Temporal changes in the most effective pollinator on a bimodal system involving bees and hummingbirds (#35011)

First submission

Guidance from your Editor

Please submit by **25 Apr 2019** for the benefit of the authors (and your $200 publishing discount).

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Raw data check
Review the raw data. Download from the materials page.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files
Download and review all files from the materials page.

5 Figure file(s)
3 Table file(s)
1 Raw data file(s)

Custom checks

Field study

- Have you checked the authors field study permits?
- Are the field study permits appropriate?

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- Data is robust, statistically sound, & controlled.
- Speculation is welcome, but should be identified as such.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Temporal changes in the most effective pollinator on a bimodal system involving bees and hummingbirds

Roberta L B Leal [Corresp., 1], **Marina M Moreira** [1, 2], **Alessandra R Pinto** [3], **Julia O Ferreira** [1], **Miguel Rodriguez-Girones** [4], **Leandro Freitas** [1]

[1] Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

[2] Centro de Ciências Agrárias, Universidade Federal do Espírito Santo, Alegre, Espírito Santo, Brazil

[3] Programa de Pós-Graduação em Ecologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

[4] Estación Experimental de Zonas Áridas, Almería, Spain

Corresponding Author: Roberta L B Leal
Email address: robertaleal@jbrj.gov.br

A generalist pollinator system is identified through the interaction of a plant species with two or more species or functional groups of pollinators. The spatio-temporal variation of the most effective pollinator is the factor most frequently advocated to explain the emergence and maintenance of generalist pollination systems. Studies comprising both temporal variation on floral visitor assemblages and the effectiveness on pollination by different functional groups are restricted to a few systems. Thus, there are gaps in knowledge about generalist species concerning the variation of pollinators and their effectiveness over time. In this study we evaluated the pollination effectiveness (i.e., frequency associated with efficacy) of the floral visitors of *Edmundoa lindenii* (Bromeliaceae) across four reproductive events. We analysed the frequency of floral visitors (large bees, small bees, and hummingbirds) through focal observations and their single-visit efficacy (seed set). Pollen limitation (PL) index was estimated comparing seed set after hand cross and natural pollination. *Edmundoa lindenii* is self-incompatible and parthenocarpic, requiring the action of pollinators for reproduction. Hummingbirds have greater efficacy than large bees, and small bees act as pollen robbers. The frequency of floral visitors varied among the years, and overall hummingbirds were more effective than large bees. The PL index varied among the years, with limitation only occurring in the reproductive event of 2017, when hummingbirds were scarce. Our results allow us to conclude that a generalist species can suffer or not PL in different reproductive events, in response to variations in the pollinator assemblage. Although the evolution of a generalized pollination system is expected when different pollinators play the same role as selective agents, our results support that generalization may also be favoured when pollinators with lesser efficacy provide reproductive assurance, lightening fluctuations of the most effective pollinators, such as could be the case for large bees and *E. lindenii*.

Temporal changes in the most effective pollinator on a bimodal system involving bees and hummingbirds

Roberta Luísa Barbosa Leal[1], Marina Muniz Moreira[1,2], Alessandra Ribeiro Pinto[3], Julia de Oliveira Ferreira[1], Miguel Angel Rodríguez-Gironés[4], Leandro Freitas[1]

1 Jardim Botânico do Rio de Janeiro, Rio de Janeiro, RJ, Brazil
2 Universidade Federal do Espírito Santo, Centro de Ciências Agrárias, Alegre, ES, Brazil
3 Universidade Federal do Rio de Janeiro, Programa de Pós-Graduação em Ecologia, Rio de Janeiro, RJ, Brazil
4 Estación Experimental de Zonas Áridas, Almería, Spain

Corresponding Author:
Roberta Leal[1]
Rua Pacheco Leão, 915, Jardim Botânico, Rio de Janeiro, Brasil.
Email address: robertalbleal@gmail.com

ABSTRACT

Background

A generalist pollination system is identified through the interaction of a plant species with two or more species or functional groups of pollinators. The spatio-temporal variation of the most effective pollinator is the factor most frequently advocated to explain the emergence and maintenance of generalist pollination systems. Studies comprising both temporal variation on floral visitor assemblages and the effectiveness on pollination by different functional groups are restricted to a few systems. Thus, there are gaps in knowledge about generalist species concerning the variation of pollinators and their effectiveness over time. In this study we evaluated the pollination effectiveness (i.e., frequency associated with efficacy) of the floral visitors of *Edmundoa lindenii* (Bromeliaceae) across four reproductive events.

Methods

We analysed the frequency of floral visitors (large bees, small bees, and hummingbirds) through focal observations and their single-visit efficacy (seed set). Pollen limitation (PL) index was estimated comparing seed set after hand cross and natural pollination.

Results

Edmundoa lindenii is self-incompatible and parthenocarpic, requiring the action of pollinators for reproduction. Hummingbirds have higher efficacy than large bees, and small bees acted only as pollen robbers. The frequency of floral visitors varied among the reproductive events, and overall hummingbirds were more effective than large bees. The PL index varied among the reproductive events, with limitation only occurring in the reproductive event of 2017, when hummingbirds were scarce.

INTRODUCTION

In most plants, flowers are visited by a diverse assemblage of animals (*Waser et al., 1996*). These floral visitors may differ in their contribution to the plant reproductive success, as they can vary in the efficacy of pollen transfer (*Shuttleworth & Johnson, 2008*; *Freitas, 2013*; *Ollerton, 2017*). Moreover, these visitors can be arranged in different functional groups of pollinators based on their behaviour and morphological traits (*Fenster et al., 2004*). Plants with bimodal systems are examples of generalist systems, in which flowers are attractive to and pollinated by two functional groups of pollinators (*Waser & Price, 1990*; *Castellanos, Wilson & Thomson, 2003*; *Manning & Goldblatt, 2005*; *Shuttleworth & Johnson, 2008*).

Based on the principle of "the most effective pollinator," plants with a bimodal generalist system can be interpreted as an intermediary stage in the transition from one specialized pollinator to another, in which both vectors are able to pollinate the flower (*Stebbins, 1970*). Alternatively to this explanation, plants with bimodal generalist systems may be favoured in certain scenarios, for example, under unpredictable pollination environments (*Herrera, 1988*; *Waser et al., 1996*; *Ollerton et al., 2007*). Accordingly, the spatio-temporal variation of the most effective pollinator is the factor most frequently advocated to explain the emergence and maintenance of generalist pollination systems (*Herrera, 1996*; *Armbruster et al., 2000*).

Studies encompassing generalist pollination systems only report floral visitor assemblages and visitation rates (e.g., *Thompson, 2001; Freitas & Sazima, 2006; Scrok & Varassin, 2011*), despite the expected variation on fruit and seed set (i.e., variation in fitness consequences of

flower visits) after visits by different floral visitors, and even the fact that not all visitors are actual pollinators (*Armbruster, Fenster & Dudash, 2000; Ollerton, 2017*). Several studies have gone further, exploring spatiotemporal variation in the composition and frequency of visitors (*Fenster & Dudash, 2001; Ivey, Martinez & Wyatt, 2003; Zych et al., 2018*) whereas others have quantified the contribution to plant reproduction of different pollinator species or functional groups (*Ávila Jr. & Freitas, 2011; Muchhala, et al., 2013; Amorim, Galetto & Sazima, 2013; Salas-Arcos et al., 2017*). However, information comprising both temporal variation in floral visitor assemblages and the effectiveness of pollination by different functional groups is restricted to a few systems (e.g., *Larsson, 2005; Wiggam & Ferguson, 2005*) investigated since the seminal studies by C. M. Herrera in the 1980s (*Herrera, 1987*; *1988*). Thus, there are gaps in knowledge about the variation of pollinators in generalist plants and their effectiveness over time.

