FROM DUSK TILL DAWN: NOCTURNAL AND DIURNAL POLLINATION IN THE EPIPHYTE *Tillandsia heterophylla* (BROMELIACEAE)

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Abstract:

In order to compare the effectiveness of diurnal and nocturnal pollinators, we studied the reproductive biology and pollinators of *Tillandsia heterophylla* E. Morren, an epiphytic tank bromeliad endemic to southeastern Mexico. Since anthesis in *T. heterophylla* is predominantly nocturnal but lasts until the following day, we hypothesized that this bromeliad would receive visits from both diurnal and nocturnal visitors, but that the nocturnal visitors would be the most effective pollinators, since they arrive first to the receptive flower, and that bats would be the most frequent nocturnal visitors, given the characteristics of the nectar. Flowering of *T. heterophylla* began in May and lasted until July. The species is fully self-compatible with an anthesis that lasts for approximately 15-16 h. Mean volume of nectar produced per flower was 82.21 µl with a mean sugar concentration of 6.33%. The highest volume and concentration of nectar were found at 20:00 h, with a subsequent decline in both cases to almost zero over the following 12 h period. *Tillandsia heterophylla* has a generalist pollination system, since at least four different morphospecies of visitors pollinate its flowers: bats, moths, hummingbirds and bees. Most of the pollinating visits corresponded to bats and took place in the early evening, when stigma receptivity had already begun; making bats the probable pollinator on most occasions. However, diurnal pollinators may be important as a "fail-safe" system by which to guarantee the pollination of *T. heterophylla*.

Key words: bees, chiropterophily, mixed-pollination system, humid montane forest, Mexico, ornithophily, pollinator effectiveness.

INTRODUCTION

Pollination syndromes are a set of floral characteristics shared between unrelated plant taxa that attract different floral visitors in order to utilize them as pollen vectors. These characteristics include flower morphology (size, shape, color and marks on the perianth), floral rewards (nectar, pollen, oils, resins), and floral phenology (Fenster *et al*. 2004; Willmer 2011). These floral traits presumably exploit the sensorial mechanisms, nutritional requirements, morphology and behavior (Mayfield *et al*. 2001; Fumero-Cabán & Meléndez-Ackerman 2007) of the "most effective pollinator" (*sensu* Stebbins 1970) and may shape the plant-pollinator interaction. However, other studies of pollination systems show that generalization seems to be more common than specialization (Waser *et al.* 1996 and references therein). Moreover, the pollination syndrome hypothesis may not describe the entire diversity of floral traits and spectrum of pollinators (Ollerton *et al.* 2009; Avila Jr. & Freitas 2011). In contrast, recent literature (e.g., Murúa & Espíndola 2014) supports the pollination syndrome concept, especially in tropical plants (Rosas-Guerrero *et al.* 2014 and references therein). Nevertheless, in order to determine the pollination effectiveness of a visitor, it is necessary to consider other floral traits, such as anthesis duration, pollen release timing, scent production pattern, chemical components and nectar production patterns, in relation to the morphology and behavior of the floral visitors (Willmer 2011; Amorim *et al.* 2013).

In plants with extended anthesis (24 hours or more), it is common to observe diurnal and nocturnal flower visitors. As pointed out by many authors (e.g., Herrera 1990; Groman & Pellmyr 1999; Massinga *et al.* 2005; Rymer *et al.* 2005; Ne'eman *et al.* 2010; Amorim *et al.* 2013), in these cases it is important to consider the effectiveness of the different groups of visitors based not only on their relative visitation frequency, but also on the results of exclusion experiments that aim to quantify the relative contribution of each pollinator group to overall plant reproduction success. Moreover, many studies have found that the pollination effectiveness of diurnal or nocturnal visitors is of either equal (e.g., Young 2002; Wolff *et al.* 2003; Dar *et al.* 2006; Walter 2010) or complementary importance in different aspects of pollination (quality, quantity or pollen transfer distance; e.g., Miyake & Yahara 1998, 1999; Giménez-Benavides *et al.* 2007; Amorim *et al.* 2013), indicating the presence of a complementary or mixed pollination system, in which pollination is performed by two groups of floral visitors (Jennersten & Morse 1991; Sazima *et al*. 1994; Maruyama *et al*. 2010; Walter 2010).

Among the angiosperms, Bromeliaceae is a species-rich Neotropical family, comprising approximately 3160 species from 50 genera (Zotz 2013), in which pollination by vertebrates predominates over that provided by insects and most of the species are pollinated by hummingbirds (Kessler & Krömer 2000; Canela & Sazima 2005; Krömer *et al*. 2006). There is even one example of a bimodal pollination system in the hummingbird/bee-pollinated *Aechmea*

nudicaulis Griseb (Schmid *et al.* 2011). Genera of the subfamily Tillandsioideae, such as *Guzmania, Vriesea*, and *Tillandsia*, have evolved floral characteristics that attract a wide range of pollinators, including insects, birds and even bats (Benzing 2000; Aguilar-Rodríguez *et al.* 2014).

Tillandsia heterophylla E. Morren is an epiphytic tank bromeliad, endemic to southeastern Mexico (Espejo-Serna *et al.* 2005). Gardner (1986) inferred that *T. heterophylla* was pollinated by moths due to the nocturnal anthesis, tubular corolla, whitish petals, and "sweet fragrance" presented by the species. Later, Hietz & Hietz-Seifert (1994) also suggested moth-pollination for this species, based on floral traits; however, these suppositions were never confirmed. The discovery of dilute, hexose-rich nectar in *T. heterophylla* led Krömer *et al.* (2008) to propose bat-pollination for this species. This represents a rare case in the *Tillandsia* genus, with only *T. macropetala* Wawra (a species sympatric to *T. heterophylla*) recently reported as the sole example of chiropterophily within the genus (Aguilar-Rodríguez *et al.* 2014). In common with other bat pollinated bromeliads, such as *T. macropetala*, *Werauhia gladioliflora* (H. Wendland) J.R. Grant, *Encholirium vogelii* Rauh, *E. glaziovii* Mez,, and some Brazilian species of *Vriesea* (Sazima *et al.* 1989, 1995, 1999; Cascante-Marín *et al.* 2005; Tschapka & von Helversen 2007; Christianini *et al.* 2013; Aguilar-Rodríguez *et al.* 2014), anthesis in *T. heterophylla* is predominantly nocturnal, but the flowers remain open until the following day thus enabling potential visits from both nocturnal and diurnal animals (Miyake & Yahara 1998, 1999; Wolff *et al.* 2003).

To date, only few studies (Christianini *et al.* 2013; Aguilar-Rodríguez *et al.* 2014) have assessed the effectiveness of diurnal and nocturnal pollination in Bromeliaceae, a family with important functions in tropical forest ecosystems (e.g., roles in forest succession, water and mineral cycling, and as a refuge for fauna) and in the maintenance of canopy diversity (Benzing 2000). In this study, we characterize the reproductive biology and pollinators of *T. heterophylla* in the humid montane forest of central Veracruz, Mexico. Specifically, we determine the floral phenology, reproductive system and the quantity and timing of floral reward (nectar), as well as the identity of the flower visitors and their relative effectiveness as pollinators (determined by fruit set, seed set, visitation frequency and behavior during the visit). Since anthesis in *T. heterophylla* is predominantly nocturnal but lasts until the following day, our hypothesis was that this bromeliad would receive visits from both diurnal and nocturnal visitors. We also hypothesized that nocturnal visitors would be more effective pollinators, since they arrive first to the receptive flower, and that bats would be the most frequent nocturnal visitors, given the characteristics of the nectar (Krömer *et al.* 2008).

