Journal of Arid Environments xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Journal of Arid Environments

journal homepage: www.elsevier.com/locate/jaridenv

Bats and moths contribute to the reproductive success of the columnar cactus *Pilosocereus leucocephalus*

Antonio Miranda-Jácome[a], Ricardo Rodríguez-García[a], Miguel A. Munguía-Rosas[b,*]

[a] *Instituto de Investigaciones Biológicas, Universidad Veracruzana, Xalapa, 91190, Mexico*

[b] *Laboratorio de Ecología Terrestre, Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional (Cinvestav), Carretera Antigua a Progreso km 6, Mérida, 97310, Mexico*

ARTICLE INFO

Keywords:
Bat pollination
Columnar cactus
Moth pollination
Pollination
Pollination system

ABSTRACT

The pollination systems of columnar cacti in the dry tropics are often thought to be highly specialized to bats. This specialization is generally inferred when flowers that are only exposed to the activity of nocturnal visitors set fruit and seed. Although moths are also common visitors to the flowers of columnar cacti at night, it is generally thought that their contribution to the reproductive success of this cactus is negligible. Using selective exclusions, we assessed the contribution of bats and moths to the reproductive success in a population of *Pilosocereus leucocephalus* in central Mexico. Fruit set was 100% for bat-pollinated flowers and 34% in moth-pollinated flowers. Seed number per fruit was 1473 in bat-pollinated and 836 in moth pollinated flowers. Our results clearly show that in addition to bats, moths are effective pollinators of *Pilosocereus leucocephalus* in the study area. Therefore, bats are the main pollinators of *P. leucocephalus*, and moths are the secondary pollinators.

Columnar cacti are the dominant elements in the plant communities of arid and semiarid ecosystems in the New World, where they provide valuable feeding resources and shelter to several animal species (Wolf and Rio, 2003). Some of these animals may establish a mutualistic relationship with the columnar cacti to the extent that the local extirpation of the interacting animal(s) may compromise the permanence of columnar cacti in the ecosystem (Fleming and Holland, 1998). Columnar cacti are animal-pollinated and because self-incompatibility is widespread among these plant species, columnar cacti strongly depend on their pollinators for sexual reproduction (e.g. Ibarra-Cerdeña et al., 2005; Valiente-Banuet et al., 1997).

Most of the columnar cacti in tribes Pachycereeae and Cereeae have chiropterophilic flowers (i.e. flowers with nocturnal anthesis, pale coloration, a musty smell and are bell-shaped); however, the degree of specialization in the pollination system varies widely and a variety of animals such as birds, bees and moths may visit and pollinate their flowers (Valiente-Banuet, 2002). A geographic pattern in the degree of specialization in plant–pollinator relationships has been described: tropical chiropterophilic columnar cacti are highly specialized to bat pollination while extratropical cacti are pollinated by bees and birds in addition to bats (Munguía-Rosas et al., 2010; Valiente-Banuet et al., 1996). Most studies supporting this geographic pattern were exclusion experiments (diurnal vs. nocturnal visitors) in which bat-specialization was simply inferred when flowers only exposed to nocturnal visitors set fruit (e.g. Ibarra-Cerdeña et al., 2005; Valiente-Banuet et al., 1997; Valiente-Banuet et al., 2004). However, more detailed observations with the aid of video cameras equipped with infrared light have revealed that nocturnal moths are also frequent visitors to the flowers of columnar cacti in the tropics (Ibarra-Cerdeña et al., 2005; Munguía-Rosas et al., 2010). These studies assumed that nocturnal moths were not effective pollinators based on their foraging behavior on the flowers; i.e. apparently, the moths did not touch reproductive organs. Even though moths are globally relevant pollinators, assessing their effectiveness is challenging because direct observation of their behavior is difficult at night and may be biased when assisted by artificial light (Macgregor et al., 2019). A highly accurate method for assessing pollinator effectiveness is selective experimental exclusion of visitors in the field; however, to our knowledge, until now, no study has evaluated experimentally whether the moths are effective visitors of columnar cacti (i.e. contribute to the fruit and seed set).

