}$ *E. rhodotricha* = 0.67; $O_{jk}$ *H. balansae* = 0.9) and an intermediate overlap between *E. rhodotricha* and *H. balansae* ($O_{jk}$ = 0.45).

**Table 1.** Number of observations throughout the year (N), mean vector (μ) and mean date, mean vector SD, mean vector length (r) and Rayleigh (Z) test of reproductive phenophases of *E. rhodotricha*, *H. balansae* and *P. saxicola* in a remnant of Chaquenian vegetation, Mato Grosso do Sul, Brazil, from December 2014 to November 2015.

| phenophase | duration (months) | N | μ (mean date) | mean vector SD | r | Z | P |
|---|---|---|---|---|---|---|---|
| *Echinopsis rhodotricha* | | | | | | | |
| Flowering | 9 (Aug–Mar/May) | 128 | 330.01° (1 Dec) | 52.83° | 0.65 | 54.70 | **<0.001** |
| Fruiting | 7 (Sep–Oct/Dec–Apr) | 25 | 25.67° (26 Jan) | 52.63° | 0.66 | 10.75 | **<0.001** |
| *Harrisia balansae* | | | | | | | |
| Flowering | 7 (Sep–Mar) | 38 | 333.97° (4 Dec) | 34.42° | 0.84 | 26.49 | **<0.001** |
| Fruiting | 5 (Dec–Apr) | 17 | 25.85° (26 Jan) | 44.79° | 0.74 | 9.23 | **<0.001** |
| *Praecereus saxicola* | | | | | | | |
| Flowering | 7 (Sep–Mar) | 165 | 349.10° (20 Dec) | 49.67° | 0.69 | 77.83 | **<0.001** |
| Fruiting | 7 (Nov–May) | 102 | 12.84° (13 Jan) | 37.41° | 0.81 | 66.60 | **<0.001** |

Significant values *P* < 0.05 are shown in bold.
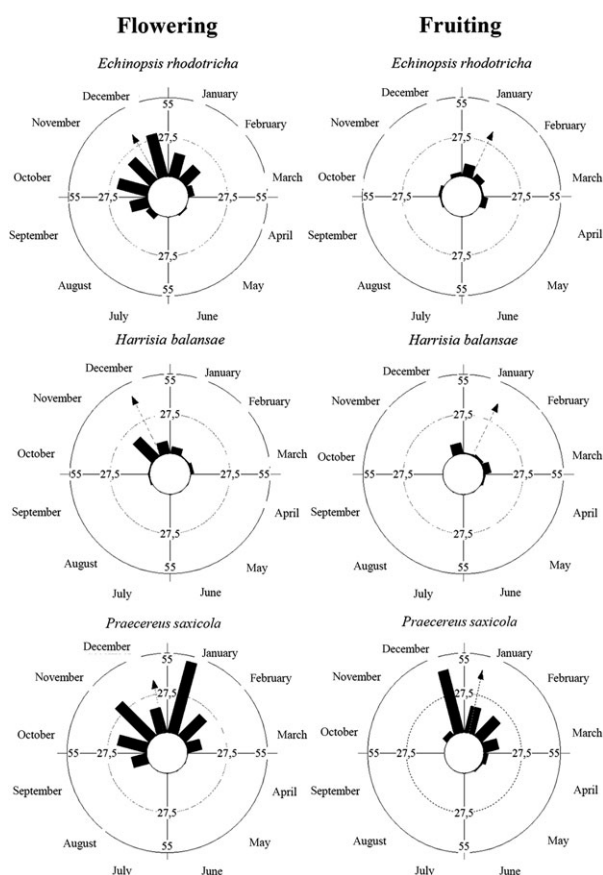
**Fig. 1.** Circular diagrams for flowering (left) and fruiting (right) of *E. rhodotricha*, *H. balansae* and *P. saxicola* (Cactaceae) in a remnant of Chaquenian vegetation (Arboreal Steppe Savannah), Porto Murtinho City, Mato Grosso do Sul, Brazil, from December 2014 to November 2015.

The best model related to flowering presented differences across the species was photoperiod/relative air humidity, rainfall and mean temperature showed a positive relationship with the flowering of *E. rhodotricha*, *H. balansae* and *P. saxicola*, respectively (Table 2). The model that best explained fruiting was only positively related to mean temperature in *E. rhodotricha* (Table 2). This is because the intensity of fruiting increases with the average temperature.

## Morphology and floral biology

In the study area, individuals of *E. rhodotricha* are shorter (approx. 40-cm high) than those of *P. saxicola* at ~1.5 m and *H. balansae* reaching >2 m (Table 3). The flowers of *E. rhodotricha* and *P. saxicola* occupy an upright (horizontal) position in relation to the stem (Fig. 2A, B), whereas those of *H. balansae* are in an inclined position (Fig. 2C). In all three species, the petaloid lobes are white, and the floral tube is narrow and long in the flowers, which are externally light brown (*E. rhodotricha*) or greenish (other species; Fig. 3). The flowers are either actinomorphs (*E. rhodotricha*, *P. saxicola*) or zygomorphs (*H. balansae*). In *H. balansae*, zygomorphy is conferred by the numerous stamens, which concentrate mainly in the lower portion of the floral throat (Fig. 2D), whereas in the other two species they form a homogeneous circle around the floral throat.

In general, *H. balansae* has the largest flowers, followed by *E. rhodotricha* and *P. saxicola* (Table 3). The PCA found little overlap among the species, evidencing that they occupy different morphometric spaces, albeit still insufficient to separate them into different groups (Fig. 3). *P. saxicola* has shorter stamens, style/stigmata and flowers (total length). *E. rhodotricha* has a longer floral tube and more stamens. *H. balansae* presents higher values for perianth and floral throat diameters and has a higher number of ovules and, therefore, a lower P/O ratio (Table 3).

In the studied species, anthers were basified, ripe, white and produce pollen with high viability (between 80% and 100%; Table 3). The gynoecium has a long style and stigma with a variable number of lobes (Table 3) positioned in the lower portion (*H. balansae*) or in the centre of the flower (other species). Nectar is produced in nectariferous tissue that lines the floral tube and is stored in the lower third of this tube. *E. rhodotricha* presents the least amount of energy per flower by producing less concentrated nectar in smaller quantities compared to the other species. *H. balansae* has the highest volume of nectar, and the nectar in *P. saxicola* has the highest concentration (Table 3).

Each night, usually one to three flowers per plant would open up, with higher mean values recorded for *H. balansae* (Table 3). The flowers lasted for about 15 h and had greater longevity at night. In *H. balansae* and *P. saxicola*, the flowers start to open at dusk, around 17:00 and 18:00 h, respectively, while in *E. rhodotricha*, the beginning of floral opening occurred around 20:00 h. At the beginning of floral anthesis,

**Table 2.** Akaike Information Criterion (AIC). Regression model between total monthly rainfall, mean temperature, photoperiod and relative air humidity, and reproductive phenophases of *Echinopsis rhodotricha*, *Harrisia balansae* and *Praecereus saxicola* in remnant of Chaquenian vegetation, Porto Murtinho city, Mato Grosso do Sul, Brazil, from December/2014 to November/2015. ΔAIC = initial AIC - final AIC. Number of observations = 12 months. Significant values ($P - 0.05$) are shown in bold.

| specie | phenophase | ΔAIC | intercept | $r^2$ | F | P | rainfall | mean temperature | photoperiod | relative humidity |
|---|---|---|---|---|---|---|---|---|---|---|
| *Echinopsis* | Flowering | 3.08 | −150.86 | 0.92 | 66.4 | **<0.001** | – | – | 12.01 | 0.24 |
| *rhodotricha* | Fruiting | 4.33 | −19.08 | 0.41 | 8.60 | **0.014** | – | 0.79 | – | – |
| *Harrisia* | Flowering | 2.23 | −48.75 | 0.55 | 7.89 | **0.01** | 0.03 | – | – | – |
| *balansae* | Fruiting | 1.96 | −30.08 | 0.31 | 2.66 | 0.11 | 0.22 | 0.84 | 0.16 | 0.16 |
| *Praecereus* | Flowering | 2.15 | −160.30 | 0.60 | 9.85 | **0.005** | – | 3.44 | – | – |
| *saxicola* | Fruiting | 1.27 | −163.41 | 0.44 | 3.88 | 0.05 | −0.01 | 5.12 | 0.80 | 0.80 |

Significant values $P < 0.05$ are shown in bold.