Generalized pollination systems have ecological and evolutionary dimensions (*Armbruster, Fenster & Dudash, 2000*), therefore the effect of several pollinators in the process of evolutionary generalization depends on the selective pressures exerted by those floral visitors. In this sense, differences in pollination efficacy (sensu *Freitas, 2013*) among functional groups may be enhanced if the variations in the pollination environment affect the plant reproductive success. Pollen limitation (PL), the lower fruit and/or seed production due to inadequate pollen receipt, is widespread in angiosperms (*Ashman et al., 2004; Knight et al., 2005*), and similarly to the pollinator effectiveness, its magnitude varies at several scales (*Bennett et al., 2018*). However, how temporal variations in the pollination environment and LP levels are related is a fundamental but poorly understood aspect to a better understanding of the mechanisms that lead to the maintenance of generalized pollination systems (see *Koski et al., 2018*).

Here, we focused on the temporal variation of pollinator effectiveness in a tropical Bromeliaceae species with bimodal pollination system. We measured the efficacy of floral visitors and investigated whether the frequency of visits between different functional groups of pollinators varied along four years of observation. Furthermore, we tested the PL on different reproductive events to evaluate the effect of the frequency of each pollinator group on reproductive success. In addition, we measured the reflectance of attractive floral parts over a range of relevant wavelengths, as well as nectar production and the effects of nectar removal, in order to understand how this species attracts its major pollinators.

MATERIALS AND METHODS

Study site and species

This study was conducted in an area covered by montane Atlantic Forest, located in the Serra dos Órgãos National Park (PARNASO), Rio de Janeiro state, Brazil (22° 52'- 22° 54' S and 42° 09'- 45° 06' W, ca. 960 m a.s.l.). The total annual rainfall at the study site is 2,436 mm, with the rainiest period between December and March and colder and drier months from June to August. The mean annual temperature is 18.6 °C, with minimum and maximum monthly temperatures of 13.7 ºC and 22.9 ºC (climate data for 2015 to 2018 from the meteorological station located inside the PARNASO). The field research reported here was performed using the required permit (SISBIO No. 34882, No. 432793).

Edmundoa lindenii (Regel) Leme (Bromeliaceae - Bromelioideae) is a terrestrial, saxicolous, or epiphyte herb, endemic to the Atlantic Forest in south and southeastern Brazil (*Martinelli et al., 2008; BFG, 2015*). In the study area, this species flowered between December and February, and produced fruits between March and April, and its flowers were visited by bees and hummingbirds (*R.L.B. Leal, pers. obs.*). The study was carried out in four reproductive events (from 2014 to 2018).

Floral biology

We obtained the number of individuals with open flowers in December (n = 10 individuals), January (n = 74 individuals), and February (n = 24 individuals). We measured inflorescences of *E. lindenii* (n = 16 individuals) directly in the field with a pachymeter, considering the following traits: scape length, inflorescence diameter, and bract length. Flowers (n = 73) from 28 individuals were collected in the field, stored in 70% alcohol, and measured in the laboratory with a pachymeter considering the following structures: corolla tube length (i.e., from septal nectary to the opening of the corolla) and the width of the corolla tube opening. We counted the number of ovules in 25 flowers (n = 15 individuals).

To analyse the colour quantitatively, we measured the spectral reflectance of petals, sepals, and bracts. For this, 12 flowers (n = 6 individuals) were collected in the field, stored in thermal bags containing moist paper, and brought to the laboratory, where they were immediately measured (*Lunau et al., 2011*). We measured the reflectance using an USB2000

spectrophotometer (OceanOptics, Inc., Dunedin, FL, USA) coupled with a deuterium–halogen light source (DH-2000; OceanOptics, Inc., Ostfildern, Germany), with a light emission range between 215 and 1700 nm. We took all reflectance measurements at a 45° angle in relation to the plant structure, and we used barium sulphate as the white standard and black paper as the black standard (*Chittka & Kevan, 2005*).

We used the logarithm version of the receptor noise-limited model to compare the colours of the petals, sepals, and bracts from the bee and hummingbird subjective view, since it suits tri- and tetrachromatic colour vision systems (*Vorobyev & Osorio, 1998*). The chromatic distances were calculated with the model of Vorobyev. We modelled spectral sensitivity curves using data from *Sephanoides sephaniodes* (*Herrera et al., 2008*) to estimate hummingbird colour distances, and from *Bombus terrestris* for bees *(Telles & Rodriguéz-Gironés, 2015*). In all cases, we used standard daylight illumination (D65 – *Wyszecki & Stiles, 1982*). Using these models, we determined the spectral location of each structure in a colour space for each pollinator.

The distance between two points in a colour space provides an approximation of the perceived colour difference (*Endler & Mielke, 2005*). We evaluated colour distances between sepals, petals, and bracts. Using the receptor noise-limited model, we estimated that two colours were discriminable if their distance was greater than 0.27 units for bees (*Telles & Rodríguez-Gironés, 2015*) and 1.0 for hummingbirds (*Vorobyev et al., 1998*). For representation, we also calculated the colour loci of the flower colours in the respective colour space models: the colour hexagon for bees (Chittka 1992) and the colour tetrahedron for hummingbirds (*Vorobyev et al., 1998*).

Nectar

We measured the nectar volume in flowers previously bagged in bud stage, with a graduated microliter syringe (Hamilton, Nevada, USA), and the concentration with hand-held refractometer (Bellingham + Stanley Eclipse, UK). To evaluate the nectar production along time of anthesis, 36 flowers (n = 10 individuals) previously bagged at the bud stage were measured every one hour and half intervals after the onset of anthesis. In total, we performed measurements at four different times of the day (7:00, 8:30, 10:00, and 11:30). To evaluate if the removal of the nectar stimulates the secretion, 24 flowers (n = 6 individuals) were submitted to four removal treatments (R = no removal, R1 = one removal, R2 = two removals, and R3 = three removals).

We calculated the total amount of sugar (mg) per flower by multiplying nectar volume (μL) by its corrected concentration (mg/μL) according to *Dafni et al.* (*2005*).

Breeding system and pollen limitation

We evaluated the breeding system and the pollen limitation (PL) through manual pollination treatments. For this, floral buds of different individuals were previously bagged with "voile" bags, and the flowers submitted to the following treatments: spontaneous self-pollination (n = 20 flowers were bagged and not manipulated); manual self-pollination (49 flowers were supplemented manually with pollen from the same flower and bagged); cross-pollination (130 flowers were supplemented with pollen from different individuals, located at 10 m, and bagged). A total of 131 flowers not bagged were marked and maintained under natural conditions, of which 49 were marked in 2016, 20 were marked in 2017, and 62 were marked in 2018. At the end of treatments, the number of seeds produced per fruit was evaluated. We assessed the self-incompatibility by the index of incompatibility (ISI) based on *Zapata and Arroyo* (*1978*). According to this index, species with ISI > 0.30 are classified as self-incompatible (*Ramirez & Brito, 1990*). We calculated the index of PL for each year according to *Larson and Barrett* (*2000*). Values of IPL ≥ 0.20 indicate absence of PL, whereas values above 0.80 indicate a strong PL (*Freitas et al., 2010*).

Frequency and efficacy of floral visitors

In order to evaluate the identity of floral visitors and their frequency of visits, we performed focal observations (sensu *Dafni, 1992*), by censuses of 30-min per individual (n = 143 individuals) between 6:00 and 12:00, totalizing 184 hours of observation in the four reproductive events (in the years: **2015** = 43.5h; **2016** = 39.0h; **2017** = 51.0h; and **2018** = 50.5h). Images and videos were captured during the visits to evaluate the foraging behaviour and the floral resources obtained. The visits were identified as legitimate or illegitimate by the expected mode of pollination, considering the shape and arrangement of the flower parts (sensu *Irwin et al., 2010, Freitas, 2018*). Specimens of insects were collected for posterior identification. We grouped the floral visitors into three functional groups based on body size and foraging behaviour, as following: large bees, small bees, and hummingbirds.

