UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA VEGETAL)

PADRÕES DE CORES DE FLORES E A POLINIZAÇÃO EM VEGETAÇÕES SAZONAIS

AMANDA EBURNEO MARTINS

Dezembro - 2019

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Dissertação apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Biologia Vegetal)

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RESUMO

A visão é o principal sentido utilizado pelos polinizadores para forragear, no qual a cor das flores é a primeira característica de atração. A percepção dos sinais de cores depende da coloração do segundo plano das flores, composto principalmente pelas folhas. Diferenças na composição das espécies e na estrutura da vegetação, e também na sazonalidade climática, podem interferir na cor do segundo plano de folhas em diferentes vegetações e estações, interferindo como os polinizadores percebem a flor. Portanto, a diversidade de cores das flores e os padrões de floração de uma comunidade podem estar relacionados com a composição de polinizadores e condições ambientais. Desta maneira, utilizando comunidades vegetais e considerando o sistema visual das abelhas, nós descrevemos e comparamos a diversidade de cores das flores e seus sinais, dando importância para a cor do segundo plano de folhas em duas vegetações sazonais tropicais e uma vegetação sazonal temperada. Em seguida, para vincular os sinais florais com a sazonalidade, nós analisamos a importância das síndromes de polinização levantadas, o padrão de floração, a influência da cor do segundo plano de folhas no padrão de cor das flores entre as estações. Nós encontramos diferenças na diversidade de cores das flores e confirmamos a influência da coloração do segundo plano de folhas, juntamente com a estrutura da vegetação e a intensidade da sazonalidade nos sinais florais exibidos em diferentes vegetações sazonais, de acordo com a visão das abelhas. Altos valores dos sinais cromáticos foram encontrados na vegetação temperada e na estação seca da comunidade tropical sazonal, enquanto os valores mais altos de sinais acromáticos, foram encontrados nas vegetações tropicais e na estação chuvosa da comunidade tropical sazonal. As abelhas foram o principal polinizador da comunidade estudada, porém outros animais também contribuem para a polinização, caracterizando a comunidade com padrão generalista de polinização. Amarelo e *bluegreen* foram as cores predominantes de flores, de acordo com a visão humana e das abelhas, respectivamente. Apesar destas cores geralmente estarem relacionadas com a melitofilia, nós não encontramos uma clara relação entre cores das flores e síndromes de polinização. Por fim, a sazonalidade molda os padrões de floração e a diversidade de cores e sinais florais, promovendo sinais distintos ao longo do ano e maximizando a detecção de flores pelos polinizadores ao longo das estações. Nós oferecemos novas perspectivas na área da ecologia sensorial, reforçando a importância de considerar a cor do segundo plano e o sistema visual das abelhas.

Palavras-chave: polinização, diversidade de flores, sazonalidade, padrão de floração, segundo plano de folhas, cores das flores, comunicação visual, sinais florais, vegetação sazonal, interação planta-polinizador.

ABSTRACT

Vision is the main sense used to forage by pollinators being the flower colour the primary feature of attraction. Colour signals perception depend on the flower background colouration, mainly composed by leaves. Differences in species composition and vegetation structure, and also the seasonality, may change the leaf-background colouration of different vegetations and seasons, interfering how the pollinators perceive a flower. Therefore, flower colour diversity and flowering patterns of a community may be related to the pollinators' composition and environmental conditions. Using a community level-approach and according to bee visual system, we described and compared the flower colour diversity and the signals of a temperate and two tropical seasonal vegetations, considering their leaf background colouration. Then, to link flower signals to seasonality and using the cerrado *sensu stricto* as a model of seasonal vegetation, we analysed the importance of the surveyed pollination syndromes, the community flowering pattern, flowering patterns according to the colour of flowers and the influence of seasonal changes in the background colouration in the flower colour signals between seasons. We found differences in flower colour diversity and confirmed the influence of the leaf-background colouration, along with the vegetation structure and seasonality intensity, in flower colour signals displayed in different seasonal vegetations according to the colour vision of bees. Higher values of chromatic signals were found in the temperate vegetation and higher values of achromatic signals in tropical vegetations. We confirmed the bees as the dominant pollinator in the cerrado *sensu stricto* but with a high diversity of animals as pollinators, featuring a generalist pattern of pollination. Yellow and bluegreen were the predominant flower colours, according to the colour vision of humans and bees, respectively. Although these colours are generally related to melittophily, we did not find a clear relationship between colours and syndromes. Finally, we confirmed that seasonality shaped the community flowering patterns and flower colour diversity over time, promoting distinct flower signals and maximizing flower detection by pollinators over seasons. Higher values of chromatic signals were promoted by the background of the dry season and higher values of achromatic signals by the background of the wet season. We offered news perspectives in sensorial ecology, reinforcing the importance of consider the background colour and the pollinators' visual system when analysing flower colours at community-level.