**Table 3.** Characteristics of plants and flowers of *E. rhodotricha*, *H. balansae* and *P. saxicola* (Cactaceae) in a remnant of Chaquenian vegetation, Mato Grosso do Sul, Brazil. x̄ = arithmetic mean ± SD.

| characteristic | *Echinopsis rhodotricha* x̄ ± SD | *Harrisia balansae* x̄ ± SD | *Praecereus saxicola* x̄ ± SD |
|---|---|---|---|
| Plant height (m) | 0.4 ± 0.3[a] | 2.2 ± 0.9[b] | 1.3 ± 0.7[b] |
| Number of open flowers day⁻¹·plant⁻¹ | 1.4 ± 0.6[a] | 2.6 ± 1.7[b] | 1.4 ± 0.6[a] |
| Perianth | | | |
| Flower length (CF) (cm) | 17.5 ± 1.9[a] | 17.5 ± 2.2[a] | 9.9 ± 2.2[b] |
| Perianth diameter (DP) (cm) | 7.8 ± 1.4[a] | 11.2 ± 2.8[b] | 7.4 ± 1.3[a] |
| Throat diameter (DG) (cm) | 2.0 ± 0.4[a] | 5.3 ± 1.6[b] | 2.0 ± 0.6[a] |
| Floral tube | | | |
|   Length (CT) (cm) | 13.1 ± 2.8[a] | 8.9 ± 1.2[b] | 6.3 ± 1.9[b] |
|   Diameter (DT) (cm) | 2.6 ± 0.3[a] | 4.4 ± 0.8[b] | 1.5 ± 0.4[a] |
| Androecium | | | |
| Number of stamens | 518 ± 37[a] | 290 ± 60[b] | 226 ± 16[b] |
| Anther height (cm) | 13.59 ± 1.3[a] | 13.8 ± 1.6[a] | 7.1 ± 1.0[b] |
| Pollen viability (%) | 87 ± 9[a] | 96 ± 3.5[a] | 89 ± 20[a] |
| Gynoecium | | | |
| Number of stigmatic valves | 11 ± 2[a] | 13 ± 2[a] | 12 ± 2[a] |
| Height of stigma (cm) | 12.6 ± 1.85[a] | 13.8 ± 1.7[a] | 7.83 ± 0.9[b] |
| Number of ovules | 2203 ± 452[a] | 5022 ± 642[b] | 1552 ± 298[a] |
| Pollen–ovule ratio | 198 ± 36[a] | 109 ± 10.5[b] | 166 ± 13[a] |
| Nectar | | | |
| Volume (ml) | 0.08 ± 0.02[a] | 0.2 ± 0.04[b] | 0.13 ± 0.12[ab] |
| Solute concentration (%) | 10.1 ± 4.5[a] | 12.9 ± 1.2[ab] | 16.3 ± 4.3[b] |
| Energy/flower (cal/flower) | 32.2 ± 12.5[a] | 101.2 ± 20.7[b] | 90.0 ± 105.3[ab] |

Values followed by different letters on the same line differ significantly (*P* < 0.05).



**Fig. 2.** Flower of *Echinopsis rhodotricha* (A). *Xylocopa* sp. when approaching the flower of *Praecereus saxicola* (B). Side (C) and frontal (D) view of the flower of *Harrisia balansae*. *Melipona* cf. *rufiventris* collecting pollen from flowers of *E. rhodotricha* (E). Fluorescent marker is mainly present on petals of *P. saxicola* (F). Bars = 1 cm.

the anthers are open, except in *P. saxicola*; however, the stigmas are not receptive, and the stigmatic lobes are close together. After 1 (*E. rhodotricha*), 2 (*H. balansae*), 3 or 4 h (*P. saxicola*), the flowers are fully open, *i.e.* the perianth and androecium elements are positioned, and the stigmatic lobes are fully distended and receptive. The flowers of *E. rhodotricha* and *P. saxicola* exude a mild sweet odour, whereas those of *H. balansae* by 21:00 h present a scent similar to that of bleach (sodium hypochlorite). The flowers of *H. balansae* and *P. saxicola*, respectively, begin to wither and close, a process that may extend up to 08:00 h (*H. balansae*) and 09:00 h. (*P. saxicola*). In *E. rhodotricha*, the flowers remain open until almost the end of the morning, closing and wilting around 11:00 h.

### Reproductive system

Fruiting after spontaneous self-pollination with a high number of viable seeds was recorded only in *P. saxicola* (Table 4). All species started fruiting after hand self-pollination, but in
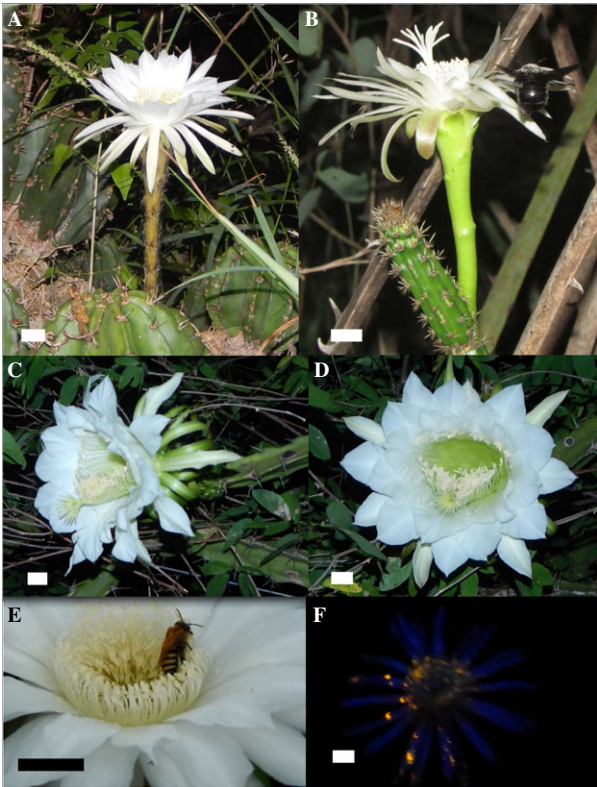
*P. saxicola* the percentage of fruiting after this treatment was low (n = 1; 7.7%), lower in fact than that registered under spontaneous self-pollination. In *H. balansae* the only fruit that formed contained a few aborted (wilted, deformed, yellowish-brown) ovules (Table 4).

