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ORIGINAL RESEARCH ARTICLE

Disentangling the path of pollinator attraction in temporarily colored flowers

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Abstract

Plants may use different strategies to attract pollinators in long distance (e.g. floral display) and in short distance (e.g. ratio between differentially colored flowers) scales. The Verbenaceae *Lantana canescens* Kunth is a wide spread species in open sites of the Brazilian Pantanal wetland. Individuals of this generalist species can produce a variable number of open inflorescences with yellow and white flowers that are organized in whorls. In this study we tested the hypothesis that increased floral display (long distance attraction) and the ratio between yellow and white flowers (short distance attraction) enhances the number of pollinator species and individuals. We observed flower visitors and calculated floral parameters in 38 plots of 1 m^2 each, that contained a varying number of flowering *L. canescens* individuals. Non-metric multidimensional scaling and Bray-Curtis distances were used to account for flower visitor composition and the relative visitation rate, respectively. We used a structural equation model to test the power of each predictor variable on the visitation rate and a covariance analysis to disentangle the effect of each independent variable on the frequency of plant-pollinator interactions. We found that the number of flower visitors and the visitation rate increased with increasing number of inflorescences. Disentangling long and short distance attraction indicated that the number of inflorescences (per plot) and the number of yellow flowers (yellowing effect) contributed most to flower visitation at long and short distance, respectively.

Keywords Floral display · *Lantana canescens* · Pantanal wetland · Honey bees · Neotropical region · Pollinator attraction

Introduction

Flowering plant species differ in floral trait combinations and trait complexity and use these as strategies to attract pollinators for reproduction (Endress 1996). Two mechanisms that have been suggested to enhance visitation rate by pollinators are: i) increasing long distance perception through the production of aromatic volatiles (Heiduk et al. 2017) with increased flower size (Glaettli and Barrett 2008) and number of flowers (Grindeland et al. 2005); ii) improving resource detection by potential pollinators at a short distance scale, based on fine traits such as flower architecture (Faegri and van der Pijl 1979; Santos-Gally et al. 2013) or color patterns (Weiss 1991, 1995; Weiss and Lamont 1997; Brito et al. 2015; van der Kooi et al. 2015; Reverté et al. 2016).

Some plant species change the color of their petals as an indication of fertilization and senescence (Barrows 1976; Weiss 1995), others produce flowers with mixed colors. Displaying differentially colored patterns has been indicated to increase attractiveness for pollinators (Stanton 1987). However, bees do not discriminate fine color patterns at a long distance scale, but instead it has been suggested that they see a single mixed color composed by color pattern and ratio (Brito et al. 2015).

Bees are dependent on the floral resources such as nectar and pollen of angiosperms. They search, sense, detect, collect and feed on floral resources to maintain their basic metabolic demand and to feed their offspring (Woodard and Jha 2017). Because these behaviors are energetically costly, it may pay

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off to optimize and improve their cognition capacity e.g. by focusing only on the most rewarding flower types and by rejecting flowers that they have learned to be poorly rewarding (Niggebrügge and De Ibarra 2003; Chittka 2017). Honey bees for example show a mechanism of unsuccessful attempt avoidance which means that they recognize rewardable flowers and discard unrewardable ones. Plants may support this mechanism by signaling a lack of floral resources (e.g. by changing flower color pattern) after fertilization and during senescence (Weiss 1991; Dyer et al. 2007).

Additionally, biotic and abiotic conditions seem to be assessed by floral visitors during foraging behavior. While bees seem to optimize their foraging behavior by visiting flowers with high pollen quality (Vaudo et al. 2016) they also seem to prefer foraging on flowers that are standing in the sun since warm flowers provide a metabolic advantage (Whitney and Chittka 2007).

