The Science of Nature (2022) 109:3
https://doi.org/10.1007/s00114-021-01771-9

ORIGINAL ARTICLE

Müllerian mimicry between oil-producing orchids and Malpighiaceae? An old hypothesis finally tested

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Received: 7 June 2021 / Revised: 28 October 2021 / Accepted: 5 November 2021

Abstract

The concept of mimicry has been developed for animals, but it also applies to plants. Plant species may be Müllerian mimics if they have similar reproductive traits and offer similar rewards to the pollinators. Several Oncidiinae orchids offer floral oils to their pollinators and have been suggested to form a Müllerian complex with species of Malpighiaceae. We provide a test of this hypothesis using *Gomesa flexuosa* (Orchidaceae) and *Janusia guaranitica* (Malpighiaceae), which are sympatric and phenologically synchronous, secrete the same floral resource (oils), and show similar flower morphology. We exposed individuals of *Gomesa* near and far from individuals of *Janusia* and monitored floral visitation. Both species were exclusively pollinated by oil-collecting bees, sharing *Centris trigonoides* as a pollinator. Nevertheless, the probability of bee visitation, number of contact approximations, and number of visits to flowers of *Gomesa* were similar when individuals were near and far from *Janusia*. These findings do not support the Müllerian mimicry hypothesis in these two species. Their resemblances can be better explained by the "exploitation of perceptual biases" hypothesis. According to this hypothesis, pre-existing traits in Oncidiinae orchids (e.g. colour, shape, rewards) may coopt oil-collecting bees that usually search for rewards in Malpighiaceae species with similar flower traits.

Keywords Exploitation of perceptual biases · Floral visitation · Müllerian mimicry · Oil-collecting bees · Oil flowers · Pollination

Introduction

In the middle of the nineteenth century, Henry W. Bates (1862), studying butterflies from the Neotropics, proposed that palatable species could resemble unpalatable ones when in sympatry, exhibiting an aposematic (advertising) colouration and gaining protection against predation. This pattern of phenotypic convergence was later named as Batesian mimicry in honour of its discoverer. In Batesian mimicry, the harmful species is called *model* and has a true aposematic colour, while the *mimic* displays a "disguise" (Ruxton et al. 2004). The greater the similarity between the phenotypes of the species, the greater the predator's difficulty in distinguishing between palatable (i.e. the mimic) and unpalatable (i.e. the model) species. A few years later, naturalist Fritz Müller (1878) formulated an explanation for the similarities between phenotypes of unpalatable species, which could reduce mortality costs as a result of learning by naive predators. This pattern of phenotypic convergence between unpalatable species was later named as Müllerian mimicry, also in honour of its discoverer. In Müllerian mimicry, all species within a mimetic complex should be considered *co-mimics* (or *co-models*), because there is a mutual convergence in appearance between all forms (Ruxton et al. 2004). Müller (1878) himself stated that knowing which one is the original and which one is the copy is an irrelevant question, assuming this mutual convergence. Another difference between these two types of mimetism is the frequency-dependent selection: in Batesian mimicry, the higher the frequency of mimics in the population, the lower their fitness advantage, whereas in Müllerian mimicry, the higher the frequency of co-mimics

Communicated by: Tatiana Cornelissen

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Published online: 07 December 2021

in the population, the higher their fitness advantage (Ruxton et al. 2004).

Although the concepts of Batesian and Müllerian mimicry were developed based on animals as study systems, they may also be applied to plants (Wiens 1978; Dafni 1984; Roy and Widmer 1999). As it happens to animals, cases of Batesian mimicry in plants should include a model, a mimic, and a signal-receiver, which is an individual that cannot properly differentiate between models and mimics (Schaefer and Ruxton 2009). Based on this general framework, we can include as cases of Batesian mimicry those species in which pollination is based upon deceit. In this case, species that do not offer resources (i.e. mimics) may attract floral visitors (i.e. signal-receivers) by mimicking the general appearance, chemical signals, and/or reflectance spectra of resource-offering plants (i.e. models). The floral visitors, usually looking for food, apparently are not able to discriminate between species with or without floral resources (Roy and Widmer 1999; Schaefer and Ruxton 2009). In species of Orchidaceae, for instance, deceptive, non-rewarding flowers are generally aromatic, and these fragrances are pointed as the main attraction factors for potential insect pollinators (Vereecken and McNeil 2010). Examples of this type of Batesian mimicry have already been described for several orchids of the genera *Disa* (Johnson 1992, 1994, 2000; Johnson et al 2003a; Anderson et al. 2005; Johnson and Morita 2006), *Anacamptis* (Johnson et al. 2003b), and *Orchis* (Galizia et al. 2005). Moreover, Carmona-Díaz and García-Franco (2009) have observed an increase in the fruit set of *Trichocentrum luridum* (as *Oncidium cosymbephorum*) when in proximity to *Malpighia glabra* (Malpighiaceae). In the same way, *Cyrtopodium punctatum* presented an increased rate of fructification when growing near the oil-rewarding malpigh *Byrsonima lucida* (Pemberton and Liu 2008). Both orchids mentioned above are food deceit species and offer no resources to the pollinators. Sexual deceit is another example of Batesian mimicry widely reported in orchids. In this case, male polinators are lured by orchid flowers that mimic the sexual morphology and/or the sexual pheromones of their female counterparts. By trying to copulate with the "females", males pollinate the orchids (e.g. Singer et al. 2004; Vereecken et al. 2012; Martel et al. 2016).