The efficacy of the different groups of pollinators was evaluated through experiments of selective exposition, in which flowers previously bagged at the bud stage were exposed to a single visit, then marked and bagged. The number of flowers exposed to visitors of the three functional groups was 48 flowers to small bees in 2016, 20 flowers to hummingbirds and 20 flowers to large bees in 2017, and 65 flowers to hummingbirds and 60 flowers to large bees in 2018. *Edmundoa lindenii* is parthenocarpic, which means that flowers develop into fruits independent of pollination; therefore, the measure of efficacy was estimated for each treatment (i.e., pollinator group) from the product between the proportion of fruits with seeds formed after visits by each pollinator group and the number of seeds in each fruit.

Data analyses

We evaluated the production of nectar along the anthesis and the effect of nectar removal in nectar secretion by analyses of variance (one-way ANOVA), using the function *aov*. We assessed the differences between treatments (time of anthesis and number of removals) by Tukey HSD post-hoc test, using the function *TukeyHSD*.

To evaluate whether hummingbirds and large bees differ in their efficacy, we conducted a linear mixed-effects model. For this, we used the functional group of pollinators (two levels: hummingbirds and large bees) as fixed effects, and the year when the treatments were conducted (two levels: 2017 and 2018) as random intercept terms. We fitted all the linear mixed-effects models using the *lmer* function from the lme4 package (*Bates et al., 2015*). We calculated the significance of each term in the model using the function *anova* from the *lmer* package (*Kuznetsova et al., 2017*) and the differences between levels of categorical factors using the *lsmeans* package (*Lenth, 2016)*. We did not compare the efficacy of small bees as no seeds were produced after their visits.

To evaluate the association between functional pollinator groups (hummingbirds and large bees) and the reproductive events (in the years 2015, 2016, 2017, and 2018), we conducted a chi-squared test using the function *chi.test* (2 X 4 contingency table). We performed all the analyses in R version 3.4.4 (*R Development Core Team, 2016*).

RESULTS

Floral biology

The flowers of *E. lindenii* are grouped in a compound corymboid inflorescence with 100–150 flowers, inserted in the leaf rosette (Fig. 1). Inflorescence diameter reached 121.32 ± 17.01 mm and scape length 296.87 ± 23.86 mm (mean ± SD throughout the text). The flowers are hermaphrodite, with the androecium presenting six stamens included in the corolla and anthers with longitudinal dehiscence (Fig. 1). The gynoecium is also included in the corolla and the style ends in a three-lobed stigma (Fig. 1). The inferior and trilocular ovary contains 197.9 ± 54.12 ovules. The length of bracts and sepals was 55.15 ± 6.99 mm and 26.0 ± 4.0 mm, respectively. The corolla is tubular (length: 17.95 ± 2.92 mm) with a narrow opening (3.11 ± 1.17 mm). The flowers have diurnal anthesis (*ca.* 06:00-12:00h), characterized by the presence of exposed pollen grains and receptive stigma. The production of nectar starts in the beginning of anthesis and does not increase over time ($F = 0.338$; $df = 3$; $p = 0.798$; Fig 3A). However, the removal of nectar stimulated new secretion ($F = 6.632$, $df = 3$, $p = 0.00273$, Fig. 3B).

The bracts reflect red wavelengths, whereas the corolla is UV-reflecting white, and the sepals are UV-absorbing white (Fig. 2). The colour of petals, sepals, and bracts, as well as open or closed flowers, is distinguishable by bees and hummingbirds. Flower colour was 1–7 times above the discrimination criteria (0.27) for bee vision (petals-sepals 4 ± 1, bracts-sepals 4 ± 2) and 5–15 times above the discrimination criteria (1.0) for hummingbirds (petals-sepals 8 ± 2, bracts-sepals 12 ± 4, bracts-petals 15 ± 7).

Breeding system and pollen limitation

Edmundoa lindenii is self-incompatible (ISI = 0.08; Table 1) and parthenocarpic (Table 1), requiring the action of pollinators for sexual reproduction. The PL index varied between years, with PL only occurring in the reproductive event of 2017 (2016 = -0.07, 2017 = 0.70, 2018 = -0.03).

Floral visitors and temporal variation

The flowers of *E. lindenii* were visited by 11 species of animals belonging to three functional groups (hummingbirds, large bees, and small bees). Hummingbirds were the group with the highest species richness (Table 2; Fig.1). All of animals that approached the flowers, only the small bee *T. spinipes* conducted illegitimate visits, resulting in damage of corolla and/or anthers

by chewing. Hummingbirds and large bees foraged for nectar acting as legitimate visitors, and small bees collected pollen.

Hummingbirds had greater effectiveness than large bees (contrast: $t = 7.708$, $df = 74$, $p < 0.001$; Fig. 4; Table 3), whereas small bees did not acted as pollinators, as no flowers visited by them produced fruit with seeds. The frequency of floral visits by each functional group varied among the reproductive events (Fig. 5). The variation between years was more pronounced for large bees and small bees than for the hummingbirds, whose frequency decreased in 2017 (Fig. 5). Overall, hummingbirds were more effective than large bees (Table 3). However, variations in both cumulative and relative frequency of visits by each group-between the reproductive events were remarkable (Fig. 5), and this was reflected in the values of the effectiveness of hummingbirds and large bees each year (Table 3). Chi-square analysis indicated an association between the frequency of the groups of pollinators and the reproductive events of each year ($\chi^2 = 70.356$, $df = 3$, $p < 0.010$).

DISCUSSION

Through observational and experimental approaches, we have shown that the frequency of functional groups of flower visitors (hummingbirds, large bees, and small bees) varied between reproductive events of *E. lindenii*, and this influenced the plant's reproductive success. Among the three functional groups of visitors, hummingbirds and large bees acted as pollinators, with hummingbirds exhibiting greater efficacy than large bees. The existence of year-to-year changes in the composition of floral visitor species has been found in several systems (*Schemske & Horvitz, 1984; Traveset & Sáez, 1997; Price et al., 2005; Olesen et al., 2008; Petanidou et al., 2008*), while in others, pollinator efficacy between different years was studied (*Fishbein & Venable, 1996; Stoepler et al., 2012*). However, there are fewer studies that consider the relationship between variations in pollinator assemblages and plant reproductive success in different reproductive seasons (*Herrera, 1990; Fleming et al.,2001; Salas-Arcos et al., 2017*). Our results allow us to conclude that a generalist species can suffer or not PL in different reproductive events, in response to variations in the pollinator assemblage.

The occurrence of PL is a common phenomenon in angiosperms (*Larson & Barrett 2000, Bennett et al. 2018*) and is modulated by several factors, such as floral attributes, environmental conditions, and population demography (*Ashman et al., 2004*). Generalization in pollination is

expected to alleviate PL, because having different pollen vectors may buffer fertility reduction associated with unpredictability of a certain pollinator between years. Accordingly, more generalized plants are less prone to show PL in the Brazilian Atlantic Forest (*Wolowski, Ashman & Freitas, 2014)*. However, pollinators belonging to different functional groups may vary in their pollination effectiveness (e.g., *Dar, Arizmendi & Valiente-Banuet, 2006; Ávila & Freitas, 2011*), so pollinator shifts among years may lead to different levels of reproductive success. The occurrence of PL was recorded in *E. lindenii* only in the year in which there was a strong decrease in the frequency of visits by hummingbirds, when large bees were the most frequent visitors. Thus, the effectiveness of large bees alone was not enough to achieve the potential seed set fully. The evolution of a generalized pollination system is expected when different pollinators play the same role as selective agents (*Gómez & Zamora, 2006*). However, generalization may also be favoured when less efficacious pollinators provide reproductive assurance, sustaining fluctuations of the most effective pollinators, such as could be the case for large bees and *E. lindenii*. Lastly, combined measurements of PL and pollinator assemblages along time and space is an interesting approach to evaluate the effects of variable pollination environments (see *Gómez & Zamora, 2006* for other suggestions in this regard).

