Arthropod-Plant Interactions (2020) 14:409–419
https://doi.org/10.1007/s11829-020-09759-4

ORIGINAL PAPER

Effect of diurnal vs. nocturnal pollinators and flower position on the reproductive success of *Echium simplex*

Julia Jaca[1] · **Manuel Nogales**[2] · **Anna Traveset**[1]

Received: 5 June 2019 / Accepted: 18 March 2020 / Published online: 27 March 2020

Abstract

Nocturnal pollination plays an important role in sexual plant reproduction but has been overlooked, partially because of intrinsic difficulties in field experimentation. Even less attention has received the effect of within-inflorescence spatial position (distal or proximal) on nocturnal pollinators of columnar plants, despite numerous studies examining the relationship between such position and reproductive success. Woody endemic *Echium simplex* possesses large erect inflorescences bearing thousands of flowers which are visited by a wide array of diurnal and nocturnal animals. In this study, we identified nocturnal visitors and compared their pollination effectiveness with that of diurnal pollinators in different inflorescence sections by means of selective exclosures in NE Tenerife (Canary Islands). Nocturnal visitors included at least ten morphospecies of moths (such as *Paradrina rebeli* and *Eupithecia* sp.), two coleopteran species (mainly *Alloxantha* sp.), neuropterans (*Chrysoperla carnea*), dictyopterans (*Phyllodromica brullei*), dermapterans (*Guanchia* sp.) and julidans (*Ommatoiulus moreletii*). In general, plants excluded from pollinators set less fruits than open-pollination (control) plants which set fruits homogeneously across sections. Diurnally pollinated plants set more fruit in their upper parts whereas nocturnally pollinated plants set fruit in both upper and bottom sections. We conclude that although the frequency and diversity of diurnal pollinators is far higher than that of nocturnal pollinators, both exhibit different foraging behaviour that generates complementary effects on the reproductive success of *E. simplex*.

Keywords Canary Islands · Pollination effectiveness · Reproductive biology · Resource allocation

Introduction

Plant reproductive success is the result of the interactions of both biotic (e.g. pollination, herbivory, disease) and abiotic components (e.g. resource availability such as nutrients or water, physical environment such as cloudiness, wind, humidity or temperature) of the ecological context with maternal constraints (Lee 1988). Mutualistic interactions between plants and their pollinators are of particular interest. Although most studies have focused on diurnal pollinators, nocturnal pollination plays a more important role in sexual plant reproduction than previously suspected, since pollen is carried over greater distances by moths than by diurnal insect pollinators (Macgregor et al. 2018). Nocturnal pollination has been overlooked partially because of the intrinsic difficulty of field experimentation at night; moreover, such process may easily be affected by artificial light at night (Knop et al. 2017).

Nocturnal pollinators include a variety of taxa including insects, bats, birds, and even rodents (Baker 1961; von Helversen and Winter 2003; Knop et al. 2017). Some floral traits are usually associated with nocturnal pollination and form a particular pollination syndrome (Faegri and van der Pijl 1966; Fenster et al. 2004; Reynolds et al. 2009). This idea has been a central theme in pollination biology for many years (Faegri and van der Pijl 1966) and suggests that certain floral traits enhance the pollination efficiency of a particular pollinator type, leading to specialization

---

Handling Editor: Christina Mogren.

✉ Julia Jaca
JuliaJaca@hotmail.com

[1] Global Change Research Group, Institut Mediterrani D'Estudis Avançats IMEDEA (CSIC- UIB), C/Miquel Marqués 21, E07190-Esporles, Mallorca, Balearic Islands, Spain

[2] Island Ecology and Evolution Research Group, Instituto de Productos Naturales Y Agrobiología (CSIC-IPNA), C/Astrofísico Fco. Sánchez 3, E38206, La Laguna, Canary Islands, Spain

in that pollination type. The flower characteristics traditionally associated with nocturnal pollination syndrome include: opening at dusk/night (Baker 1961; Van Doorn and Van Meeteren 2003), pale colour or white (Baker 1961; Lunau and Maier 1995), attracting scent (Jürgens et al. 2002; Raguso 2008) and copious nectar (Fenster et al. 2004). Other specific floral traits involved in the attraction of nocturnal animal visitors include CO_2 gradients, tactile cues, thermogenesis and humidity gradients (Borges et al. 2016). However, most plants are visited by a broad range of morphologically and taxonomically diverse species (Waser 1982; Elam and Linhart 1988; Haber and Frankie 1989; Thompson and Pellmyr 1992; Sahley 1996; Nassar et al. 1997), indicating that flower morphology may not be an accurate predictor of the type of animal visiting the flowers. Moreover, further observations and experiments addressed at evaluating the contribution of pollination to plant fitness are needed to differentiate pollinators from other visitors, since many species are nectar and/or pollen thieves (Schemske and Horvitz 1984; Waser et al. 1996).

In plants in which the flowers are grouped in inflorescences, numerous studies have examined the relationship between reproductive success and flower anthesis (early or late) and/or within-inflorescence spatial position (distal or proximal) (for a review, see Stephenson 1981; Wyatt 1982; Lee 1988; or Diggle 1995). For example, in species with columnar inflorescences with acropetal flower opening, higher fruit and seed set are often found in proximal flowers (Solomon 1988; Herrera 1991; Ehrlén 1992, 1993; Karoly 1992; Guitian 1994; Guitián and Navarro 1996; Navarro 1996) than in intermediate flowers (Sutherland 1987) or proximal flowers (Goldingay and Whelan 1993). Three non-exclusive hypotheses have been proposed to explain these patterns of within- inflorescence variation regarding reproductive success:

1. The 'resource competition hypothesis', focused on abiotic components, postulates that the ovaries compete for a limited amount of resources (Stephenson 1981 and references therein; Klein et al. 2015).
2. The 'architectural effect hypothesis', related to maternal constraints, postulates that there is a constraint on the translocation of nutrients to reproductive organs due to the inherent structural features of an inflorescence, such as the waning of the vasculature in distal structures or the variation in the diameter of supporting structures (Diggle 1995 and references therein).
3. The 'non-uniform pollination hypothesis', with biotic components, postulates that there is a variation in pollen receipt along the inflorescence and differences may be attributable to insufficient quantity or quality of pollen (Lee 1988; Thomson 1989a; Berry and Calvo 1991; Goldingay and Whelan 1993; Kudo et al. 2001).