Unlike deceptive pollination systems, resource-offering plants that mimic each other's flowers can be regarded as cases of Müllerian mimicry because all species included in the mimetic complex offer resources to the floral visitors. Co-mimic species have a convergent morphology, and the signals emitted to the pollinators are not deceptive (Dafni 1984). This convergence in morphology increases the fitness of all co-mimics, so that their similarity should be regarded as adaptive (Schaefer and Ruxton 2009). Müllerian mimicry is relatively rare in plants, and to our knowledge, several cases have been suggested, but no experimental demonstration in support to this hypothesis has been formally provided (see references in Bierzychudek 1981; Dafni 1984; Roy and Widmer 1999; Chase et al. 2009; Neubig et al. 2012). In the 1960s (e.g. van der Pijl and Dodson 1966; Table S1), some authors highlighted the overall resemblance between Oncidiinae orchids and species of Malpighiaceae. More recently, several authors have suggested that many South American oil-offering Oncidiinae orchids should be part of Müllerian mimetic complexes also involving species of Malpighiaceae (e.g. Chase et al. 2009; Pemberton 2010; Neubig et al. 2012; Table S1). These putative Müllerian mimics show similar morphology, colouration, and light absorption spectra (Chase et al. 2009). In the case of species of the genus *Gomesa* (Oncidiinae), the flowers of several species have oil-secreting glands, known as elaiophores (Chiron et al. 2009; Aliscioni et al. 2009; Gomiz et al. 2013; Pansarin et al. 2016; Gomiz et al. 2017; reviewed by Castro and Singer 2019), which provide a reward similar to that offered by many species of Malpighiaceae (Renner and Schaefer 2010; Gomiz et al. 2017).

Oil-collecting bees of the family Apidae play a major role in the pollination of oil-offering South American plant species (Machado 2004; Alves-dos-Santos et al. 2007). These insects have even developed specialized structures for both the extraction of floral oils and their deposition in fore tarsal oil-storage structures essentially made of branched hairs on their hind legs (Neff and Simpson 1981; Buchmann 1987; Machado 2004; Alves-dos-Santos et al. 2007). Oil-collecting bees can chemically modify the oils and use them as provisions for their larvae or employ these resources for water-resistant cell linings (Buchmann 1987; Simpson and Neff 1981; Alves-dos-Santos et al. 2007; Renner and Schaefer 2010; Possobom and Machado 2017). Given that oil-collecting bees visit both Malpighiaceae and some Oncidiinae species (Singer and Cocucci 1999; Reis et al. 2006; Sigrist and Sazima 2004; Pansarin et al. 2016), sympatric species of these two taxa may have common pollinators, which should be able to move freely between individuals of both groups. Besides morphological similarity and overlapping phenologies and distributions, to share the same pollinators is a necessary condition for the existence of Müllerian mimicry in plants (Roy and Widmer 1999).

Suggestions of Müllerian mimicry involving Oncidiinae orchids and Malpighiaceae species can be found in the literature since 1966 (e.g. van der Pijl and Dodson 1966; Table S1). In the last 10 years, several papers reinforced this suggestion with observational data (see references in Table S1). To our knowledge, however, there is no formal test to the hypothesis that Oncidiinae orchids and Malpighiaceae species form a Müllerian mimicry complex. The main goal of this study is to fill this gap and provide the first experimental test of this long-lasting hypothesis. Particularly, we tested whether the South American *Gomesa*

flexuosa (Oncidiinae) and *Janusia guaranitica* (Malpighiaceae) are Müllerian co-mimics. These two species were chosen because they are sympatric, there is considerable overlap in their geographical distribution and flowering periods, their flowers are similar in shape and colour, and preliminary field observations indicated that they were visited by similar-sized *Centris* bees. Given that both plant species are oil-offering species (Lorenzo 1981; Gomiz et al. 2013), the hypothesis of Müllerian mimicry predicts that they would share at least one common oil-collecting bee pollinator. Moreover, if the two species form a Müllerian complex, it would also be expected that their co-occurrence would positively affect each other's visitation by potential pollinators.

Methods

Studied species

Gomesa is a highly diverse genus in the Atlantic Rainforest and Cerrado (Brazilian savannah), with the current number of accepted species ranging from 80 (Reflora 2021) to 125 (The Royal Botanic Gardens (Kew) and Missouri Botanical Garden 2021). *Gomesa flexuosa* occurs specially in the southern and southeastern coast of Brazil, also reaching the Northeast region (GBIF 2021; Fig. 1). The genus *Janusia*, in turn, has only 14–15 accepted species (Reflora 2021; The Royal Botanic Gardens (Kew) and Missouri Botanical Garden 2021;). One of the most widespread species of the genus is *J. guaranitica*, which occurs through all Uruguay, Northern Argentina, southern Brazil (Fig. 1), Paraguay, and most parts of Bolivia (GBIF 2021). In Porto Alegre, State of Rio Grande do Sul, Southern Brazil, *G. flexuosa* occurs naturally in sympatry to *J. guaranitica* (Fig. 1).

The flowering period of *G. flexuosa* lasts from October to December. The vivid yellow flowers (Fig. 2A-C) are aggregated in lateral multi-flowered panicles (rarely racemes) that come out of the base of the pseudobulb. The labellum (modified median petal) presents a tubercular callus (Fig. 2B). The elaiophores are located at the callus or in structures formed by the folding of the labellum's edges, near the base of the column (Gomiz et al. 2017; Fig. 2B). All the orchid pollen content of a flower is "packaged" and is withdrawn from the flower as a translatory unit called pollinarium (Dressler 1993; Fig. 2C). The base of the column is thickened, and this prominent structure is called tabula infrastigmatica (Dressler 1993).

The flowering period of *J. guaranitica* lasts from late October to mid-March. The umbelliform inflorescences (Fig. 2D-F) are composed of four (sometimes three) flowers that diverge from the same point at the apex of the peduncle. The flowers have a vivid yellow-coloured pentamerous corolla with free, unguiculate petals (Fig. 2D). One of the petals is slightly larger and has a more elongated nail (narrow portion), known as the standard or flag petal. Four pairs of elaiophores are attached to the calyx (Fig. 2E). These elaiophores are placed externally to the sepals, being exposed even in floral buds. The pistil is central, with rounded, apically exposed stigma (Fig. 2F), and surrounded by five stamens containing the loose pollen. Overall, both studied species present similarly coloured yellow flowers, with similar flower morphology, including narrow, slender petal bases (Fig. 2).