Keywords: pollination, flower diversity, seasonality, flowering pattern, leaf-background, flower colour, visual communication, floral signals, seasonal vegetation, plant-pollinator interaction.

SUMMARY

GENERAL INTRODUCTION

The Angiosperms group feature a high diversity and richness of species, reaching to 295.000 species described so far (Christenhusz & Byng 2016). Biotic pollination is responsible for 80% of flowering species reproduction in temperate communities and around 94% in tropical communities, providing a key ecosystem service of plant-pollinator interaction (Kremen 2005, Ollerton et al. 2011). Plants-pollinators interactions are mediated by a high variety of stimulus produced by flowers which are driven by biotic and abiotic factors. Plants are subjected to a selective pressure exerted by the pollinators with an adjustment of flower signals to the cognitive system of the pollinators, prioritizing the attraction of effective pollinators (Chittka et al. 1999, Rosas-Guerrero et al. 2014). The evolution of the plant-pollinator adjustment may have led to the current Angiosperms flower types diversity, as well as their high species richness (Hiscock 2011, Raven et al. 2014, Ashworth et al, 2015). Plant-pollinator must also match in space and time which involves pollinators behaviours, environmental limitations for the occurrence of plants and animals' species and flowering patterns. Flowering patterns are important to understand communities' temporal structure, influencing the plants-pollinators diversity and maintenance in a community (Gentry 1974, Lieth 1974, Ramirez 2006). The flowering time and the pollinator's activity are dependent on weather conditions, and the climate seasonality is the main element conditioning the flowering patterns (Morellato et al. 2016, Donnelly et al. 2011). In tropical seasonal vegetations, the flowering response has been linked to distinct abiotic factors, such as temperature, rainfall, day length and cloud cover (Abrahamczyk et al. 2011, Morellato et al. 2013). Furthermore, biotic interactions also influence the community flowering patterns, such as, the plants-pollinators interactions and seed dispersers may structure the flowering time and the fruit production, affecting the reproductive season extension and the species synchrony (Frankie et al. 1974, Camargo et al. 2013, Morellato et al., 2016). The response for biotic and abiotic factors could create distinct flowering patterns, addressed by competition or facilitative interactions (Feinsinger et al. 1987, Mitchell et al. 2009, Staggemeier et al. 2010). For example, when a community has an aggregate blooming with high resources availability for the floral visitors, the temporal overlap may facilitate and increase pollination, by increasing the flowers conspicuousness (Moeller, 2004, Ramirez, 2006, Morellato et al. 2016).

Flowering plants use various stimulus to attract pollinators, such as colour, scent, size and shape, but flower colour is the primary feature to attract pollinators (Fenster et al. 2004, Warren & Billington 2005, Arnold et al. 2009, Arnold 2010, Shrestha et al. 2014). The flower colours are composed by pigments, mainly the carotenoids and anthocyanin's, signalizing the resource availability, such as nectar and pollen (Arnold 2010). The vision is the main sense used by pollinators to forage and depending on the cognitive sensibility, they may have preferences for certain colours and also by learning, associate a colour with a specific reward (Chittka et al., 1999, Raine & Chittka 2007, Arnold 2010, Brito et al., 2014). Additionally, in general, flowers more contrasting against the background are easily detected and more visited than those that are less conspicuous (Spaethe et al. 2001, Arnold 2010). Besides on pollinators' visual system, the colour perception and contrasts may also be influenced by the background colour and illuminance conditions (Endler, 1993; Menzel, 1997; Binkenstein and Schaefer, 2015; Bukovac et al., 2017). The background is characterized by temporal variability and spatial heterogeneity and, along with the cognitive capacity of pollinators, is a driver of flower constancy (Lunau el al., 1996; Camargo et al., 2014, Bukovac et al., 2017).