Woody endemic *Echium* species in the Canary Islands, both candelabra shrubs and monocarpic rosette 'trees', possess large erect inflorescences often carrying thousands of flowers visited by a wide range of animals. The patterns of female reproductive success within inflorescences have never been assessed. Previous studies with *Echium simplex* revealed that despite being visited by diurnal insects, birds and lizards, flying insects were responsible for most of the pollination. Flowers visited by flying insects (mainly Hymenoptera) set more fruits and also their seeds germinated more than those coming from unvisited flowers (Jaca et al. 2019). However, *E. simplex* might also be visited at night, as its flowers possess traits associated with the moth pollination syndrome (phalaenophily): they open at night, produce pale-coloured or white flowers with a heavy scent, offering rewards (nectar and pollen) in tubular corollas (Baker 1961; Kevan and Baker 1983).

In the present study, we aimed to investigate the reproductive success of both nocturnal and diurnal pollinators in different inflorescence sections. Our specific questions were: (1) what are the nocturnal pollinators of *E. simplex* in each inflorescence section and how frequent are they relative to diurnal pollinators? (2) what is the pollination effectiveness of nocturnal and diurnal pollinators in each inflorescence section, in terms of fruit and seed set, seed weight, and germination?

Materials and methods

Study species

The giant rosette plant *E. simplex* DC. (Boraginaceae), locally known as 'tajinaste blanco', is endemic to the Anaga Biosphere Reserve in NE Tenerife (Canary Islands). This area encompasses a 4.9–3.9 million-year-old basaltic massif (Guillou et al. 2004). It is considered a vulnerable species in the red list of Spanish vascular flora (Moreno 2008), with very few, reduced and isolated populations. The species is one of the three monocarpic *Echium* species in the Canary Islands, together with *E. wildpretii* on La Palma and Tenerife, and *E. pininana* on La Palma, and it grows for 5–9 years before producing a single large inflorescence (Stöcklin and Lenzin 2013). Reproductive individuals reach a height of up to 3 m, of which the prolonged inflorescence—composed of scorpioid cymes—can contribute up to 1.5 m. The inflorescence height is directly proportional to the rosette diameter and it flowers acropetally (from bottom/proximal to upper/distal parts). The cymes are double coiled and the largest plants may show 3–4 branches per cyme. Each cyme presents a new flower every second day, mostly opening in the morning and living for 2.5–3 days. Flowers are protandrous with transitional and male phases producing more nectar than the female phase (Jaca et al. 2019). After a successful pollination event, a flower develops into a fruit which consists of a maximum of four nutlets. The number of cymes and flowers per cyme increases along the inflorescence. The smallest of our examined plants had an average of 12 flowers per cyme whilst the largest had 51. The number of mature subfruits per flower (from one to three, on average) also increased along the inflorescence. Hence, the number of potential seeds produced increases enormously with the size of the inflorescence, ranging from 4560 to 234,000 (Stöcklin and Lenzin 2013).

Flowers are protandrous and are open for two to three days. The carpel elongates and splits, becoming taller than the anthers during the female phase. The flowers open successively from the proximal to the distal part of the cyme. The total flowering time of an individual plant is 3–5 weeks. Nectar standing crop varies during flower ontogeny with male and transitional flowers producing more nectar than in the female phase (approx. 2 µl vs. 1 µl) but sugar concentration remains constant (~ 17%) (Jaca et al. 2019).

Study area

The study site is located at the north-west of Chamorga village, northeastern Tenerife (Canary Islands). The population of *E. simplex* is found at an altitude around 250 m a.s.l. and occupies an area of about 1 km^2. There are also scattered individuals along the north coast trails. The location has a warm coastal climate with average temperatures between 17 and 19 °C in winter and 20 and 25 °C in summer. The summer is very dry and most rain falls in winter, but only in small quantities. The area is exposed to the moist northeastern trade wind, which is responsible for the lush green vegetation of Anaga mountains. The vegetation is shrubby-herbaceous, dry-Mediterranean and characterized by numerous endemic species such as *Artemisia thuscula, Descurainia millefolia, Aeonium canariense, Asphodelus tenuifolius, Achyranthes aspera* and *Galactites tomentosa.* Fieldwork was conducted once a week during a 5-week period at the peak of the flowering season of *E. simplex*, between 10th May and 8th June 2016.

Flower visitors and visitation frequency

Data on diurnal visitors and visitation frequency was available from our previous study on this plant (Jaca et al. 2019). To identify nocturnal flower visitors and determine their visitation frequency, a total of 18 haphazardly chosen individual plants were observed during focal censuses for a total of 35 h. A census consisted in the observation of individual plants for 60 min, for a total of ca. 2 h per plant at a shorter distance (0.5 m). Observations started after dusk (between 21:30 h and 22:00 h) and lasted up to midnight (between 23:30 h and 00:00 h), as nocturnal pollination composition does not usually change during the night (Jennersten 1988; Cordeiro et al. 2017) and visitation frequency is more related to weather conditions than night-time, as observed by video recordings (Jaca et al. 2019). During the days of the experiments (8th-11th May 2016), sunrise occurred about 07:15 h and sunset about 20:45 h (information taken in the capital, Santa Cruz de Tenerife, similar to the fieldwork site due to its proximity). Insects of all species or morphospecies were captured and taken to the lab for identification. Animals were considered as flower visitors whenever they touched the flower, as the sexual organs are exerted from the corolla. For each flower visitor, we recorded species identity (sometimes at family or order level), number of flowers and section of the plant visited (i.e. high, intermediate or low section).

Relative effectiveness of night and day flower visitors as pollinators

We conducted experiments to study the importance of pollination by diurnal and nocturnal flower visitors. Prior to flowering, the inflorescences of 21 haphazardly selected plants were bagged with muslin cloth to exclude any type of flower visitor and randomly assigned to day (‘diurnally pollinated plants’) or night (‘nocturnally pollinated plants’) time exposure treatment. Once per week, diurnally pollinated plants were unbagged during all the hours of the day (from 06:00 h to 21:00 h), while nocturnally pollinated plants were unbagged all the hours of the night (from 21:00 h to 06:00 h the next day), and kept bagged the rest of the time. Additionally, 12 plants were permanently bagged to assess the level of autogamy, while 13 individuals were left open to pollinators, i.e. acting as a control group.

Five cymes from upper, intermediate and lower sections of each inflorescence and plant were collected once ripe and taken to the laboratory. Fruit set was calculated as the proportion of flowers that develop into fruits, and seed set as the amount of viable seeds produced per fruit. Seeds were regarded as non-viable (aborted) based on a characteristic smaller size and greyness. Previous germination trials confirmed that such seeds are indeed not viable (Jaca et al. 2019).

Germination trials were later carried out to test for differences among treatments (i.e., control, autogamy, diurnal pollination and nocturnal pollination). A total of 1105 viable seeds (at least 18 seeds per plant, i.e., six seeds per inflorescence section per plant) were sown in early October 2016 into trays filled with a 1.2.1 mixture of peat, common agricultural soil and ravine sand in a greenhouse in Tacoronte (North Tenerife), as in Jaca et al.

