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Floral traits and Foraging Behavior of Bee Species visiting *Martynia annua* L. (Martyniaceae) in a Coastal Habitat

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Abstract

Floral visitors are often overlooked in those plants considered invasive and widespread weed species. *Martynia annua* L. is an example of an introduced species to the old world being native from tropical America, however, information of its endemic pollinators in the Neotropical region is missing. In this study, the floral visitors of *M. annua* were evaluated in Chamela Field Station (Chamela-Cuixmala Biosphere Reserve) in Jalisco, Mexico. Our aim was to provide information of the breeding system of *M. annua* and then indicate the potential pollinators. We included morphological and sexual features to estimate the outcrossing index (OCI). The frequency, behavior, and pollen loads were considered to find the potential pollinator. Despite the evidence of protandry and OCI indicating a xenogamous breeding system, the lack of herkogamy suggests *M. annua* is a facultative xenogamous species. The highest frequency of visits corresponded to the maximum diameter of corolla. *Euglossa viridissima* Friese was the most recurrent visitor. However, this species often carried a high proportion of heterospecific pollen and did not touch any sexual structure of the flower. In contrast, *Centris agilis* Smith performed as the most likely pollinator. Flowers of *M. annua* offer valuable rewards to its visitors and may be a good source of energy to those foragers capable of reaching the nectaries, though small bees are apparently unable to penetrate the flowers. We consider that there are evidence that suggests *M. annua* is a specialized melittophilic plant, pollinated by moderate to large-sized hairy bees throughout its distribution.

Introduction

Martynia annua L. (Martyniaceae) is an herbaceous perennial plant native of America. It ranges from 350 to 950 m of elevation from southern Arizona, through the Neotropical area of Mexico, Central America and the Antilles (Calderón, 1998). It was introduced in the old world around the 18th century and now occurs in all of its continents where is considered an invasive species (Calderón, 1998; Khuroo et al., 2012; CABI, 2018). *M. annua* reaches 1–3 m height and is covered with dense glandular sticky hairs, almost glabrous when adult. Stems are branched and could be lignified at the base in old individuals. Leaves are simple, blades reniform to broadly ovate, margins entire to deeply lobed ending in an acute apex. Petioles are 3–25 cm long. The inflorescence is a short raceme with 10–20 purplish white flowers, which are campanulate, zigomorph, sympetalae, pedicels are 1–2 cm long; flower tubes slightly to strongly ventricose. Calyx is greenish, 1.5 cm long with five free sepals. The corolla is five-lobed, with pink to reddish spots; the basal lobe widened to form a landing surface and presenting a yellow nectar guide. Two fertile stamens, two staminodes, and sometimes one rudimentary (Calderón, 1998). These morphological features are commonly associated to melittophily (bee

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pollination) and are shared with most genera of Martyniaceae: *Ibicella, Martynia,* and *Proboscidea* (Phillippi & Tyrl, 1979). Nonetheless, the breeding system of *M. annua* has never been studied and data on floral biology is poor.

Assuming the type of breeding system based on floral traits (i.e. pollination syndrome hypothesis) should be addressed with caution (Fenster et al., 2004). Since the spatiotemporal plant-pollinator assemblage can play a role in the outcrossing process (Herrera, 1988; Lázaro et al., 2008), it becomes imperative to evaluate the assemblage of pollinators in correspondence to multiple floral traits (Johnson & Steiner, 2000). Moreover, studies on the pollinator systems of invasive species may incorporate useful information (e.g. ecological predictor data) for risk modelling of invasive species (Jiménez-Valverde et al., 2011), thus effective eradication programs (Gardener et al., 2010).

Only carpenter bees, digger bees and hawkmoths have been reported visiting flowers of *M. annua* in India, where this plant is considered invasive (Raju & Reddi, 2000). In Mexico, *Trigona fulviventris* Guérin-Méneville is the only known bee that visits *M. annua* (Ayala, 2004). Therefore, literature about floral visitors of *M. annua* along its natural rage is scarce.

Our work focused on the bee community that visited *M. annua* in a biosphere reserve located within the natural distributional range of the plant. Because of its external morphology, we hypothesized that (a) *M. annua* attracts only visitors of the bee community (melittophilous) and that (b) larger and hairy bees may perform a better pollination fitness.

Material and Methods

Study area.

The study was conducted in September 2017 in the surrounding facilities of Chamela Biological Station, UNAM, part of the "Reserva de la Biósfera Chamela-Cuixmala (RBCC)". It is placed in Jalisco State, Mexico, located 1.7 km from the nearest point to the Pacific coast (19.49891 N, 105.04441 W). It belongs to a tropical subhumid region with contrasting dry and rainy season. Annual mean temperature is 25.2°C, averaging 745.1 mm/year of rainfall (Trejo, 1999). The predominant vegetation is deciduous tropical dry forest (Miranda & Hernández, 1963).