The flower conspicuousness is related to the pollinator's visual sensitivity but the contrast against the background may affect the pollinators' perception and their efficiency to find flowers (Endler 1993, Spaethe et al. 2001, Arnold 2010, Hansen et al. 2012, Bukovac et al. 2017). According to Camargo et al. (2014), the climatic seasonality alters the leaf-background colouration. This is because the leaves lose chlorophyll and become more yellowish, brown or reddish in the dry season when compared to the predominant green colour of the young and mature leaves of the rainy season. Hence, changes in the background may interfere the flower colour perception and, consequently, the flowers' contrasts, detection and visitation by pollinators (Endler 1993, Binkenstein & Schaefer 2015). Flowers are expected to be more conspicuous for bees when contrasting against the background of their own environment (Arnold & Chittka 2012, Binkenstein & Schaefer (2015). Therefore, differences in background colouration, vegetation structure and composition and climatic seasonality also exert selective pressures on the flower colour signals and diversity in communities.

The cognitive sensibility of floral visitors is different from the humans (Briscoe & Chittka 2001, Arnold et al. 2009). For example, a blue flower in the human vision could be considered violet or yellow for bees and white for flies (Arnold et al. 2009). This happen because humans, despite presenting a trichromatic vision like most of insects, are sensible to distinct wavelengths. Bees visual systems, present sensitive photoreceptors to green, blue and ultraviolet (UV) wavelengths (three channels), while humans detect wavelengths in red, green and blue (three channels) and birds' photoreceptors perceive from the UV to the red wavelengths (four channels) (Mc Call and Primarck 1992, Chittka and Menzel 1992, Briscoe & Chittka 2001, Arnold et al. 2009, Ohashi et al. 2015). There are works such as Warren and Billington (2005), which classify the flower colours according to the human vision perception, but due to differences in colour perception by floral visitors, it becomes essential to study and classify the flower colours according to the pollinators' visual system. Therefore, temporal and spatial flowering patterns, animal and plant morphology, resources availability and the pollinators' cognitive system are the main structuring factors in pollinator's communities (Morellato et al. 2016, Camargo et al. 2019).

In this context, using a community level-approach the present master theses had as goals: (i) according to the bee visual system, analyse and compare the flower colour diversity and signals of bee-flowers among three seasonal plant communities using the Fabaceae family as a model: the tropical campo rupestre (rupestrian grassland), and cerrado *sensu stricto* (woody savanna) and the temperate shrubland vegetation in Mediterranean Basin; (ii) analyse the flowering pattern, the pollinators diversity, flower colour diversity and signals by bees visual system using the cerrado *sensu stricto* vegetation as a model.

In the first chapter we investigated the Fabaceae flower colour diversity and signals among the three communities considering the leaf background colouration, the distinct degree of seasonality, from tropical to temperate, and vegetation structure, from dominant woody to grassy landscape. We discussed how the flower colour differences among communities are important for pollinators' discrimination, detectability and flower conspicuousness. In the second chapter we analysed the cerrado flower colours diversity according to humans and bees, the floral signals variation to bees' visual system among wet and dry season's and the pollination syndromes in the cerrrado community. Finally, linking the flower contrasts to seasonality, we discussed the community flowering patterns, the background colouration influence in the temporal pattern of flower colours and in the floral contrasts between the seasons.

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CHAPTER 1.

ORIGINAL ARTICLE

Colour signals in bee-pollinated flowers: the significance of seasonality and leaf-background.