(2019). Trays were watered every two days to ensure that the soil was constantly moist, and seedling emergence was registered every 5 days for 3 months until January 2017, when the germination experiment concluded after no seeds germinated during the next 25 days. Germinability (fraction of seeds that germinate) and germination rate (days to germination) were recorded for each seed (although we use the term germination we actually refer to the seedling time emergence). Seeds sown under each treatment were previously weighed to the nearest 0.1 mg.

Statistical analyses

We used generalized linear mixed models (GLMM) in R software version 3.5.0 (R Core Team 2018), followed by a Tukey test of multiple comparisons. Census observations were clustered into functional groups of visitors for the analysis. The model was adjusted to a gamma error distribution with a negative inverse link function, using the number of probed flowers per unit time and per flower as response variables and observation ID, nested in 'individual plant', as random effect. For the diurnal vs. nocturnal pollination and germination experiments, each estimate of plant reproductive success (i.e. fruit set, seed set, seed weight, germinability, and germination rate) was analysed separately as an independent variable. In these models, the response variables used were treatment and inflorescence section, whereas individual plant was used as random effect to control for lack of independence among flowers on the same individual plant. Differences in fruit set and germinability were estimated using a binomial error distribution and logit link function, whereas a Poisson family with a log link function was used to test for differences in seed set and germination rate (as the data were a discrete count of seeds or days, respectively). Seed weight was normally distributed and, for this variable, we thus adjusted errors to a Gaussian distribution and identity link function.

Results

Floral visitors and visitation rates at night

Nocturnal insects visiting flowers of *E. simplex* were clustered into 6 groups: (1) moths, at least ten morphospecies, of which only two (*Paradrina rebeli* and *Eupithecia* sp.) could be identified, (2) beetles, mainly *Alloxantha* sp., with one unidentified, (3) neuropterans (*Chrysoperla carnea*, F. Chrysopidae), (4) dictyopterans *(Phyllodromica brullei*, F. Blattellidae), (5) dermapterans, (*Guanchia* sp. F. Forficulidae), and (6) julidans (*Ommatouilus moreletii,* F. Julidae) (Table 1). Visitation rates exhibited differences among insect groups ($\chi^2 = 142.03$, df = 5, $P < 0.001$). The most frequent insect groups were lepidopterans (Fig. 1), visiting higher (distal) sections within the inflorescences, followed by coleopterans at intermediate and low positions, and other species mainly at the low sections (Table 1).

Comparative reproductive effectiveness of nocturnal and diurnal pollination in the three inflorescence sections

Fruit set was affected by pollination treatment and inflorescence section (pollination treatment × section: $\chi^2 = 33.34$, df = 6, $P < 0.001$, Fig. 2). The number of fruits produced per flower was higher in the control plants open to pollinators, compared to those excluded from all pollinators and to those only visited by nocturnal pollinators. Within a plant, the number of fruits produced was higher in upper and bottom inflorescence sections in nocturnally pollinated plants, whereas it was higher in the upper section in diurnally pollinated plants (Fig. 2).

There was no interaction effect of pollination treatment x inflorescence section on seed set ($\chi^2 = 12.38$, df = 6, $P = 0.054$). Seed set was influenced by pollination treatment ($\chi^2 = 17.25$, df = 3, $P < 0.001$, Fig. 3) but not by inflorescence section ($\chi^2 = 1.93$, df = 2, $P = 0.38$). Diurnally pollinated plants produced more seeds per fruit than nocturnally pollinated ones and also than control plants (Fig. 3).

Table 1 Number of visits per inflorescence section by each group of nocturnal visitors

Visitor group	No. visits	Position within the inflorescence		
		Upper	Intermediate	Bottom
Lepidoptera (at least 10 moth morphospecies)	69	41	23	5
Coleoptera (mainly *Alloxantha* sp.)	19	0	8	11
Chrysoperla carnea (Neuroptera)	1	0	1	0
Phyllodromica brullei (Blattaria)	1	0	0	1
Guanchia sp (Dermaptera)	4	0	1	3
Ommatouilus moreletii (Julidae)	3	0	0	3

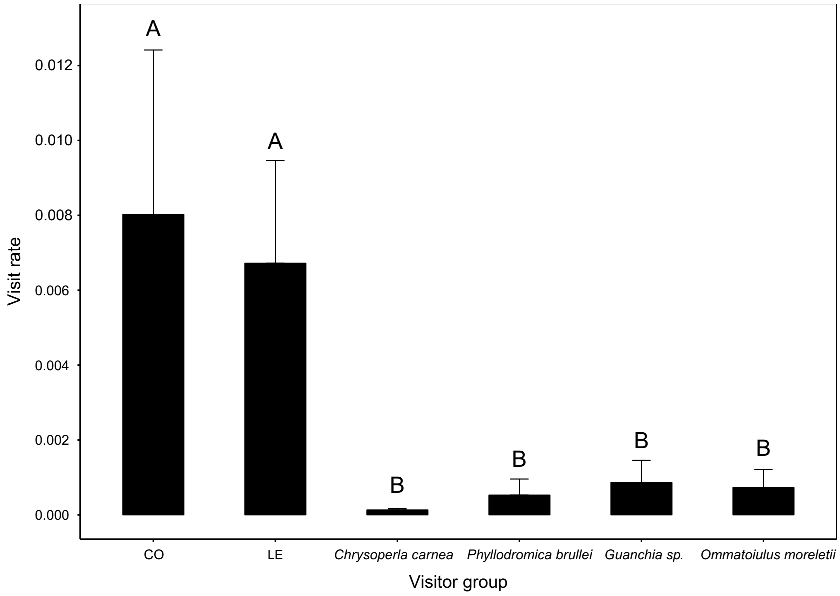

Fig. 1 Flower night visitation rate (visits h^{-1} $flower^{-1}$) of *E. simplex* by different insect groups (*CO* coleoptera, mainly *Alloxantha* sp., *LE* lepidoptera). Letters indicate significant differences using Tukey's test after GLM

