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Effect of mutualistic and antagonistic bees on floral resources and pollination of a savanna shrub☆

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ABSTRACT

Since Darwin, cheaters have been described in plant-pollinator mutualisms. Bignoniaceae species have a wide interaction network with floral visitors, and most of those interactions are established with cheaters. Thus, our objective was to determine which role each floral visitor plays in a system composed by bees and a Bignoniaceae savanna species. So, here we described the bees' behaviour and defined experimentally who are the mutualists and cheaters, we described the temporal sequence of interactions, quantified pollen and nectar removal, and checked for the potential effect of robbery damages on pollinator behaviour. Pollinators visited a small number of flowers, mainly in the early morning, while the most frequent cheaters (robbers and thieves) visited the flowers throughout the day, increasing visitation at midmorning, when pollinators had already visited the flowers. We considered medium-sized bees as pollinators, small bees acted only as cheaters, not contributing to seed production. Pollen thieves reduced the amount of male gametes available for pollination, while nectar thieves and robbers were associated with nectar depletion. We did not find any significant difference in the number of pollen grains deposited on the stigmas of flowers with and without robbery damages. In conclusion, when pollinators visit *J. caroba* flowers in the early morning, there might be a smaller competition pressure among visitors due to the greater amount of resources available, especially in recently opened flowers, and due to the low frequency of cheaters visits, which probably prevents a major negative impact of cheaters on pollination and plant reproduction in this species.

1. Introduction

Floral communicative traits, such as scent (Vogel, 1990) and flower colours (Chittka and Thomson, 2001) may attract animals with similar sensory abilities (Schaefer and Ruxton, 2011). However, these visual cues and signals do not attract exclusively mutualistic animals searching for floral rewards, so in nature, floral resources are frequently exploited by a variable range of animals. Indeed, plant-pollinator mutualisms are cooperative interactions mediated by floral resources that can be susceptible to exploitative species acting as cheaters, without providing benefits to plants (Darwin, 1841; Thompson, 1982; Bronstein, 1994).

Cheaters can be classified in different ways due to their behaviour and impact on plants. In particular, thieves are visitors that enter the flower tube, by the same opening used by pollinators to access the floral resources, without causing damage to the flower, but a mismatch of morphologies precludes pollination; while robbers can access floral resources only through damages performed to the floral structure (Inouye, 1980). In both cases, floral resources, like pollen and nectar, can be depleted without pollen transfer services. Additionally, the intensity of cheaters' impact on mutualistic systems may depend on the temporal sequence in which these interactions take place (Barker and Bronstein, 2016). For example, cheaters visiting flowers earlier than pollinators can decrease floral resources, reducing flower attractiveness, which may lead to changes in pollinator behaviour and, consequently, negatively affect plant reproduction (Hargreaves et al., 2009).

Nectar is the main floral reward in angiosperms, and it is essential in determining the interactions between flowers and their visitors, both pollinators and cheaters (Willmer, 2011). Nectar robbery and theft may cause various effects on plants' reproductive

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success (Maloof and Inouye, 2000; Irwin et al., 2010). The impact of nectar robbers on pollinator behaviour is supposed to differ from the impact caused by nectar thieves because, besides the nectar depletion caused by both groups, the robbers may create visual signs (damages) that could be recognized by pollinators.

On the other hand, pollen thieves are associated with the reduction of pollen that, in other situations, could be removed and dispersed by pollinators (Hargreaves, 2007; Hargreaves et al., 2009). It may lead to a reduction of the amount of male gametes available for fertilization, directly and negatively affecting plants' sexual reproduction (Carmo et al., 2004). In some *Jacaranda* species, small bees, generally described as pollen thieves, may occasionally touch flowers' reproductive structures during pollen collection manoeuvres (Guimarães et al., 2008; Maués et al., 2008; Milet-Pinheiro and Schlindwein, 2009). Indeed, it was evidenced, for other biological systems, that small bees may transfer pollen grains to the stigma and, thus, occasionally act as pollinators (Hargreaves et al., 2009; Ballantyne et al., 2015). However, the effectiveness of floral visitors has been based, for example, on the frequency of interactions, presence of pollen on the insect's body, pollen deposition on the stigma, and pollen tube development (Castro et al., 2013). So, an experimental approach that tests the role of small bees, previously classified as pollen thieves, on the sexual reproduction of these plant species is lacking.