The goal of this paper was to assess the relative contribution of bats and moths to fruit set and seed production in a population of the columnar cactus *Pilosocereus leucocephalus* (Cactaceae, tribe Cereeae) in central Mexico using a field experiment. *P. leucocephalus* is a tropical cactus endemic to Mesoamerica with typically chiropterophilic flowers (bell-shaped, whitish to pinkish, that release an unpleasant odor). The flowers are hermaphroditic but unable to set fruit without pollinators (Munguía-Rosas et al., 2010). Birds and bees visit the flowers of this

* Corresponding author.
E-mail address: munguiarma@cinvestav.mx (M.A. Munguía-Rosas).

https://doi.org/10.1016/j.jaridenv.2019.06.001
Received 22 February 2019; Received in revised form 28 May 2019; Accepted 8 June 2019

cactus but their contribution to fruit set is minor (11.9%) relative to that of nocturnal pollinators (88.1%). Bats (87%) and moths (13%) visit the flowers of *P. leucocephalus* at night and have been observed to touch its reproductive organs (Munguía-Rosas et al., 2010). However, currently we do not know whether moths contribute to the reproductive success of *P. leucocephalus*. Because moths visit the flowers of *P. leucocephalus* and have been observed to touch the reproductive organs flowers, we predicted that moths could be contributing to the fruit and seed set of this species in the study area.

The population of *P. leucocephalus* we study is located at the lowest part (950 m a.s.l.) of the municipality of Xalapa in central Veracruz, Mexico (19° 35° 20" N, 96° 50' 38" W). Vegetation is tropical dry forest, dominant plant species are *Lysiloma microphylla* (Fabaceae), *L. acapulcensis* (Fabaceae), *P. leucocephalus* (Cactaceae) and *Dodonaea viscosa* (Sapindaceae) (Ortega, 1981). The weather is the driest of the sub-wet group with a mean annual temperature of 24 °C and 519 mm rainfall (Ortega, 1981).

During the spring and summer of 2014, we visited the study area and tagged 100 accessible and reproductive cacti (≥ 2 m tall), from which we randomly selected a subsample of 25 individuals. Before anthesis, four accessible buds per cactus that were about to open were bagged and allocated to one of the following treatments: (i) bat pollination or (ii) moth pollination. To have a balanced block design, two buds on each plant were allocated randomly to one of the two treatments. The final sample size was 100 flowers, 50 per treatment. The bat pollination treatment consisted of spraying naphthalene around the flower to prevent moths from visiting. The moth pollination treatment consisted of placing a cage made of a metallic mesh (0.5×0.5 cm square openings) around the flowers. The cages had a circular window of about 3.5 cm in diameter in the front which prevented bat visitation but allowed moths to visit. Naphthalene has been traditionally used to repel moths in domestic settings and is relatively specific. Naphthalene is not thought to affect flower development but, as a precaution, we did not apply naphthalene directly to the flowers, but rather, only on the trunk and pseudocephalia. The wingspan of the bat species that visit *P. leucocephalus* in the study area (*Choeroniscus godmani*, *Glossophaga soricina, Leptonycteris nivalis*) is 18 to more than 45 cm, while the average wingspan of common moth species in the area is only about 5–7 cm. Before sunrise (≈ 5 a.m.), experimental flowers were protected with cages made of the same metallic mesh, but totally closed to prevent any diurnal animals from visiting the flowers and to prevent seed removal until the fruit had ripened or aborted. Aborted and ripe fruits were harvested, dissected to assess ovule maturation and to count apparently viable seeds (black, hard and fully developed seeds).

To ensure that our treatments effectively filtered the target visitors, we filmed a subsample of 10 flowers on five different plants (two flowers per plant, one for each treatment) with a video camera under an infrared light (CuddeBack, Model 1231) that was set up about 2 m away from the flowers for 4 h (1900–2300 h). We filmed two flowers on a single plant per night on five consecutive nights. The number of visits by target and non-target visitors seen in the video were counted. Simultaneously, nearby we placed 1 mist net (6×2 m) from 1900 to 0500 h to catch bats and used entomological nets to catch moths in the proximity of the filmed plants.

Differences between bat and moth pollination treatments in terms of fruit set (binary response variable) and the number of seeds per fruit were assessed using generalized linear mixed models (GLMM) with a binomial (fruit set) or Poisson error distribution (seed number per fruit). In both models, the plant was included as a random factor. Visit frequency was compared between pollination treatments with a chi square test. Statistical analyses were run in R 3.5.1 (R Core Team, 2018).