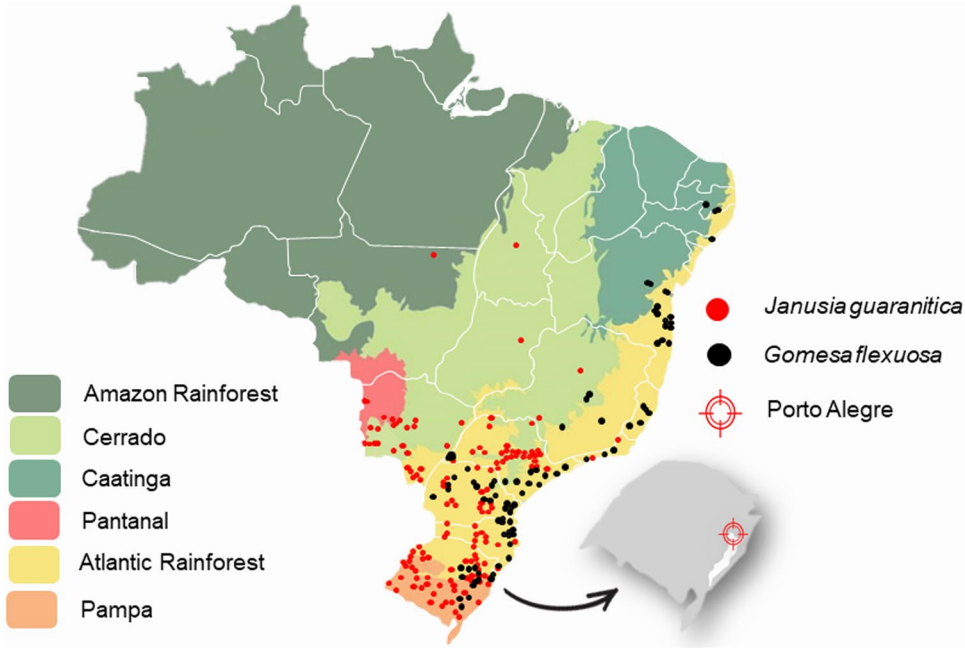

Fig. 1 *Gomesa flexuosa* and *Janusia guaranitica*'s geographical distribution in Brazil, showing the main biomes in the country. Note that both species are widespread and co-occur in Porto Alegre, State of Rio Grande do Sul, Southern Brazil, where our study was performed. Species occurrences used to build the map were obtained from SpeciesLink database (CRIA - Centro de Referência e Informação Ambiental 2021). The data were optimized by removing duplicate coordinates and plotting only Brazilian occurrences in the map (see Tables S2 and S3 in Supplementary Information)

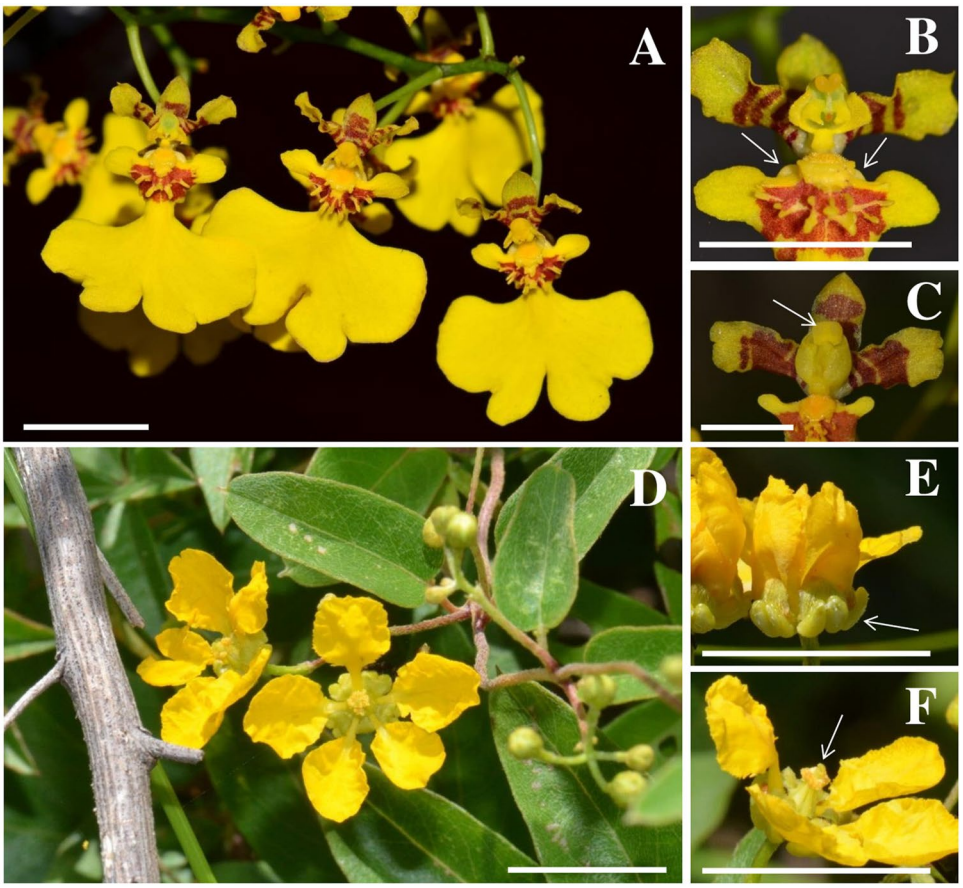

Fig. 2 Reproductive structures of *Gomesa flexuosa* (**A**–**C**) and *Janusia guaranitica* (**D**–**F**). **A** Set of flowers. **B** Detail of column, pollinarium, callus, and "sac-like" elaiophores (arrows). **C** Thickened column of a pollinated flower, sealing stigmatic cavity and keeping the anther cap (arrow). **D** Flowers and their unguiculate petals. **E** Floral buds and elaiophores (arrow). **F** Detail of stamens and the central pistil with exposed stigma (arrow). Scale bars = 1 cm