In fact, the putative role of each floral visitor on seed production remains unclear for most systems. Bignoniaceae species, for example, have shown a rich and wide interaction network with floral visitors, with 75% of the interactions established with cheaters (Genini et al., 2010). However, the real impact of cheaters on plant pollination remains poorly understood, probably due to the complexity of this communication network. Thus, our objective was to determine which role each floral visitor plays in a system composed by a Bignoniaceae species, *Jacaranda caroba*, and the bees that visit its flowers. For this purpose, we described the behaviour of each floral visitor and we tested the effectiveness of all the bees that entered the flower tube on the production of viable seeds; we characterized the temporal sequence of interactions displayed by all bees that visited *J. caroba* flowers; we quantified pollen and nectar removal by pollinators and cheaters; and we verified the potential effect of nectar robbery damages to the corolla tube on pollen grains deposition. Specifically, we asked: (i) who acts as cheaters and as pollinators in this system; (ii) how is the visitation pattern of cheaters and pollinators throughout the day; (iii) do cheaters affect pollen and nectar availability to pollinators; (iv) does nectar robbery damage negatively affect pollen deposition on the stigma?

2. Material and methods

2.1. Plant species and study site

We selected *Jacaranda caroba* as the focal plant species because it is involved in a rich network of interactions, including several cheaters species acting as thieves and robbers, with only two bee species recognized as pollinators due to their morphology and behaviour by Guimarães et al. (2008). *Jacaranda caroba* is the accepted name and *J. oxyphylla*, as referred in Yanagizawa and Maimoni-Rodella (2007) and in Guimarães et al. (2008), is currently treated as synonym (The plant list, 2013).

Jacaranda caroba (Vell.) DC. (Bignoniaceae) is a plant species endemic to Brazil that occurs mainly in cerrado *lato sensu* and shows a habit varying from shrub to small trees (0.7–3.0 m) (Gentry and Morawetz, 1992). This species is characterized by opposite phyllotaxis and bipinnate composed leaves; panicle inflorescence; tubular-campanulate flowers with dorsoventrally flat corollas, varying from light to dark purple; the reproductive structures are located in the centre of the flower tube; the androecium has didynamous stamens, with dithecate anthers and one long glandular staminode; the gynoeceum is composed of a pistil with a swollen ovary above the cylindrical nectary; fruits are elliptical capsules with membranous winged seeds that are wind-dispersed (Gentry and Morawetz, 1992). The specimens' vouchers are deposited in the Herbarium BOTU "Irina Delanova de Gemtchujnicov" (voucher numbers 32538, 32539 and 32540).

This study was conducted at the "Estação Ecológica de Santa Bárbara" from the "Instituto Florestal do Estado de São Paulo", located in São Paulo state, Brazil (approximately between 22°46′to 22°50′S and 49°10′ to 49°15′W). The reserve has 2712 ha of cerrado *sensu lato* and seasonal forest vegetation. The climate is seasonal with average temperatures of 16 °C and 23 °C in the coldest and hottest months, respectively. Annual rainfall varies from 1000 to 1300 mm; the soil is usually deep, acidic and nutrient-poor (Melo and Durigan, 2011).

In the cerrado *sensu stricto* areas (savanna like vegetation) we mapped and labelled 230 individuals of *J. caroba* distributed in an area of approximately 8 km^2. During the flowering period, we randomly sampled several subsets of these labelled plants to use in different types of further detailed experiments.

2.2. Cheaters or pollinators? Bee behaviour and bee-flower dimensions

To describe bees' behaviour and visitation frequency throughout the day, we randomly assigned 30 previously labelled individuals, and conducted focal observations based on the protocol by Dafni et al. (2005). We performed observations throughout the daylight period, so that by the end of the flowering period we had 60 h of observation. During each interval of time, we recorded how many flowers were visited by each bee species per plant, the time the bees spent in each flower and the type of resource they collected. Later, we classified the bee visitors as pollinators or as different sorts of cheaters according to Inouye (1980). Additionally, we measured the thorax's diameter of the five specimens of each bee species and the tube diameter in the corolla region where the anthers and stigma are placed using 27 flowers from 9 plants (three flowers/plant) to describe the morphological adjustment between the bees' bodies and the floral tubes.

2.3. Cheaters or pollinators? Bee efficiency in the production of viable seeds

Considering that nectar-robbing bees never touch the flowers' reproductive structures, since they access nectar from the outside of the flower, we created two field experimental treatments to evaluate the role of bees that access floral resources from inside the flower, on seed formation: 'natural visitation' (natural seed set from all potential bee visitors) and 'small bees' (seed set excluding medium-sized bee visits). We also created one additional experiment: 'floral visitor exclusion' in order to check the need of a pollen transfer vector in the studied population.

The treatment in which the flowers were exposed to 'natural visitation' allowed all bee species to visit flowers of 10 different plants. These plants showed a mean of 3.1 ± 1.9 inflorescences and each inflorescence had 126.3 ± 55.9 flowers. In this treatment all the flowers produced by all the inflorescences of each plant were exposed to floral visitors during the entire reproductive period, which lasted around 90 days. In the 'small bees' treatment, we prevented the occurrence of medium and large-sized bees' visits by covering 14 inflorescences from 14 plants (one inflorescence/plant) with a wire mesh box with holes of 10 mm of diameter that only allowed the entrance of small bees. In this treatment all the flowers produced by the caged inflorescences (68.3 ± 34.9 flowers) were