The Verbenace *Lantana canescens* Kunth produces inflorescences with yellow and white flowers organized in whorls. Fertile flowers are typically yellow and lose their color to become white when they are older, as an indication of senescence. We tested the effect of long and short distance attraction on the visitation rate of insect floral visitors of *L. canescens* in an open site. We hypothesized that i) higher number of inflorescences increases the number of floral visitors as well as the visitation rate (long distance attraction) since a higher number of flowers may represent a greater source of resources. ii) visitation rate increases with increasing number of yellow flowers (net amount of floral resources) iii) bees visit more flowers in the sun than in the shade.

Materials and methods

Study site

The study was conducted at Passo do Lontra (19°34′19.01” S, 57°01′53.05” W) in the world’s biggest tropical wetland, the Pantanal, in the state of Mato Grosso do Sul, Brazil in August 2018. Data were collected during three consecutive days from 7:30 to 11:00 am and from 1:00 to 4:30 pm during the dry season of a flooding site. We randomly assigned 38 independent plots of 1 m^2 located in our study site and counted the number of *L. canescens* individuals, inflorescences and the number of yellow flowers per plot. Plots were either located in the sunlight or in the shade.

Study species

Lantana canescens flowers are united in inflorescences with fertile yellow flowers and infertile white flowers that are two to three days old. Flowers are zygometric and petals are lobed. Flower production starts during the dry season in August and lasts until the beginning of the wet season in January (Pott and Pott 1994). The plant produces scents (Pino et al. 2011), that seem to be attractive to a large set of insects, especially to bees (Pott and Pott 1994; Boff et al. 2013).

Floral visitors

Each of the 38 plots was observed by two collectors for 10 min and all floral visitors were collected. Rarefied species accumulation curve and variation in species composition between plots was evaluated by non-metric multidimensional scaling (NMDS) with the R package vegan (Oksanen et al. 2019). We calculated the Bray-Curtis distances between all pairwise combinations of plots by considering the relative frequency of visits.

Individual and populational aspects

We considered the total number of inflorescences (floral display) per plot ($n = 38$) as a parameter for long-distance attraction (lda). Thus, the higher the number of inflorescences (whorls) inside a plot, the bigger the floral display.

The effect of floral color pattern on the frequency of flower visitors was assessed first by calculating the ratio between yellow and white flowers in 39 randomly chosen inflorescences within our 38 study plots. The number of yellow flowers varied between inflorescences. It ranged from zero “0” (absence of yellow flowers) to a maximum of eight yellow flowers per whorl. In 25 of the 39 inflorescences we found fewer yellow flowers than white flowers. In the remaining 14 inflorescences the number of yellow flowers was equal or higher than the number of white flowers (Fig. 1). On average the number of white flowers in the population was three times higher than the number of yellow flowers (t-test: $t = 4.26$, $df = 38$, $P < 0.001$), in other words 75% of the flowers were white and 25% yellow.

We used a linear model (lm: number of yellow flowers per inflorescence ~ number of white flowers per inflorescence) to assess the ratio between yellow and white flowers per inflorescence in the whole population, using the car package in R (Fox and Weisberg 2019). The residuals of this model were used to estimate short-distance attraction (sda) which indicates the number of yellow flowers in relation to the number of white flowers. Higher residuals indicate a higher number of yellow flowers (yellowing effect of flowers in the plot), lower residuals indicate a higher number of white flowers, and a residual value of 0 indicates an equal number of white and yellow flowers (Electronic Supplementary Material 1).