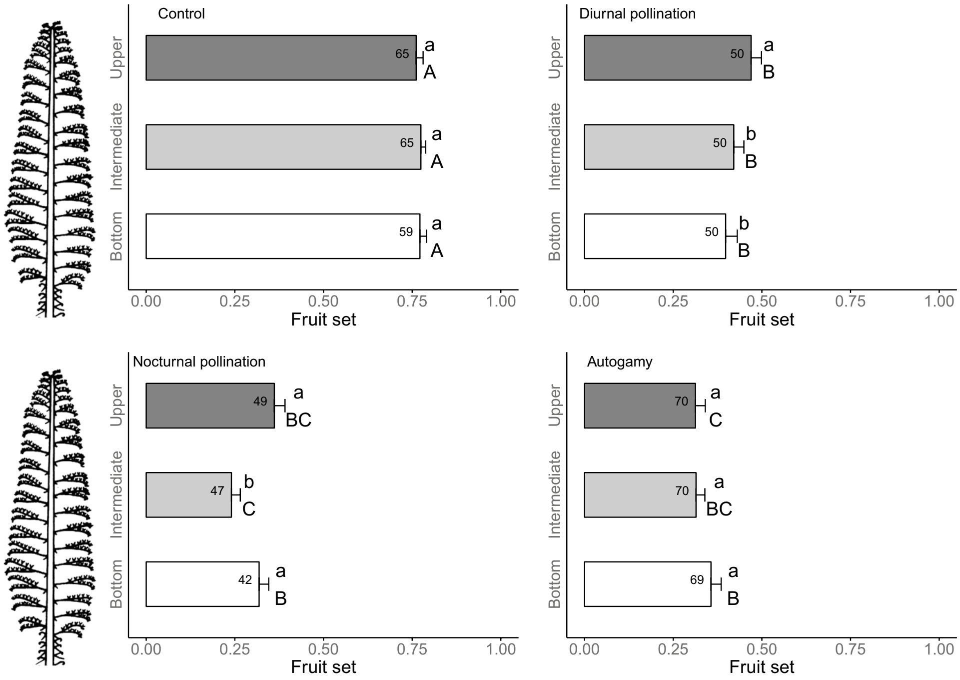

Fig. 2 Mean (+SE) proportion of flowers that set fruit per pollination treatment and inflorescence section. Numbers inside each bar are sample sizes (number of cymes). Lower case letters indicate significant differences between inflorescence sections within each treatment, and capital letters indicate significant differences between treatments within-inflorescence sections using Tukey's test after GLM

Similarly, there was no interactive effect on seed weight between pollination treatment and inflorescence section ($\chi^2 = 10.67$, df = 6, $P = 0.10$). Seed weight was affected by both pollination treatment and inflorescence section ($\chi^2 = 8.96$, df = 3, $P = 0.03$; and $\chi^2 = 24.51$, df = 2, $P < 0.01$, respectively, Fig. 4). Seeds from selfed flowers were significantly heavier than those from control flowers (Fig. 4A). Moreover, bottom inflorescence sections produced lighter seeds than upper and intermediate sections (Fig. 4B).

Regarding germination patterns, both germinability and germination rate were influenced by an interactive effect among seed weight, inflorescence section and pollination treatment ($\chi^2 = 16.01$, df = 6, $P < 0.05$, and $\chi^2 = 104.30$, df = 6, $P < 0.001$, respectively, Figs. 5 and 6).

In all inflorescence sections, most of the heavier seeds from control plants germinated. However, seeds from other treatments and inflorescence sections behaved differently.

The heavier seeds of the diurnally pollinated plants germinated more when seeds were from the high sections of the inflorescence. The opposite occurred with seeds from the intermediate and low inflorescence sections, i.e. heavier seeds germinated less. Furthermore, the heavier seeds of

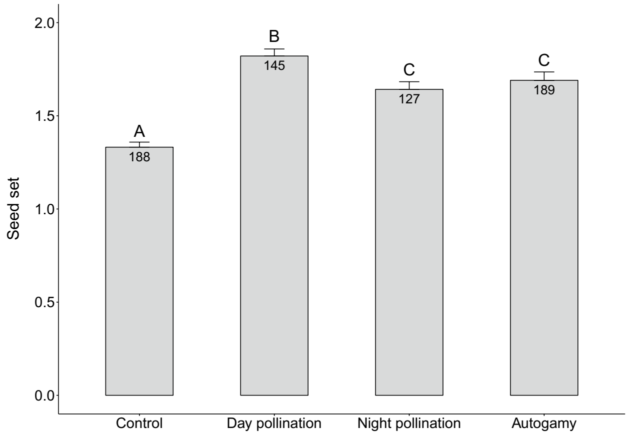

Fig. 3 Mean (+SE) number of seeds per fruit for each pollination treatment. Numbers inside each bar are fruit sample sizes. Letters indicate significant differences using Tukey's test after GLM

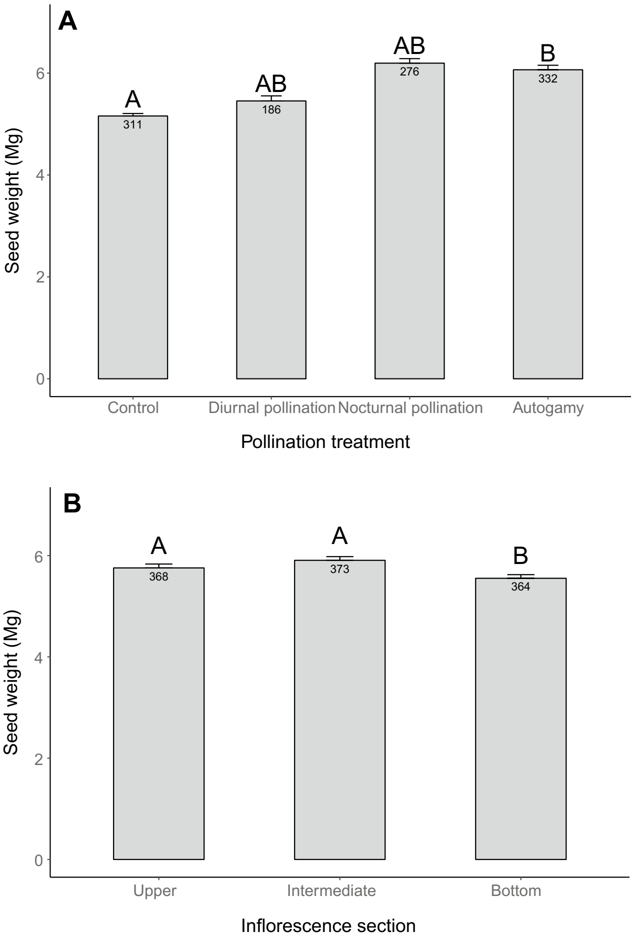

Fig. 4 Mean (+SE) seed weight per **A** pollination treatment, and **B** inflorescence section. Numbers in each bar are sample sizes. Letters indicate significant differences using Tukey's test after GLM

the nocturnally pollinated plants in the high and intermediate sections germinated slightly more than the lighter ones, whereas the opposite happened with seeds from the low sections, i.e. germinated less than lighter ones. Finally, for the autogamy treatment, we found that the heavier seeds had a higher germinability than the lighter ones, but this was only with seeds from the intermediate section and we found the opposite in the low and high sections, i.e. lighter seeds germinated more (Fig. 5).