Higher fruiting percentages were recorded after xenogamic cross-pollination and in the treatment for geitonogamy in *E. rhodotricha* (Table 4). The highest percentage of fruiting under natural conditions (control) was recorded for *H. balansae* (n = 6; 66.6%), followed by *P. saxicola* (n = 10; 50.0%) and *E. rhodotricha* (n = 6; 30.0%). *H. balansae* presented higher seed formation and fecundity after xenogamy treatment, *E. rhodotricha* after geitonogamy and in *P. saxicola* after spontaneous self-pollination, similar to the control group (Table 4). Seeds of fruits obtained from different treatments of pollination presented high viability (95.0–99.6% average), except in *H. balansae* treated with hand self-pollination in which the ovules were aborted.

### Floral visitors

We recorded 12 species of visitor in the flowers of *E. rhodotricha*, six in *H. balansae* and nine in *P. saxicola*. These included a species of spider and 16 species of insects belonging to the orders Hymenoptera (n = 5 spp.), Coleoptera (7), Orthoptera (2), Hemiptera (1) and Lepidoptera (1). Almost half of the species (47%) were observed during the diurnal
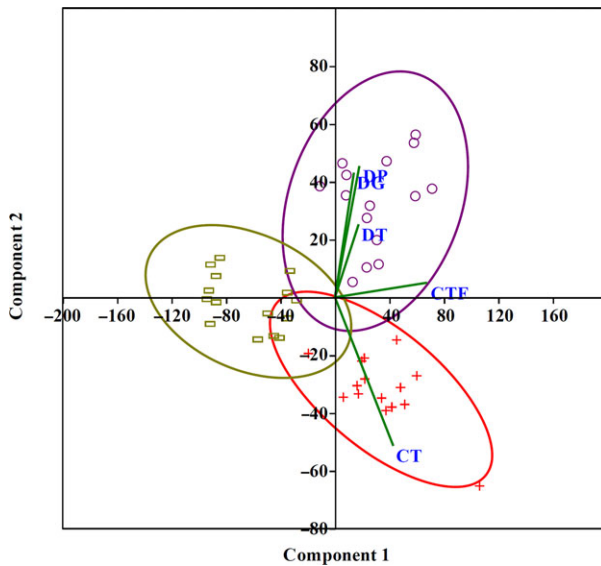
**Fig. 3.** Two-dimensional dispersion diagram showing morphometric data for flowers of *Echinopsis rhodotricha* (red), *Harrisia balansae* (purple) and *Praecereus saxicola* (green) from a matrix of variance and covariance in PCA. CTF, flower length; DP, perianth diameter; DG, throat diameter; CT, tube length; DT, floral tube diameter.

period (Hymenoptera, Araneae, two species of Coleoptera), and the others were all nocturnal (Table S1). The *Xylocopa* sp. bee (Fig. 2B) and the *M. rustica* sphingid were recorded only in *P. saxicola*. The Alticini (indeterminate species 2) beetle was observed only in *H. balansae*. The bees *Melipona* cf. *rufiventris* (Fig. 2E) and *Trigona* cf. *fuscipennis*, the beetles *Iphimeis* sp. and *Camptotes vittata*, as well as species of Hemiptera were recorded only in *E. rhodotricha*. The remaining visitors (n = 9 spp.) were shared between two or three species (the Alticini beetle only in one; Table S1, Fig. 4).

We observed the sharing of potential pollinators only between *E. rhodotricha* and *P. saxicola*, which generally showed greater similarity in floral visitors (5 spp.) compared to visitors between *H. balansae* and *P. saxicola* with 3 spp. or *H. balansae* and *E. rhodotricha* with 2 spp. (Fig. 4, Table S1). *Apis mellifera*, the second most frequent floral visitor in *E. rhodotricha*, also visited *P. saxicola*.

Floral visitors sought flowers to collect pollen and/or nectar, to eat floral parts (florivory), to copulate (*Cyclocephala* sp. and *Iphimeis* sp. beetles) or, in the case of the spider, probably to prey on other visitors (Fig. 4). Pollen was collected by four species of bee in the flowers of *E. rhodotricha* and/or *P. saxicola* (Table S1). Bees collected pollen (i) by vibration (Buchmann 1983) (*Xylocopa* sp.), remaining a few seconds on the flower, or by (ii) picking up a 'cluster' of stamens, often with the mouth parts (*M.* cf. *rufiventris*), and then collecting pollen with the first two pairs of legs, generally repeating this behaviour for all stamens, except for *T.* cf. *fuscipennis*, which collected pollen only on the stamens most external to the throat of the floral tube, remaining on average 40 s on each flower. In both cases, the bees, except *T.* cf. *fuscipennis*, contacted the stigmatic flower lobes and generally visited all the flowers of the plant, as well as nearby plants.

Nectar was collected by *Manduca rustica*, *Augochoropsis* sp. and by the species of Hemiptera (Table S1). To collect nectar

**Table 4.** Results of treatments to test the reproductive system of *E. rhodotricha*, *H. balansae* and *P. saxicola* (Cactaceae) in a remnant of Chaquenian vegetation, Mato Grosso do Sul, Brazil. n. fruit, number of fruits obtained; n. flower, number of flowers used in each treatment; n. seed, number of seeds; x̄ n. ovules, mean number of ovules that the species presents. ± separates arithmetic mean (left) and SD (right).

| treatment | Echinopsis rhodotricha | | | | Harrisia balansae | | | | Praecereus saxicola | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % (n. fruit/n. flower) | n. seed | fecundity (n. seed/x̄ n. ovule) | seed viability (%) | % (n. fruit/n. flower) | n. seed | fecundity (n. seed/x̄ n. ovule) | Seed viability (%) | % (n. fruit/n. flower) | n. seed | fecundity (n. seed/x̄ n. ovule) | seed viability (%) |
| Self-pollination | | | | | | | | | | | | |
| Spontaneous | 0 (0/20) | – | – | – | 0 (0/7) | – | – | – | 20[a] (2/10) | 794 ± 194[a] | 0.42 ± 0.59[a] | 97.4 ± 0.99[a] |
| Hand | 27.8[a] (5/18) | 272 ± 70[a] | 0.12 ± 0.03[a] | 98.8 ± 0.8[a] | 50[a] (1/2) | 70* | 0.013 | 0 | 7.7[b] (1/13) | 232 | 0.14 | 100 |
| Cross-pollination | | | | | | | | | | | | |
| Geintonogamy | 63.6[b] (7/11) | 308 ± 208[a] | 0.13 ± 0.09[a] | 98.9 ± 0.5[a] | 100[b] (2/2) | – | – | – | 0 (0/6) | – | – | – |
| Xenogamy | 60[b] (6/10) | 515 ± 351[a] | 0.23 ± 0.15[a] | 95 ± 0.5[a] | 66.6[c] (4/6) | 546 ± 42[a] | 0.10 ± 0.007[a] | 99.6 ± 0.53[a] | 66.6[c] (4/6) | 322 ± 211[a] | 0.2 ± 0.13[b] | 99.5 ± 0.5[a] |
| Open pollination | | | | | | | | | | | | |
| Control | 30[a] (6/20) | 427 ± 135[a] | 0.19 ± 0.06[a] | 97.7 ± 1.18[a] | 66.6[c] (6/9) | 695 ± 385[a] | 0.13 ± 0.07[a] | 95.1 ± 4.6[a] | 50[d] (10/20) | 416 ± 203[ab] | 0.26 ± 0.13[ab] | 97.8 ± 2.14[a] |