MATERIALS AND METHODS

Study site and species characterization

This study was conducted between May and July of 2011 in the municipality of Tlalnelhuayocan, located in the central region of Veracruz State, Mexico. The site has an

elevation of 1500 to 1700 m a.s.l. (Mehltreter *et al.* 2005), an annual precipitation of 1650 mm and an average temperature of 14°C (Williams-Linera 2002). The predominant vegetation is humid montane forest (Williams-Linera 2007); however, most of the landscape has been highly fragmented by human activities (Cruz-Angón *et al.* 2010; Toledo-Aceves *et al.* 2011). Fieldwork was conducted in a *Cedrela odorata* (Meliaceae) and *Quercus* spp. (Fagaceae) plantation of *ca.* 5.3 ha, surrounded by patches of secondary forest (19° 31´ 53.3" N, 96° 58´ 40.7" W) with naturally occurring individuals of *T. heterophylla* growing on the tree trunks at a height of 1.5-2 m from ground level.

Tillandsia heterophylla has a stemless rosette of around 50 cm in diameter, a terminal inflorescence of approximately 1.5 m in height and about one to six spikes, each of which bears eight to 16 flowers (Fig. 1A). The flowers are actinomorphic, with a bell-shaped, slightly tubular corolla. The petals (*ca.* 10 cm in length) are white (Fig. 1B) and the stamens (*ca.* 7 cm) are approximately the same length as the style (Espejo-Serna *et al.* 2005). This species is endemic to the states of Chiapas, Hidalgo, Puebla and Veracruz (Espejo-Serna *et al.* 2005; Martínez-Meléndez *et al.* 2009; Ceja-Romero *et al.* 2010) and occurs at elevations of between 600-1700 m a.s.l. in dry deciduous forests, but mainly in humid montane forests. It is also commonly found in secondary forest, live fences, urban parks and agroforestry systems such as coffee plantations.

Floral phenology and breeding system

Phenology was assessed by examining the inflorescences of 16 plants, recording the number of flowers per spike and per day. Floral anthesis was observed in flowers marked on the bract to enable continued monitoring of the development of individual flowers until fruiting. Floral condition was inspected every day for three weeks, recording flower growth, initiation of anthesis, opening of the corolla and withering (Cascante-Marín *et al.* 2005; Martén-Rodríguez & Fenster 2008). Stigmatic receptivity was determined by direct observation of the presence of mucilage on the stigma surface in seven flowers (Escobedo 2007). In order to confirm their receptivity, the stigmas of another six flowers were dipped in an aqueous solution of hydrogen peroxide (Kearns & Inouye 1993) at 1, 6 and 12 hours following the initiation of anthesis (Martén-Rodríguez & Fenster 2008).

To determine the breeding system of *T. heterophylla*, we carried out standard manual pollination treatments on the inflorescences of 10 individuals placed in pots with gravel in a structure covered with mosquito netting to prevent visits to the flowers (hereafter referred to as the "planthouse"). The planthouse was located *ca.* 200 m from the study site, and provided shade and temperatures similar to that found in the plantation. Irrigation was carried out sporadically. In the planthouse, the virgin flowers were subjected to four treatments at no more than two hours after initiation of anthesis (for details see Aguilar-Rodríguez *et al.* 2014): a) Emasculation (Apomixis) (*n* = 16 flowers); b) Spontaneous self-pollination (*n* = 18 flowers); c) Cross-pollination (Xenogamy) (*n* = 17 flowers); and d) Self-pollination (Autogamy) (*n* = 14 flowers). The presence of fruits was recorded and quantified eight weeks after applying the treatments.

Percentage of fructification (fruit set) was calculated for each treatment and, once the fruits were ripe, the number of seeds per fruit (seed set) was recorded. We subsequently calculated the self-compatibility (SC) and the autogamy (AI) indices (Zapata & Arroyo 1978; Wendt *et al.* 2001; Kamke *et al*. 2011) in order to quantify the self-compatibility of *T. heterophylla*; AI was multiplied by 100 in order to express this value as a percentage (Martén-Rodríguez & Fenster 2008).

Nectar secretion pattern

Nectar volume and concentration were measured in 20 flowers chosen randomly from the 10 individuals at the planthouse (two flowers per plant). No pollination treatment was applied to these flowers and all the measurements were taken on nights without rain. Nectar volume production was recorded by extracting all the accumulated nectar in each flower every 2 hours using 80 µl and 10 µl capillary tubes. This continued until no more nectar could be removed. Total nectar volume per flower was calculated as the sum of the hourly values (Tschapka & von Helversen 2007). The percentage of sugars contained in each nectar sample was measured using a field refractometer (Mod. HRT32, range: 0-32%, weight/weight, precision: 0.2%; A. Krüss Optronic, Germany).

Record of floral visitors

For the purposes of this study, we considered any animal that made contact with the corolla of the flower to be a floral visitor (*sensu* Schmid *et al*. 2011*b*). In order to be considered as a legitimate pollinator of *T. heterophylla*, the visitor had to enter the corolla (Muchhala 2006) and come into contact with the reproductive organs of the flower (either stamina or stigma, or both). If a visitor moved the whole inflorescence when placing its head inside the flower, it was considered that contact had been made with the reproductive organs of the flower (Slauson, 2000). Recordings of the floral visitors were made between March and April 2011.

For the recording of nocturnal visitors, we used a video camera (DCR-SR65 Sony Corporation, Japan) in night vision mode, equipped with an infrared light (HVL-HILR Sony Corporation, Japan). The camera was placed on a tripod 1.3-1.5 m from the ground and at a distance of 1.5 m from the flower. Nocturnal recording took place from 19:00 h to 23:30 h or 00:00 h, as dictated by a combination of equipment capacity and environmental conditions. Diurnal direct observation of floral visitors took place over two periods during the day; in the early morning (06:00 to11:00 h) and during the late afternoon (17:00 to 19:00 h) at a distance of around 3 m from the flowers. In addition, we checked for arthropods in individual flowers of *T. heterophylla* in all the observed plants for 10 minutes every hour. We recorded visitor species (or morphospecies), time of visit, number of flowers visited, and the reward sought by the visitor. The video recordings were analyzed using the program Final Cut Pro 7 (Apple Inc. 2009) at a speed of 3 fps (10% of real time).

In addition, we carried out nocturnal bat sampling using two mist-nets (6 x 12 m) for a period of four nights. The nets were placed at least 1 m from a different *T. heterophylla* individual in flower per night, and checked every 30 min. Nets were opened at dusk (19:00 h) and closed after 6 hours. Captured species were identified using field guides (Reid 2009) and following the taxonomy of Simmons (2005) and Velazco and Patterson (2013).

Effectiveness of nocturnal *vs.* diurnal visitors

In order to assess the pollination effectiveness of nocturnal and diurnal floral visitors, we conducted exclusion experiments in 16 individual plants between March and April 2011.