Pollinators identify and select flowers using a variety of characteristics, including size and contrast of colouring, which serve as a guide for floral visitors (*Papiorek et al., 2016*). The attributes measured in *E. lindenii* were detectable and allowed access to resources by both pollinators. Petals had UV reflection, sepals absorbed UV, and bracts were red. These results correspond to the expected pattern for attraction of bees and hummingbirds, as bees have a spectrum of vision that includes UV wavelengths, around 300–400 nm (*Kevan et al 2001*), and hummingbirds are known for their preference for red-coloured flowers that mostly are UV-absorbent (*Lunau et al. 2011*). Trade-offs between selective pressures exerted by different pollinators could occur if they differ in preference for floral traits (*Gervasi & Schiestl 2017*). However, fluctuation in the frequency between reproductive events may reduce the probability of pollinators exerting consistent selective pressures on the floral traits of the plants, suppressing plant specialization towards a pollinator type (*Schemske & Horvitz, 1984; Gómez & Zamora, 2006*). The same condition may be favoured by repeated visits to the same flower by different pollinators, and this is consistent with the nectar secretion pattern of *E. lindenii* where, although nectar production did not increase during anthesis, nectar removal stimulated its secretion.

The mechanisms of self-incompatibility in plants are complex and diverse in their physiological, morphological, biochemical, and genetic aspects (*Nettancourt, 2001*). The population of *E. lindenii* at PARNASO was self-incompatible, but self-compatibility has been registered in other populations of this species (*Matallana et al., 2010*). Variations between self-incompatibility and self-compatibility within species are common in plant evolution and may indicate transitions between reproductive systems (*Igic, Lande & Kohn, 2008*). Studies have shown that compatibility barriers can be broken by genetic changes (such as mutations) (e.g., *Sassa et al., 1997*), physiological factors, elevated temperatures, and stress (e.g., *Tazuka et al., 1997*), allowing for self-pollination. Moreover, breeding systems may be related to the degree of pollination generalization, linking shifts in pollination and incompatibility systems. For instance, Wessinger and Kelly (*2018*) found a relationship between self-compatibility and attributes related to the attraction of hummingbirds, including red flowers and loss of floral aroma and UV-absorbing pigments. In *E. lindenii*, self-incompatibility served as a barrier to autogamous pollination, since our records indicate that small bees access the anthers, make long visits to the flower, and manipulate the pollen. In fact, pollinators usually do not operate independently of herbivores (florivores in this case), which may generate a trade-off between the fitness functions by each kind of organism (*Ashman, 2002; Gómez & Zamorra, 2006; Gélvez-Zúñiga et al., 2018*).

This paper contributes to our knowledge about variable pollination environments, which may lead to generalization of pollination systems. The factors that influence the temporal variation in pollinator effectiveness are not as well understood, and consequently, cannot yet be predicted. After conducting a temporal analysis, we were able to establish the importance of the secondary pollinators for reproductive assurance in a generalist species. This is a starting point toward a better understanding of the ecological processes that drive the evolution of generalist pollination systems.

CONCLUSION

Our results allow us to conclude that a generalist species can suffer or not PL in different reproductive events, in response to variations in the pollinator assemblage. Although the evolution of a generalized pollination system is expected when different pollinators play the same role as selective agents, our results support that generalization may also be favoured when

pollinators with lesser efficacy provide reproductive assurance, lightening fluctuations of the most effective pollinators, such as could be the case for large bees and *E. lindenii*.

ACKNOWLEDGMENTS

The authors thank Gabriel C. Rocha for field and data analysing assistance, and the staff of the Serra dos Órgãos National Park for logistics support and for allowing access to Park facilities; the authors thank Cristovão Albuquerque (Publicase, Brazil) for language editing.

REFERENCES

Amorim FW, 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* 5(2): 317-327.

Armbruster S, Fenster C, Dudash M. 2000. Pollination 'principles' revisited: specialization, pollination syndromes, and the evolution of flowers. In: Totland Ø, ed. *The Scandinavian Association for Pollination Ecology honours Knut Fægri*. Oslo: Det Norske Videnskaps-Akademi, 179-200.

Ashman, T. (2002. The role of herbivores in the evolution of separate sexes from hermaphroditism. *Ecology* 83: 1175 1184.

Ashman T-L, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, 85:2408-2421. DOI:10.1890/03-8024

Ávila Jr. RS, 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. DOI: 10.1071/bt10280

Bates D, Maechler M, Bolker B, Walker S. 2015. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1–7. 2014.

BFG. 2015. Growing knowledge: an overview of seed plant diversity in Brazil. Rodriguésia 66: 1085-1113.

Bennett JM, Steets JA, Burns JH, Durka W, Vamosi JC, Arceo-Gomez G, Burd M, Burkle LA, Ellis AG, Freitas L, Li J, Rodger JG, Wolowski M, Xia J, Ashman T, Knight TM. 2018. GloPL, Global data base on pollen limitation of plant reproduction.

Castellanos MC, Wilson P, Thomson JD. 2003. Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution* 57: 2742-2752. DOI:10.1111/j.0014-3820.2003.tb01516.x

Chittka L. Kevan PG. 2005. Flower colour as advertisement. In Dafni A, Kevan PG, Husband BC, eds. *Practical Pollination Biology*. Cambridge, Canada: Enviroquest, 157–196.

Dar S, Arizmendi MDC, Valiente-Banuet A. 2006. Diurnal and nocturnal pollination of *Marginatocereus marginatus* (Pachycereeae: Cactaceae) in Central Mexico. *Annals of Botany* 97(3): 423-427. DOI: 10.1093/aob/mcj045

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35: 375-403. DOI: 10.1146/annurev.ecolsys.34.011802.132347

Fenster CB, Dudash MR. 2001. Spatiotemporal variation in the role of hummingbirds as pollinators of *Silene virginica*. *Ecology* 82(3): 844-851.

Fishbein M, Venable DL. 1996. Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology* 77*(*4): 1061-1073. DOI: 10.2307/2265576

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(4), 511-530.

Freitas L. 2013. Concepts of pollinator performance: is a simple approach necessary to achieve a standardized terminology?*Brazilian Journal of Botany*36: 3-8. DOI: 10.1007/s40415-013-0005-6

Freitas L. 2018. Precisamos falar sobre o uso impróprio de recursos florais. *Rodriguésia* .

Freitas L, Sazima M. 2006. Pollination biology in a tropical high-altitude grassland in Brazil: interactions at the community level. *Annals of the Missouri Botanical Garden* 93: 465-516. DOI: 10.3417/0026-6493(2007)93[465:PBIATH]2.0.CO;2

Freitas L, Wolowski M, Sigiliano MI. 2010. Ocorrência de limitação polínica em plantas de Mata Atlântica. *Oecologia Australis* 14: 251-265.

Gélvez-Zúñiga I, Teixido AL, Neves AC, Fernandes GW. 2018. Floral antagonists counteract pollinator-mediated selection on attractiveness traits in the hummingbird-pollinated Collaea cipoensis (Fabaceae). *Biotropica* 50: 797-804. DOI:10.1111/btp.12574

Gervasi DD, Schiestl FP. 2017. Real-time divergent evolution in plants driven by pollinators. *Nature Communications* 8, 14691. DOI: 10.1038/ncomms14691

Gómez JM, Zamora R. 2006. Ecological factors that promote the evolution of generalization in pollination systems. In Waser N, Ollerton J. eds. *Plant-pollinator interactions: from specialization to generalization.* Chicago: University of Chicago Press, 145-165.

Herrera CM. 1987. Components of pollinator "quality": comparative analysis of a diverse insect assemblage. *Oikos* 50: 79-90. DOI: 10.2307/3565403

Herrera CM. 1988. Variation in mutualisms - the spatio-temporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society* 35: 95-125. DOI: 10.1111/j.1095-8312.1988.tb00461.x

Herrera CM. (1990). "Daily Patterns of Pollinator Activity, Differential Pollinating Effectiveness, and Floral Resource Availability, in a Summer-Flowering Mediterranean Shrub." *Oikos* 58(3): 277-288.