Study area

We conducted a field experiment at Porto Alegre Botanical Garden (30° 03′ 06.07″ S; 51° 10′ 37.95″ W), in a place surrounded by an open area predominantly occupied by cespitose herbs and lianas, with the presence of some shrubs and trees, such as *Cecropia pachystachya* (Urticaceae) and *Schinus terebinthifolia* (Anacardiaceae). The study site has a locally abundant, natural population of *J. guaranitica* and a scarce population of *G. flexuosa*.

Field observations and experimental design

To evaluate the possible existence of Müllerian mimicry between the study species, we conducted a field experiment in which we brought cultivated individuals of *G. flexuosa* close to individuals of a natural population of *J. guaranitica*. The experiment was conducted between November–December in 2018/2019 and 2019/2020 using two individuals of *G. flexuosa* at each time, each one with a sole inflorescence, aiming to accurately monitor the flow of pollen from one individual to another (Fig. 3). In total, we used five pairs, comprising ten individuals of *G. flexuosa*. Given that the specimens of *G. flexuosa* present a gradual flowering, the experiments consisted of subjecting each pair of inflorescences to two types of exposure (Fig. 3): between 1–2 m (hereafter, "near" exposure) and 20 m (hereafter, "far" exposure) from an individual of *J. guaranitica*. In a literature search, we found no study on the size of the foraging area in solitary bees (the shared pollinator of both species studied here; see "Results"); thus, we based our decision about the distances of 1–2 m and 20 m on two main criteria. Considering the small size of the malpighs (herbaceous plants with only dozens of flowers), we assumed that any possible magnet effect (sensu Laverty 1991) on the pollinators would be restricted to the adjacent areas of the focal individual (i.e. no more than 5-m radius). In fact, our preliminary field observations indicated that individuals of *Centris* bees frequently moved from the malpigh to the orchids in the near exposure, but this movement has never been recorded in the far exposure.

Each type of exposure was performed for three sunshiny days (not necessarily consecutive), keeping a 3-day interval between exposures (Fig. 3). During this interval, the individuals of *G. flexuosa* were maintained inside a green house at the Orchidarium of the Porto Alegre Botanical Garden, where the bees do not have access to the flowers. Thus, the minimum time required for monitoring each pair was 9 days:

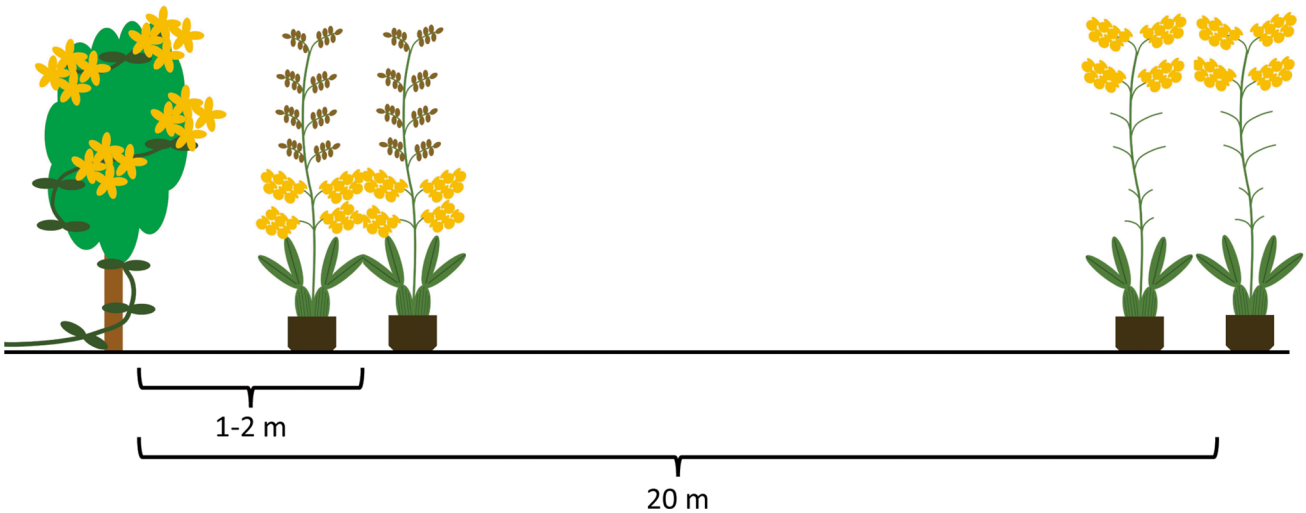

Fig. 3 Scheme of the experimental design used to test the hypothesis of Müllerian mimicry between the orchid *Gomesa flexuosa* and the malpigh *Janusia guaranitica*, which is a cespitose herb. Each pair of orchids (one inflorescence per individual) was exposed near (1–2 m) and far (20 m) from the focal malpigh. In the near exposure, which lasted for three sunshiny days (consecutive or not), both orchids had only one-third of their flowers open. In the scheme, we are depicting the first flowers to bloom in the inflorescence, while the remaining inflorescence contains only buttons. After the near exposure, the pair of orchids was maintained inside a green house for three consecutive days, during which one-third of the flowers opened, but had no access to pollinators. Finally, in the far exposure, which also lasted three sunshiny days (consecutive or not), both orchids had only one-third of their flowers open. In the scheme, we are depicting the last flowers to bloom in the inflorescence. We highlight, however, that the order of exposure (near vs. far) was randomly assigned to the experimental orchids to avoid any possible bias in the results. Both in the near and far exposures, the total number of open flowers was similar between the orchids and the focal malpigh