These treatments were conducted as follows (see Aguilar-Rodríguez *et al.* 2014 for details): a) Diurnal exposure (DE) (n = 46); b) Nocturnal exposure (NE) (n = 40); c) Emasculated diurnal exposure (EDE); (n = 26); d) Emasculated nocturnal exposure (ENE) (n = 24); and e) Control (n = 41). To determine the effectiveness of the visitors, as well as the fruit set (as a percentage) and seed set produced by these treatments (eight weeks after treatments), we considered the frequency of visits (in percentage of the total number of visits), number of legitimate visits, and the behavior of each visitor during its visit to the flower (Montalvo & Ackerman 1986).

Statistical analysis

A Chi-square test was used to compare percentages of fructification in all pollination treatments, including those conducted to assess the breeding system and pollination effectiveness. Analysis of variance was performed to measure the influence of pollination treatments on seed set. As the data were not normally distributed (Kolmogorov-Smirnov test), a non-parametric Kruskal-Wallis test with Tukey type comparisons was used. A Pearson correlation was conducted to determine the relationship between nectar volume and concentration of sugars and the period during which the measurements were taken. All analysis was carried out using the program Statistica (ver. 7, StatSoft Inc. 2004) with a level of significance of $P \leq 0.05$.

RESULTS

Floral phenology and breeding system

Flowering in *T. heterophylla* began during the second week in May and lasted until the second week in July. The flower began lengthening at 11:00 h while anthesis began around 18:30 h (range: 17:00 to 20:30 h, n = 120 flowers). Anthesis lasted for *ca.* 15-16 h (n = 132 flowers), coinciding with the stigmatic receptivity (*ca.* 15 h, n = 13 flowers; Fig. 2). At the beginning of anthesis, pollen was already present on the anthers and, in an untouched flower, pollen on the anthers lasted until 06:00 h the following day (Fig. 2). Each individual *T. heterophylla* produces 0.4 ± 0.2 flowers per day (mean ± SD, range 0 to 3 flowers, n = 298 flowers in 27 individuals), over a period of 17 days (17.13 ± 9.51 days, n = 16 inflorescences).

The pollination treatments carried out in the planthouse produced a fruit set of around 60%, with a difference of only 5.36% found between the treatment with the highest (Emasculation) and lowest fruit set (Self-pollination) (Table 1). Seed set did not differ significantly between treatments in the planthouse (H=0.198, df=3, P>0.05; $n = 65$ fruits from 10 individuals; Table 1). According to the SC and AI indexes, *T. heterophylla* is a fully self-compatible species (ISI=0.95, or 95%, IAS=1.17).

Nectar secretion pattern

Mean volume of nectar in the flowers of *T. heterophylla* was 82.21 µl ($\pm$ 48.13, $n = 20$ flowers from 10 individuals) per plant per night, while mean sugar concentration was 6.33% ($\pm$ 4.90%; range 0 to 19%, $n = 20$ flowers from 10 individuals). Nectar production started at the beginning of anthesis and lasted for 12 h, at a rate of 7 µl/h (Fig. 2). The highest nectar volume (50.38 $\pm$ 35.32 µl) and concentration (13.23% $\pm$ 3.12%) was found at 20:00 h and had reduced in both cases to nearly zero 12 h later (0.13 $\pm$ 0.32 µl and 0.19% $\pm$ 0.75 respectively). The Pearson correlation showed that a significant negative relationship existed between the time at which nectar was extracted (conducted at intervals of 2 h) and the volume produced (r = -0.60, P<0.05), as well as between the time and the concentration of dissolved sugars (r = -0.82, P<0.05).

Record of floral visitors

Over a total of 167 hours of observation (62 nocturnal and 105 diurnal) of 301 flowers of *T. heterophylla* (235 nocturnal, 66 diurnal), we recorded 99 visits (40 nocturnal, and 59 diurnal) from eight different flower visitors (Table 3).

Nocturnal visitors: We recorded 40 nocturnal visits; nine from noctuid moths (Noctuidae) and 31 from bats (Fig. 1C-D). Most of the moth visits were observed between 20:00 to 23:30 h. During these visits, the moths landed on the corolla and, on seven occasions, they entered into the corolla and remained there for 91 $\pm$ 99 s ($n = 7$ visits) before leaving (Fig. 1C). We consider that, on those seven visits, the moth made contact with the anthers and stigma simultaneously.

We captured bats on two non-consecutive nights, recording nine individuals from four species: *Carollia sowelli* Baker, Solari y Hoffmann, *Desmodus rotundus* Geoffroy, *Sturnira parvidens* Geoffroy and *Sturnira hondurensis* Anthony. None of these species carried pollen of *T. heterophylla*; however, during the video recordings, we recorded a medium-sized tail-less bat with a longer snout than that of the specimens captured, indicating a nectarivorous habit (Fig 1D). In a study realized two months earlier at the same site (Aguilar-Rodríguez *et al.* 2014), we captured specimens of the nectarivorous bat *Anoura geoffroyi* Gray (Phyllostomidae: Glossophaginae), a species that coincides with the traits observed in the bats recorded visiting *T. heterophylla*. Another nectarivorous bat species reported in the zone, *Glossophaga soricina* Pallas (Sosa *et al.* 2008), has a conspicuous uropatagium and tail, unlike *A. geoffroyi*. We therefore consider that *A. geoffroyi* performed 31 visits to *T. heterophylla* flowers, beginning one

hour after sunset and until 00:00 h, with the majority of visits taking place between 20:00 h and 21:30 h. The recorded bats always inserted their entire head into the corolla, moving the whole inflorescence, thus indicating full contact with the reproductive organs of the flower. Mean visit duration was 0.15 ± 0.09 s ($n = 5$ visits), in each case making contact with the anthers at the forehead and neck.

Diurnal visitors: We observed 59 visits to the flowers, comprising three species of hummingbirds, a bee (Fig. 1E-F), an ant and a fly.

The hummingbird species that visited *T. heterophylla* were *Amazilia cyanocephala* Lesson (Fig. 1E)*, Amazilia beryllina* Deppe and *Lampornis* sp., with 14, two and 13 visits, respectively. Mean visit duration was 8 ± 21 s ($n = 8$ visits) for *A. cyanocephala*, and 1 ± 0.5 s for *Lampornis* sp. ($n = 13$ visits). The three hummingbird species foraged in a similar manner, approaching from the front or upper part of the flower, introducing then rapidly withdrawing the beak. Both *Amazilia* species visited *T. heterophylla* flowers during the morning, between 07:00 h and 09:30 h. At the beginning of the observations, we observed a fight above the flowers between two individuals of *A. cyanocephala* and *A. beryllina*, after which we only observed *A. cyanocephala* during the morning. This species always visited *T. heterophylla* flowers after visiting co-occurring individuals of ornithophilous *T. limbata* Schltdl and *T. kirchhoffiana* Wittmack (P. A. Aguilar-Rodríguez, pers. obs.). *Lampornis* was the only hummingbird species to visit *T. heterophylla* before dawn, at around 18:30 h, generally opening the blossom with its beak in order to extract nectar before anthesis occurred.

Sweat bees (Halictidae: Augochlorini) began visiting *T. heterophylla* flowers later in the flowering season (Fig. 1F). This bee species visited the flowers early in the morning, just after dawn (05:50 to 07:20 h) and in late afternoon, before dusk (18:00 h). Mean visit duration was 346 ± 246 s ($n = 17$ visits). At each visit, the bees only collected pollen, covering most of the inner surface of the petals with pollen in the process. The bees then abandoned the flower and flew out of sight or to another *Tillandsia* species in the vicinity. In addition, we observed three individual ants and two flies consuming nectar from the base of the corolla.