Herrera CM. 1996. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In Lloyd DG., Barrett SCH. eds. *Floral biology: studies on floral evolution in animal-pollinated plants*. New York: Chapman & Hall, 65-87.

Herrera G, Zagal JC, Diaz M, Fernández MJ, Vielma A, Cure M, Martinez J, Bozinovic F, Palacios AG. 2008. Spectral sensitivities of photoreceptors and their role in colour discrimination in the green-backed firecrown hummingbird (*Sephanoides sephaniodes*). *Journal of Comparative Physiology A* 194(9): 785-794. DOI: 10.1007/s00359-008-0349-8.

Igic B, Lande R, Kohn JR. 2008. Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences* 169(1): 93-104. DOI: 10.1086/523362

Ivey CT, Martinez P, Wyatt R. 2003. Variation in pollinator effectiveness in swamp milkweed, *Asclepias incarnata* (Apocynaceae). *American Journal of Botany* 90(2): 214-225.

Irwin RE, Bronstein JL, Manson JS, Richardson L. 2010. Nectar robbing: ecological and evolutionary perspectives. *Annual Review of Ecology, Evolution, and Systematics* 41: 271-292.

Kevan PG, Chittka L, Dyer AG. 2001. Limits to the salience of ultraviolet: lessons from colour vision in bees and birds. *Journal of Experimental Biology 204*(14), 2571-2580.

Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman T. 2005. Pollen limitation of plant reproduction: pattern and

process. *Annual Review of Ecology, Evolution, and Systematics* 36: 467-497. DOI: 10.1146/annurev.ecolsys.36.102403.115320

Koski MH, Ison JL, Padilla A, Pham AQ, and Galloway LF. 2018. Linking pollinator efficiency to patterns of pollen limitation: small bees exploit the plant–pollinator mutualism.*Proceedings of the Royal Society B: Biological Sciences* 285(1880): 20180635. DOI: 10.1098/rspb.2018.0635

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. Lmer Test package: tests in linear mixed effects models. *Journal of Statistical Software* 82(13): 1–26. DOI: 10.18637/jss.v082.i13

Larson BM, Barrett SC. 2000. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* 69(4): 503-520. DOI: .1111/j.1095-8312.2000.tb01221.x

Larsson M. 2005. Higher pollinator effectiveness by specialist than generalist flower-visitors of unspecialized *Knautia arvensis* (Dipsacaceae). *Oecologia* 146: 394-403. DOI: 10.1007/s00442-005-0217-y

Lenth RV. 2016. Least-squares means: the R package lsmeans. *Journal of Statistical software* 69(1): 1-33. DOI: 10.18637/jss.v069.i01

Lunau K, Papiorek S, Eltz T, Sazima M. 2011. Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *Journal of Experimental Biology* 214(9): 1607-1612. DOI: 10.1242/jeb.052688

Manning JC, Goldblatt P. 2005. Radiation of pollination systems in the Cape genus *Tritoniopsis* (Iridaceae: Crocoideae) and the development of bimodal pollination strategies. *International Journal of Plant Sciences* 166: 459-474. DOI: 10.1086/428703

Martinelli G, Vieira CM, Gonzalez M, Leitman P, Piratininga A, Costa AF, Forzza RC 2008. Bromeliaceae da Mata Atlântica brasileira: lista de espécies, distribuição e conservação. *Rodriguésia* 59(1): 209-258.

Matallana G, Godinho MAS, Guilherme FAG, Belisario M, Coser TS, Wendt T. 2010. Breeding systems of Bromeliaceae species: evolution of selfing in the context of sympatric occurrence. *Plant Systematics and Evolution* 289(1-2): 57-65. DOI: 10.1007/s00606-010-0332-z

Muchhala N, Caiza A, Vizuete JC, Thomson JD. 2009. A generalized pollination system in the tropics: bats, birds and *Aphelandra acanthus*. *Annals of Botany* 103(9): 1481-1487.

462 Nettancourt D. 2001. *Incompatibility and incongruity in wild and cultivated plants*. Berlin:
463 Springer. 322. DOI: 10.1007/978-3-662-04502-2
464 Olesen JM, Bascompte J, Elberling H, Jordano P. 2008. Temporal dynamics in a pollination
465 network. *Ecology* 89(6): 1573-1582. DOI: 10.1890/07-0451.1
466 Ollerton J, Killick A, Lamborn E, Watts S, Whiston M. 2007. Multiple meanings and modes: on
467 the many ways to be a generalist flower. *Taxon* 56: 717-728.
468 Ollerton J. 2017. Pollinator diversity: distribution, ecological function, and conservation. *Annual*
469 *Review of Ecology, Evolution, and Systematics* 48: 353-376. DOI: 10.1146/annurev-ecolsys-
470 110316-022919
471 Osorio D, Vorobyev M. 2008. A review of the evolution of animal colour vision and visual
472 communication signals. *Vision research* 48(20): 2042-2051.
473 Papiorek S, Junker RR, Alves-dos-Santos I, Melo GA, Amaral-Neto LP, Sazima M, Wolowski
474 M, Freitas L, Lunau K. 2016. Bees, birds and yellow flowers: pollinator-dependent
475 convergent evolution of UV patterns. *Plant Biology* 18(1): 46-55. DOI: 10.1111/plb.12322
476 Petanidou T, Kallimanis AS, Tzanopoulos J., Sgardelis SP, Pantis JD. 2008. Long-term
477 observation of a pollination network: fluctuation in species and interactions, relative
478 invariance of network structure and implications for estimates of specialization. *Ecology*
479 *Letters* 11(6): 564-575. DOI: 10.1111/j.1461-0248.2008.01170.x
480 Price MV, Waser NM, Irwin RE, Campbell DR, Brody AK. 2005. Temporal and spatial
481 variation in pollination of a montane herb: a seven-year study. *Ecology* 86(8): 2106-2116.
482 DOI: 10.1890/04-1274
483 Ramirez N, Brito Y. 1990. Reproductive biology of a tropical palm swamp community in the
484 Venezuelan llanos. *American Journal of Botany* 77(10): 1260-1271. DOI: 10.1002/j.1537-
485 2197.1990.tb11378.x
486 Salas-Arcos L, Lara C, Ornelas JF. 2017. Reproductive biology and nectar secretion dynamics of
487 *Penstemon gentianoides* (Plantaginaceae): a perennial herb with a mixed pollination system?
488 *PeerJ* 5: e3636. DOI: 10.7717/peerj.3636
489 Sassa H, Hirano H, Nishio T, Koba T. 1997. Style-specific self-compatible mutation caused by
490 deletion of the S-RNase gene in Japanese pear (*Pyrus serotina*). *The Plant Journal* 12(1):
491 223-227. DOI: 10.1046/j.1365-313X.1997.12010223.x