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ABSTRACT

- **Background and Aims**: Flower colour is a primary feature attracting pollinators. Since pollinators have different visual system, floral colours must be adjusted to their cognitive system. In addition, seasonal changes on leaf-background colouration, and vegetation structure, may affect the attractiveness of flowers. Using bee-pollinated Fabaceae species as a model we compared flower colour signals and diversity, considering the leaf-background colour, among three seasonal plant communities: a tropical rupestrian grassland (*campo rupestre*), a tropical woody savanna (cerrado), and a temperate Mediterranean shrubland vegetation. We asked whether colour parameters differed among these communities as they show different bee composition, degrees of seasonality, and vegetation structure (woody to grassy).
- **Methods:** To describe flower colours and leaf-background of each community, we measured flower and leaf reflectance spectrum of Fabaceae species. Using the visual system of bees, we described and compared the leaf-background colouration, flower colour diversity, chromatic (colour contrast and spectral purity) and achromatic (green contrast and brightness) variables among each community and between temperate and tropical sites.
- **Key Results**: Leaf-background differed among communities: Mediterranean shrubland showed higher values of reflectance, followed by the cerrado and the *campo rupestre* communities. Bee-uvgreen and bee-bluegreen colours predominated in tropical communities, while in the Mediterranean shrubland all bee-colours occurred, preponderating bee-uvgreen and bee-green. Additionally, flowers from the Mediterranean shrubland showed higher values of chromatic variables, whereas flowers from tropical communities showed higher achromatic variables values.
- **Conclusions**: The dominant background associated to each community structure and seasonality, act as a selective pressure driving the diversity of flower colours in Fabaceae species. Therefore, colour signals face local environmental pressures with the background coloration ensuring flower conspicuousness for pollinators.
- **Keywords**: Fabaceae, background colour, bee visual system, seasonality, flower colour diversity, pollination, visual communication, plant-pollinator interaction, floral signals, cerrado, campo rupestre, Mediterranean Basin.

INTRODUCTION

During the course of evolution, plant-pollinator interactions have provided plants with ways to reach reproductive success by offering rewards to pollinators (Kearns *et al.*, 1998; Hiscock, 2011; Raven *et al.*, 2014). Flowers contain several stimuli that attract pollinators, such as colour, shape, size, and scent, but colour signals are considered the most important (Fenster *et al.*, 2004; Warren and Billington, 2005; Shrestha *et al.*, 2014). Most floral visitors rely on their vision to forage and flower colour signals are generally adjusted to the cognitive system of pollinators (Chittka *et al.*, 1999; Gumbert *et al.*, 1999; Fenster *et al.*, 2004; Reverté *et al.*, 2016). Among different colour signals produced by flowers, bees show a preference for more saturated colours and rely more in colour contrasts against the background to distinguish and detect flowers (van der Kooi *et al.*, 2019; Rohde *et al.*, 2013; Bukovac *et al.*, 2017; but see Hempel de Ibarra *et al.*, 2014). Perception of colour signals is influenced not only by the visual system of pollinators, but also by the predominant colour in the background (Endler, 1993; Menzel *et al.*, 1997; Binkenstein and Schaefer, 2015; Bukovac *et al.*, 2017). Therefore, differences in the background colouration that are related to seasonality may also exert selective pressures on flower colour diversity and colour signals of plant communities. Even though flower colour signals are key to understand the evolutionary process of pollination, little is known about how it varies between plant communities and what colour parameters are more relevant to the evolution of flower colour (van de Kooi *et al.*, 2018).

Although bees are the major pollinators in tropical and temperate systems, social bees and vertebrate pollinators predominate in tropical systems, while solitary bees and flies are more frequent in temperate systems (Petanidou and Vokou, 1990; Dafni and O’toole, 1994; Potts *et al.*, 2005; Schleuning *et al.*, 2012; Camargo *et al.*, 2019). Additionally, environmental conditions also differ between tropical and temperate systems, mainly due to vegetation structure and a stronger seasonality in temperate systems (Petanidou and Lamborn, 2005). To address those issues, we used bee-pollinated Fabaceae as a model to analyse flower colour diversity and signals across three different seasonal plant communities: two tropical, the *campo rupestre* and the cerrado savanna, and one temperate, the Mediterranean shrubland.

The tropical rupestrian grasslands, or *campo rupestre* is a montane vegetation mosaic dominated by open grasslands with undershrubs, small sclerophyllous evergreen shrubs and rare small trees on rock outcrops (Silveira et al., 2016; Morellato and Silveira, 2018), with high incidence of solar radiation. The strong seasonal climate did not characterize a seasonal reproductive season, being the flowers available all year long, and the pollinator activity the mediator for flowering strategies (Rocha *et al.*, 2016; Le Stradic *et al.*, 2018). The cerrado is a woody savanna composed mostly by deciduous and semi-deciduous shrubs and trees species, with lower incidence of solar radiation when compared to open grassy and shrub physiognomies (Coutinho, 2006) and the *campo rupestre*. The flowering is marked seasonal, peaking in the transition between the dry to rainy season, although there are species flower year-round (Oliveira & Gibbs 2000, Gottsberger & Silberbauer-Gottsberger 2006). The temperate Mediterranean shrubland is composed by an herbaceous layer sparsely covered by scattered sclerophyllous trees, and a dense scrub layer, with high incidence of solar radiation and the climate characterized by a strong seasonality (Rundel *et al.*, 2016). Both the water stress during the summer and the low temperatures during the winter limit the reproductive season mainly to the spring (Arroyo, 1990; Petanidou et al., 1995; Bosch et al., 1997; Flo *et al.*, 2018).