Regarding germination rate, heavier control seeds from the upper and intermediate sections germinated earlier, whereas those from the bottom section were later. The germination rate of seeds in relation to their weight in diurnally vs. nocturnally plants showed the opposite patterns, i.e. heavier seeds from the upper and bottom sections of diurnally pollinated plants germinated faster, but not those from intermediate sections, and heavier seeds from the upper and bottom sections of nocturnally pollinated plants took longer to germinate, while those from intermediate sections germinated faster (Fig. 6). Finally, heavier selfed seeds germinated faster than the lighter ones from all sections of the plant.

Discussion

Ours is the first study that combines the effect of type of pollinators (nocturnal vs. diurnal) and inflorescence section on the reproductive success of a plant species. *Echium simplex* exhibited a uniform fruit set along the inflorescence, suggesting absence of competition among sections or maternal constraints, and uniform pollination. Although the species is mostly pollinated during the day, we found that nocturnal and diurnal pollinators displayed a complementary pollination behaviour which translated into a complementary reproductive success.

Diversity of flower visitor groups

At night, *E. simplex* flowers are visited by six different functional groups of animals. This is a higher number than the usually reported in nocturnal pollination studies, where mostly moth visits are reported (Stephenson and Thomas 1977; Jennersten and Morse 1991; Jürgens et al. 1996; Ghazoul 1997; Groman and Pellmyr 1999; Martinell et al. 2010, but see Brantjes and Leemans 1976). However, the attractiveness of this plant for insect visitors is greater during daytime, with up to 90 species of flower visitors identified (Jaca et al. 2019). This pattern of higher species diversity during the day is found in some plants (Jennersten and Morse 1991; Ghazoul 1997), though diversity is higher at night in others (Brantjes and Leemans 1976; Stephenson and Thomas 1977; Jürgens et al. 1996; Groman and Pellmyr 1999; Martinell et al. 2010). Some nocturnal insects are also

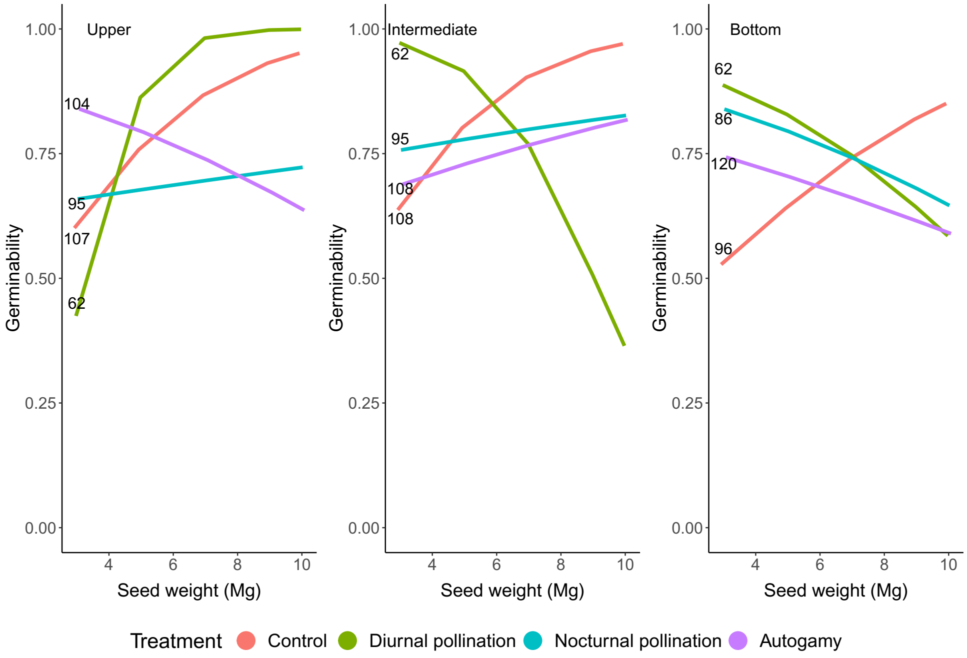

Fig. 5 GLMM predicted probabilities of germinability along seed weight for each exclusion treatment and inflorescence section. Numbers besides each line are sample sizes

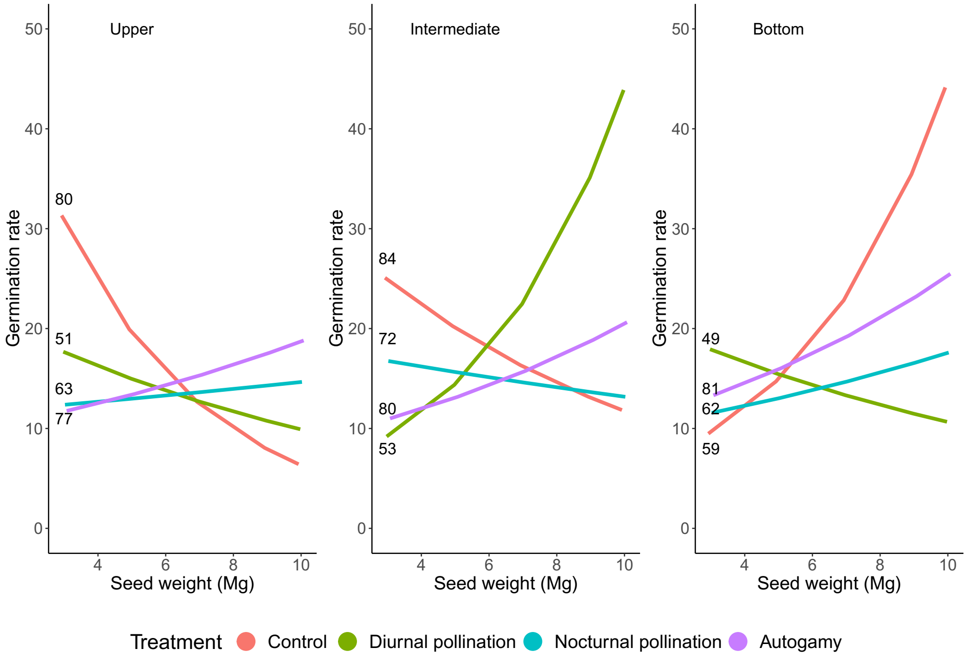

Fig. 6 GLMM predicted probabilities of germination rate according to seed weight for each exclusion treatment and inflorescence section. Numbers beside each line are sample sizes

observed in day censuses (Knop et al. 2017), as in our study. Indeed *Chrysoperla carnea, Guanchia* sp. and *Phyllodromica brullei* were also recorded in diurnal censuses (Jaca et al. 2019), as these animals can have diurnal activity or rest/hide within the flowers.