Effectiveness of nocturnal *vs*. diurnal visitors

The Emasculated nocturnal exposure treatment produced the lowest fruit set at 66.66%, which was only 15% lower than the Control treatment. In contrast, in the Diurnal exposure treatment, the fruit set was 94.87%, nearly 28% higher than the lowest Emasculated nocturnal exposure treatment (Table 2). Pollination treatment had no influence on seed set (H=9.14, df=4, P>0.05; n = 148 fruits from 16 individuals). In general, there is a significant effect of treatment on the fruit set, both in the treatments conducted in the planthouse and those performed *in situ* (X^2=10.082, df=4, P<0.05).

DISCUSSION

The epiphytic bromeliad *T. heterophylla* is a self-compatible species with a generalist pollination system, since at least four different floral visitors pollinate its flowers: bats, moths, hummingbirds and bees (Fig. 1, Table 3). Moreover, fruit and seed set were statistically similar among treatments (Table 1; Table 2), indicating that *T. heterophylla* produces similar quantities of fruits and seeds regardless of the pollen vector and the origin of such pollen.

Similar fructification would be expected in a complementary or mixed pollination system between diurnal and nocturnal floral visitors (Jennersten & Morse 1991; Sazima *et al*. 1994; Maruyama *et al*. 2010; Walter 2010; Amorim *et al*. 2013). In addition, we consider that the daily production of a single flower by *T. heterophylla* (the most commonly observed situation in the field) promotes some degree of outcrossing in this self-compatible species.

It is worth noting that our findings suggest that *T. heterophylla* presents reproductive apomixis, a beneficial trait for a wind-dispersed species spreading clonal seeds over a vast area, but also one that reproduces via vegetative clonal growth, which produces aggregated individuals forming clusters (see Barbará *et al*. 2009); both of these spatial patterns are present in *T. heterophylla*. Apomixis has evolved as a response to the scarcity of pollinators and/or the colonization of new areas, especially in monocarpic weeds (e.g., species producing fruits only once; Baker 1974; Shivanna 2014). Furthermore, the low quantity of nectar found in *T. heterophylla* compared to other chiropterophilous species (see below), may be explained as a strategy to economize resources in an apomictic species (Shivanna & Tandon 2014) while maintaining enough nectar to promote animal pollination.

The notion described above could affect our results regarding pollinator effectiveness; however, it is known that even if a species is capable of producing fruits and seeds without pollination, floral traits in autogamous flowers do not completely prevent outcrossing when suitable pollinators are present (Shivanna & Tandon 2014 and references therein). This could help to mitigate the disadvantages of apomixis, such as the accumulation of non-adaptive mutants and inbreeding depression (Charlesworth & Charlesworth 1987; Shivanna & Tandon 2014). In the case of *T. heterophylla*, a diverse group of animals function as pollinators (Table 3), each with differences in visitation frequency, timing and behavior during visits, probably contributing in different degrees to the sexual reproduction of *T. heterophylla*. We cannot discount the possibility of self-pollination in this bromeliad in place of apomixis, since some flowers in the field had pollen on the stigma at anthesis, and fruit sets in the Emasculation and the Spontaneous self-pollination treatments were similar (Table 1). On the other hand, despite the fact that the planthouse was close (ca. 200 m) to the field study site, some cryptic environmental factor could be responsible for the lower fruit set recorded in the planthouse compared to the *in situ* treatments.

Considering only the *in situ* treatments, the fruit set in un-emasculated flowers was higher than in any other pollination treatment performed in the field, regardless of the time of pollinator activity, but with highest fruit set in the diurnal pollination treatments (Diurnal exposure: 94% *vs.* Nocturnal exposure: 90%; Emasculated diurnal exposure: 82% *vs.* Emasculated nocturnal exposure: 66%). This indicates that the pollination services performed by diurnal and nocturnal floral visitors have an extra reproductive input to the self-pollination capabilities of *T. heterophylla*, with a slight advantage presented in diurnal pollination, in terms of fruit set.

Autogamy seems to be common among bromeliads (Matallana *et al.* 2010), but without understanding the breeding system, as well as the identity and behavior of flower visitors (Montalvo & Ackerman 1986) in relation to the pollen dehiscence and stigma receptivity, the plant could be characterized as more "generalized" in its effective pollination than it actually is (Willmer 2011). In the case of *T. heterophylla*, our first hypothesis was proved, since the flowers received visits from both nocturnal and diurnal floral visitors.

While fruit set in the diurnal exposed treatments was higher, most of the bat recordings occurred early in the night (when stigma receptivity had already begun, along with anther dehiscence) before the visits of most of the diurnal visitors, even those of the moths. This indicates that bats are the probable pollinators on most occasions. In this sense, the timing and frequency of the bat visits suggest that these were the most effective pollinators of *T. heterophylla*, since the moth visits were far too infrequent (nine *vs.* 31 visits) to be characterized as the principal pollinator of *T. heterophylla*, as was suggested originally by Gardner (1986) and Hietz & Hietz-Seifert (1994). Furthermore, the presence of predators such as frogs and spiders near the flowers (P. A. Aguilar-Rodríguez, pers. obs.) may reduce the presence of moths around the *T. heterophylla* flowers (see for example Arango *et al.* 2012).

The white and bell-shaped corolla, in addition to the size of the anthers (Gardner 1986), anthesis duration (mainly nocturnal), nectar production pattern and the nectar-sugar composition (Krömer *et al.* 2008) and concentration, fitted the characteristics assumed for nocturnal pollinated plants, specifically those of bat-pollinated species (according to Krömer *et al.* 2008 and Fleming *et al.* 2009). In support of our second hypothesis, a nocturnal visiting bat, considered to be *A. geoffroyi*, was the most frequent visitor to *T. heterophylla* flowers, probably pollinating the flower on each occasion.

Taken together, the three species of hummingbirds accounted for 29% of the total number of visits to *T. heterophylla* flowers, probably also pollinating on each occasion (except for *Lampornis*). *Amazilia cyanocephala* always visited *T. heterophylla* after visiting other co-occurring *Tillandsia* species, such as *T. limbata* and *T. kirchhoffiana*. Since the nectar volume at the time of visits of *A. cyanocephala* was almost zero (0.13 ± 0.32 µl), the hummingbird was probably more attracted to other species nearby and was visiting *T. heterophylla* flowers in an opportunistic manner (unless a hummingbird foraged on a flower that had not been previously

visited by a nocturnal animal, in which case it would have the whole amount of nectar at its disposal). Co-occurring species offering higher rewards may alter the potential pollinator assemblage near a flowering plant, increasing opportunities for pollination (Gómez 2002). While we did not measure the nectar amount/concentration of *T. limbata* and *T. kirchhoffiana*, we observed many hummingbirds foraging at these species (P. A. Aguilar-Rodríguez, pers. obs.). The hummingbird *Lampornis* visited *T. heterophylla* flowers during the evening, even opening the floral buds before anthesis (similar behavior was noted by Sazima *et al.* 1994), when it may have found a most abundant reward, but with no pollen released or no stigmatic receptivity. As pollinators, bats disperse more conspecific pollen than hummingbirds (Muchhala & Thomson 2010) and also over larger areas (attributed to the more territorial behavior of hummingbirds; Buzato *et al.* 1994, Sazima *et al.* 1994; Arizaga *et al.* 2000; Moraes & Sebbenn 2011).