3 days for the near exposure, 3 days of interval between exposures, and 3 days for the far exposure (Fig. 3). For each pair of individuals, the order of exposure was randomized. There was no other *J. guaranitica* individual (besides the focal one) within a radius of at least 100 m, so that the visual stimulus received by the pollinators was restricted to the plants used in the experiment. When the individuals of *G. flexuosa* were near *J. guaranitica* (i.e. near exposure), visits in both species were recorded. When individuals of *G. flexuosa* were far from *J. guaranitica* (i.e. far exposure), only visits to the orchids were recorded because they were the focus of our study. The records of visits to the plants were performed between 09:00 h–12:00 h and 13:00 h–16:00 h (i.e. 6 h daily), totaling 180 h and 30 days of field observations. For each visit, we recorded the hour and time of permanence of the floral visitor in the inflorescences. Arrivals of insects were classified as follows: (1) *visits*, when individuals landed and manipulated the flowers, or (2) *contact approximations*, when individuals only touched the flowers and then left. To estimate the permanence time of individuals on the flowers, only visits were considered (contact approximations were excluded). The visits to the flowers were recorded with both a digital camera (Nikon D5100) and a camcorder (Sony DCR-SR21E). Video recordings allowed us to accurately document visitation activities and permanence time on the flowers. At the beginning of each stage (i.e. a new pair of orchids and a new exposure), the number of available flowers was recorded. At the end of each day, orchid flowers were checked for confirming pollinaria removals and depositions. Due to the removal of the pollinaria (that hold the entire pollen content of each flower) and the sealing mechanism of the columns, we can confidently know how many flowers donated pollen and how many of them were pollinated, respectively.

Plant vouchers were deposited in the ICN Herbarium, at Universidade Federal do Rio Grande do Sul. Vouchers of the pollinators were captured and deposited in the Entomological Collection at Universidade Federal do Paraná (UFPR), Curitiba, Brazil.

Statistical analyses

To test if the proximity to *J. guaranitica* influences the *probability of bee visitation* to *G. flexuosa*, we used a generalized linear mixed model (GLMM) in which the predictor variable was the type of exposure (near vs. far from *J. guaranitica*) and the response variable (with binomial distribution of errors) was the occurrence (yes vs. no) of visits to the orchids per day during each exposure period of 3 days. To test if the proximity to *J. guaranitica* influences the *number of contact approximations* and the *number of visits* to flowers of *G. flexuosa* we also used GLMMs in which the predictor variable was the type of exposure and the response variables (both with negative binomial distribution of errors to

account for overdispersion of the data) were, respectively, the number of contact approximations and the number of visits to the orchids per day during each exposure period of 3 days. For all models described above, each pair of individuals of *G. flexuosa* provided six datapoints: 3 days in the near exposure and 3 days in the far exposure. Finally, to test if the proximity to *J. guaranitica* influences the *time of permanence* of visitors on the flowers of *G. flexuosa*, we used a linear mixed model (LMM) in which the predictor variable was the type of exposure and the response variable (with Gaussian distribution of errors) was the time of permanence (in seconds) of each visitor on the flowers of the orchids per day during each exposure period of 3 days. Because the data of the near exposure showed much higher variation than that of the far exposure, we corrected the analysis for heteroscedasticity. We did not include the number of open flowers in any analysis because paired *t*-tests indicated no statistical difference between (i) the total number of open flowers of the orchids in the near and far exposures and (ii) the total number of open flowers of the orchids and the malpigh in near and far exposures ($t = 0.953$; $df = 19$; $p = 0.366$).

In all models we built (GLMMs and LMM), the experimental pairs were included as a random variable because each inflorescence of the pair was exposed both near and far from an individual of *J. guaranitica*. The GLMMs were performed using the packages nlme (Pinheiro et al. 2019) and MASS (Venables and Ripley 2002). The LMM was performed using the package lme4 (Bates et al. 2015) of the software R 3.6.3 (R Core Team 2021).

Results

Pollinators, floral visitors, and overall visitation

In agreement with our preceding observations Castro et al. (in press), *Gomesa flexuosa* and *J. guaranitica* share a pollinator species: female bees of *Centris* (*Hemisiella*) *trigonoides* (Apidae: Centridini), which visited the flowers throughout our observation period (Fig. 4). Overall, bees worked the flowers in a similar way (Fig. 4). The individuals landed on the flowers and attached themselves to the petals, usually biting their tabula infrastigmatica (orchids) (Fig. 3C) or the nails of the petals (malpighs) (Fig. 4E). We only observed flights between flowers of the orchids and the malpighs during the near exposure. *Janusia guaranitica* also had other floral visitors (Fig. S1 in Supplementary Information): *Paratetrapedia fervida* (Apidae: Tapinotaspidini), *Arhysoceble picta* (Apidae: Tapinotaspidini), *Epicharis* (*Hoplepicharis*) *fasciata* (Apidae: Centridini), and *Centris* (*Melanocentris*) *obsoleta* (Apidae: Centridini). All these taxa are oil-collecting bees. Regarding the orchids, individuals of *P. fervida* and an unidentified Halictidae species made only contact approximations, leaving the flowers shortly after landing and never reaching their reproductive structures.