Values followed by different letters in the same column differ significantly (*P* < 0.05).
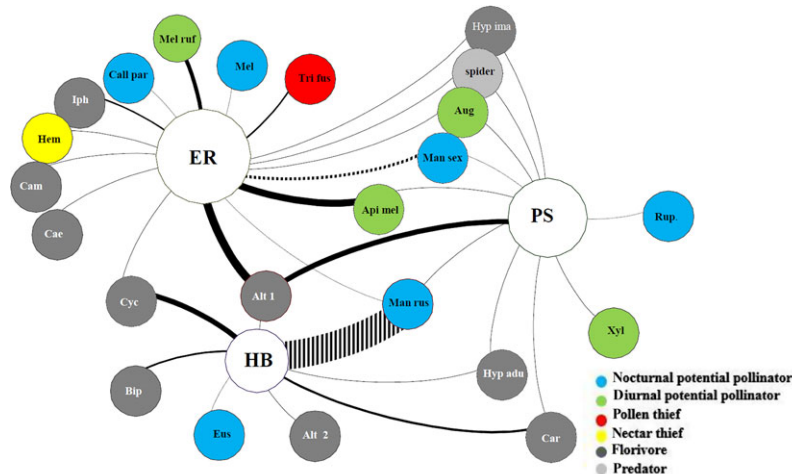
**Fig. 4.** Kamada–Kawai graph showing network of interactions among *Echinopsis rhodotricha*, *Harrisia balansae* and *Praecereus saxicola*, their floral visitors and a species of spider camouflaged among the petals. The animals were categorised according to their use of the flower and/or performance in pollination, taking into account their visit frequency (continuous vertices) or pollen load on the proboscis (dashed vertices). Api mel, *Apis mellifera*; Mel ruf, *Melipona* cf. *rufiventris*; Xyl, *Xylocopa* sp.; Aug, *Augochloropsis* sp.; Man rus, *Manduca rustica*; Man sex, *Manduca sexta*; Cal par, *Callionima* cf. *parce*; Eus, *Euscirrhopterus* sp.; Mel, *Melipotis* sp.; Rup, *Rupela* sp.; Tri fus, *Trigona* cf. *fuscipennis*; Bip, Biphylidae 1; Iph, Iphimeis sp.; Alt 1, *Babiohaltica* sp.; Alt 2, Alticini 2; Cam, *Camptodes vittata*; Car, *Carpophilus* sp.; Cyc, *Cyclocephala* sp.; Hyp adu, *Hyperophora* (adults); Hyp ima, *Hyperophora* (unripe); Cae, Caelifera (nimph); Hem, Hemiptera; Spider, spider.

in the flowers of *P. saxicola*, *M. rustica* approached the flower and, while in flight, inserted its proboscis into the floral tube, contacting anthers and stigmas with this structure. After 1–2 s, they abandoned the flower. The bee *Augochloropsis* sp. entered the floral tube of *E. rhodotricha* to collect nectar, contacting stigmas and anthers. Several nymphs of Hemiptera species pierced the medial portion of the floral tube of a flower of *E. rhodotricha* and sucked the floral nectar through the orifice formed.

Species of beetles, Orthoptera and the bee *T.* cf. *fuscipennis* fed on floral parts, especially the stamens, and eventually the petal edges (*T.* cf. *fuscipennis*, Caelifera nymphs, Alticini beetles). In general, they damaged the flower, acting as florivores, especially beetles that remained on the flower all night, sometimes in large numbers (*Babiohaltica* sp., unripe stage of *Hyperophora*). Also, they often used the flower as a copulation site (*Cyclocephala* sp., *Iphimeis* sp.). Spider species remained camouflaged among petaloid lobes, expecting to prey on other floral visitors.

Twenty-seven specimens of moths (Lepidoptera, Heterocera) were captured with the aid of a light trap. These belong to 23 different species, subdivided into five families: Sphingidae (5 sp., 7 specimens), Saturnidae (6 sp., 7 specimens), Noctuidae (10 sp., 11 specimens), Erebidae (1 sp., 1 specimen) and Crambidae (1 sp., 1 specimen). Among them, three species of Sphingidae (one specimen of *Callionima* cf. *parce*, *Manduca sexta* and *M. rutica*) and another three moth species (one specimen of each) presented pollen of the species studied on the proboscis. All species of Sphingidae presented pollen of *E. rhodotricha*, especially *M. sexta* and *M. rustica*, which also presented pollen of *P. saxicola* (n = 4 grains) and *H. balansae* (n = 259 grains), respectively (Fig. 4, Table S2). No species of Crambidae, Erebidae or Noctuidae was shared among the studied Cactaceae (Fig. 4, Table S2).

## Pollen flow

Using fluorescent dye particles, we were not able to confirm interspecific pollen flow. Specifically, fluorescent dye particles were only recorded on stigmas and/or petals of the same flowers on which they were applied (n = 5/6 flowers in *E. rhodotricha* and n = 7/19 flowers in *P. saxicola*) and once on the stigma of another flower of the same plant in *P. saxicola*. Fluorescent dye particles were not recorded on stigmas or petals of *H. balansae*.

## DISCUSSION

The sum of the flowering and fruiting period of the species studied corresponds to 9 and 8 months, respectively, a fact that guarantees resources for the maintenance of both anthophilous (nectar, pollen, floral parts) and frugivorous fauna for an extended time during the rainy season in the study area. This has been reported for other studies (*e.g.* Talora & Morellato 2000; Souza *et al.* 2016). In this sense, *H. balansae* and *P. saxicola* presented the highest caloric nectar considering the energy/flower value, even though *E. rhodotricha* blooms for a longer time (9 months). In the tropical region, most species with zoochoric dispersal show fruit maturation in the rainy season (Rathcke & Lacey 1985) at a time when a higher number of dispersers are available (Leck 1972; Kimura *et al.* 2001). According to Batalha *et al.* (1997) and Reis-Sinval (2011), yields in the rainy season show better quality (size, weight) and time of viability.

Flowering in the rainy season probably contributed to the positive relationship between this phenophase and the climatic variables tested, although these factors diverged among the studied species, perhaps as a result of the variation in the beginning and end of the flowering period and/or duration of this phenophase between them. According to Rathcke & Lacey

(1985), photoperiod, temperature and humidity are the main abiotic factors associated with the beginning of flowering in tropical regions. In fact, in the study area, the longest, warmest and rainiest days occur in the rainy season (Freitas *et al.* 2013). Fruiting was apparently less influenced by the tested abiotic factors. Rathcke & Lacey (1985) argue that environmental factors rarely stimulate the onset or development of fruit, which are usually controlled by endogenous factors. In *H. portoricensis*, temperature and rainfall showed a generally positive correlation with the production of buds, flowers and fruit (Rojas-Sandoval & Meléndez-Ackerman 2011), diverging from that observed for *M.* cf. *smithiana* in which these reproductive variables were apparently not influenced by abiotic factors (Ruiz *et al.* 2000).

The similarity in flowering pattern between the three species (extended, seasonal, mean dates in December during the rainy season) generated a high flowering overlap between them, thus resulting in interspecific pollen flow. However, our experiment with fluorescence dye particles did not allow us to suggest any flow of pollen among *E. rhodotricha*, *H. balansae* and *P. saxicola*, suggesting the presence of some mechanism among them, possibility associated with flower morphology and functionality, that acts to reduce or evade this flow (see below). On the other hand, we observed a sharing of pollinators, which may favour a heterospecific flow among the three species, as recorded from the Sphingidae specimens collected in a light trap. In addition to carrying pollen on the proboscis, these insects may act as pollinators when they visit Cactaceae flowers, especially other *Echinopsis* species (*e.g. E. ancistrophora*, *E. chiloensis* subsp. *chiloensis* and *E. terscheckii*), which are also pollinated by *Manduca sexta* (Schlumpberger & Raguso 2008; Ortega-Baes *et al.* 2010b; Walter 2010). Floral characteristics mentioned for sphingophilous species, such as white flowers, hypocrateriform floral type and long tube, with mainly nocturnal anthesis and emitting odour (Faegri & van der Pijl 1980; Endress 1994; Proctor *et al.* 1996), were observed in all three species studied. In addition, nectar characteristics, such as volume and solute concentration, were similar to those of other Cactaceae pollinated by sphingids (*e.g. Stenocereus euruca, Cereus peruvianus*; Silva & Sazima 1995; Nassar *et al.* 1997), including species of the studied genera (*e.g. Echinopsis terscheckii, Harrisia portoricensis*; Rojas-Sandoval & Meléndez-Ackerman 2009; Ortega-Baes *et al.* 2010b).