Data collection. During the last week of September 2017, we monitored the floral visitors of *M. annua* from 06:00 to 00:00 h within a span of three consecutive days. Every two hours, three observers recorded information during 10 min (i.e. 900 min of field observations). *In situ* data considered information on the foraging conduct, beginning and end of activity, visit, recognition fly, and time spent inside the flower. Since individuals of *M. annua* usually contained less than four opened flowers, each observer often recorded information on groups of 2-4 different flowers distributed in small plots containing 3-4 flowering plants (N=10/plants/day). Each monitoring round, observers switched places to minimize individual perception bias.

Individual flowers pre-anthesis were selected from 10 different plants to collect biological (i.e. anthesis period and floral lifespan) and morphological data. The latter included 12 morphological attributes of reproductive and non-reproductive floral traits.

The total nectar production and sugar concentration per individual flower were evaluated in flowers covered with soft tulle mesh to prevent the entrance of any visitor (Fig 1A).

The bees that visited the flowers were caught and killed in separated tubes. Then we used Fuchsin jelly to remove all pollen grains from the vertex and scutum of the bees. We made preparations of each individual bee to observe and count the pollen grains under 40 – 100X. Previously, we collected pollen from virgin flowers of *M. annua* as a reference for the species and identify the different pollen morphotypes by using Palacios-Chavez et al. (1989) and Quiroz-García et al. (1994).

Data analysis. Morphometric and sexual data were used to estimate the outcrossing index of *M. annua* (OCI), to give an approximation of the plant´s breeding system (Cruden, 1977). The OIC ranges from 0 to 4; where 0 = cleistogamy, 1 = obligate autogamy, 2 = facultative autogamy, 3 = facultative xenogamy and 4 = xenogamy.

Considering the spatial separation between stigma and anthers, we used a paired *t* test to proof herkogamy in flowers. Changes in the frequency of visitation rate through time were evaluated with a chi-square test of Goodness of fit. Statistical tests were done with R v3.4.4 (R Core Team, 2018).

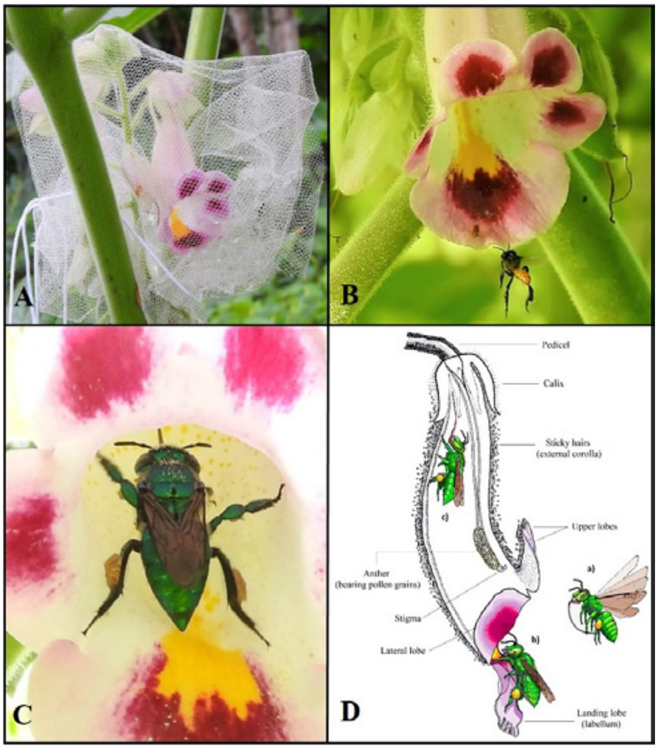

Fig 1. Exclusion and foraging behavior during floral anthesis of *Martynia annua*. **A.** Floral exclusion with soft tulle mesh. **B.** *Trigona fulviventris;* **C.** *Euglossa viridissima*; **D.** Representation of foraging behavior of *E. virissima* on *M. annua*: **a)** Recognition flight, **b)** landing, and **c)** obtaining nectar. Diagram by Falcón-Brindis, A.

Results

Floral traits and floral resources

Flowers of *M. annua* opened between 05:00 and 08:00 h, reaching the maximum diameter of the corolla at midday (3.46 ± 0.77 cm, hereafter standard error (SE), N=10). Anthesis varied from 1–2 days. Flowers kept partially opened at night (i.e. lobes of corolla gently retracted inward), even when plants exhibited nyctinasty (upward folding position of leaves) when sunlight started vanishing at 17:00 –18:00 h. Leaves returned to their position until morning. Each plant displayed 2–3 opened flowers simultaneously during blooming (N=12). Nectar production averaged 0.021 ± 0.086 ml with a concentration of 36.16 ± 2.28 °Bx/flower (N= 6).