Visits to the flowers occurred from early morning until around 15:30 h. The bees *C. trigonoides* and *P. fervida* together accounted for 92.9% of the total number of visits to *J. guaranitica* (172 of 185 visits), with *C. trigonoides* responsible for 70.2% (130 of 185 visits) and *P. fervida* for 22.7% (42 of 185 visits). The total time of permanence of *C. trigonoides* and *P. fervida* on the flowers of *J. guaranitica* was 582 min and 256 min, respectively. The time of permanence of all other species totalled only 30 min. In both types of exposure, pollination (pollen transfer) was strictly performed by individuals of *C. trigonoides*. These bees visited the orchids for 16 times in the near exposure and 11 times in the far exposure, staying in the flowers for a total of 33 and 9 min, respectively. Then, they comprised 27 visits, remaining on the flowers for a total of 42 min.

Both the number of visits and the total time of permanence of visitors on *J. guaranitica* flowers were much higher than those observed in *G. flexuosa*. In total, we recorded 185 visits and 869 min of permanence of floral visitors on the malpigh flowers. Throughout the experiment, 10 orchid flowers had their pollinaria removed and only three were pollinated. Remarkably, individuals of *G. flexuosa* had their pollinaria removed and were pollinated only in the far exposure. Throughout the experiment, only one orchid fruit was developed, also in the far exposure.

Field experiment

The probability of bee visitation to *G. flexuosa* in the far exposure was similar to the near exposure ($z = 0.431$; $p = 0.667$; Fig. 5A). The number of contact approximations did not differ between the far and the near exposures ($t = 0.184$; $df = 24$; $p = 0.855$; Fig. 5B). The number of visits to flowers also did not differ between the far and the near exposures ($t = 0.893$; $df = 24$; $p = 0.381$; Fig. 5C). Finally, the time of permanence of the bees on the flowers was higher in the near than in the far exposure ($t = 2.437$; $df = 57$; $p = 0.018$; Fig. 5D).

Discussion

Mimicry, be it Batesian or Müllerian, is frequently referred as the main process responsible for the pollination of many South American Oncidiinae orchids. In fact, there are several empirical studies that provide evidence of Batesian mimicry in Orchidaceae (e.g. Nierenberg 1972; Johnson 1992, 1994, 2000; Johnson et al. 2003a, b; Anderson et al. 2005; Galizia et al. 2005; Johnson and Morita 2006; Pemberton and Liu 2008; Carmona-Díaz and García-Franco 2009), but the

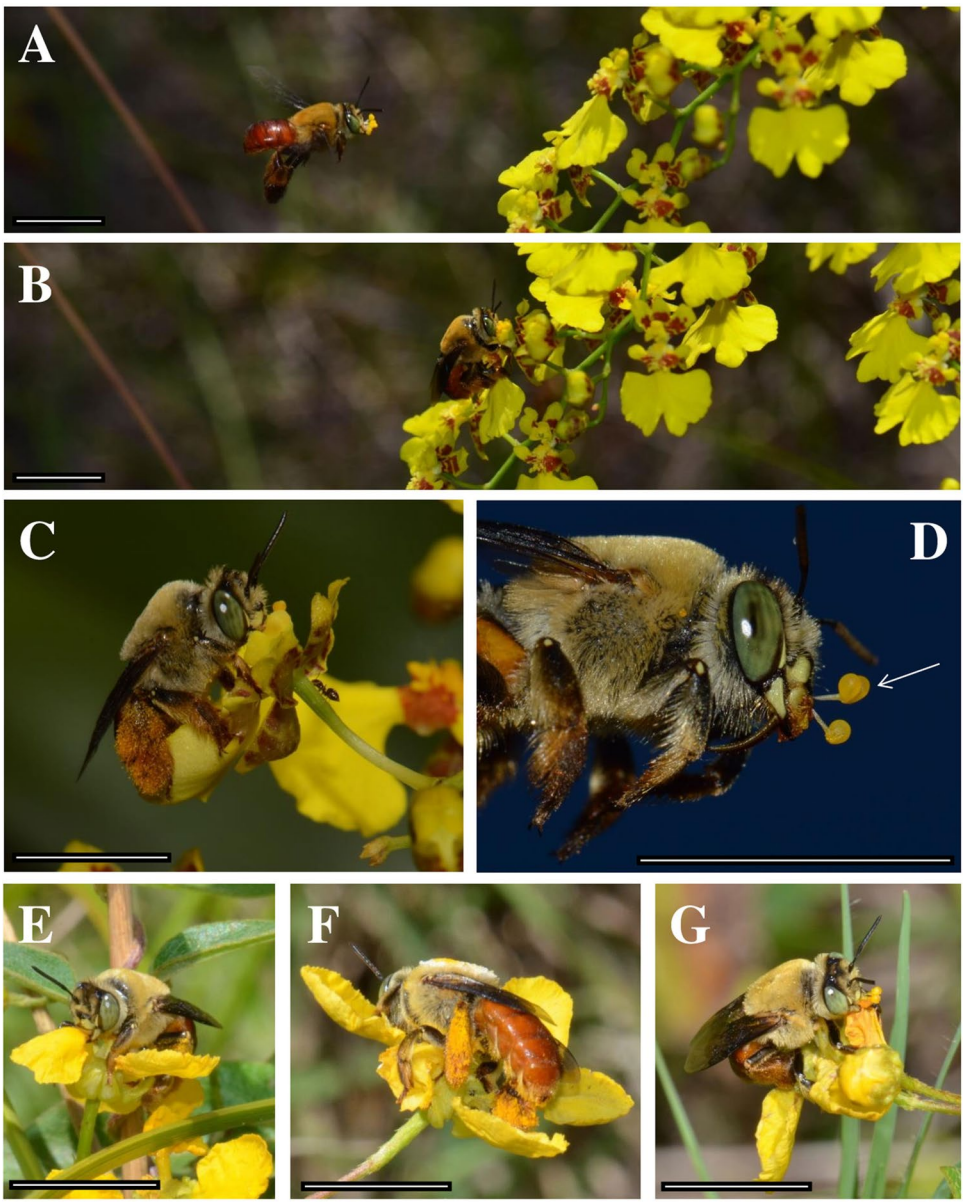

Fig. 4 *Centris trigonoides* pollinating *Gomesa flexuosa* (**A**–**D**) and *Janusia guaranitica* (**E**–**G**). **A**–**C** Sequential pictures of the bee approaching and landing on orchid flowers. **D** Site of pollinarium (arrow) attachment, the clypeus. **E** Bee biting the nail of the petal to stabilize itself on the flower. **F** Pollen-laden individual. **G** Bee with orchid pollinarium attached to its head. Scale bars = 1 cm