We recorded morphological and/or functional differences that could, in turn, generate differences in the pollination mechanism of the species studied. For example, the inclined position in the plant and the zygomorphy of *H. balansae* flowers is conferred by the grouped arrangement of the stamens (and stigmas) on the lower portion of the floral throat, which are most expanded in this species and may, therefore, allow hawkmoths to enter the throat when visiting the flowers, as observed for *Agrius cingulatus* and *M. rustica* upon visiting flowers of *Cereus peruvianus* and *Hylocereus costaricensis*, respectively (see Fig. 4 in Silva & Sazima 1995; Fig. 4 in Haber & Frankie 1989). In the other species, hawkmoths probably visit in flight, hovering above the flower, as observed for *M. rustica* in *P. saxicola*. This would explain the high pollen load of *H. balansae* on the *Manduca sexta* specimen collected in a light trap relative to the pollen load of *E. rhodothrica* and *P. saxicola* sampled in this and other hawkmoth specimens collected using the same method. It could also reduce the

deposition of heterospecific pollen because of the difference in position of the stigmas. Thus, it can be concluded that the hawkmoths sampled in flowers (*M. rustica*) and/or collected in a light trap were the main pollinators of the studied species. The non-registration or the low frequency of visits to the cactus flowers studied is linked to nocturnal insects, which are typically difficult to observe (Ávila Junior *et al.* 2010), suggesting the need for greater sampling effort with less intrusive technologies, such as filming.

On the other hand, anthesis, which extended beyond the nocturnal period in flowers of *E. rhodotricha* and *P. saxicola*, allowed for the occurrence of diurnal visitors. Among them, a few less effective ones may act as secondary pollinators (*sensu* Wolf & Stiles 1989), as in the case of most bees in the flowers of *E. rhodotricha* (*Melipona* cf. *rufriventris*), *P. saxicola* (*Xylocopa* sp.) or both species (*Apis mellifera, Augochloropsis* sp.). Bees of various genera (*e.g. Bombus, Colletes, Megachile, Xylocopa, Apis mellifera*) and Trochilinae hummingbirds (*Amazilia chionogaster, Chlorostilbon aureoventris*) were considered as secondary pollinators of *Echinopsis chiloensis* spp. chiloensis (Walter 2010) and *E. schickendantzii* (Alonso-Pedano & Ortega-Baes 2012) in semi-arid areas or in Argentinean and Chilean deserts. In this case, we have a well established mixed or generalist pollination system in species of plants with extended anthesis, including cacti (*e.g.* Alonso-Pedano & Ortega-Baes 2012), allowing primary pollination by nocturnal pollinators (*e.g.* sphingids, bats) and secondary pollination by visitors (*e.g.* hummingbirds, bees; Queiroz *et al.* 2016; and references therein). These systems may be beneficial, because they increase the number of pollen vectors throughout the lifespan of the flower (Queiroz *et al.* 2016), as well as act as a 'safety mechanism' to ensure pollinating service in the absence of more 'adapted' nocturnal pollinators (Fleming *et al.* 1996, 2001). Indeed, *E. rhodotricha* and *P. saxicola*, presented a similar pattern of removal/deposition (in flower and/or plant) of fluorescent dye particles that may be related to the occurrence of day visitors in both species. This fact may be especially important for *E. rhodotricha*, which does not produce fruits by spontaneous self-pollination, although it is self-compatible and can 'take advantage' of self-pollination and geitonogamic pollination. According to Baker (1955), self-compatible species can guarantee the production of seeds in the absence of specialised floral visitors. However, experiments would need to be performed to test the efficiency of bees on the reproductive success of these species, for example by checking for fruit and/or seed production (*e.g.* Spears 1983).

Self-compatibility in *E. rhodotricha* was probably favoured by the action of diurnal pollinators. That is, when they visit another flower in the same individual, they end up performing both self-pollination and geitonogamic pollination. This fact, in association with the most effective pollinator deficit, may have contributed to the establishment of self-compatibility in the study population. Similarly, *E. rhodotricha* appears to be the first case of self-compatibility in the genus because, up to now, we have only observed self-incompatibility in *E. chamaecereus*, *E. atacamensis* subsp. *pasacana*, *E. chiloensis* subsp. *chiloensis*, *E. terscheckii*, *E. thelegona* and *E. schickendantzii* (Boyle & Idnurm 2001; Ortega-Baes *et al.* 2010b; Walter 2010; Ossa & Medel 2011; Alonso-Pedano & Ortega-Baes 2012; Ortega-Baes & Gorostiague 2013). In the Brazilian Chaco, six populations of *E. rhodotricha* showed low genetic diversity,

heterozygous deficits and moderate inbreeding (Fava 2015), which may result from crosses within and between related individuals, mainly carried out by secondary pollinators.

*Harrisia balansae*, although self-sterile and self-incompatible, had a higher fruiting rate in natural conditions (control = 66.6%) compared to *E. rhodotricha* (control = 30.0%), which is self-compatible and has nocturnal and diurnal pollination. However, partial self-compatibility diverges from the cactus species (n = 12) of several self-incompatible genera (n = 7) studied in Argentina, Mexico, Venezuela and the United States (see Table 2 in Rojas-Sandoval & Meléndez-Ackerman 2009). Therefore, the fruits/seeds of *H. balansae* obtained in the control treatment probably result from Sphingidae (*e.g. Manduca rustica*) pollination, as recorded for *H. portoricensis* (Rojas-Sandoval & Meléndez-Ackerman 2009). *Praecereus saxicola*, unlike *E. rhodotricha* and *H. balansae*, recorded fruiting by spontaneous self-pollination (20.0%). Fruiting after manual self-pollination was significantly lower than that after any other treatment in *P. saxicola*, suggesting that self-incompatibility is frequent in the studied population. However, spontaneous self-pollination may have contributed to higher fruit yields in *P. saxicola* (control = 50.0%) compared to *E. rhodotricha* (control = 30.0%). The P/O ratio of the three species characterises them as facultative autogams (*sensu* Cruden 1977), even in *H. balansae* whose P/O ratio was significantly lower, but our study points to different breeding systems for such species.

In *H. balansae*, fecundity (seed/ovule) was lower than that recorded for *E. rhodotricha* and *P. saxicola*, probably because of the high number of ovules in the former species. However, the fecundity observed in most of the treatments for the three species (usually <0.5) was low when compared to other species of Cactaceae (*e.g. Pachycereus schottii, Pilosocereus moritzianus, Stenocereus griseus, Subpilocereus repandus, Subpilocereus horrispinus*; Nassar *et al.* 1997; Holland & Chamberlain 2007). Holland & Chamberlain (2007) considered the value of fecundity to be low (0.61) in *Pachycereus schottii*, and argue that the investment in the production of many ovules possibly ensures large seed production if the population presents a low fruiting rate.