492 Schemske DW, Horvitz CC. 1984. Variation among floral visitors in pollination ability: a
493 precondition for mutualism specialization. *Science* 225(4661): 519-521. DOI:
494 10.1126/science.225.4661.519
495 Scrok GJ, Varassin IG. 2011. Reproductive biology and pollination of *Aechmea distichantha*
496 Lem. (Bromeliaceae). *Acta Botanica Brasilica* 25(3): 571-576.
497 Shuttleworth A, Johnson SD. 2008. Bimodal pollination by wasps and beetles in the African
498 milkweed *Xysmalobium undulatum*. *Biotropica* 40: 568-574. DOI: 10.1111/j.1744-
499 7429.2008.00418.x
500 Skorupski P, Chittka L. 2010. Differences in photoreceptor processing speed for chromatic and
501 achromatic vision in the bumblebee, *Bombus terrestris*. *Journal of Neuroscience* 30(11):
502 3896-3903. DOI: 10.1523/JNEUROSCI.5700-09.2010
503 Stebbins GL. 1970. Adaptive radiation of reproductive characteristics in angiosperms, I:
504 Pollination mechanisms. *Annual Review of Ecology and Systematics* 1: 307-326. DOI:
505 10.1146/annurev.es.01.110170.001515
506 Stoepler TM, Edge A, Steel A, O'Quinn RL, Fishbein M. 2012. Differential pollinator
507 effectiveness and importance in a milkweed (*Asclepias*, Apocynaceae) hybrid zone.
508 *American Journal of Botany* 99(3): 448-458. DOI: 10.3732/ajb.1100272
509 Team, R. C. 2016. Vienna: R Foundation for Statistical Computing, 2016. *R: A language and*
510 *environment for statistical computing*.
511 Telles, F. J., & Rodríguez-Gironés, M. A. 2015. Insect vision models under scrutiny: what
512 bumblebees (Bombus terrestris terrestris L.) can still tell us. The Science of Nature, 102(1-
513 2), 4.
514 Tezuka T, Tsuruhara A, Suzuki H, Takahashi SY. 1997. A connection between the self-
515 incompatibility mechanism and the stress response in lily. *Plant and Cell Physiology* 38(2):
516 107-112. DOI: 10.1093/oxfordjournals.pcp.a029139
517 Thompson JD. 2001. How do visitation patterns vary among pollinators in relation to floral
518 display and floral design in a generalist pollination system? *Oecologia* 126(3): 386-394.
519 Traveset A, Sáez E. 1997. Pollination of *Euphorbia dendroides* by lizards and insects: spatio-
520 temporal variation in patterns of flower visitation. *Oecologia* 111(2): 241-248. DOI:
521 10.1007/PL00008816

Waser NM, Price MV. 1990. Pollination efficiency and effectiveness of bumble bees and hummingbirds visiting *Delphinium nelsonii*. *Collectanea Botanica* 19: 9-20.

Waser NM, Chittka L, Price MV, Williams NM, and Ollerton J. 1996. Generalization in pollination systems, and why it matters. *Ecology* 77: 1043-1060. DOI: 10.2307/2265575

Wessinger CA, Kelly JK. 2018. Selfing can facilitate transitions between pollination syndromes. *American Naturalist* 191(5): 582-594. DOI: 10.1086/696856

Wiggam S, Ferguson CJ. 2005. Pollinator importance and temporal variation in a population of *Phlox divaricata* L. (Polemoniaceae). *American Midland Naturalist* 154(1): 42-54. DOI: 10.1674/0003-0031(2005)154[0042:PIATVI]2.0.CO;2

Wolowski M, Ashman T-L, Freitas L. 2014. Meta-analysis of pollen limitation reveals the relevance of pollination generalization in the Atlantic forest of Brazil. *PLoS One* 9(2): e89498. DOI: 10.1371/journal.pone.0089498

Zapata TR, Arroyo MTK. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica* 10(3): 221-230. DOI: 10.2307/2387907

Zych M, Junker RR, Nepi M, Stpiczyńska M, Stolarska B, Roguz K. 2018. Spatiotemporal variation in the pollination systems of a supergeneralist plant: is *Angelica sylvestris* (Apiaceae) locally adapted to its most effective pollinators? *Annals of Botany* [Epub [Epub ahead of print]

Figure 1

Edmundoa lindenii is visited by three functional groups:hummingbirds, large bees, and small bees.

Examples of visitation by small bees – Trigona spinipes (A); large bees – Bombus morio (B); and hummingbirds – Amazilia fimbriata (C). All observations were made in the montane Atlantic Forest at Serra do Órgãos National Park southeastern Brazil in 2016 – 2018.

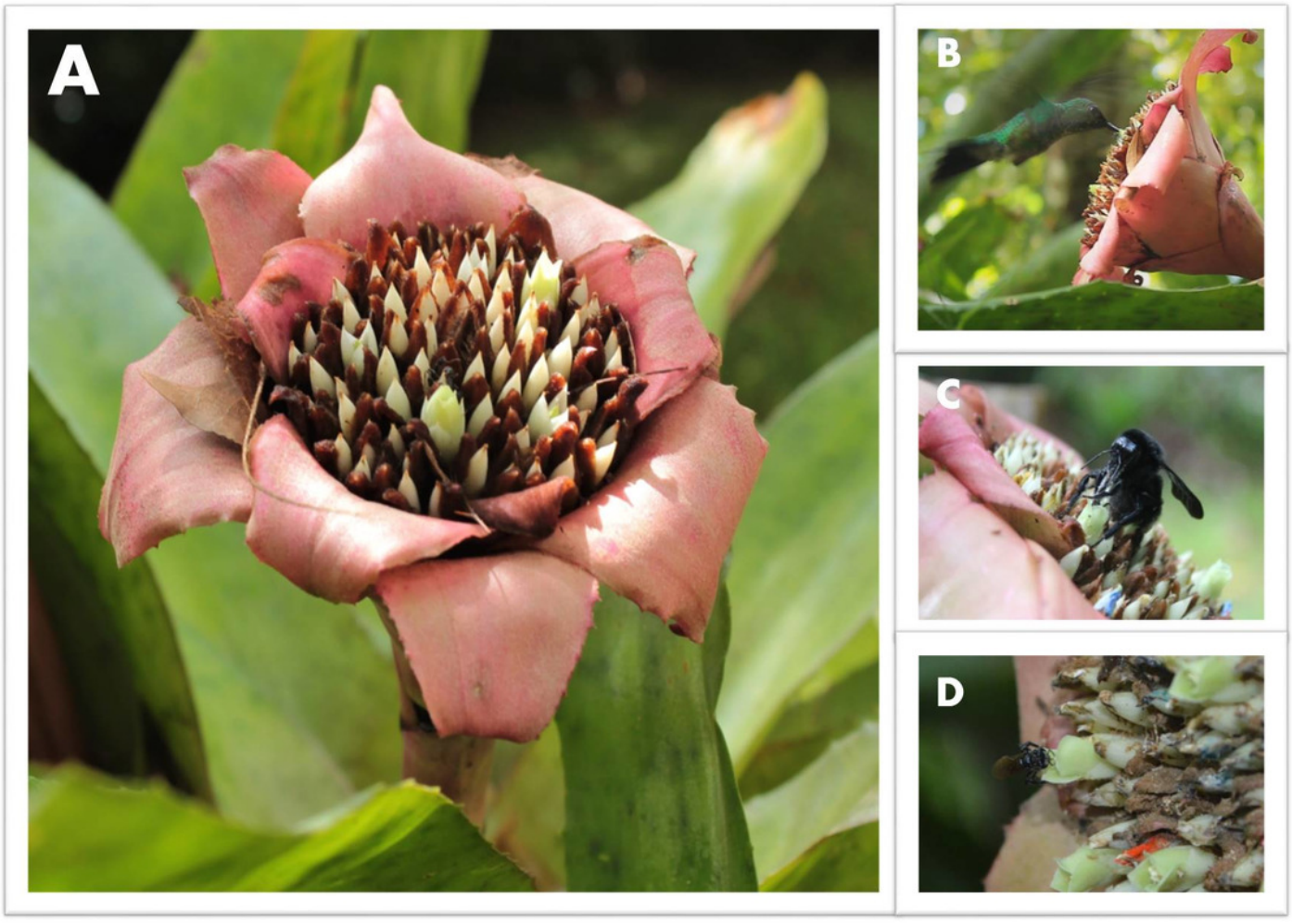

Figure 2

Nectar production by *Edmundoa lindenii* did not increase over anthesis time, but removalof nectar stimulated new secretion.

(A) Nectar production along the time of anthesis. (B) Nectar production after experimental removal of nectar (R = no removal, R1 = one removal, R2 = two removals, and R3 = three removals). For both boxplots, the thick horizontal line represents the median values, the upper and lower sides of the box represent the corresponding quartiles, and vertical lines are minimum and maximum values of the data range. Dots are outliers. Different letters indicate statistical significance between pairs of years ($p < 0.05$) by ANOVA post-hoc test (TukeyHSD).