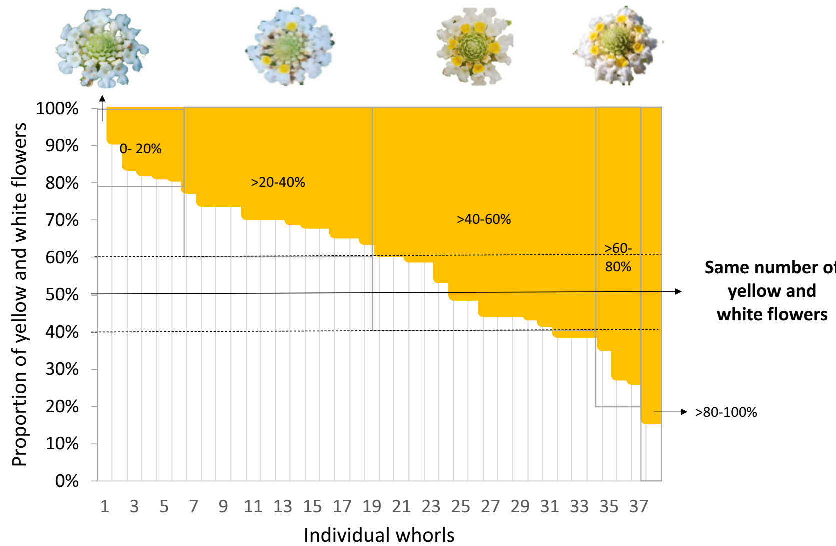

Fig. 1 The number of yellow and white flowers in individual whorls varied among plants in the population. The number of white flowers was on average three times higher than the number of yellow flowers

Disentangling long and short distance pollinator attraction

We performed two analyses of covariance (ancova) to test the effect of each independent variable, number of inflorescenses (lda), ratio between yellow/white flowers (sda) and their interaction with sunlight, on the visitation rate. Interactions between lda and sunlight as well as the interaction between sda and sunlight were both not significant ($F_{1,34} = 0.046$ with $P = 0.83$ and $F_{1,34} = 0.791$ with $P = 0.38$, respectively) and were removed from the models. Furthemore, we tested the effect of lda on the number of flower visits in sunny and in shaded plots with linear regression models (lm: number of visits per plot ~ lda + number of floral visitor species in sunny plots; and lm: number of visits per plot ~ lda + number of floral visitor species in shaded plots, respectively).

Structural equation model (SEM)

SEM provided a visual representation of our system with statistical key parameters that describe the relationships between the response variable (visitation rate) and each predictor variable (sda, lda, and sunlight) using the lavaan and semPlot R packages (Rosseel 2012; Epskamp and Stuber 2017).

Results

We collected a total of 185 flower visitors on *L. canescens* inflorescences during 380 min of observations (0.43 visitor min $^{-1}$); 163 of them belonged to the group of Apidae (*Apis mellifera* Linnaeus and *Ceratina* aff. *morrensis* Strand, 1910), 20 specimen were other hymenopterans and two specimen were dipterans. Plots with a higher visitation rate had a greater diversity of flower visitor species (Species richness: rarefied species accumulation by randomization of floral visits number by insects of 13 species). Species richness of flower visitors was higher in sunny plots compared to shaded plots (Fig. 2).

Apis mellifera accounted for more than 80% of the visits ($n = 150$), with similar foraging activities throughout the day. This species had a higher visitation rate than all other species together (Fig. 3). Ordination (NMDS) recovered 96% of variance in Bray-Curtis distances (linear fit, $r^2 = 0.96$). *A. mellifera* dominated almost all plots but was absent in one where we observed only a single species of Vespidae. *Ceratina* aff. *morrensis*, which was observed only in the morning, performed 5% of the total number of visits ($n = 9$). Other Hymenoptera performed 13% ($n = 24$) and Diptera 1% (n = 2) of the visits. Flower visitation behavior of both