The most common nocturnal visitors in *E. simplex* were moths and the beetle *Alloxantha* sp. (Oedemeridae). This contrasts with other studies that report beetle visits as merely anecdotal (Stephenson and Thomas 1977; Groman and Pellmyr 1999; Martinell et al. 2010, but see Knop et al. 2017). When moths land on the inflorescence of *E. simplex* they sometimes walk over the flowers while probing them, and may remain on them for a short period. All body parts can contact the exerted anthers and pistils, and thus they are potentially effective pollinators (Ghazoul 1997). The moth diversity we found on *E. simplex* is much lower than that reported in other studies in both paleartic and neartic realms, such as those on *Manfreda virginica* or *Silene otitis* and *S.*

sennenii (Brantjes and Leemans 1976; Groman and Pellmyr 1999; Martinell et al. 2010), but is similar to *Catalpa speciosa* or *S. vulgaris* and others (Stephenson and Thomas 1977; Jürgens et al. 1996). Beetles feed on pollen and move within the flowers but are probably irrelevant pollinators. In fact, their presence may indeed be deleterious, reducing final reproductive success by removing pollen from the stigmas (Kevan and Baker 1983; Jaca et al. 2019). As for other flower visitors, these nocturnal beetles were seen only anecdotally in other studies on night pollination, without being considered as pollinators (Crumb et al. 1941; Brantjes and Leemans 1976; Thien 1980; Schneemilch et al. 2011; Knop et al. 2017). However, specific floral cues like scent components involved in attracting nocturnal animal visitors are unknown for this plant and may be worth addressing in future studies,

Regarding visitation frequency, nocturnal visitors were less frequent than diurnal ones (Jaca et al. 2019). This pattern is consistent with that found in most nocturnal pollination studies, despite the target species having a nocturnal syndrome (Stephenson and Thomas 1977; Ghazoul 1997; Young 2002 for a comparative table among studies; Martinell et al. 2010). It has been suggested that nocturnal visitors are scarcer because of their energetics, as they might require a larger nectar reward because of the cooler night temperatures (Morse and Fritz 1983); it has also been suggested that they could increase their length of visit during the night compared to diurnal pollinators (McMullen 2009).

Reproductive effectiveness of night and day pollination in the inflorescence sections

In our previous studies on *E. simplex,* we found that diurnal flying hymenopterans are the main pollinators responsible for its reproductive success (Jaca et al. 2019). In general, control plants set more fruits than diurnally or nocturnally pollinated, and than selfed plants, while diurnally pollinated plants set more fruits than nocturnally pollinated and selfed plants. This result is consistent with other studies (Bertin and Willson 1980; Morse and Fritz 1983; Jennersten and Morse 1991; Guitian et al. 1993; Navarro 1999), but not with others in which no differences have been found (McMullen 2009) or where a higher fruit set in nocturnally pollinated plants compared to diurnally pollinated plants has been reported (Martinell et al. 2010).There was no difference in fruit set among plant sections in either control or selfed plants, suggesting absence of competition among sections or maternal constraints, and uniform pollination in *E. simplex*, hence refuting the three hypotheses raised in the introduction, unlike most studies of reproductive success patterns in inflorescences (Diggle 1995 for a review; Tremblay 2006; Torices and Méndez 2010). It is generally thought that perennial monocarpic species use stored reserves for fruit development rather than resources obtained during the flowering season, even more so than annually fruiting species (Stephenson 1981; Udovic and Aker 1981). However, day- and night-pollinated plants showed a fruit production pattern indicating non-uniform pollination (Karoly 1992; Kudo et al. 2001; Tremblay 2006). Some studies (Lee 1988; Tremblay 2006) have reported higher reproductive success in bottom positions due to the behaviour of pollinators; these move distally upward on inflorescences, may become satiated with the resources and thus may leave the plant before visiting the upper flowers; alternatively, the bottom of the inflorescence may be more likely than the distal parts to receive cross pollen. We found that diurnally pollinated plants set more fruits in upper inflorescence sections. One explanation might be that if diurnal insects (mostly bees) do indeed move upwards, upper positions may avoid stigma clogging to some extent (Brown and Mitchell 2001) if *E. simplex* competes with other co-flowering plants for pollinators. By contrast, other studies found higher pollen deposition in the upper flowers of inflorescences, with no relation to directional pollinator foraging and bee preference for higher flowers (Roubik et al. 1982; Lortie and Aarssen 1999). The deposition of large amounts of self-pollen, however, may also clog up the stigma and prevent effective pollination (Kikuzawa 1989; Thomson 1989b).

Nocturnally pollinated plants were found to set less fruits in intermediate compared to bottom and upper parts. The presence of *Alloxantha* sp. consuming the pollen in intermediate sections might reduce final reproductive success; previous studies have documented beetles reducing plant fitness due to pollen consumption (Kevan and Baker 1983).

Diurnally pollinated plants set more seeds per fruit than control plants. This finding in *E. simplex* is consistent with studies by Jennersten (1988) and Martinell et al. (2010) who found higher or equal seed set in controls and day-pollinated plants. However, the reduced seed set in control plants may be compensated by the greater fruit production. Although some studies also found higher seed set in diurnally compared with nocturnally pollinated flowers (Jennersten 1988), most studies actually found the opposite (Jürgens et al. 1996; Groman and Pellmyr 1999; Young 2002; Martinell et al. 2010) or no effect (Jennersten and Morse 1991). In addition, no differences were detected between seed set of nocturnal and selfed fruits, indicating a low effectiveness of nocturnal pollinators, as documented by Jennersten (1988) for *Viscaria vulgaris*.

Seeds of *E. simplex* coming from selfed flowers were heavier than those resulting from cross-pollination. The reason is that the former have a thicker coat, whilst embryo size is similar between the two treatments (Jaca et al. 2019). Comparing seed weight between inflorescence sections, bottom seeds were lighter than upper and intermediate ones. This contrast with other studies that have found basal seeds to be heavier (Byrne and Mazer 1990; Navarro 1996; Vallius 2000).

In accordance with findings from other germination studies (Schemske 1983; Navarro and Guitián 2002), heavy seeds showed higher germinability and germinated faster than light ones in all treatments, except those from the bottom sections of inflorescences. The thicker seed coat produced by selfed flowers is probably what slows germination (Crocker 1906; Miyoshi and Mii 1988). Indeed, this was previously reported in at least one species, *Sinapis arvensis* (Paolini et al. 2001).