same is not true for Müllerian mimicry (e.g. Bierzychudek 1981). Although the hypothesis of Müllerian mimicry in orchids is widely evoked in the literature on pollination biology (Table S1), a formal empirical test was still lacking. To support the existence of Müllerian mimicry, the species should present the following traits, besides sharing at least one pollinator: overall similarity in flower morphology, similar chemical compounds (e.g. floral oils), spatial co-occurrence, and phenological synchrony. Considering that *G. flexuosa* and *J. guaranitica* are oil-offering species, have similar floral morphology and chemical compositions of oils (Reis et al. 2007), occur in the same place (Fig. 1), and bloom synchronously, they fulfill some of the criteria to be considered Müllerian co-mimics. However, the results of our field experiment suggest that bees can differentiate between the two plant species, contrasting with an important expectation of the Müllerian mimicry hypothesis that states that pollinators are not able to discriminate species of a mimetic complex. We show that the probability of bee visitation, the number of contact approximations, and the number of visits to the *G. flexuosa*'s flowers were similar when individuals were close and far from individuals of *J. guaranitica*. Only the time of permanence of the bees on the orchid flowers was higher when the individuals of both plant species were close to each other, but this increase did not reflect in higher fruiting success. Fruiting in *G. flexuosa* was minimal, with only one fruit developed throughout the experiment. This result does not provide support for a key prediction of Müllerian mimicry, which establishes that the fitness of the co-mimics should increase in sympatry (Schaefer and Ruxton, 2009).

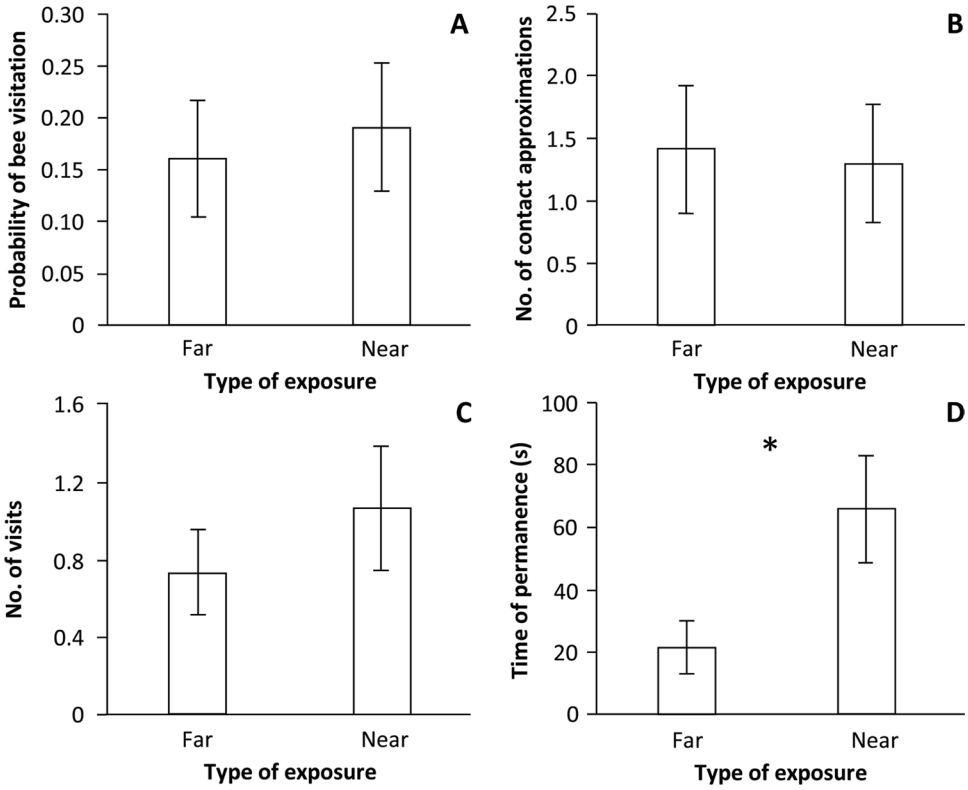

Fig. 5 Results of the field experiment to test the existence of Müllerian mimicry between the orchid *Gomesa flexuosa* and the Malpighiaceae *Janusia guaranitica*. Individuals of the orchid were exposed far (20 m) and near (1–2 m) the malpigh. The response variables gathered in the experiment were **A** probability of bee visitation, **B** number of contact approximations, **C** number of visits, and **D** time of permanence of each bee on the flowers. Bars represent means and errors are standard errors estimated by the models. The asterisk denotes a significant difference ($p < 0.05$) between the types of exposure

Taken together, our findings suggest that the Müllerian mimicry is not the best explanation for the similarities between the flowers of *G. flexuosa* and *J. guaranitica*.