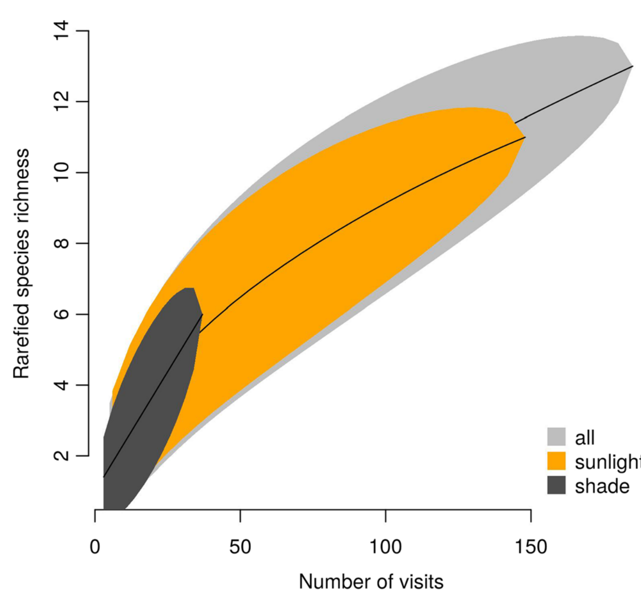

Fig. 2 Rarefied species accumulation curves by randomization of number of flower visits on flowers of *Lantana canescens*. The polygons correspond to the 95% confidence intervals

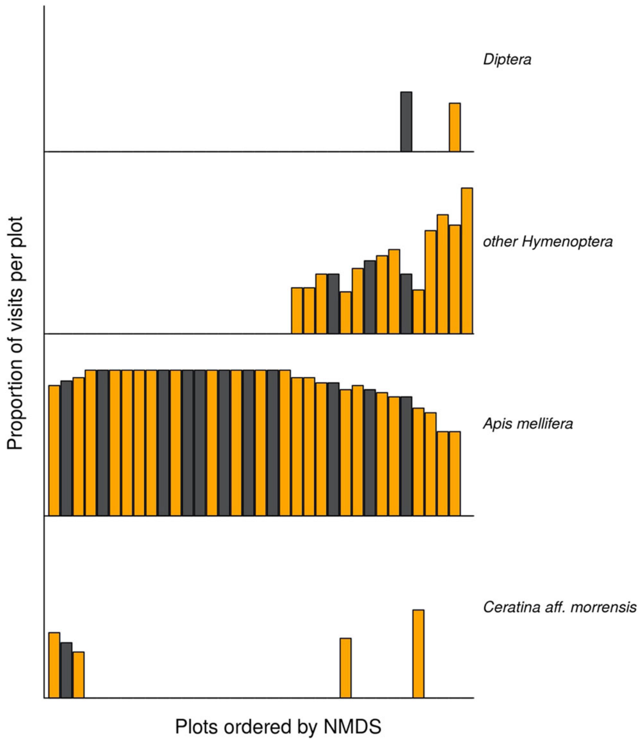

Fig. 3 Composition of flower visitor species on flowers of *Lantana canescens* in 38 plots, ordered by non-metric multidimensional scaling (NMDS) from Bray-Curtis distances, based on relative visitation rate. The honey bees *A. mellifera* were the most frequent visitor in sunny plots (orange bars) and in shaded plots (dark gray bars)