Fabaceae has a cosmopolitan distribution, high species diversity (770 genera and 19500 species), a huge variety of habits, types of flowers, and fruits (Koenen *et al.*, 2013; Lewis *et al.*, 2013; LPWG, 2017), and most Fabaceae species rely on bee as pollinators (Potts *et al.*, 2005; Gottsberger and Silberbauer-Gottsberger, 2006; Guerra *et al.*, 2016). In Brazil, Fabaceae is the most diverse plant family and the second most important in the cerrado and *campo rupestre* (BFG, 2015; Flora do Brasil, 2020). Fabaceae is also one of the most diverse families throughout the Mediterranean vegetation, with 56 genera and 513 species (Thompson, 2005; Rundel *et al.*, 2016).

Considering the background colouration of vegetation types with different degrees of seasonality (from temperate to tropical) and vegetation structures (from woody to grassy areas), we aimed to answer the following questions: (i) what is the flower colour diversity and how does it vary among the three communities? (ii) do flower colour signals differ among communities? Due to differences in vegetation structure among the three communities, we expect differences on light, mainly related to the incidence of UV-light, and in the background colouration contrast. Flowers will be under higher UV-light incidence mostly in open areas, such as, in the *campo rupestre* and in the Mediterranean shrubland. However, we expect that differences in background coloration contrasts will be higher in closed vegetations, as the cerrado, to Mediterranean shrubland and the *campo rupestre* open grasslands. As a consequence, flower colour signals will probably differ among the three seasonal vegetations. These differences should be related to the most important colour signals for bees, with high signals of both colour contrast and colour purity, as they are probably under a higher selective pressure, supporting the hypothesis that bee flowers are adapted to the Hymenopteran vision

(Hempel de Ibarra *et al.*, 2001; 2002; Spaethe *et al.*, 2001; Dyer *et al.*, 2011; Rohde *et al.*, 2013). We also expect a higher diversity of flower colours among tropical Fabaceae species in the Mediterranean region, because seasonality is stronger in this system and, when many species are flowering at the same time, a high flower colour variation is expected as a way to avoid reproductive interference (Petanidou and Lamborn, 2005; Rundel *et al.*, 2016). Finally, the chromatic variables will be higher in the Mediterranean vegetation and the achromatic variables will be higher in the cerrado vegetation.

MATERIALS AND METHODS

Study areas and species survey

We sampled bee-pollinated Fabaceae species from three seasonal communities: two tropical communities, the *campo rupestre* and the cerrado, and a temperate community, the Mediterranean shrubland (Table 1, Supplementary Information).

The *campo rupestre* vegetation is an old, highly diverse vegetation mosaic dominated by a grassland matrix (Silveira *et al.*, 2016). The study area comprises the National Park of Serra do Cipó and its surrounding buffer zone (Morro da Pedreira, an Environmental Protection Area), located in the south part of the Espinhaço mountain range, Minas Gerais, Southeast Brazil (Rocha *et al.*, 2016) (Figure 1a, b). We surveyed the rupestrian grasslands located in the higher altitudes (above 900m), denominated as *campo rupestre stricto senso* (Silveira *et al.*, 2016), characterized by a dominant grassy layer with scattered small, sclerophyllous evergreen shrubs and herbs, surrounding the typical rocky outcrops dominated by herbs, shrubs and small trees (Giulietti *et al.*, 1987; Alves and Giulietti *et al.*, 1997; Silveira *et al.*, 2016; Morellato and Silveira, 2018; Camargo *et al.*, 2019, Mattos et al. 2019). The dominant herbaceous layer is composed mainly by Poaceae, Cyperaceae, Xyridaceae, Eriocaulaceae, and Velloziaceae, while the most representative sub-shrub families are Asteraceae, Melastomataceae, Fabaceae, and Malpighiaceae (Mattos et al. 2019). The climate of the region consists of a cold dry season (from May to September) and a warm wet season (from October to April), with frequent fires and strong winds in the dry to wet season transition (Fernandes *et al.*, 2016; Alvarado *et al.*, 2017).