According to the video recordings, experimental devices successfully allowed the visits of target visitors to experimental flowers, visits of non-target visitors were zero. The visit rate of moths (71 visits) was greater than that of bats (43 visits) (Table 1). However, bats touched

Table 1
Visiting frequency (total number of visits and number of effective visits [i.e., the visitor touched the reproductive organs]) of target nocturnal visitors to flowers of *Pilosocereus leucocephalus* in two exclusion treatments: bat pollination (naphthalene-sprayed flowers) and moth pollination (flowers in a 0.5×0.5 metallic mesh cage) assessed using a video camera with infrared light. The % of effective visits (Effectiveness) is shown in the last row. Visits by non-target visitors (i.e., moths visiting flowers in the bat-pollination treatment and vice versa) was zero for the two treatments.

	Treatment	
Visits	Bat pollination	Moth pollination
Total	43	71
Effective	41	20
Effectiveness (%)	95	28

the reproductive organs far more frequently (95%) than moths did (28%) ($\chi 2 = 12.69$, $P < 0.01$) (Table 1). Bats touched the style and anthers with their anterior body (head, neck, shoulders, and the upper part of the chest and back) and moths touched these structures with their legs, proboscides and, more rarely, their wings. Sometimes it was unclear whether or not the fine appendages of moths touched the reproductive organs of flowers in the videos, and so the percentage of effective visits may have been underestimated. During the experiment, three species of phyllostomid bats (subfamily Glossophaginae) visited the flowers in the bat pollination treatments: *Choeroniscus godmani, Glossophaga soricina* and *Leptonycteris nivalis*, species that were also caught in the mist net. These bat species were also found in a previous study with the pollen of *P. leucocephalus* on their fur in this and other populations in Mexico (Munguía-Rosas et al., 2010). Flowers in the moth pollination treatment were visited by *Agrius cingulata, Isognathus rimosa inclitus* and *Pseudosphinx tetrio,* all of which belong to the Sphingidae family.

From our sample of 100 flowers, 73% set fruit and the remaining 27% aborted. Of these, 30% (8) aborted at the flower stage, and 70% (19) exhibited a variable engrossment of the ovary. All of the flowers (100%) in the bat pollination treatment set fruit, and while significantly lower ($\chi_1 = 5.56$, $P = 0.02$), a relevant proportion of flowers in the moth pollination treatment also set fruit (34%) (Fig. 1). Interestingly, some of the fruit that aborted early in the moth pollination treatment were infested with fly or Lepidoptera larvae, insects that probably were repelled by the naphthalene around the flowers assigned to the bat pollination treatment. Therefore, the contribution of moths to fruit set may be even greater if larval infestation is controlled. Fruit developed from flowers in the bat pollination treatment had 55% (1473.54 ± 135.82, n = 50 fruits) more seeds than fruit from flowers in the moth pollination treatment (836.18 ± 87.11, n = 17 fruits) (Fig. 1). The standard deviation of the random factor was particularly high for seed number (904.55), suggesting that, for some plants, seed production per fruit attributable to moths was similar to or even greater, than in fruits developed from exclusively bat-pollinated flowers. The standard deviation of the random term for fruit set was 0.47.

Our results confirm that bats are the main pollinators of *P. leucocephalus* in the study area. This is probably due to their capacity to carry large quantities of pollen on their fur, and their high degree of mobility (Fleming et al., 2001). Although the role of moths as effective pollinators of chiropterophilic columnar cacti in the tropics has long been considered negligible (e.g. Munguía-Rosas et al., 2010; Valiente-Banuet et al., 2004), our experiment clearly shows that they can make an important contribution to the reproductive success of the study species (34% of fruit set, 836 seeds per fruit), at least in this study area. Even though we studied a single population over a single flowering season, previous studies conducted in this and other populations of *P. leucocephalus* in Mexico have reported that moths visit the flowers over two consecutive years (2006 and 2007; Munguía-Rosas et al., 2010). In