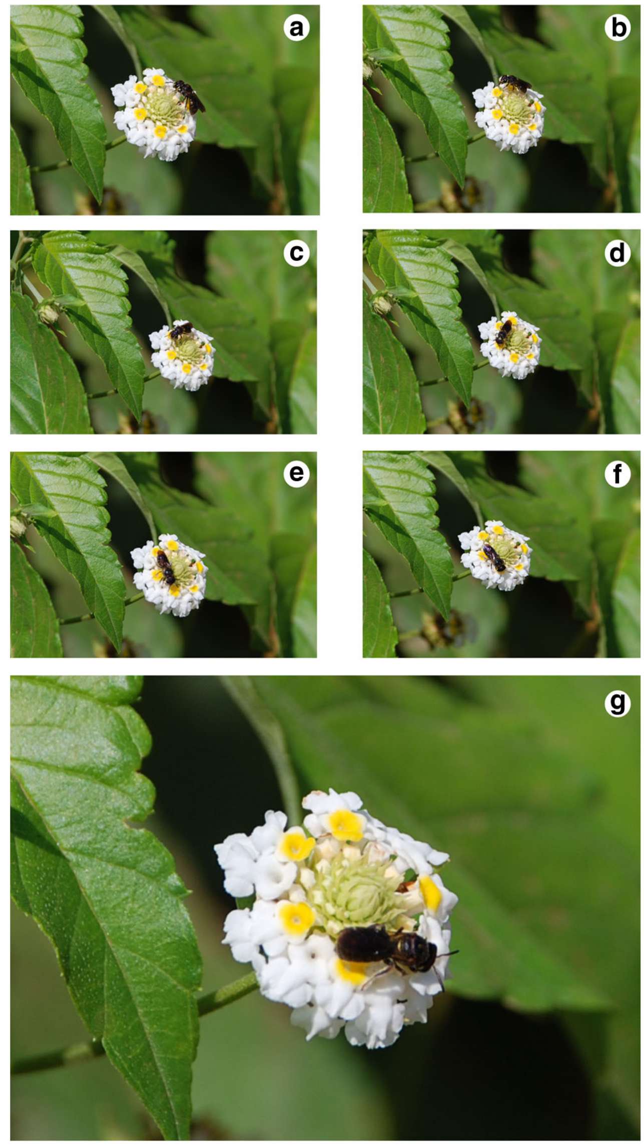

Fig. 4 Centripetal foraging behavior (a to g) performed by *Ceratina* aff. *morrensis* exclusively on yellow flowers of *Lantana canescenses*

A. mellifera and *Ceratina* aff. *morrensis* were similar: the bees used the flower whorl as a landing platform. After anthesis, both bee species performed centripetal movement and foraged at yellow flowers (Fig. 4 and see Electronic Supplementary Material 3). All floral visitor species ignored white unfertile flowers, except the honeybees which were observed visiting white flowers before anthesis of yellow fertile flowers.

Long-distance attraction variance explained 30% of the visitation rate ($F_{2,\ 35} = 9.01$, $P < 0.001$, adjusted $r^2 = 0.3023$) with a significantly higher frequency of visits in plots exposed to sunlight ($t = 3.18$, $P = 0.003$) (Fig. 5a). The variance of short distance attraction explained 22% of the visitation rate (adjusted $r^2 = 0.2191$) and was positively associated with the yellowing effect ($F_{2,\ 35} = 6.19$, $P = 0.005$). Visitation rate was higher in sunny plots compared to plots in the shade ($t = 2.53$, $P = 0.016$) (Fig. 5b). However, the slope coefficient was higher in shaded plots ($b_{sunlight} = 0.01$, $b_{shade} = 0.04$).

Our structural equation model showed a strong positive effect of floral display, long-distance attraction - lda ($Z = 2.70$, $P = 0.007$), and sunlight ($Z = 2.81$, $P = 0.005$) on the visitation rate (Fig. 6). Other factors such as the residuals of the ratio between yellow and white flowers (see linear regression model), and the short-distance attraction – sda ($Z = 1.16$, $P = 0.12$), did not show a significant effect on the visitation rate. Sunlight had a marginally significant effect on sda ($Z = 1.91$, $P = 0.056$) and did not have an significant effect on lda ($Z = 0.32$, $P = 0.751$).

Discussion

In this study we found that the most frequent flower visitor of *Lantana canescens* was the exotic honey bee *A. mellifera*. The visitation rate was higher than that of all other flower visitor species together. Plants that were located in the sunlight had more different flower visitor species and a higher visitation rate than flowers in the shade. SEM analysis indicated a strong positive effect of long distance attraction and sunlight on the