Concluding remarks

Despite the relatively abundant literature on nocturnal vs. diurnal pollination, and on fruiting patterns along the inflorescences, this is the first study that examined both effects simultaneously. We found that *E. simplex* was visited at night—mainly by moths and beetles—but at lower rates than during the day. The exclusion experiment indicated that fruiting patterns along the inflorescences in open-pollinated and selfed plants show no variation, revealing absence of competition among sections or maternal constraints, and uniform pollination. By contrast, differences were found between nocturnally and diurnally exposed plants, suggesting different behaviour between nocturnal and diurnal pollinators. This may generate complementarity effects in *E. simplex* pollination services. However, presumably because of the extremely high visitation frequency, diurnally pollinated plants set more fruits and seeds than nocturnally pollinated plants. Seeds from selfed flowers were heavier than those resulting from cross-pollination and showed reduced germinability and germination rate. This is because the former have a thicker coat, whilst embryo size remains similar. On the other hand, crossed seeds showed increased germinability and germination rate than lighter ones in all exclusion treatments and in the upper and intermediate sections.

Acknowledgements The authors thank Marcos Báez and Antonio Pérez Delgado for insect identification, Benito Pérez Vispo for his technical assistance in the field, and Juana Pérez López for providing logistical support in Chamorga. We are also grateful to Servicio Administrativo de Medio Ambiente, Excmo. Cabildo Insular de Tenerife for permission (2016-01704) to work in Anaga Biosphere Reserve, Tenerife. The company Tagoro Medioambiente provided its greenhouse to perform the seedling emergence experiments; Manuel Martín helped us in the follow-up of the experiment. Julia Jaca was funded by a predoctoral fellowship from the Ministerio de Educación, Cultura y Deporte (FPU13/05880) and by the unemployment benefit from the Ministerio de Trabajo, Migraciones y Seguridad Social. The study was framed within a project financed by the Ministerio de Economía, Industria y Competitividad (CGL2017-88122-P) to Anna Traveset.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Baker HG (1961) The adaptation of flowering plants to nocturnal and crepuscular pollinators. Q Rev Biol 36:64–73

Berry PE, Calvo RN (1991) Pollinator limitation and position dependent fruit set in the high Andean orchid *Myrosmodes cochleare* (Orchidaceae). Plant Syst Evol 174:93–101

Bertin RI, Willson MF (1980) Effectiveness of diurnal and nocturnal pollination of two milkweeds. Can J Bot 58:1744–1746

Borges RM, Somanathan H, Kelber A (2016) Patterns and processes in nocturnal and crepuscular pollination services. Q Rev Biol 91:389–418

Brantjes NBM, Leemans JAAM (1976) *Silene otites* (Caryophyllaceae) pollinated by nocturnal lepidoptera and mosquitoes. Acta Bot Neerl 25:281–295

Brown BJ, Mitchell RJ (2001) Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia 129:43–49

Byrne M, Mazer SJ (1990) The effect of position on fruit and of yield in relationships among components of yield in *Phytolacca rivinoides* (Phytolaccaceae). Biotropica 22:353–365

Cordeiro GD, Pinheiro M, Dötterl S, Alves-dos-Santos I (2017) Pollination of *Campomanesia phaea* (Myrtaceae) by night-active bees: a new nocturnal pollination system mediated by floral scent. Plant Biol 19:132–139

Crocker W (1906) Role of seed coats in delayed germination. Contributions from the Hull Botanical Laboratory. LXXXV Bot Gaz 42:265–291

Crumb SE, Eide PM, Bonn AE (1941) The European earwig. USDA Tech Bull 766:76

Diggle PK (1995) Architectural effects and the interpretation of patterns of fruit and seed development. Annu Rev Ecol Syst 26:531–552

Ehrlén J (1992) Proximate limits to seed production in a herbaceous perennial legume, *Lathyrus vernus*. Ecology 73:1820–1831

Ehrlén J (1993) Ultimate functions of non-fruiting flowers in *Lathyrus vernus*. Oikos 68:45–52

Elam DR, Linhart YB (1988) Pollination and seed production in *Ipomopsis aggregata*: differences among and within Flower color morphs. Am J Bot 75:1262–1274

Faegri K, van der Pijl L (1966) The principles of pollination ecology. Pergamon Press, Oxford

Fenster CB, Armbruster WS, Wilson P et al (2004) Pollination syndromes and floral specialization. Annu Rev Ecol Evol Syst 35:375–403

Ghazoul J (1997) The pollination and breeding system of *Dipterocarpus obtusifolius* (Dipterocarpaceae) in dry deciduous forests of Thailand. J Nat Hist 31:901–916

Goldingay RL, Whelan RJ (1993) The influence of pollinators on fruit positioning in the Australian shrub *Telopea speciosissima* (Proteaceae). Oikos 68:501–509

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

Guillou H, Carracedo JC, Paris R, Torrado FJP (2004) Implications for the early shield-stage evolution of Tenerife from K/Ar ages and magnetic stratigraphy. Earth Planet Sci Lett 222:599–614

Guitian J (1994) Selective fruit abortion in *Prunus mahaleb* (Rosaceae). Am J Bot 81:1555–1558

Guitián J, Navarro L (1996) Allocation of reproductive resources within inflorescences of *Petrocoptis grandiflora* (Caryophyllaceae). Can J Bot 74:1482–1486

Guitian P, Guitian J, Navarro L (1993) Pollen transfer and diurnal versus nocturnal pollination in *Lonicera etrusca*. Acta Oecol 14:219–227

Haber WA, Frankie GW (1989) A tropical hawkmoth community: Costa Rican dry forest sphingidae. Biotropica 21:155–172

Herrera J (1991) Allocation of reproductive resources within and among inflorescences of *Lavandula stoechas* (Lamiaceae). Am J Bot 78:789–794

Jaca J, Nogales M, Traveset A (2019) Reproductive success of the Canarian *Echium simplex* (Boraginaceae) mediated by vertebrates and insects. Plant Biol 21:216–226

Jennersten O (1988) Pollination of *Viscaria vulgaris* (Caryophyllaceae): the contributions of diurnal and nocturnal insects to seed set and seed predation. Oikos 52:319–327

Jennersten O, Morse DH (1991) The quality of pollination by diurnal and nocturnal insects visiting common milkweed, *Asclepias syriaca*. Am Midl Nat 125:18–28

Jürgens A, Witt T, Gottsberger G (1996) Reproduction and pollination in Central European populations of *Silene* and *Saponaria* species. Bot Acta 109:316–324

Jürgens A, Witt T, Gottsberger G (2002) Flower scent composition in night-flowering *Silene* species (Caryophyllaceae). Biochem Syst Ecol 30:383–397

Karoly K (1992) Pollinator limitation in the facultatively autogamous annual, *Lupinus nanus* (Leguminosae). Am J Bot 79:49–56

Kevan PG, Baker HG (1983) Insects as flower visitors and pollinators. Annu Rev Entomol 28:407–453

Kikuzawa K (1989) Floral biology and evolution of gynodioecism in *Daphne kamtchatica* var. jezoensis. Oikos 56:196

Klein AM, Hendrix SD, Clough Y et al (2015) Interacting effects of pollination, water and nutrients on fruit tree performance. Plant Biol 17:201–208