The corolla diameter of *M. annua* was greater than 6 mm (Table 1) and did not exhibit herkogamy, since anthers and stigma were not spatially separated (t = 0.00064, df = 6, p = 0.49). Dehiscence of anthers occurred since flower opening and before stigma receptivity, which denoted protandry. The outcrossing index (OCI), *M. annua* displays a xenogamous reproduction system (Supplementary Material). The floral measurements of *M. annua* are described in Table 1.

Table 1. Floral attributes of *Martynia annua* in the studied location. SE = standard error.

Floral attributes	Mean (cm)	SE
Non-reproductive		
Flower lenght	5.76	0.66
Corolla diameter	2.96	0.86
Inner diamater of corolla	1.81	0.82
Pedicel length	1.48	0.79
Pedicel diameter	0.21	0.20
Reproductive		
Gynoecium length	2.44	0.73
Stigma lenght	1.95	0.42
Ovary (polar)	0.48	0.20
Upper length of ovary	0.26	0.17
Ovary (equatorial)	0.26	0.22
Stamen length	2.53	0.84
Anther length	1.86	0.33

Floral visitors and foraging behavior

Six floral visitors were identified, including five species of bees: *Centris (Melanocentris) agilis* Smith, *Euglossa viridissima* Friese, *Frieseomelitta nigra* (Cresson), *T. fulviventris*, and *Caenaugochlora* sp., and one species of moth (Lepidoptera: Noctuidae).

Most of the visits occurred between 10:00-12:00 h. The frequency of floral visitors differed along the day (χ2 = 81.91, d.f. = 8, p< 0.001), presenting the highest rates of visitation at noon with 8.66 ± 2.60/individuals, which agrees with the maximum diameter of corolla (Fig 2).

The most abundant visitor was *E. viridissima* (70.6 % of total records), the vast majority were males (93.3% N=15) occurring within 6:00 and 18:00 h. The other recurrent visitor was *T. fulviventris*, occurring between 10:00 and 16:00 h. Individuals of this species, along with *F. nigra* frequently slipped at the entrance of the corolla while trying to make their way inside the flowers (Fig 1B). Thus, they failed the attempts to reach the nectar in >90% of observations (n = 13). Noctuid moths were observed reaching the nectar with their proboscis while standing on the landing lobe of the corolla.

None of the floral visitors were seen removing pollen from the anthers. Only *C. agilis* was observed touching the reproductive organs of *M. annua*. In general, the visitation period had short duration (< 5.5 s), though *E. viridissima* spent 2-12 s in recognition flights around the flower, and then stayed 2-15 s inside the flower during the nectar consumption (Fig 1C-D).

Pollen loads

In total, 12 different pollen morphotypes were found on the scutum surface among the floral visitors of *M. annua* (Supplementary Material). Moreover, the composition and amount of pollen loads were variable (Table 2). In general, the amount of pollen extracted from the scutum of *C. agilis* was overwhelming if compared with the rest of individuals collected of other species. *E. viridissima* transported 50 to 80% of heterospecific pollen.

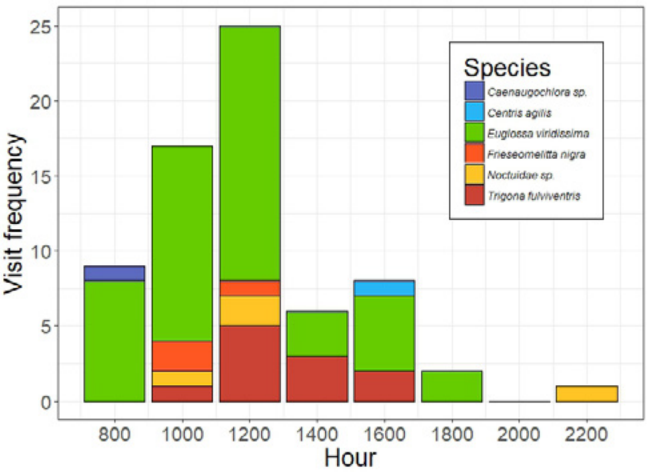

Fig 2. Assemblage of floral visitors and their frequency per hour in *Martynia annua*.