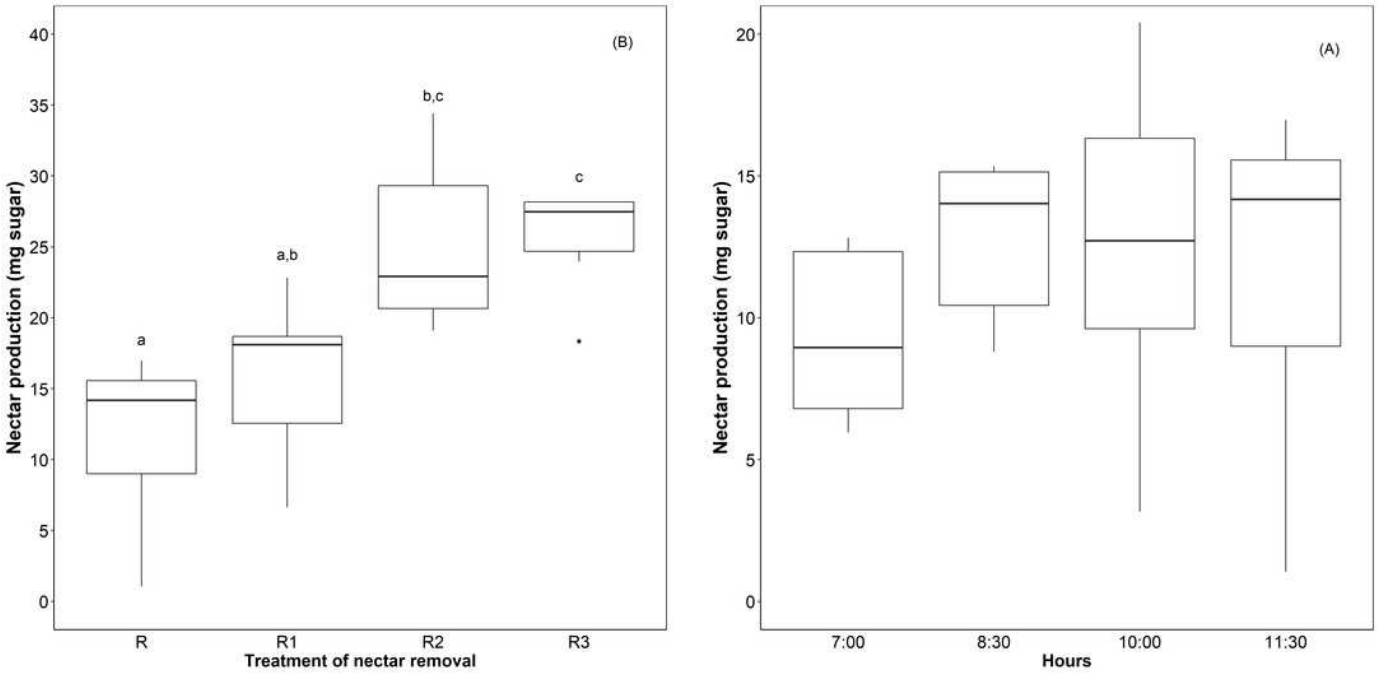

Figure 3

Attractive structures of *E. lindenii* include red bracts, UV- reflecting white petals, and UV-absorbing white sepals, and can be detected by bees and hummingbirds.

(A) Spectroscopic analysis of reflectance by typical attractive structures in *E. lindenii* inflorescences. For each structure, the coloured line represents the mean reflectance and the corresponding colour shading represents the standard deviation. In red, bract reflectance (n = 12 individuals); in blue, sepal reflectance; and in green petal reflectance (n= 12 flowers of 6 individuals for both petals and sepals). (B) Hexagon model for bee vision based on the photoreceptors of *Bombus terrestris*.(C)Tetrahedron model for bird vision based in the photoreceptors of *Sephanoides sephaniodes*. In both models, the gray point represents achromatic center, witch the red point represents mean loci for bracts, the purple point indicates the mean loci for sepals, and the blue point represents mean loci for bracts.

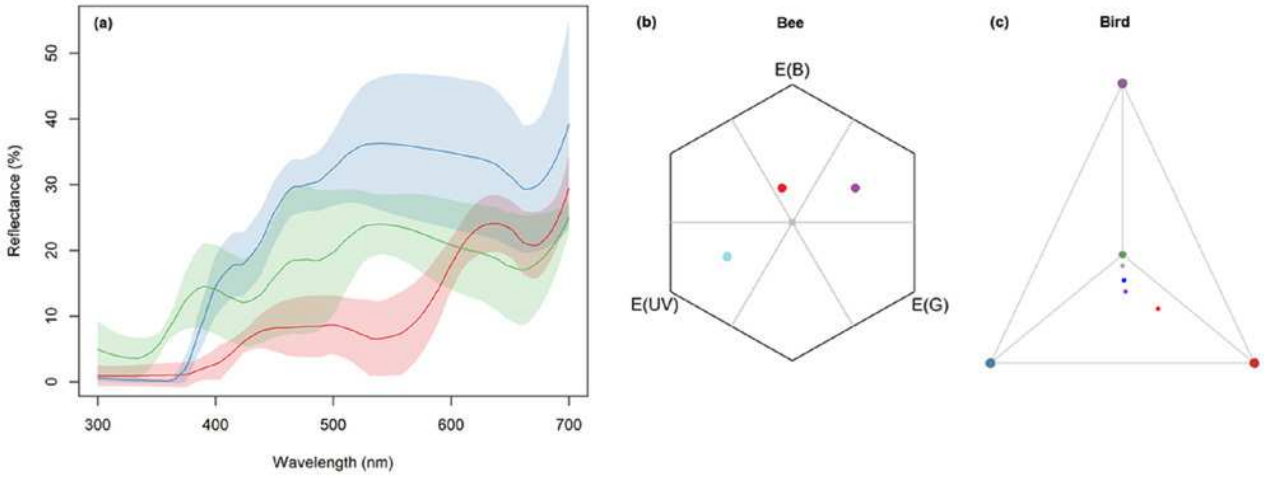

Figure 4

Hummingbirds have greater efficacy than large bees in two reproductive events.

(A) 2017 and (B) 2018. HB = hummingbirds, LB = large bees. Different letters show statistically significant difference ($p < 0.05$) of linear mixed effect model.