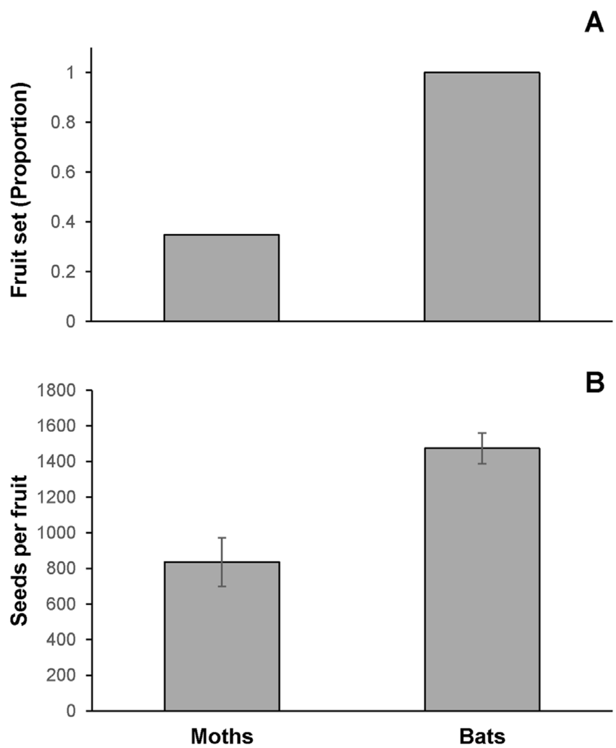

Fig. 1. Fruit set (A) and number of seeds per fruit (B) for the columnar cactus *Pilosocereus leucocephalus* in central Mexico under two experimental conditions: Flowers exposed to nectar-feeding bats (Bats) and flowers exposed to nocturnal moths (Moths). Fruit set is the proportion of flowers that set fruit and seed production is the mean number of seeds per fruit (± 1SE). In both cases (A & B) differences between treatments were statistically significant.

addition, the moth species that visit flowers of *P. leucocephalus* in the study area have an extensive overlap with this species of cactus (Ballesteros-Mejia et al., 2011; Ignativ et al., 2011; Seifert, 1974); therefore, moth pollination in other populations of *P. leucocephalus* is likely, but needs to be confirmed.

Previous studies have suggested that the pollination systems of chiropterophilic columnar cacti in the tropics may be highly vulnerable owing to their high degree of specialization on bats (Valiente-Banuet, 2002). However, it has also been suggested that other visitors may play an important role as secondary pollinators in chiropterophilic columnar cacti in the tropics when nectar-feeding bats are scarce or unpredictable (Rivera-Merchand and Aeckerman, 2006). In light of our findings, the high degree of specialization on bats in other chiropterophilic columnar cacti should be reviewed because nocturnal vs. diurnal exclusions cannot separate the contribution of bats from that of moths to the reproductive success of cacti. When fruit and seeds are only produced as a result of the activity of nocturnal visitors, this does not necessarily imply that these cacti are exclusively pollinated by bats.

Currently, it is recognized that specialized pollination systems are relatively rare in nature (Waser et al., 1996), and this is because plant species with highly specialized pollination systems are prone to extinction, and therefore, selected against. Also, other studies have shown that pollination syndromes are not reliable predictors of the main pollinators (Ollerton et al., 2009). Because the chiropterophilic pollination syndrome is widespread among species in the columnar cactus tribes Pachycereeae and Cereeae, we cannot rule out the possibility that chiropterophily is a phylogenetically conserved character and not the result of pollinator-mediated selection and adaptation in these columnar cacti. Also, this syndrome does not preclude the participation of other nocturnal visitors that may play a role as secondary pollinators.

In conclusion, moths are effective pollinators of *P. leucocephalus* in the study area. While bats are the main pollinators, given that 34% of the flowers visited exclusively by moths set fruit and these fruits have on average 836 seeds per fruit, this suggests that moths play a role as secondary pollinators.

Authors' contributions

AM-J, RR-G designed the study and collected data, MAM-R analyzed data and wrote the first draft. All authors revised and approved final version.

Declaration of conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

We are grateful to T Carmona-Valdovinos for comments made to RR-G on his undergraduate thesis from which this paper was derived. R Matus Guzmán and S Vázquez Castillo provided valuable field assistance. The owners of Rancho San Ignacio, E Gómez and C Caraza, kindly allowed us to work on their land. This study was funded by SEP-PRODEP (PTC-795 funds to AM-J).