The other diurnal pollinators, the bees, were the third most frequent floral visitors (25%), visiting before dusk and early in the morning. Other studies have documented bees as bromeliad pollen thieves (including from species pollinated by bats; Kaehler *et al.* 2005), and have even considered them as complementary pollinators in bimodal pollinator systems (Schmid *et al.* 2011a, b). During visits, the bees collected pollen from the anthers, saturating the stigma with pollen, possibly causing self-pollination and removal of the remnant pollen from the anthers (depleting the pollen for subsequent visitors). In this sense, bees could affect the male fitness of *T. heterophylla* by promoting selfing and limiting pollen transfer (Vivarelli *et al.* 2011). Furthermore, the bees were only recorded near the end of the *T. heterophylla* flowering season, reducing their relative contribution to the fruit set of diurnal pollination treatments.

Considering that bats were the most frequent visitors, and the work of Krömer *et al.* (2008), which clearly distinguishes the nectar traits of *T. heterophylla* from other ornithophilous bromeliads, we compared *T. heterophylla* to other bat-pollinated bromeliads. However, it does not fit well the nectar traits reported in chiropterophilous species, such as *W. gladioliflora*, which is the best documented case of chiropterophily and produces 1129 µl of nectar with a concentration of 17% sugars (Tschapka & von Helversen 2007), in contrast to the 82 µl with 6% sugar in *T. heterophylla*. Moreover, *T. macropetala*, which is sympatric to *T. heterophylla*, offers 400 µl of nectar at a sugar concentration of 7% (Aguilar-Rodríguez *et al.* 2014). Nevertheless, the amount of nectar in *T. heterophylla* is higher than those of terrestrial *Encholirium vogelii*, which produces only 4 µl with 18% sugar (Christianini *et al.* 2013); and more concentrated than the nectar produced by *E. glaziovii* (4.65%; Sazima *et al.* 1989). In addition, other bat-pollinated species produce small amounts of nectar over two nights; the terrestrial herb *Irlbachia alata* (Aubl.) Maas (Gentianaceae) produces 42 µl at a concentration of 19.5% in the first night and 19.5 µl and concentration of 8.7% in the second. This nectar production is between 25-50% of the volume produced by *T. heterophylla* but at higher concentrations (Machado *et al.* 1998).

In common with *W. gladioliflora*, *T. macropetala*, *E. glaziovii*, and *E. vogelii*, *T. heterophylla* receives diurnal visitors, specifically hummingbirds; however, in the former cases, it transpired that these were nectar thieves. Interestingly, *E. vogelii* in Brazil is effectively pollinated by a

sphinx-moth and at least four hummingbirds, as well as a bat species, and thus shows a similar pollination pattern to that of *T. heterophylla*. In fact, the reduced amounts of nectar in both species can be interpreted as "left-over" nectar, or even as a transitional reward that is coincidently similar to the less copious nectar supply of hummingbird-pollinated species (Christianini *et al.* 2013). Hummingbirds prefer sucrose-rich nectar, while *T. heterophylla* nectar is hexose-rich (Krömer *et al.* 2008) and also more dilute than that preferred by hummingbirds (20-25%; Nicolson and Fleming 2003). However, it is similar in volume and concentration to that of other hummingbird-pollinated bromeliads, such as *Tillandsia multicaulis* Steud (2-12 µl, 8-18% sugar; Ordano & Ornelas 2004).

Considering all visitors, *T. heterophylla* featured two distinct assemblages of pollinators, nocturnal (bats) and diurnal (hummingbirds and, to a lesser extent bees), as a result of extended anthesis and stigma receptivity. In addition, it has a floral morphology that enables visitors like hummingbirds to pollinate this plant (in contrast, *T. macropetala* is also visited by hummingbirds, but features an "open" corolla that does not facilitate contact between reproductive parts of the flower and the birds; Aguilar-Rodríguez *et al.* 2014). This results in the first reported case of mixed pollination between bats and diurnal pollinators in *Tillandsia* (but see reports for *Guzmania*, Krömer *et al.* 2006). Since overall pollinator effectiveness varies both spatially and temporally (Bustamante *et al.* 2010; Amorim *et al.* 2013), diurnal pollinators may be important for a night-blooming species with extended anthesis when the nocturnal pollinators fail to visit (e.g., on rainy nights), as long as pollen is present in the anthers and they visit different individuals; if not, the diurnal visitors could only promote selfing or waste of nectar and pollen.

Longer flower longevity benefits the male fitness of the plant, by promoting the dispersion of pollen (Aximoff & Freitas 2010), as referred to in the "pollen donation hypothesis" (see Broyles & Wyatt 1990). Most plant species synchronize their rewards to attract the most effective pollinator (Stebbins 1970), but some may offer sufficient rewards to attract secondary pollinators (Willmer 2011); this seems to be the case for *T. heterophylla*, which probably originated from a bird or an insect pollinated antecessor, based on the phylogenetic data and the preponderance of those syndromes in *Tillandsia* (see Gardner 1986; Barfuss *et al.* 2005; Givnish *et al.* 2011; but see Aguilar-Rodríguez *et al.* 2014 for a chiropterophilous *Tillandsia*). Overlapping traits between ornithophilous and chiropterophilous syndromes are suggested as indicating specialization towards bat pollination (Gottsberger 1986; Buzato *et al.* 1994; Muchhala & Thomson 2010), probably due to the overall effectiveness of bats as pollen dispersers (von Helversen & Winter 2003; Muchhala & Thomson 2010).

In addition to the considerable capacity of this species for self-pollination, the mixed pollination system of *T. heterophylla*, where different animals may constitute a "fail-safe" system (*sensu* Dar *et al.* 2006) to guarantee pollination in situations when certain pollinators may fail, could constitute an advantage for survival in the fragmented landscape where this species is found. This situation could affect our results by changing the identity and abundance of visitors in our study area, but the influence of fragmentation on the foraging of large-sized pollinators, such as bats, varies depending on species (e.g., Quesada *et al.* 2004). Since *T. heterophylla* is abundant in secondary forests and live fences, we believe that our study reflects the current situation of this pollination system.

Finally, since *T. heterophylla* may be in a transition from or towards a complementary pollination system, further studies are required to confirm this assumption. These should include other indicators of fitness, such as percentage of germination and levels of inbreeding depression. In addition, it may be valuable to obtain additional data (e.g., pollen tube growth, ovule fertilization, and seed germination from different *in situ* treatments (for the latter, see Cascante-Marín *et al.* 2005) to clarify the role of pollinators in this possibly apomictic species. It would be also important to determine possible differences in the floral visitors among other habitats (e.g., natural forests) within the distribution range of *T. heterophylla* and to study the significance of this and other bromeliad species to the maintenance of pollinator populations, especially those in areas that have undergone significant anthropogenic land use changes.

ACKNOWLEDGEMENTS

REFERENCES

Aguilar-Rodríguez P.A., MacSwiney G.M.C., Krömer T., García-Franco J.G., Knauer A., Kessler M. (2014). First record on bat-pollination in the species-rich genus *Tillandsia* (Bromeliaceae). *Annals of Botany*, **113**, 1047-1055.

Amorim F.W., Galetto L., Sazima M. (2013) Beyond the pollination syndrome: nectar ecology and the role of diurnal and nocturnal pollinators in the reproductive success of *Inga sessilis* (Fabaceae). *Plant Biology*, **15**, 317-327.