The cerrado study site is located at Itirapina, São Paulo, Southeast Brazil (22º 10' 31.41"S; 47° 52' 26.1 3"W) (Figure 1c, d). The vegetation is denominated a cerrado *sensu stricto*, the widespread cerrado physiognomy in Brazil (Coutinho 2006, Reys et al. 2013). The cerrado is a semi-deciduous vegetation characterized by a discontinuous woody layer that reaches around 6 - 7 m high (but some individuals can reach up to 12 m), scattered shrubs, and a continuous herbaceous layer (Reys *et al.*, 2013; Camargo *et al.*, 2018). The most abundant and species rich families are Myrtaceae, Malpighiaceae, and Fabaceae, with *Anadenanthera*, *Bahunia*, and *Stryphnodendron* as the most representative Fabaceae genera (Reys *et al.*, 2013). The climate is marked by a warm rainy season (from October to March) and a cold dry season (from April to September) (Reys *et al.*, 2013).

The Mediterranean shrubland, located in the Mediterranean Basin, consists of a mosaic of vegetation types characterized by a dense scrub layer, scattered sclerophyllous trees, and a diverse herbaceous layer in open areas (Blumler, 1991; Pausas, 1997; Blondel and Aronson, 1999; Rundel *et al.*, 2016). The study site was located at the Doñana Natural Park (37º 17’ N, 06º 25’ W) and sampling was carried out at Pinares de Hinojos (37º16’56.4” N, 6º23’7.3” W) and Pinares de Aznalcázar (37º14’27.7”N, 6º10’45.3” W) (Figure 1e, f). The tree layer consists mainly of *Quercus suber* L. (Fagaceae) and *Pinus pinea* L. (Pinaceae), while the shrub layer is composed mainly of Cistaceae, Lamiaceae, Fabaceae, Ericaceae, and Myrtaceae, families that dominate post fire forest succession (Talavera *et al.*, 2001; Arista and Ortiz, 2007; Rundel *et al.*, 2016). The climate is characterized by warm dry summers (from May to September) and cold wet winters (from December to March) (Rundel *et al.*, 2016).

We selected Fabaceae species, filtering by bees as the main pollinator (Dafni and O’toole, 1994; Potts *et al.*, 2005; Gottsberger and Silberbauer-Gottsberger, 2006; Guerra *et al.*, 2016); these species and pollinators were identified with specialized bibliography and herbarium collections, or based on previous studies developed at the same study sites (Valdés *et al.*, 1987; Camargo *et al.*, 2018, 2019; Rocha *et al.*, 2016). Vouchers specimens were lodged in the Rioclarense Herbarium (HRCB) of the São Paulo State University (UNESP) and in the Herbarium of the Universidad de Sevilla (Herbario SEV). We choose to work with Fabaceae not only because it is one of the most important families in all three vegetation types, but also because by using species from the same botanical family we avoid possible phylogenetic bias due to differences in plant composition and diversity.

Colour reflectance and flower signals

We collected reflectance data of 58 bee-pollinated Fabaceae species with a high variety of flower colours and morphologies (Figure 2). We collected flower colour data of 14 *campo* *rupestre* species (data collected by Camargo et al. 2019), 10 cerrado species, and 34 Mediterranean shrubland species (random sampling in the communities) (Table 1, Supplementary Information). To analyse leaf-background, we collected data of 391 *campo rupestre* species, 80 cerrado species, and 22 Mediterranean shrubland species. We analysed flower colours and leaf-background according to Camargo *et al.* (2019). We obtained the mean reflectance spectra calculated from the reflectance data of 10 flowers of each species and 10 leaves of each Fabaceae species but also of other species of the community. We measured the flower reflectance spectrum between 300 and 700 mm, including ultraviolet light (UV), with a spectrometer (Ocean Optics – Jaz Modular Optical Sensing Suite). For all species, we considered the flower colour as the predominant colour in its floral display, which generally corresponds to the petals. The leaf-background was determined by the mean reflectance spectra of the community leaves, according to Camargo et al. (2014) - 316 species in the *campo rupestre*, 80 species in the cerrado and 22 species in the Mediterranean shrubland. We also calculated and compared the flower variables from tropical vs temperate in a standard background from *Pavo* package, as a control treatment to understand if the differences in flower signals that we found will kept or changed due to the background changed (Menzel *et al*. 1997; Maia *et al.*, 2016; 2018).