Discussion

This is the first study describing the reproductive system of *M. annua* in the area of its native distribution and summarizing aspects on floral biology, visitors, and their behavior. Our results indicate that *M. annua* has hermaphroditic flowers with absent herkogamy but with temporary separation between the release of pollen and the receptivity of the stigma (i.e. protandry). According to the OCI, *M. annua* has a xenogamous reproductive system (Cruden, 1977), which is usually present among plants who require outcrossing

Table 2. Pollen loads on floral visitors and average time spent within the flowers of *Martynia annua.* Pg = pollen grains of *M. annua* (Mean ± SE); Hpg = heterospecific pollen grains; Sa = scutum area. SE = standard error.

Family	Species	*Pg*	*Hpg*	*Sa (cm²)*	*Time*
Apidae	*Euglossa viridissima*	10 ± 9	2 ± 10	0.25 ± 0.01	6.4 ± 5.3
	Centris agilis	798	3	0.49	3.4
	*Frieseomelitta nigra**	1	0	0.04	4.1
	*Trigona fulviventris**	12 ± 3	2.2 ± 1.3	0.04	3.8 ± 0.88
Halictidae	*Caenaugochlora* sp*	1	3	0.09	3.7

* Never entered to the flower.

pollen transfer either by biotic or abiotic vectors (Fenster et al., 2004). Despite protandry being a method to avoid self-pollination (Richards, 1986), the absence of herkogamy could eventually allow autogamy in *M. annua* in the absence of the appropriate vectors that promote outcrossing. This alternative is also present in *Proboscidea louisianica* (Mill.) (the closest relative of *M. annua*), which is a facultative xenogamous species (Phillippi & Tyrl, 1979).

Floral traits as predictors of the expected type of visitors (i.e. pollination syndromes), could depend on the plant-pollinator assemblage co-existing in a particular space and time (Herrera, 1988; Lázaro et al., 2008). However, syndrome concepts have been broadly tasted, supporting that adaptations in the most effective pollinator group can drive the convergent floral evolution (Wolfe & Krstolic, 1999; Lázaro et al., 2008; Gong & Huang, 2009; Rosas-Guerrero et al., 2014). Therefore, some important traits in *M. annua,* such as reward, color, fragrance, and zygomorphism may have such convergence ruled by specialized visitors.

Plants with zygomorphic flowers may restrict the approaching angle of desired pollinators, supporting the idea of the ecological coherence between the flower and the suitable floral visitors (Fenster et al., 2004; Sargent, 2004). In all visitation records, we noticed that small bees slipped on when intended to alight and climb up to take the reward. Thus, they were unable to reach the nectar. Probably both the thin waxy layer in the internal surface of the corolla and the flower bearing angle (>70°) cloud restrict the access to small bees.

Among the most common and generalist bee species occurring at the RBCC, *E. viridissima* has been reported interacting with 24 plant species (Ayala, 2004). That would explain why *E. viridissima* transported a great diverse pollen grains. However, heterospecific pollen transfer can reduce the fitness of plants by loss of pollen and physical blocking (Raine et al., 2007). Moreover, this euglossine species was not necessarily transporting conspecific pollen nor touching reproductive organs of *M. annua*.

Despite the usual lower frequency of large bees, the higher rate of pollen deposition (>50 times that of small bees) makes them important pollinators of wild plants (Liu & Huang, 2013). In our results, the amount of pollen grains carried by an individual of *C. agilis* was stunning. Its size and abundant pilosity allowed for transportation of large pollen loads on the scutum. Similarly, in *P. louisianica,* the bee species *Melissodes communis* Cresson and *Bombus pensylvanicus* (De Geer) were found to be the main pollinators (Phillippi & Tyrl, 1979). Both bee species are moderate to large sized and densely clothed with long hairs. Our results suggest that *C. agilis* would have the potential to be among the few specialized effective pollinators of *M. annua.*

In terms of our two proposed hypotheses we conclude that: (a) the reproductive biology of *M. annua* suggests a strong relationship with some specialized bees to perform outcrossing; (b) the effective outcrossing must be driven by large hairy bees that touch reproductive organs during visits hand have high rates of pollen deposition. In addition, some bees such as *E. viridissima* may use *M. annua* as an energetic supply rather than a protein source. Considering the wide range of distribution of *M. annua*, it is likely that bee species of the genera *Centris*, *Bombus*, *Xylocopa* or the tribe Eucerini, may be the associated pollinators in other regions of America. Finally, since *M. annua* is tolerant to human disturbance (Calderón, 1998), it is conceivable to think that the pollinator community vary along gradients of conservation and fragmentation, but also to have reproductive adaptations that allow their prevalence.

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Supplementary Material

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Authors' contribution

D Cárdenas-Ramos contributed with the floral biology and botanical analysis of the studied plant. A Falcón Brindis is responsible of the analysis, structure, and integration of this work. R Badillo-Montaño contributed with ecological and botanical interpretation. I Hinojosa-Díaz and R Ayala provided information and interpretation regarding the biology and behavior of the studied bees.

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