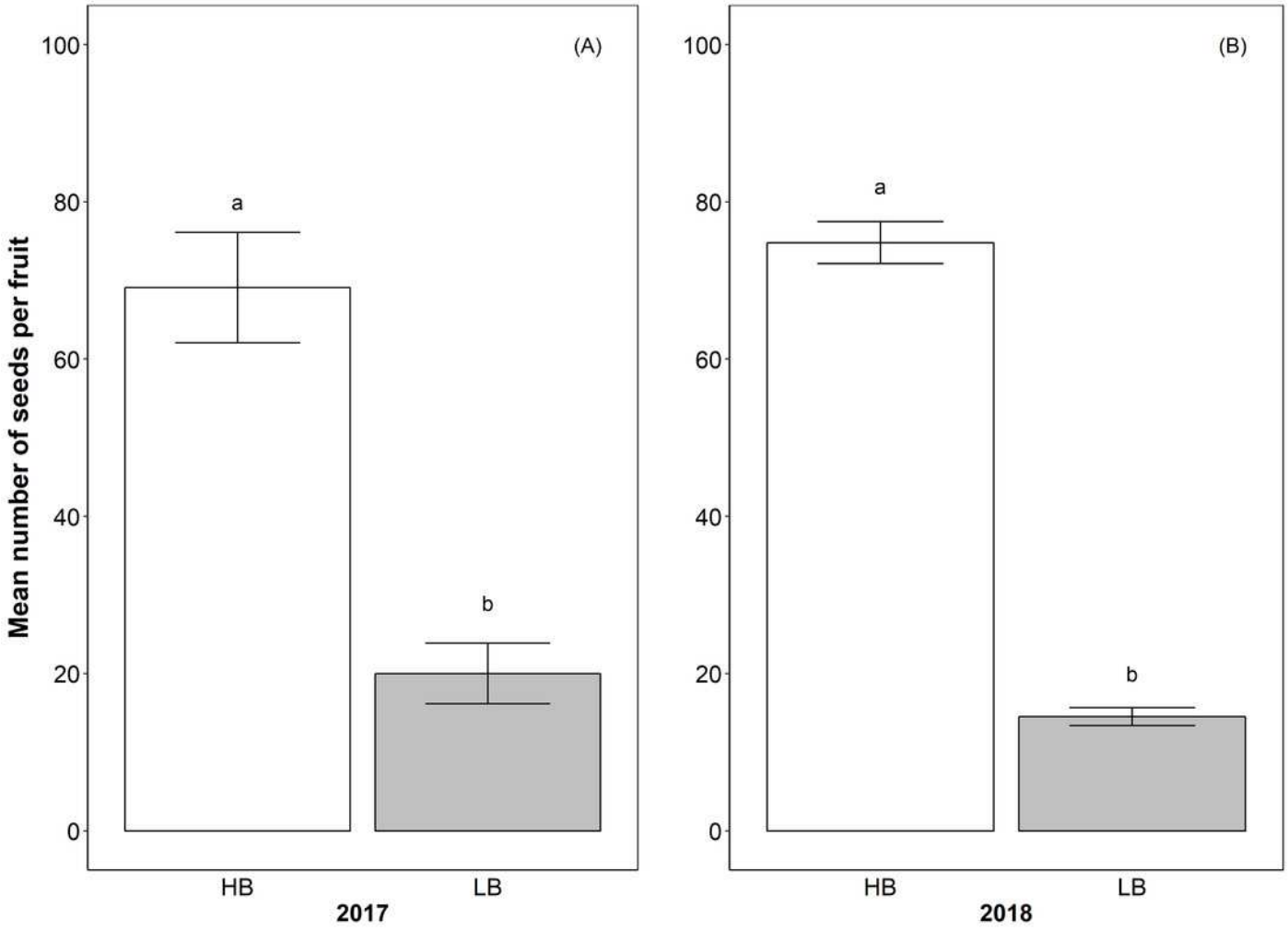

Figure 5

The two groups of pollinators displayed variation in the frequency of visits during reproductive events.

Visitation records were made throughout the flowering period in each reproductive event.

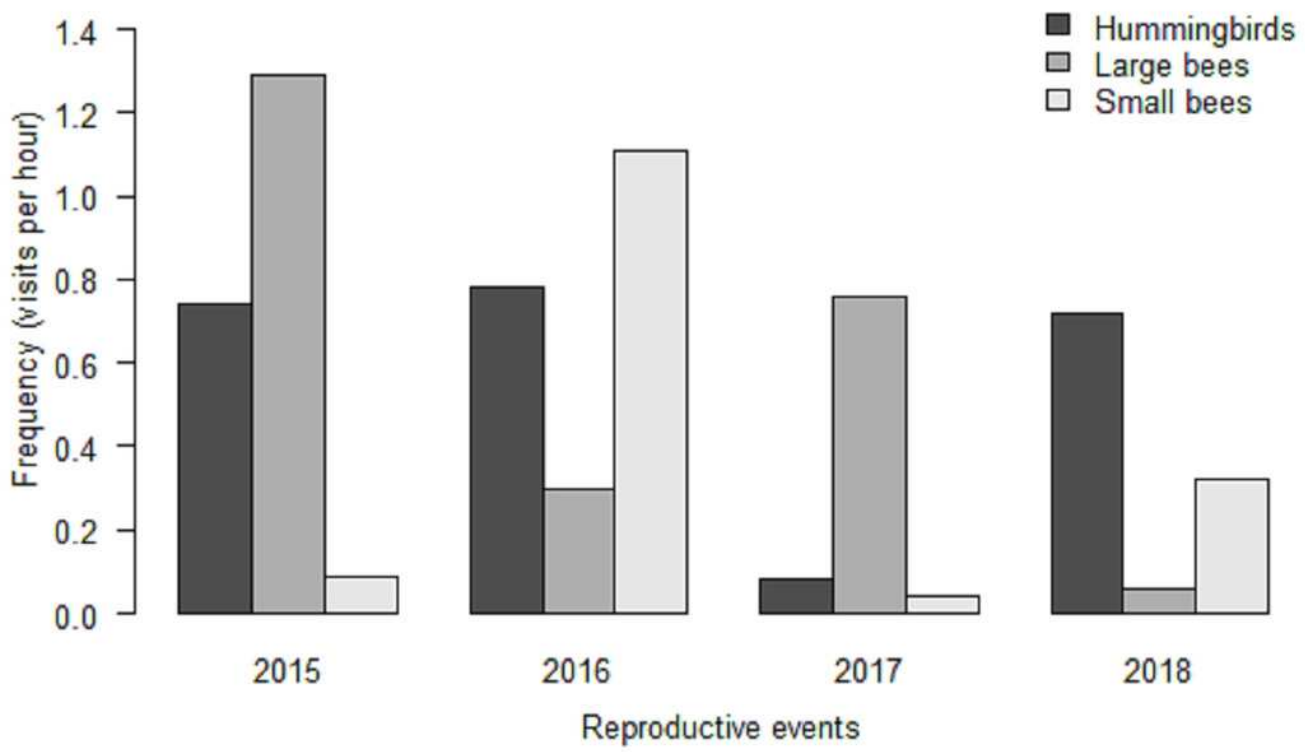

Table 1(on next page)

The population of *E. lindenii* in PARNASO is self-incompatible and parthenocarpic.

With cross-pollination and natural conditions, most flowers produced large amounts of fruit with seeds, whereas autonomous and self-pollination resulted in few fruits with seeds. All treatments were made in the montane Atlantic Forest at Serra do Órgãos National Park, southeastern Brazil.

Treatments	Fruits (n)	Fruits with seeds (n)	Seeds (mean ± sd)
Cross-pollination	130	127	116.10 ± 62.29
Hand self-pollination	49	4	10.29 ± 37.77
Autonomous	20	1	1.3 ± 5.81
Natural conditions	131	108	97.85 ± 75.50

1

2

Table 2(on next page)

In *Edmundoa lindenii* we registered 11 floral visitors belonging to three functional groups: HB = hummingbirds, LB = large bees, SB = small bees.

Rewards taken by the visitors: P = pollen, N = nectar. All records were made in the montane Atlantic Forest at Serra do Órgãos National Park in southeastern Brazil in 2016 – 2018.

Family	Species	Functional group	Resource
Trochilidae	*Amazilia lactea* (Lesson, 1832)	HB	N
	Amazilia versicolor (Vieillot, 1818)	HB	N
	Leucocholoris albicollis (Viellot, 1818)	HB	N
	Phaethornis eurynome (Lesson, 1832)	HB	N
	Ramphodon naevius (Dumont, 1818)	HB	N
	Thalurania glaucopis (Gmelin, 1788)	HB	N
	Amazilia fimbriata (Gmelin, 1788)	HB	N
Apidae	*Bombus morio* (Swederus, 1787)	LB	N / P
	Bombus brasiliensis (Lepeletier, 1835)	LB	N / P
	Euglossa sp.	LB	N
Apidae	*Trigona spinipes* (Fabricius,1793)	SB	P

1

Table 3(on next page)

Hummingbirdswere most effective in three of four reproductive events measured in *Edmundoa lindenii.*

Functional groups of pollinators	2015	2016	2017	2018
Hummingbirds (HB)	1726.8	1672.9	215.9	1942.7
Large bees (LB)	904.1	193.7	629.6	48.4
Proportional effectiveness (HB x LB)	0.66 x 0.34	0.90 x 0.10	0.25 x 0.75	0.98 x 0.02

1