To calculate flower chromatic and achromatic variables based on the visual system of bees, we used the bee-hexagon proposed by Chittka (1992). In the hexagon, each colour is defined by a point (colour loci) and the position of this point is given by the x and y coordinates. These coordinates are defined according to the excitation of each photoreceptor promoted by the light reflected from a surface against its background based on a previously specified visual system (Chittka 1992). Here, we used the model proposed for *Apis mellifera* (Menzel and Backhaus, 1991).

Based on regular photos, on the reflectance data, and on specialized literature of the studied flowers, we sought for categorical and quantitative variables that have been described as important visual signals for bees. We calculated the colour contrast against the natural and standard background (the contrast between two colours detected by all photoreceptors) and considered it as a quantitative variable (Van der Kooi *et al.*, 2019). The colour contrast is the r-vector value in the bee-hexagon, which is the distance between the colour loci and the hexagon centre (Chittka, 1992; Spaethe *et al.*, 2001; Rohde *et al.*, 2013). To calculate the green contrast against the natural and standard background (the perceptual contrast between two colours detected by the green photoreceptor; Chittka, 1992; Spaethe *et al.*, 2001; Van der Kooi *et al.*, 2019), we subtracted the green photoreceptor excitation by 0.5 (Spaethe *et al.*, 2001; Dyer et al. 2016). We calculated the brightness, or colour intensity, as the sum of the three photoreceptors excitations, the dominant wavelength, and the spectral purity (Lunau *et al.*, 1996; Spaethe *et al.*, 2001; Rohde *et al.*, 2013).

We also calculated the colour contrasts between flowers, represented by the Euclidian distance between their colour loci (Arnold and Chittka, 2012), being a reference metric for colour discrimination and diversity. According to previous studies, bees can distinguish two colours when the Euclidian distance between their colour loci is higher than 0.1 hexagon units (HU) (Chittka *et al.*, 1993; Dyer, 2006; Arnold and Chittka, 2012). However, in natural conditions, flower colours that have distances higher than 0.2 HU favour colour discrimination improving in 50% the probability to promote flower constancy (Chittka *et al.*, 2001b). Values between 0.04 and 0.1 are not easily discriminated and values below 0.04 are not discriminated by bees (Chittka, 1992; Spaethe *et al.*, 2001b; but see Garcia *et al.*, 2018).

Flowers were classified based on human-colours, as yellow, white, and pink, and based on bee-colours, according to Chittka *et al.* (1994), as bee-blue, bee-green, bee-uv, bee-bluegreen, bee-uvgreen, and bee-uvblue. We also determined the presence of colour patterns (a floral display composed by more than one colour or reflectance pattern), which can be represented by the combination of the main flower colour and floral guides, anthers, or stamens (Camargo *et al.*, 2019).

Data analyses

Quantitative variables (brightness, dominant wavelength, spectral purity, colour, and green contrasts) were compared among the studied plant communities (campo rupestre, cerrado and Mediterranean shrubland) with a Kruskal-Wallis test followed by a Dunn's post-hoc test. To compare the background reflectance spectra between the three communities, we used the non-parametric matched-pairs Friedman test followed by a post-hoc test. All analyses related to reflectance data and colour variables were performed using the *Pavo* package (Maia *et al.*, 2016; 2018) in R software (R Development Core Team). We also made comparisons between tropical and temperate seasonally dry ecosystems by joining the data of the tropical communities (*campo rupestre* and cerrado) and balancing the number of species analysed between the areas.