The subtribe Oncidiinae is highly diverse and geographically widespread in the neotropical region (Chase 2009; Neubig et al. 2012). Therefore, species belonging to this clade may rely on equally diverse pollination processes and vectors of pollination. As we showed here, Müllerian mimicry, in particular, is unlikely to be the main explanation for species presenting "oncidioid" flowers (see Fig. 2A-C), which are morphologically similar to the flowers of Malpighiaceae and offer the same type of reward to the pollinators (i.e. oil). Many Oncidiinae species have the so-called oncidioid flowers (Neubig et al. 2012), but do not occur sympatrically with malpighs (Caiafa and Silva 2005; Araújo et al. 2021). In the case of the two species studied here, there is great overlap in their geographic distributions, especially in Southern and Southeastern Brazil, but there are also several localities where *G. flexuosa* occur in the absence of *J. guaranitica* (Fig. 1). In the localities of allopatry, the floral morphology of the orchid is indistinguishable from the localities of sympatry (J.B. Castro, *pers. obs.*), which does not support the possibility of convergent evolution of floral morphology in response to Müllerian mimicry as a general explanation to the similarities between several floral traits of *G. flexuosa* and *J. guaranitica*. Although we do not discard the possibility that some sympatric species of Oncidiinae and Malpighiaceae may form mimetic complexes, our data do not support this hypothesis for *G. flexuosa* and *J. guaranitica*.

An alternative explanation for the overall resemblance between *G. flexuosa* and *J. guaranitica* is provided by the "exploitation of perceptual biases" (EPB) hypothesis (Schaefer and Ruxton 2009; de Jager and Anderson 2019). According to this hypothesis, both plant species suffer a selective pressure imposed by a signal-receiver's sensory system. Different from mimicry, in which the mimics and models should converge to a similar phenotype and confuse the visitors, the EPB hypothesis proposes that some signal-receivers present pre-existing perceptual biases that favour species possessing any trait that could possibly match these biases. In other words, a given plant species could already present traits that favour its relationship with a signal-receiver even before the interaction begins. The perceptual biases may be driven by sensory (i.e. an innate "perception" of the individual) and/or cognitive (i.e. the "learning" of the individuals) biases (Schaefer and Ruxton 2009). Sensory biases are inherent of the perceptual system of the individuals and encompass innate properties and generalized preferences, such as the attraction for more odoriferous flowers or for larger floral displays when animals forage on rewarding plants (Martin 2004). Cognitive biases may shift after each experience passed by the signal-receivers because they are based on associative learning processes.

The vision of many insects presents some inhibitory interactions between green and blue receptors, which causes in

these animals a remarkable preference for yellow structures (Kelber 2001). Many Oncidiinae orchids have a general phenotype of yellow flowers that is shared by several species within the subtribe. Allied to yellow flowers, other features seen in many Oncidiinae orchids, such as elaiophores (Chase et al. 2009; Neubig et al. 2012) and tabula infrastigmatica (Dressler 1993), may be pre-existing traits that facilitate the visitation of oil-collecting bees searching for rewards in Malpighiaceae species. Note that the above-mentioned features were not developed due to co-occurrence with a putative model because they are shared between *G. flexuosa* and its closest relatives (Chase et al. 2009; Neubig et al. 2012). This argument reinforces that the EPB hypothesis may be a better explanation than Müllerian mimicry to the general resemblance between *G. flexuosa* and *J. guaranitica*.

Conclusion

Our experiment does not provide empirical support to the hypothesis that oil-producing orchids and species of Malpighiaceae form are Müllerian mimics. We argue that the EPB hypothesis provides the better explanation for the general resemblance between *G. flexuosa* and *J. guaranitica*. EPB is possibly more widespread in plants than mimicry, since it does not rely on long-term spatio-temporal association between species. The mimicry and the EPB hypotheses use different explanations for the different degrees of resemblance between plant species. Only the mimicry hypothesis predicts that signal-receivers confuse the identity of models and mimics—a pattern that was not observed in our study. Following the EPB hypothesis, the identification of perceptual biases of pollinators is an important step toward a broad understanding of the dynamics and evolutionary trajectories of plant-pollinator relationships. Future studies involving Müllerian mimicry in orchids should search for points of adaptive divergence between putative mimics and their closest non-mimetic relatives by comparing phenotypic traits across a phylogeny. This approach allows the identification of derived traits that were likely result of long-term spatio-temporal associations between models and signal-receivers. In turn, if a given trait selected by a signal-receiver is shared between the putative mimic and its closest phylogenetic relatives, it suggests that EPB could be the driver of the pollinator-plant relationship rather than mimicry.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00114-021-01771-9.

Acknowledgements We thank Gabriel Melo, from Universidade Federal do Paraná (Brazil), for the identification of the bee species; Rosana Farias-Singer, Andréia Maranhão Carneiro, Natividad Fagundes, and Priscila Porto Alegre Ferreira, from the Porto Alegre Botanical Garden (Brazil), for the availability of equipments and study locations and for the permit for using the research area; Oscar Perdomo for the assistance in the development of the map; and Matthias Waltert and two anonymous reviewers for useful comments on an early draft of the manuscript.

Author contribution JBC, GM, and RBS conceived the ideas; JBC and GM designed methodology; JBC collected the data; JBC and GM analysed the data; GM performed the statistical analyses; JBC led the writing of the manuscript. All authors contributed to the revision of drafts.

Funding This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)/Brazil – Finance Code 001 (grant number 88882.439424/2019–01). JBC gratefully acknowledges his CAPES grant. This contribution is part of the first author's Ph.D. Thesis (in Botany) at the Programa de Pós-graduação em Botânica of the Universidade Federal do Rio Grande do Sul (UFRGS).

Data availability The data used in the analyses will be available in Mendeley Data upon acceptance.

Code availability The codes used in the data analyses will be available in Mendeley Data upon acceptance.

Declarations

Ethics approval According to the Brazilian law and the rules of Universidade Federal do Rio Grande do Sul, this research does not require ethical approval. Collection permits were granted by Instituto Chico Mendes de Conservação da Biodiversidade – Sistema de Autorização e Informação em Biodiversidade (numbers 40448–5 and 64504–2).

Conflict of interest The authors declare no competing interests.

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