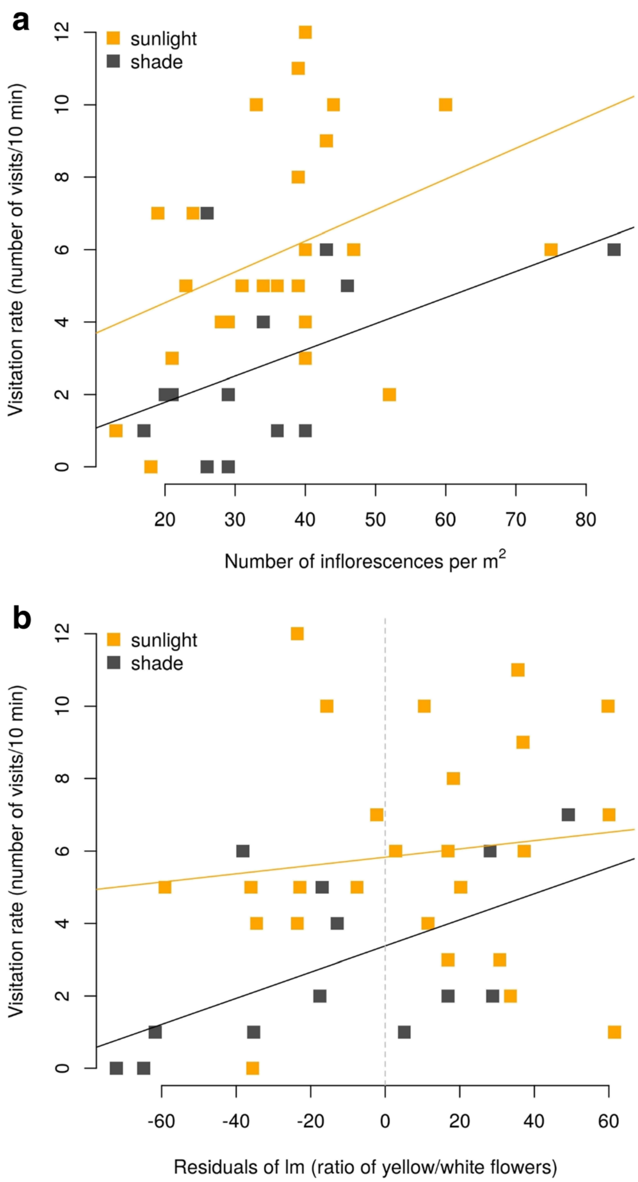

Fig. 5 Pollinator attraction measured as visitation rate at different scales (**a**) Long distance pollinator attraction (floral display) in flowers of *Lantana canescens* was stronger in sites with higher number of inflorescences, especially in sunny plots. (**b**) Short distance pollinator attraction (residuals of the linear model-ratio between yellow and white flowers) had a positive effect on the visitation rate of *L. canescens*. The vertical dashed line indicates an equal number of yellow and white flowers