Florivores (beetles, crickets) damaged the flowers and stood out in number (58.8%) and frequency of visits. This could compromise the reproductive success of the studied species in several ways, such as reducing the number of stamens (pollen), damaging stigmas and reducing floral attractiveness. Gomes *et al.* (2016) reported that florivory decreased the fruit set in a population of *E. rhodotricha* in the Brazilian Chaco. In other cacti, such as *Ariocarpus fissuratus* and *Opuntia microdasys*, high levels of florivory have limited the amount of fruit and seed (Piña *et al.* 2007; Martínez-Peralta & Mandujano 2011). Schlumpberger *et al.* (2009) argue that *Cyclocephala* beetles greatly damaged the nocturnal flowers of *Echinopsis ancistrophora* that are not 'reinforced' to withstand the destructive behaviour of these insects.

## CONCLUSIONS

Three species have hawkmoths (nocturnal vectors) as primary pollinators (*sensu* Wolf & Stiles 1989), corroborating

the pollination syndrome. However, *E. rhodotricha* and *P. saxicola* present a mixed system of pollination by nocturnal and diurnal (secondary) visitors (bees) because they have extended floral anthesis, unlike *H. balansae*. Sympatry and overlap in flowering favour the sharing of primary and/or secondary pollinators, although we observed differences in certain floral attributes (*e.g.* symmetry, size of structures, arrangement in the plant, floral lifespan and anthesis) mainly between *H. balansae* and *E. rhodotricha–P. saxicola*, which may generate differences in the pollination mode of these species. In addition, they differ in terms of reproductive system. *E. rhodotricha* is self-compatible, *H. balansae* is self-incompatible, and both are self-sterile. *P. saxicola* is pseudo-self-incompatible and partially self-fertile. In view of the above, we conclude that three species of Cactaceae present different reproductive strategies that ensure fruiting and production of viable seeds.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Climate diagram for Porto Murtinho, Mato Grosso do Sul. Data for (a) day length (photoperiod), (b) relative air humidity, (c) distribution of accumulated precipitation (bars) and average temperatures (lines) from December 2014 to November 2015. Source: Center for Monitoring of Weather, Climate and Water Resources of MS (Cemtec).

**Table S1.** Floral visitors of *E. rhodotricha*, *H. balansae* and *P. saxicola* (Cactaceae) in a remnant of Chaquenian vegetation.

**Table S2.** Number of pollen grains of *E. rhodotricha*, *H. balansae* and *P. saxicola* recorded on the proboscis of specimens of butterfly species (Noctuidae, Sphingidae) collected in a light trap in Brazilian Chaquenian vegetation.

# REFERENCES

Alcorn S.M., Mc Gregor S.E., Olin G. (1961) Pollination of saguaro cactus by doves, nectar-feeding bats, and honey bees. *Science*, **133**, 1594–1595.

Alonso-Pedano M., Ortega-Baes P. (2012) Generalized and complementary pollination system in the Andean cactus *Echinopsis schickendantzii*. *Plant Systematics and Evolution*, **298**, 1671–1677.

Alves F.M., Sartori A.L.B. (2009) Caesalpinioideae (Leguminosae) de um remanescente de Chaco em Porto Murtinho, Mato Grosso do Sul, Brasil. *Rodriguésia*, **60**, 531–550.

Arroyo M.T.K. (1976) Geitonogamy in animal pollinated tropical Angiosperms. A stimulus for the evolution of self-incompatibility. *Taxon*, **25**, 543–548.

Ashman A. (2000) Pollinator selectivity and its implications for the evolution of dioecy and sexual dimorphism. *Ecology*, **81**, 2577–2591.

Assis J.G., Pérez-Gacía F., Gonáles-Benito M.E. (2015) Tetrazolium test for seed viability and germinability of *Melocactus ernestii* Vaupel subsp. *Ernestii* and *Melocactus zehntneri* (Britton & Rose) Luetzelb. (Cactaceae). *Gaia Scientia*, **9**, 15–16.

Ávila Junior R.S.D., Cruz-Barros M.A.V.D., Correa Â.M.D.S., Sazima M. (2010) Tipos polínicos encontrados em esfingídeos (Lepidoptera, Sphingidae) em área de Floresta Atlântica do sudeste do Brasil: uso da palinologia no estudo de interações ecológicas. *Brazilian Journal of Botany*, **33**, 415–424.

Baker H.G. (1955) Self-compatibility and establishment after 'long-distance' dispersal. *Evolution*, **9**, 347–349.

Barthlott W., Hunt D.R. (1993) Cactaceae. In: Kubitzki K. (Ed), *The families and genera of vascular plants. II Flowering plants: dicotyledons, magnoliid, hamamelid, and caryophyllid families*. Springer, Berlin, Germany, pp 161–197.

Batagelj V., Mrvar A. (1988) Pajek: program for analysis and visualization of large networks. January 2015. Available from http://90.146.8.18/en/archiv_files/20041/FE_2004_batageljmrvar_en.pdf.

Batalha M.A., Aragaki S., Mantovani W. (1997) Variações fenológicas das espécies do cerrado em Emas (Pirassununga, SP). *Acta Botanica Brasilica*, **11**, 61–78.

Boyle T.H., Idnurm A. (2001) Physiology and genetics of self-incompatibility in *Echinopsis chamaecereus* (Cactaceae). *Sexual Plant Reproduction*, **13**, 323–327.

Browers J.E. (2002) Flowering patterns and reproductive ecology of *Mammillaria grahamii* (Cactaceae), a common, small cactus in the Sonoran desert. *Madroño*, **49**, 201–206.

Buchmann S.L. (1983) Buzz pollination in angiosperms. In: Jones C. E., Little R. J. (Eds), *Handbook of experimental pollination biology*. Van Nostrand Rheinhold, New York, USA, pp 73–113.

Bustamante E., Búrquez A. (2008) Effects of plant size and weather on the flowering phenology of the Organ Pipe Cactus (*Stenocereus thurberi*). *Annals of Botany*, **102**, 1019–1030.

Colaço M.A.S., Fonseca R.B.S., Lambert S.M., Costa C.B.N., Machado C.G., Borba E.L. (2006) Biologia reprodutiva de *Melocactus glaucescens* Buining & Brederoo e *M. paucispinus* G. Heimen & R. Paul (Cactaceae), na Chapada Diamantina, Nordeste do Brasil. *Revista Brasileira de Botânica*, **29**, 239–249.

Cruden R.W. (1977) Pollen–ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution*, **31**, 32–46.

Cruz P.E., Pavón N.P. (2013) Reproductive phenology of *Isolatocereus dumortieri* (Cactaceae) in semiarid scrub in central Mexico: effect of rain during the dry season. *Journal of Arid Environments*, **92**, 53–58.

Cuéllar S.R.L. (2000) Fenología de plantas frutales importantes em la dieta de vertebrados frugívoros em el Chaco Boliviano. Manejo de fauna silvestre em Amazonía y Latinoamérica, 47–56.

Dafni A. (1992) *Pollination ecology: a practical approach*. Oxford University Press, Oxford, UK, 250 pp.

Dar S., del Coro Arizmendi M., Valiente-Banuet A. (2006) Diurnal and nocturnal pollination of *Marginatocereus marginatus* (Pachycereeae: Cactaceae) in Central Mexico. *Annals of Botany*, **97**, 423–427.

Endress P.K. (1994) *Diversity and evolutionary biology of tropical flowers*. Cambridge Univesity Press, Cambridge, UK, 511 pp.

Faegri K., van der Pijl L. (1980) *The principles of pollination ecology*. Pergamon Press, Oxford, UK, 239 pp.