Knop E, Zoller L, Ryser R et al (2017) Artificial light at night as a new threat to pollination. Nature 548:206–209

Kudo G, Maeda T, Narita K (2001) Variation in floral sex allocation and reproductive success within inflorescences of *Corydalis ambigua* (Fumariaceae): pollination efficiency of resource limitation? J Ecol 89:48–56

Lee TL (1988) Patterns of fruit and seed production. In: Lovett Doust J, Lovett Doust L (eds) Plant reproductive ecology: patterns and strategies. Oxford University Press, New York, pp 179–202

Lortie CJ, Aarssen LW (1999) The advantage of being tall: higher flowers receive more pollen in *Verbascum thapsus* L. (Scrophulariaceae). Ecoscience 6:68–71

Lunau K, Maier EJ (1995) Innate colour preferences of flower visitors. J Comp Physiol A 177:1–19

Macgregor CJ, Kitson JJN, Fox R et al (2018) Construction, validation, and application of nocturnal pollen transport networks in an agro-ecosystem: a comparison using light microscopy and DNA metabarcoding. Ecol Entomol 44:17–29

Martinell CC, Dötterl S, Blanché C et al (2010) Nocturnal pollination of the endemic *Silene sennenii* (Caryophyllaceae): an endangered mutualism? Plant Ecol 211:203–218

McMullen CK (2009) Pollination biology of a night-flowering Galápagos endemic, *Ipomoea habeliana* (Convolvulaceae). Bot J Linn Soc 160:11–20

Miyoshi K, Mii M (1988) Ultrasonic treatment for enhancing seed germination of terrestrial orchid, *Calanthe discolor*, in asymbiotic culture. Sci Hortic (Amsterdam) 35:127–130

Moreno JC (2008) Lista Roja 2008 de la Flora Vascular Española. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid, Spain

Morse DH, Fritz RS (1983) Contributions of diurnal and nocturnal insects to the pollination of common milkweed (*Asclepias syriaca* L.) in a pollen-limited system. Oecologia 60:190–197

Nassar JM, Ramírez N, Linares O (1997) Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. Am J Bot 84:918–927

Navarro L (1996) Fruit-set and seed weight variation in *Anthyllis vulneraria* subsp. *vulgaris* (Fabaceae). Plant Syst Evol 201:139–148

Navarro L (1999) Pollination ecology and effect of nectar removal in *Macleania bullata* (Ericaceae). Biotropica 31:618–625

Navarro L, Guitián J (2002) The role of floral biology and breeding system on the reproductive success of the narrow endemic *Petrocoptis viscosa* rothm. (Caryophyllaceae). Biol Conserv 103:125–132

Paolini R, Bàrberi P, Rocchi C (2001) The effect of seed mass, seed colour, pre-chilling and light on the germination of *Sinapis arvensis* L. Ital J Agron 5:39–46

R Core Team (2018) R: a language and environment for statistical computing

Raguso RA (2008) Wake up and smell the roses: the ecology and evolution of floral scent. Annu Rev Ecol Evol Syst 39:549–569

Reynolds RJ, Westbrook MJ, Rohde AS et al (2009) Pollinator specialization and pollination syndromes of three related North American Silene. Ecology 90:2077–2087

Roubik DW, Ackerman JD, Copenhaver C, Smith BH (1982) Stratum, tree, and flower selection by tropical bees: implications for the reproductive biology of outcrossing *Cochlospermum vitifolium* in Panama. Ecology 63:712–720

Sahley CT (1996) Bat and hummingbird pollination of an autotetraploid columnar cactus, *Weberbauerocereus weberbaueri* (Cactaceae). Am J Bot 83:1329–1336

Schemske DW (1983) Breeding system and habitat effects on fitness components in three Neotropical *Costus* (Zingiberaceae). Evolution (N Y) 37:523–539

Schemske DW, Horvitz CC (1984) Variation among floral visitors in pollination ability: a precondition for mutualism specialization. Science 225:519–521

Schneemilch M, Williams C, Kokkinn M (2011) Floral visitation in the Australian native shrub genus *Acrotriche* R.Br (Ericaceae): an abundance of ants (Formicidae). Aust J Entomol 50:130–138

Solomon BP (1988) Patterns of pre- and postfertilization resource allocation within an inflorescence: evidence for interovary competition. Am J Bot 75:1074–1079

Stephenson AG (1981) Flower and fruit abortion: proximate causes and ultimate functions. Annu Rev Ecol Syst 12:253–279

Stephenson AG, Thomas WW (1977) Diurnal and nocturnal pollination of *Catalpa speciosa* (Bignoniaceae). Syst Bot 2:191–198

Stöcklin J, Lenzin H (2013) *Echium simplex*, ein seltener Schopfrosettenbaum auf Teneriffa. Bauhinia 24:23–37

Sutherland S (1987) Why hermaphroditic plants produce many more flowers than fruits: experimental tests with *Agave mackelveyana*. Evolution (NY) 41:750–759

Thien LB (1980) Patterns of pollination in the primitive angiosperms. Biotropica 12:1–13

Thomson JD (1989a) Deployment of ovules and pollen among flowers within inflorescences. Evol Trends Plants 3:65–68

Thomson JD (1989b) Germination schedules of pollen grains: implications for pollen selection. Evolution (N Y) 43:220–223

Thompson JN, Pellmyr O (1992) Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. Ecology 73:1780–1791

Torices R, Méndez M (2010) Fruit size decline from the margin to the center of capitula is the result of resource competition and architectural constraints. Oecologia 164:949–958

Tremblay RL (2006) The effect of flower position on male and female reproductive success in a deceptively pollinated tropical orchid. Bot J Linn Soc 151:405–410

Udovic D, Aker C (1981) Fruit abortion and the regulation of fruit number in *Yucca whipplei*. Oecologia 49:245–248

Vallius E (2000) Position-dependent reproductive success of flowers in *Dactylorhiza maculata* (Orchidaceae). Funct Ecol 14:573–579

Van Doorn WG, Van Meeteren U (2003) Flower opening and closure: a review. J Exp Bot 54:1801–1812

von Helversen O, Winter Y (2003) Glossophagine bats and their flowers: costs and benefits for plant and pollinators. In: Kunz TH, Fenton MB (eds) Bat ecology. The University of Chicago Press, Chicago, pp 346–397

Waser NM (1982) A comparison of distances flown by different visitors to flowers of the same species. Oecologia 55:251–257

Waser NM, Chittka L, Price MV et al (1996) Generalization in pollination systems, and why it matters. Ecology 77:1043–1060

Wyatt R (1982) Inflorescence architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. Am J Bot 69:585–594

Young HJ (2002) Diurnal and nocturnal pollination of *Silene alba* (Caryophyllaceae). Am J Bot 89:433–440