Arango A.M., López-Portillo J., Parra-Tabla V., Hernández-Salazar L.T., Morales-Mávil J.E., Rico-Gray V. (2012). Effect of the spider *Peucetia viridans* (Oxyopidae) on floral visitors and seed set of *Cnidoscolus multilobus* (Euphorbiaceae). *Acta Botánica Mexicana*, **100**, 1-14.

Arizaga S., Ezcurra E., Peters E., Ramírez de Arellano F., Vega E. (2000), Pollination ecology of *Agave macroacantha* (Agavaceae) in a mexican tropical desert. II. The role of pollinators. *American Journal of Botany*, **87**, 1011–1017.

Avila Jr. R.S., Freitas L. (2011) Frequency of visits and efficiency of pollination by diurnal and nocturnal lepidopterans for the dioecious tree *Randia itatiaiae* (Rubiaceae). *Australian Journal of Botany*, **59**, 176-184.

Aximoff I.A., Freitas L. (2010) Is pollen removal or seed set favored by flower longevity in a hmmingbird-pollinated *Salvia* species? *Annals of Botany*, **106**, 413-419.

Baker H.G. (1974) The evolution of weeds. *The Annual Review of Ecology*, *Evolution, and Systematics*, **5**, 1-24.

Barbará T., Martinelli G., Palma-Silva C., Fay M.F., Mayo S., Lexer C. (2009) Genetic relationships and variation in reproductive strategies in four closely related bromeliads adapted to neotropical 'inselbergs': *Alcantarea glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* (Bromeliaceae). *Annals of Botany*, **103**, 65-77.

Barfuss M.H.J., Samuel R., Till W., Stuessy T.F. (2005) Phylogenetic relationships in subfamily Tillandsoideae (Bromelliaceae) based on DNA sequence data from seven plastid regions. *American Journal of Botany*, **92**, 337-351.

Benzing D.H. (2000) *Bromeliaceae. Profile of an adaptive radiation*. Cambridge University Press, Cambridge, USA: 354 pp.

Broyles S.B., Wyatt R. (1990) Paternity analysis in a natural population of *Asclepias exaltata*: multiple paternity, functional gender, and the "pollen donation hypothesis". *Evolution*, **44**, 1454-1468.

Bustamante E., Casas A., Búrquez A. (2010) Geographic variation in reproductive success of *Stenocereus thurberi* (Cactaceae): effects of pollination timing and pollinator guild. *American Journal of Botany*, **97**, 2020-2030.

Buzato S., Sazima M., Sazima I. (1994) Pollination of three species of *Abutilon* (Malvaceae) intermediate between bat and hummingbird flower syndromes. *Flora*, **189**, 327-334.

Canela M.B.F., Sazima M. (2005) The pollination of *Bromelia antiacantha* (Bromeliaceae) in Southeastern Brazil: ornithophilous versus melittophilous features. *Plant Biology*, **7**, 411-416.

Cascante-Marín A., Oostermeijer J.G.B., Wolf J.H.D., Dennijs J.C.M. (2005) Reproductive biology of the epiphytic bromeliad *Werauhia gladioliflora* in a premontane tropical forest. *Plant Biology*, **7**, 203-209.

Ceja-Romero J., Mendoza–Ruiz A., López–Ferrari A.R., Espejo–Serna A., Pérez–García B., García–Cruz J. (2010) Las epífitas vasculares del estado de Hidalgo, México: diversidad y distribución. *Acta Botánica Mexicana,* **93**, 1-39.

Charlesworth D., Charlesworth B. (1987) Inbreeding depression and its evolutionary consequences. *The Annual Review of Ecology*, *Evolution, and Systematics*, **18**, 237-268.

Christianini A.V., Forzza R.C., Buzato S. (2012): Divergence on floral traits and vertebrate pollinators of two endemic *Encholirium* bromeliads. *Plant Biology*, **15**, 360-368.

Cruz-Angón A., Escobar-Sarria F., Gerez-Fernández P., Muñiz-Castro M.A., Ramírez-Ramírez F., Williams-Linera G. (2010) Región V. Centro de Veracruz. In: CONABIO, *El Bosque Mesófilo de Montaña en México: Amenazas y Oportunidades para su Conservación y Manejo Sostenible*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México D.F., México: 80-87.

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

Escobedo S.G.J. (2007) Biología de la reproducción de *Tillandsia prodigiosa* (Lem.) Baker Bromeliaceae. Tesis de Maestría. Instituto Politécnico Nacional. Santa Cruz Xoxocotlán, Oaxaca, México.

Espejo-Serna A., López-Ferrari A.R., Ramírez-Morillo I. (2005) *Bromeliaceae. Flora de Veracruz. Fascículo 136*. Instituto de Ecología, A. C., Xalapa, México: 307 pp.

Fenster C.B., Armbruster W.S., Wilson P., Dudash M.R., Thomson J.D. (2004) Pollination

syndromes and floral specialization. *Annual Review of Ecology & Systematics*, **35**, 375-403.

Fleming T.H., Geiselman, C., Kress, W.J. (2009). The evolution of bat pollination: a phylogenetic perspective. *Annals of Botany*, **104**, 1017-1043.

Fumero-Cabán J.J., Meléndez-Ackerman E.J. (2007) Relative pollination effectiveness of floral visitors of *Pitcairnia angustifolia* (Bromeliaceae). *American Journal of Botany*, **94**, 419-424.

Gardner C.S. (1986) Inferences about pollination in *Tillandsia* (Bromeliaceae). *Selbyana*, **9**, 76-87.

Giménez-Benavides L., Dötterl S., Jürgens A., Escudero A., Iriondo J.M. (2007) Generalist diurnal pollination provides greater fitness in a plant with nocturnal pollination syndrome: assessing the effects of a *Silene–Hadena* interaction. *Oikos*, **116**, 1461–1472.

Givnish T.J., Barfuss M.H.J., Van Ee B., Riina R., Schulte K., Horres R., Gonsiska P.A., Jabaily R.S., Crayn D.M., Smith J.A.C., Winter K., Brown G.K., Evans T.M., Holst B.K., Lutter H., Till W., Zizka G., Berry P.E., Sytsma K.J. (2011) Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. *American Journal of Botany*, **98**, 872-895.

Gómez J. M. (2002) Generalización en las interacciones entre plantas y polinizadores. *Revista Chilena de Historia Natural*, **75**, 105-116.

Gottsberger, G. (1986) Some pollination strategies in Neotropical savannas and forests. *Plant Systematics and Evolution*, **152**, 29-45.

Groman J., Pellmyr O. (1999) The pollination biology of *Manfreda virginica* (Agavaceae): relative contribution of diurnal and nocturnal visitors. *Oikos,* **87**, 373-381.

Herrera C.M. (1990) Daily patterns of pollinator activity, differential pollinating effectiveness, and floral resource availability, in a summer-flowering Mediterranean shrub. *Oikos*, **58**, 277-288.

Hietz P. Hietz–Seifert U. (1994) *Epífitas de Veracruz. Guía ilustrada para las regiones de Xalapa y Los Tuxtlas, Veracruz*. Instituto de Ecología, A.C., Veracruz, Mexico: 229 pp.

Jennersten O., Morse D.H. (1991) The quality of pollination by diurnal and nocturnal insects visiting common milkweed, *Asclepias syriaca*. *American Midland Naturalist*, **125**, 18-28.