References

Ballesteros-Mejia, L., Kitching, I.J., Beck, J., 2011. Projecting the potential invasion of the pink spotted hawkmoth (*Agrius cingulata*) across Africa. Int. J. Pest Manag. 57 (2), 153–159.
Fleming, T.H., Holland, J.N., 1998. The evolution of obligate mutualisms: senita cactus and senita moth. Oecologia 114 (3), 368–375.
Fleming, T.H., Shaley, C., Holland, J.N., Nason, J.D., Hamrick, J.L., 2001. Sonoran Desert columnar cacti and the evolution of generalized pollination systems. Ecol. Monogr. 71 (4), 511–530.
Ibarra-Cerdeña, C.N., Iñiguez-Davalos, L.I., Sánchez-Cordero, V., 2005. Pollination ecology of *Stenocereus queretaroensis* (Cactaceae), a chiropterophilous columnar cactus, in a tropical dry forest of Mexico. Am. J. Bot. 92 (3), 503–509.
Ignativ, I.I., Janovec, J.P., Centeno, P., Tobler, M.W., Grados, J., Lamas, G., Kitching, I.J., 2011. Patterns of richness, composition and distribution of Sphingid moths along an elevation gradients in the Andes-Amazon region of Southeastern Peru. Ann. Entomol. Soc. Am. 104 (1), 68–76.
Macgregor, C.J., Kitson, J.J.N., Fox, R., Hahn, C., Lunt, D.H., Pocock, M.J.O., Evans, D.M., 2019. Construction, validation, and application of nocturnal pollen transport networks in an agro-ecosystem: a comparison using light microscopy and DNA metabarcoding. Ecol. Entomol. 44 (1), 17–29.
Munguía-Rosas, M.A., Sosa, V.J., Jácome-Flores, M.E., 2010. Pollination system of the *Pilosocereus leucocephalus* columnar cactus (tribe Cereeae) in eastern Mexico. Plant Biol. 12 (4), 578–586.
Ollerton, J., Alarcón, R., Waser, N.M., Price, M.V., Watts, S., Cranmer, L., Hingston, A., Peter, C.I., Rotenberry, J., 2009. A global test of the pollination syndrome hypothesis. Ann. Bot. 103 (9), 1471–1480.
Ortega, R., 1981. Vegetación y flora de una corriente de lava (malpaís) al noreste del Cofre de Perote. Biotica (Mex.) 6, 57–84.
R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
Rivera-Merchand, B., Ackerman, J.D., 2006. Bat pollination breakdown in the Caribbean columnar cactus *Pilosocereus royenii*. Biotropica 38 (5), 635–642.
Seifert, R.P., 1974. The Sphingidae of Turrialba, Cost Rica. J. N. Y. Entomol. Soc. 82 (81), 45–56.
Valiente-Banuet, A., 2002. Vulnerability of pollination systems of columnar cacti of Mexico. Rev. Chil. Hist. Nat. 75 (1), 99–104.
Valiente-Banuet, A., Arizmendi, M.C., Rojas-Martínez, A., Domínguez-Canseco, L., 1996. Ecological relationships between columnar cacti and nectar feeding bats in Mexico. J. Trop. Ecol. 12 (1), 103–119.
Valiente-Banuet, A., Molina-Freaner, F., Torres, A., Arizmendi, M.C., Casas, A., 2004. Geographic differentiation in the pollination system of the columnar cactus *Pachycereus pecten-aboriginum*. Am. J. Bot. 91 (6), 850–855.
Valiente-Banuet, A., Rojas-Martínez, A., Casas, A., Arizmendi, M.C., Dávila, P., 1997. Pollination biology of two winter-blooming giant columnar cacti in the Tehuacán Valley, central Mexico. J. Arid Environ. 37 (2), 331–341.
Waser, N.M., Chittka, L., Price, M.V., Williams, N.M., Ollerton, J., 1996. Generalization in pollination systems, and why it matters. Ecology 77 (4), 1043–1060.
Wolf, B.O., Rio, C.M.D., 2003. How important are columnar cacti as sources of water and nutrients for desert consumers? A review. Isot. Environ. Health Stud. 39 (1), 53–67.