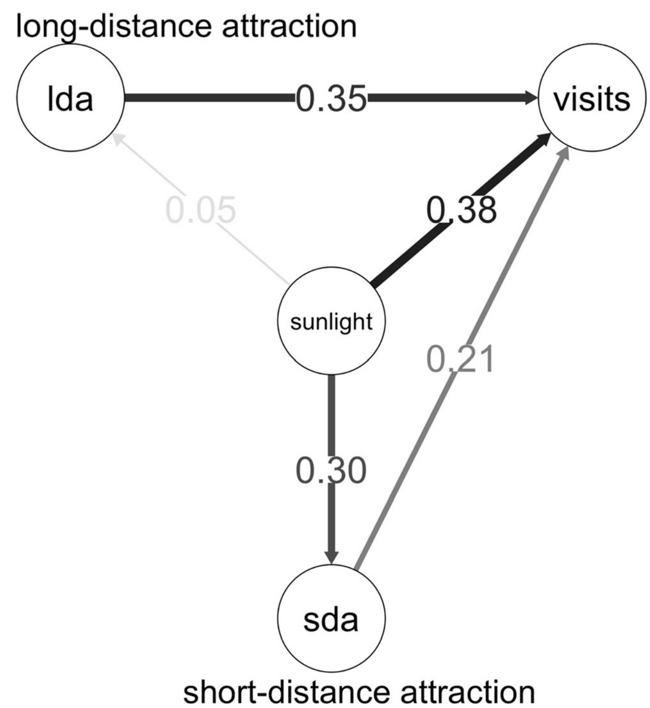

Fig. 6 Path diagram for structural equation model visualizing the effect of light exposure (sun/shade), and short and long-distance attraction on the visitation rate of *Lantana canescens* flowers. Black arrows (coefficient path $\alpha = 0.38$ and coefficient path $\alpha = 0.35$) indicate significant effects of sunlight and lda, respectively. All other factors, such as sda were non-significant (coefficient path $\alpha = 0.05$, gray arrows). Sunlight had a marginally significant effect on sda ($P = 0.056$)

visitation rate. Short distance attraction was mainly explained by the yellowing of whorls as indicated by the analysis of covariance.

In the studied population we found that *L. canescens* had on average more white flowers than colored flowers. Other plants species with flowers of different colors have also been reported in forested environments, including trees such as *Tibouchina pulchra* (Brito et al. 2015). By keeping infertile flowers (white), plants can increase their floral display. Through the increased color pattern area pollinators may detect putative floral rewards more easily from a long distance, leading to an increased visitation rate. This was found for other plant species, were an increase in inflorescence size resulted in increased number of flower visits (Weiss 1991; Glaettli and Barrett 2008). Indeed, we found in our study that a smaller floral display (lower number of inflorescences) reduced the visitation rate of *L. canescens.*

We noticed that honey bees were visiting white flowers early before anthesis, but we did not observe them visiting white flowers after anthesis. One possible explanation for this behaviour could be that the white flowers were the only ones available early in the morning. We observed that the floral tubes of some of these flowers were filled with a liquid that was probably water or nectar from the previous day. Although we did not study the nectar volume or concentration inside floral tubes throughout the day, it has been shown for other Verbenaceae species that nectar volume is reduced or absent after flower fertilization. The foraging behaviour of honey bees is likely influenced by innate preferences (Lunau and Maier 1995) and learned associations e.g. white flower ➔ no-resources, yellow flower ➔ resources (Muth et al. 2015). Thus, learning from failure and changing flower color pattern by retaining old flowers may both be mechanisms to optimize foraging.

We found that sunlight had a positive effect on the visitation rate both in long- and in short distance attraction. This may be related with the thermoregulatory capacity of insects that has been shown to be better in sunny, warm places than in colder shaded places (Whitney and Chittka 2007). Moreover, bees might gain an additional reward by visiting warm flowers when nutritional rewards are also available (Dyer et al. 2006). However the slope of the residuals, which is an indication for the proportion of yellow flowers was steeper in shaded plots. This means that a small change in the proportion of yellow flowers in shaded plots results in higher visitation rate. On the other hand, the slope of the residuals in sunny plots is flatter, meaning that a small change in the proportion of yellow flowers causes a smaller change in the visitation rate.

For the long distance pollinator attraction the floral display and the number of yellow flowers explained well the visitation rate. The ratio between yellow and white flowers however, seemed to have a weaker effect. Nityananda et al. (2014) have shown experimentally that bees make the decision of whether to land on a flower or not at short distances of around two to five centimeters. Moreover, bees have been shown to be able to count (Vasas and Chittka 2019) and they might use information on the ratio of yellow (fertile) and white (old, unfertile) flowers as an indicator of resource availability.

In this study we could disentangle factors that might influence long and short distance attraction of flower visitors of *L. canescens*, it is likely that potential pollinators use a combination of information in order to find the most efficient foraging strategy. Further research linking visual and chemical signaling and reproductive success of flowers with ephemeral color pattern may help to comprehend evolutionary mechanisms used by plants to improve pollinator attraction.

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Authors' contributions SB, AF and JAH collected the data, SB and JR performed statistical analysis, SB drafted the first version, all authors contributed to the final version and agreed on the text for publication.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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