Kaehler M., Varassin I.G., Goldenberg R. (2005) Polinização em uma comunidade de bromélias em floresta atlântica alto-montana no estado do Paraná, Brasil. *Brazilian Journal of Botany*, **28**, 219-228.

Kamke R., Schmid S., Zillikens A., Cortés-Lopes B., Steiner J. (2011) The importance of bees as pollinators in the short corolla bromeliad *Aechmea caudata* in Southern Brazil. *Flora*, **206**, 749-756.

Kearns C.A., Inouye D.W. (1993) *Techniques for pollination biologists*. Niwot, University of Colorado Press, Colorado, USA: 583 pp.

Kessler M., Krömer T. (2000) Patterns and ecological correlates of pollination modes among bromeliad communities of Andean forests in Bolivia. *Plant Biology*, **2**, 659-669.

Krömer T., Kessler M., Herzog S.K. (2006) Distribution and flowering ecology of bromeliads along two climatically contrasting elevational transects in the Bolivian Andes. *Biotropica*, **38**, 183-195.

Krömer T., Kessler M., Lohaus G., Schmidt-Lebuhn A.N. (2008) Nectar sugar composition and concentration in relation to pollination syndromes in Bromeliaceae. *Plant Biology*, **10**,

502-511.

Machado I.C.S., Sazima I., Sazima M. (1998) Bat pollination of the terrestrial herb *Irlbachia alata* (Gentianaceae) in northeastern Brazil. *Plant Systematics and Evolution*, **209**, 231-237

Martén-Rodríguez S., Fenster C.B. (2008) Pollination ecology and breeding systems of five *Gesneria* species from Puerto Rico. *Annals of Botany*, **102**, 23-30.

Martínez-Meléndez N., Pérez-Farrera M.A., Martínez-Camilo R. (2009) The vascular epiphyte flora of El Triunfo Biosphere Reserve, Chiapas, México. *Rhodora*, **111**, 503-535.

Maruyama P.K., Amorim F.W., Oliveira P.E. (2010) Night and day service: distyly and mixed pollination system in *Faramea cyanea* (Rubiaceae). *Flora*, **205**, 818-824.

Massinga P.H., Johnson S.D., Harder L.D. (2005) Heteromorphic Incompatibility and Efficiency of Pollination in two distylous *Pentanisia* species (Rubiaceae). *Annals of Botany*, **95**, 389-399.

Matallana G., Godinho M.A.S., Guilherme F.A.G., Belisario M., Coser T.S., Wendt T. (2010) Breeding systems of Bromeliaceae species: evolution of selfing in the context of sympatric occurrence. *Plant Systematics and Evolution*, **289**, 57-65.

Mayfield M. M., Waser N. M., Price M. V. (2001) Exploring the "most effective pollinator principle" with complex flowers*: bumblebees and *Ipomopsis aggregata. Annals of Botany*, **88**, 591-596.

Mehltreter K., Flores-Palacios A*., García-Franco J.G. (2005) Host preferences of low-trunk vascular epiphytes in a cloud forest of Veracruz, Mexico. *Journal of Tropical Ecology*, **21**, 651–660.

Miyake T., Yahara T. (1998) Why does the flower of *Lonicera japonica* open at dusk? *Canadian Journal of Botany*, **76**, 1806–1811.

Miyake T., Yahara T. (1999) Theoretical evaluation of pollen transfer by nocturnal and diurnal pollinators: when should a flower open? *Oikos*, **86**, 233– 240.

Montalvo A.M., Ackerman J.D. (1986) Relative pollinator effectiveness and evolution of floral traits in *Spathiphyllum friedrichsthalii* (Araceae). *American Journal of Botany*, **73**, 1665-1676.

Moraes M.L.T., Sebbenn A.M. (2011), Pollen dispersal between isolated trees in the Brazilian Savannah: A case study of the Neotropical tree *Hymenaea stigonocarpa*. *Biotropica*, **43**, 192–199.

Muchhala N. (2006) The pollination biology of *Burmeistera* (Campanulaceae): specialization and syndromes. *American Journal of Botany*, **93**, 1081-1089.

Muchhala N., Thomson J.D. (2010) Fur versus feathers: Pollen delivery by bats and hummingbirds and consequences for pollen production. *The American Naturalist*, **175**, 717-726.

Murúa M., Espíndola A. (2014) Pollination syndromes in a specialised plant-pollinator interaction: does floral morphology predict pollinators in *Calceolaria? Plant Biology*, DOI: 10.1111/plb.12225.

Ne'eman G., Jürgens A., Newstrom-Lloyd L., Potts S.G., Dafni A. (2010) A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Reviews*, **85**, 435-451.

Nicolson S.W., Fleming P.A. (2003) Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. *Plant Systematics and Evolution*, **238**, 139-153.

Ollerton J., Alarcón R., Waser N.M., Price M.V., Watts S., Cranmer L., Hingston A., Peter C.I.,

Rotenberry J. (2009) A global test of the pollination syndrome hypothesis. *Annals of Botany*, **103,** 1471-1480.

Ordano M., Ornelas J.F. (2004) Generous like flowers: nectar production in two epiphytic bromeliads and a meta-analysis of removal effects. *Oecologia*, **140**, 495-505.

Quesada M., Stoner K.E., Lobo J.A., Herrerías-Diego Y., Palacios-Guevara C., Monguía Rosas M.A., O.-Salazar K.A., Rosas-Guerrero V. (2004) Effects of forest fragmentation on pollinator activity and consequences for plant reproductive success and mating patterns in bat-pollinated Bombacaceous trees. *Biotropica*, **36**, 131-138.

Reid F. 2009. *A field guide to the mammals of Central America and Southeast Mexico*. Oxford University Press, Oxford, USA: 343 pp.

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

Rymer P.D., Whelan R.J., Ayre D.J., Weston P.H., Russell K.G. (2005) Reproductive success and pollinator effectiveness differ in common and rare *Persoonia* species (Proteaceae). *Biological Conservation*, **123**, 521–532.

Sazima I., Vogel S., Sazima M. (1989) Bat pollination of *Encholirium glaziovii,* a terrestrial bromeliad. *Plant Systematics and Evolution*, **168**, 167-179.

Sazima M., Buzato S., Sazima I. (1995) Polinização de *Vriesea* por morcegos no sudeste brasileiro. *Bromélia*, **2**, 29-37.

Sazima M., Buzato S., Sazima I. (1999) Bat-pollinated flower assemblages and bat visitors at two Atlantic forest sites in Brazil. *Annals of Botany*, **83**, 705-712.

Sazima M., Sazima I., Buzato S. (1994) Nectar by day and night: *Siphocampylus sulfureus* (Lobeliaceae) pollinated by hummingbirds and bats. *Plant Systematics and Evolution*, **191**, 236-247.

Schmid S., Schmid V.S., Zillikens A., Harter-Marques B., Steiner J. (2011a) Bimodal pollination system of the bromeliad *Aechmea nudicaulis* involving hummingbirds and bees. *Plant Biology*. **13**, 41-50.

Schmid S., Schmid V.S., Zillikens A., Steiner J. (2011*b*). Diversity of flower visitors and their role for pollination in the ornithophilous bromeliad *Vriesea friburgensis* in two different habitats in southern Brazil. *Ecotropica*, **17**, 91-102.

Shivanna K.R. (2014) Reproductive assurance through autogamous self-pollination in some annual weed species. Proceedings of the National Academy of Sciences, India, Biological Sciences, **84**, 681-687.