Fava W.S. (2015) Ecologia molecular de Leguminosae e Cactaceae no Cerrado e no Chaco: Fenologia, filogeografia e genética de populações. Thesis, Universidade Federal de Mato Grosso do Sul, Brasil.

Fenster C.B., Dudash M.R., Hassler C.L. (1996) Fluorescent dye particles are good pollen analogs for hummingbird-pollinated *Silene virginica* (Caryophyllaceae). *Canadian Journal of Botany*, **74**, 189–193.

Fleming T.H. (2000) Pollination of cacti in the Sonoran desert. *American Scientist*, **88**, 432–439.

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

Fleming T.H., Sahley C.T., Holland J.N., Nason J.D., Hamrick J.L. (2001) Sonoran Desert columnar cacti and the evolution of generalized pollination systems. *Ecological Monographs*, **71**, 511–530.

Fonseca R.B.S., Funch L.S., Borba E.L. (2008) Reproductive phenology of *Melocactus* (Cactaceae) species from Chapada Diamantina, Bahia, Brazil. *Revista Brasileira de Botânica*, **31**, 237–244.

Franck A.R. (2012) Synopsis of *Harrisia* including a newly described species, several typifications, new synonyms, and a key to species. *Haelsetonia*, **18**, 95–104.

Freitas T.G., Souza C.S., Aoki C., Arakaki L.M.M., Stefanello T.H., Sartori A.L.B., Sigrist M.R. (2013) Flora of Brazilian humid Chaco: composition and reproductive phenology. *Check list*, **9**, 973–979.

Giorgis M.A., Cingolani A.M., Gurvish D.E. (2015) Flowering phenology, fruit set and seed mass and number of five coexisting *Gymnocalycium* (Cactaceae) species from Córdoba mountain, Argentina. *Journal of the Torrey Botanical Society*, **142**, 220–230.

Gomes V.G.N., Koroiva R., Araujo A.C. (2016) Vertebrate florivory on the short-columnar cactus *Echinopsis rhodotricha* K. Schum. in the Brazilian Chaco. *Plant Ecology*, **217**, 1481–1487.

Gotelli N.J, Entsminger G.L. (2000) EcoSiM: null models software for ecology. Version 7 Acquired Intelligence Inc. & Kesey-Bear, Jericho VT05465, USA. Available from http://garyentsminger.com/ecosim/index.htm. (accessed 20 November 2015).

Haber W.A., Frankie G.W. (1989) A tropical moth community: Costa Rican dry forest Sphingidae. *Biotropica*, **21**, 155–172.

Holland J.N., Chamberlain S.A. (2007) Ecological and evolutionary mechanisms for low seed: ovule ratios: need for a pluralistic approach? *Ecology*, **88**, 706–715.

Holland N.J., Fleming T.H. (2002) Co-pollinators and specialization in the pollinating seed-consumer mutualism between senita cacti and senita moths. *Oecologia*, **133**, 534–540.

Johnson J.B., Omland K.S. (2004) Model selection in ecology and evolution. *Trends in Ecology & Evolution*, **19**, 101–108.

Kiesling R. (1975) Los géneros de Cactaceae de Argentina. *Boletín de la Sociedad Argentina de Botánica*, **16**, 197–227.

Kiesling R., Oakley L., Pin A. (2013) *Praecereus saxicola*. The IUCN Red List of Threatened Species 2013: e.T152756A674714. Available from https://doi.org/10.2305/iucn.uk.2013-1.rlts.t152756a674714.en (accessed 22 January 2017).

Kimura K., Yumoto T., Kikuzawa K. (2001) Fruiting phenology of fleshy-fruited plants and seasonal dynamics of frugivorous birds in four vegetation zones on Mt. Kinabalu, Borneo. *Journal of Tropical Ecology*, **17**, 833–858.

Lammi J. (1996–2015) Online-photoperiod calculator V 1.94 L: computation of day lengths, sunrise/sunset times, twilight and local noon. Available from http://www.sci.fi/~benefon/sol.html (accessed 20 November 2015).

Lange R.S., Scobell S.A., Scott P.E. (2000) Hummingbird-syndrome traits, breeding system, and pollinator effectiveness in two syntopic Penstemon species. *International Journal of Plant Sciences*, **161**, 253–263.

Leck C.F. (1972) Seasonal changes in feeding pressures of fruit- and nectar-eating birds in Panama. *The Condor*, **74**, 54–60.

Legendre P., Legendre L. (1998) *Numerical ecology*, 2nd edition. Elsevier Science, Amsterdam, the Netherlands, 853 pp.

Martínez-Peralta C., Mandujano M.C. (2011) Reproductive ecology of the endangered living rock cactus, *Ariocarpus fissuratus* (Cactaceae). *Journal of the Torrey Botanical Society*, **138**, 145–155.

McFarland J.D., Kevan P.G., Lane M.A. (1989) Pollination biology of *Opuntia imbricata* (Cactaceae) in southern Colorado. *Canadian Journal of Botany*, **67**, 24–28.

McIntosh M.E. (2002) Flowering phenology and reproductive output in two sister species of *Ferocactus* (Cactaceae). *Plant Ecology*, **159**, 1–13.

Molina-Freaner F., Rojas-Martínez A., Fleming T.H., Valiente-Banuet A. (2004) Pollination biology of the columnar cactus *Pachycereus pecten-aboriginum* in north-western México. *Journal of Arid Environments*, **56**, 117–127.

Moore J.A. (2011) Notes on the biology of the fragrant prickly apple cactus *Harrisia fragrans*. *The Palmetto*, **28**, 1–7.

Nadia T.L., Machado I.C., Lopes A.V. (2007) Partilha de polinizadores e sucesso reprodutivo de *Spondias tuberosa* e *Ziziplus joazeiro*, espécies endêmicas da Caatinga. *Revista brasileira de biociências*, **5**, 357–359.

Nassar J., Ramirez N., Linares O. (1997) Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual

reproduction. *American Journal of Botany*, **84**, 918–927.

Newstrom L.E., Frankie G.M., Baker H.G. (1994) A new classification for plant phenology based on flowering patterns in lowland tropical rain forest trees at La Selva, Costa Rica. *Biotropica*, **2**, 141–159.

Oakley L., Kiesling R. (2013) *Harrisia balansae*. The IUCN Red List of Threatened Species 2013: e.T152667A663592. Available from https://doi.org/10.2305/iucn.uk.2013-1.rlts.t152667a663592.en (accessed 22 January 2017).

Oakley L., Duarte W., Pin A. (2013) *Echinopsis rhodotricha*. The IUCN Red List of Threatened Species 2013: e.T152091A596143. Available from https://doi.org/10.2305/iucn.uk.2013-1.rlts.t152091a596143.en (accessed 22 January 2017).

Oksanen J., Blanchet F.G., Kindt R., O'hara R.R., Simpson G.L., Solymos P., Henry M., Stevens H., Wagner H. (2010) Vegan: Community Ecology Package. R package version 1.17-0. Disponível em. Available from http://CRAN.R-project.org/package= vegan (accessed 20 November 2015).

Ortega-Baes P., Gorostiague P. (2013) Extremely reduced sexual reproduction in the clonal cactus *Echinopsis thelegona*. *Plant Systematics and Evolution*, **299**, 785–791.

Ortega-Baes P., Aparicio-González A., Galíndez G., Fueyo P., Sühring S., Rojas-Aréchiga M. (2010a) Are cactus growth forms related to germination responses to light? A test using *Echinopsis* species. *Acta Oecologica*, **36**, 339–342.