Shivanna K.R., Tandon R. (2014) Breeding systems. In: Shivanna K.R., Tandon R., *Reproductive Ecology of Flowering Plants: A Manual*. Springer, India: 107-123.

Simmons N.B. 2005. Order Chiroptera. In: Wilson D.E., Reeder D.M. (Eds), *Mammal species of the world: a taxonomic and geographic reference*. Johns Hopkins University Press, Maryland, USA: 312-529.

Slauson L.A. (2000) Pollination biology of two chiropterophilous agaves in Arizona. *American Journal of Botany*, **87**, 825-836.

Sosa V.J., Hernández-Salazar E., Hernández-Conrique D., Castro-Luna A.A. (2008) Murciélagos. In: Manson R.H., Hernández-Ortíz V., Gallina S., Mehltreter K. (Eds), *Agroecosistemas cafetaleros de Veracruz biodiversidad, manejo y conservación*. INECOL, INE, SEMARNAT, Veracruz, México: 181-192.

Stebbins G.L. (1970) Adaptive radiation of reproductive characteristics in angiosperms I:

Pollination mechanisms. *Annual Review of Ecology and Systematics*, **1**, 307-326.

Toledo-Aceves T., Meave J.A., González-Espinosa M., Ramírez-Marcial N. (2011) Tropical montane cloud forests: current threats and opportunities for their conservation and sustainable management in Mexico. *Journal of Environmental Management*, **92**, 974-81.

Tschapka M., von Helversen O. (2007) Phenology, nectar production and visitation behaviour of bats on the flowers of the bromeliad *Werauhia gladioliflora* in Costa Rican lowland rain forest. *Journal of Tropical Ecology*, **23**, 385-395.

Velazco P.M., Patterson B.D. (2013) Diversification of the yellow-shouldered bats, Genus Sturnira (Chiroptera, Phyllostomidae), in the New World tropics. *Molecular Phylogenetics and Evolution*, **65**, 683-698.

Vivarelli D., Petanidou T., Nielsen A., Cristofolini G. (2011) Small-size bees reduce male fitness of the flowers of *Ononis masquillierii* (Fabaceae), a rare endemic plant in the northern Apennines. *Botanical Journal of the Linnean Society*, **165**, 267-277.

Von Helversen O., Winter Y. (2003) Glossophagine bats and their flowers: costs and benefits for plants and pollinators. In: Kunz T.H., Fenton M.B. (Eds), *Bat Ecology*. The University of Chicago Press, Chicago, USA: 346-397.

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

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.

Wendt T., Ferreira C.M.B., Gelli de Faria A.P., Iglesias-Rios R. (2001) Reproductive biology and natural hybridization between two endemic species of *Pitcairnia* (Bromeliaceae). *American Journal of Botany*, **88**, 1760-1767.

Williams-Linera G. (2002) Tree species richness complementarity, disturbance and fragmentation in a Mexican tropical montane cloud forest. *Biodiversity and Conservation*, **11**, 1825–1843.

Williams-Linera G. (2007) *El bosque de niebla del centro de Veracruz: Ecología, historia y destino en tiempos de fragmentación y cambio climático*. INECOL-CONABIO, Veracruz, Mexico: 204 pp.

Willmer P. (2011) *Pollination and floral ecology*. Princeton University Press, Princeton & Oxford, USA: 778 pp.

Wolff D., Braun M., Liede S. (2003) Nocturnal versus diurnal pollination success in *Isertia laevis* (Rubiaceae): a sphingophilous plant visited by hummingbirds. *Plant Biology*, **5**, 71-78.

Young H.J. (2002) Diurnal and nocturnal pollination of *Silene alba* (Caryophyllaceae). *American Journal of Botany*, **89**, 433-440.

Zapata T.R., Arroyo M.T.K. (1978) Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica*, **10**, 221-230.

Zotz G. (2013) The systematic distribution of vascular epiphytes - a critical update. *Botanical Journal of the Linnean Society,* **177**, 453-481.

Table 1. Results from the pollination treatments conducted to determine the breeding system of *Tillandsia heterophylla* (Seed set, H=0.198, df=3, P>0.05).

Treatments	Number of flowers	Number of fruits	% Fruit-set	Seed-set (mean ± SD)
Emasculation	16	10	62.50	310.50 ± 273.13
Spontaneous self-pollination	18	11	61.11	315.72 ± 247.29
Cross-pollination	17	10	58.82	281.47 ± 258.27
Self-pollination	14	8	57.14	269.71 ± 275.13

Table 2. Results of pollinator exclusion treatments performed in *Tillandsia heterophylla* in order to determine the relative effectiveness of nocturnal and diurnal pollinators (Seed set H=9.14, df=4, P>0.05; *n* = 148 fruits from 16 individuals).

Treatments	Number of flowers	Number of fruits	% Fruit-set	Seed-set (mean ± SD)
Diurnal exposure	39	37	94.87	552.17 ± 171.72
Nocturnal exposure	32	29	90.62	526.28 ± 201.63
Emasculated diurnal exposure	23	19	82.60	418.30 ± 234.55
Emasculated nocturnal exposure	21	14	66.66	379.66 ± 286.66
Control	33	27	81.81	485.39 ± 246.18

Table 3. Floral visitors recorded with video camera (nocturnal) and by direct observation

(diurnal) on *Tillandsia heterophylla* flowers.

Period of activity	Species	Relative frequency (N) %	Visit reward / objective	Visitor category
Nocturnal	*Anoura geoffroyi* (Nectarivore bat)	(31) 31.31	Nectar	Pollinator
Diurnal	*Amazilia cyanocephala* (Hummingbird)	(14) 14.14	Nectar	Pollinator
Diurnal	*Amazilia beryllina* (Hummingbird)	(2) 2.02	Nectar	Pollinator
Diurnal	*Lampornis* sp. (Hummingbird)	(13) 13.13	Nectar	Nectar thief
Diurnal	Halictidae: Augochlorini sp.1 (Sweat Bee)	(25) 25.25	Pollen	Pollinator
Diurnal	Formicidae sp.1 (Ant)	(3) 3.03	Nectar	Nectar thief
Nocturnal	Noctuidae sp.1 (Moth)	(9) 9.09	Nectar	Pollinator
Diurnal	Calliphoridae sp.1 (Fly)	(2) 2.02	Nectar	Nectar thief

Figure 1. *Tillandsia heterophylla* and its floral visitors. A) Flower of *T. heterophylla* showing the white petals and yellow stamens. B) Individual of *T. heterophylla* with inflorescence in habitat. C) A moth, a nocturnal visitor that normally only landed on the flowers without pollinating them. D) A bat, probably *Anoura geoffroyi*, the most frequent floral visitor, pollinating a *T. heterophylla* flower. E) The hummingbird *Amazilia cyanocephala* (visiting a *Tillandisa limbata*) and F) a Augochlorini bee, were two of the five diurnal pollinator species of *T. heterophylla*. Photos by M. C. MacSwiney-González (A), P. A. Aguilar-Rodríguez (B, C, D, F), and Anina Knauer (E).

Figure 2. Volume in (µl) and sugar concentration (% sugar) of nectar from *Tillandsia heterophylla*. Error bars are shown. Night duration is indicated by the open rectangle. The horizontal line ended in black points denotes the duration of stigma receptivity, while the horizontal line ended in arrows denotes the time of pollen availability on the flower.