Ortega-Baes P., Saravia M., Sühring S., Godínez-Alvarez H., Zamar M. (2010b) Reproductive biology of *Echinopsis terscheckii* (Cactaceae): the role of nocturnal and diurnal pollinators. *Plant Biology*, **13**, 33–40.

Osborn M.M., Kevan P.G., Lane M.A. (1986) Pollination biology of *Opuntia polyacantha* and *Opuntia phaeacantha* (Cactaceae) in southern Colorado. *Plant Systematic and Evolution*, **159**, 85–94.

Ossa C.G., Medel R. (2011) Notes on the floral biology and pollination syndrome of *Echinopsis chiloensis* (Colla) Friedrich & GD Rowley (Cactaceae) in a population of semiarid Chile. *Gayana Botanica*, **68**, 213–219.

Ottra J.H.L.E., Pirani J.R., Pansarim E.R. (2016) Floral biology and pollination of two sympatric species of Galipeinae (Galipeeae, Rutaceae) endemic to the Brazilian Atlantic Forest. *Flora*, **221**, 107–116.

Pennington R.T., Prado D.E., Pendry C.A. (2000) Neotropical seasonally dry forests and quaternary vegetation changes. *Journal of Biogeography*, **27**, 26–273.

Petit S. (2001) The reproductive phenology of three sympatric species of columnar cacti on Curaçao. *Journal of Arid Environments*, **49**, 521–531.

Petit S., Freeman C.E. (1997) Nectar production of two sympatric species of columnar cacti. *Biotropica*, **29**, 175–183.

Pianka E.R. (1973) The structure of lizard communities. *Annual Review of Ecology and Systematics*, **4**, 53–74.

Piña H.H., Montana C., del Carmen Mandujano M. (2007) Fruit abortion in the Chihuahuan-Desert endemic cactus *Opuntia microdasys*. *Plant Ecology*, **193**, 305–313.

Proctor M., Yeo P., Lack A. (1996) *The natural history of pollination*. Harper Collins, London, UK, 479 pp.

Queiroz J.A., Quirino Z.G.M., Machado I.C.S. (2015) Floral traits driving reproductive isolation of two co-flowering taxa that share vertebrate pollinators. *AoB Plants*, **7**, 1–13.

Queiroz J.A., Quirino Z.G.M., Lopes A.V., Machado I.C. (2016) Vertebrate mixed pollination system in *Encholirium spectabile*: a bromeliad pollinated by bats, opossum and hummingbirds in a tropical dry forest. *Journal of Arid Environments*, **125**, 21–30.

R Development Core Team (2012) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org (accessed 20 November 2015).

Rathcke B., Lacey E.P. (1985) Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics*, **16**, 179–214.

Rego J.O., Franceschinelli E.V., Zappi D.C. (2012) Reproductive biology of a highly endemic species: *Cipocereus laniflorus* NP Taylor & Zappi (Cactaceae). *Acta Botanica Brasilica*, **26**, 243–250.

Reis-Sinval F.O. (2011) Fenologia reprodutiva de pitaia vermelha no município de Lavras, MG. *Ciência Rural*, **41**, 984–987.

Rivera-Marchand B., Ackerman J.D. (2006) Bat pollination breakdown in the Caribbean columnar cactus *Pilosocereus royenii*. *Biotropica*, **38**, 635–642.

Rojas-Sandoval J., Meléndez-Ackerman E. (2009) Pollination biology of *Harrisia portoricensis* (Cactaceae), an endangered Caribbean species. *American Journal of Botany*, **96**, 2270–2278.

Rojas-Sandoval J., Meléndez-Ackerman E. (2011) Reproductive phenology of the Caribbean cactus *Harrisia portoricensis*: rainfall and temperature associations. *Botany-Botanique*, **89**, 861–871.

Rosas-Guerrero V., Aguilar R., Martén-Rodríguez S., Ashworth L., Lopezaraiza-Mikel M., Batisda J.M., Quesada M. (2014) A quantitative review of pollination syndromes: do floral traits predict effective pollinators? *Ecology Letters*, **17**, 388–400.

Ruiz A., Santos M., Cavelier J., Soriano P.J. (2000) Estúdio fenológico de Cactáceas em el Enclave Seco de la Tatacoa, Colombia. *Biotropica*, **32**, 397–407.

Schlumpberger B.O., Raguso R.A. (2008) Geographic variation in floral scent of *Echinopsis ancistrophora* (Cactaceae): evidence for constraints on hawkmoth attraction. *Oikos*, **117**, 801–814.

Schlumpberger B.O., Susanne S.R. (2012) Molecular phylogenetics of *Echinopsis* (Cactaceae): polyphyly at all levels and convergent evolution of pollination modes and growth forms. *American Journal of Botany*, **99**, 1–15.

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

Scogin R. (1985) Nectar constituents of the Cactaceae. *Southwestern Naturalist*, **30**, 77–82.

Silva W.R., Sazima M. (1995) Hawkmoth pollination in *Cereus peruvianus*, a columnar cactus from southeastern Brazil. *Flora*, **190**, 339–343.

Souza C.S., Aoki C., Alcantara D.M.C., Laroca S., Sazima M., Pott A., Sigrist M.R. (2016) Diurnal anthophilous fauna in Brazilian Chaco vegetation: phenology and interaction with flora. *Brazilian Journal of Botany*, **4**, 1–11.

Spears E.E. (1983) A direct measure of pollinator effectiveness. *Oecologia*, **57**, 196–199.

Talora D.C., Morellato P.C. (2000) Fenologia de espécies arbóreas em floresta de planície litorânea do sudeste do Brasil. *Revista brasileira de Botânica*, **23**, 13–26.

Valiente-Banuet A., Arizmendi M.D.C., Rojas-Martínez A., Domínguez-Canseco L. (1996) Ecological relationships between columnar cacti and nectar-feeding bats in Mexico. *Journal of Tropical Ecology*, **12**, 103–119.

Valiente-Banuet A., Rojas-Martinez A., Arizmendi M.D.C., Davila P. (1997) Pollination biology of two columnar cacti (*Neobuxbaumia mezcalaensis* and *Neobuxbaumia macrocephala*) in the Tehuacan Valley, central Mexico. *American Journal of Botany*, **84**, 452–452.

Veloso H.P., Oliveira-Filho L.D., Vaz A.M.S.F., Lima M.P.M., Maquete R., Brazão J.E.M. (1992) *Hand técnico da vegetação brasileira*. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro, Brasil, 544 pp.

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

Waser N.M. (1978) Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology*, **59**, 934–944.

Waser N.M., Chittka L., Price M.V., Williams N.M., Ollerton J. (1996) Generalization in pollination systems, and why it matters. *Ecology*, **77**, 1043–1060.

Weiss J., Nerd A., Mizrahi Y. (1994) Flowering behavior and pollination requirements in climbing cacti with fruit crop potential. *HortScience*, **29**, 1487–1492.

Werneck F.P. (2011) The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. *Quaternary Science Reviews*, **30**, 1630–1648.

Wolf L.L., Stiles F.G. (1989) Adaptations for the 'failsafe' pollination of specialized ornithophilous flowers. *The American Midland Naturalist*, **121**, 1–10.

Wolowski M., Freitas L. (2010) Sistema reprodutivo e polinização de *Senna multijuga* (Fabaceae) em Mata Atlântica Montana. *Rodriguésia*, **61**, 167–179.

Zar J.H. (2010) *Biostatistical analysis*, 5th edition. Prentice-Hall, Upper Saddle River, NJ, USA, 944 pp.