RESULTS

The reflectance spectra of the leaf-background differed significantly between the three communities (Chi-squared= 790.04, df= 2, p <0.001) for all the paired comparisons, being highest in the Mediterranean shrubland (Figure 3). Colour variables differed mainly between temperate and tropical communities (Figure 4). Flowers from both the *campo rupestre* and the cerrado showed higher values of green contrast and brightness than flowers from the Mediterranean shrubland (Figure 4 a and c, Table 2 [Supplementary Information]). Colour contrast, spectral purity, and dominant wavelength did not differ between the three study sites. When comparing tropical and temperate regions, colour and green contrasts, brightness, and spectral purity differed significantly, with higher values of green contrast and brightness in the tropical region and higher values of colour contrast and spectral purity in the temperate region (Figure 4 b and d, Table 2 [Supplementary Information]). No differences were found with the standard background when comparing tropical and temperate colour variables (Figure 1 [Supplementary Information]).

Yellow, as perceived by the human eye, was the most frequent flower colour found in all vegetation types and regions (tropical and temperate), but white and pink flowers were also found in all three plant communities (Figure 5a). When flowers were analysed as perceived by bees colour vision, marked colour differences were found among the three studied sites: at the *campo rupestre*, most species were bee-uvgreen (Figure 5b, Figure 6a); at the cerrado, most species were bee-bluegreen (Figure 5b, Figure 6b); and at the Mediterranean shrubland, a more scattered pattern was observed, with a similar percentage of bee-uvgreen, bee-green, and bee-blue flowers (Figure 5b, Figure 6c).

The distance of flower colour loci of 20% of bee-pollinated flowers at the *campo rupestre* was higher than 0.1 hexagon units (HU) and of 57% of species, the distance was higher than 0.2 HU (Figure 6d). In the cerrado, most species were concentrated in the bee-blue and bee-bluegreen categories of the hexagon, with 35% of bee-flowers showing a distance between their colour loci higher than 0.1 HU and 38% of species showing a distance higher than 0.2 HU (Figure 6e). Mediterranean species were more widely distributed throughout the hexagon (Figure 6f), with 16% of flowers showing a distance between their colour loci higher than 0.1 HU and 75% of species showing a distance higher than 0.2 HU.

Flowers with colour patterns predominated in the tropical plant communities (83% of the species), with most of the *campo rupestre* and cerrado flowers showing more than one colour (93% and 70%, respectively). However, in the Mediterranean shrubland, only 50% of flowers had more than one colour.

DISCUSSION

We presented a description and comparison of Fabaceae flower signals and diversity from two tropical and one temperate plant communities. The diversity of flower colours was explained as perceived by human and pollinator visual systems, by the ability of colour discrimination by bees, and by the proportion of colour pattern in each community. Yellow was the most common flower colour in all communities. Mostly of yellow flowers from *campo rupestre* and Mediterranean shubland presented UV-reflectance, an expected pattern in open areas with high UV-light incidence. The Mediterranean flowers also had higher flower colour diversity with flowers in all hexagon colour categories, representing the higher colour diversity among the communities. The colour signals were distinct due to the differences in the natural background, which the tropical region showing significantly higher values of brightness and green contrast, while higher values of colour contrast and spectral purity were found among Mediterranean species.

As expected, leaf-background colouration differed significantly among the three communities, confirming that the observed differences in flower colour signals were due to the natural background of each community (Menzel *et al.*, 1997). Differences in leaf-background were probably related to species composition, vegetation structure, leaf-exchange strategies, and changes in leaf colouration between seasons (Camargo *et al.*, 2013). Additionally, leaf reflectance could also be associated to physiological traits. For instance, Mediterranean background presented the higher spectral indices, which could be a consequence of losing cell wall elasticity in response to water stress (Peñuelas *et al.*, 1993). Hence, differences in leaf reflectance among studied sites could be related to water leaf content, water stress during the flowering season and shifts on leaf colouration between seasons.

In all communities, the predominant Fabaceae colours, as perceived by the human eye, was yellow followed by white and pink, similar to the colours of bee-pollinated flowers found in previous studies, both in tropical and temperate communities (Arnold *et al.*, 2009; Petanidou and Lamborn, 2005; Gottsberger and Silberbauer-Gottsberger, 2006; Camargo *et al.*, 2019). For the *campo rupestre* and the Mediterranean shrubland, we observed a predominance of yellow flowers, probably related to species composition, such as the dominance of widely recognized yellow flower genera (*Chamaecrista* in the *campo rupestre* and *Genista* in the Mediterranean shrubland; Irwin and Barneby, 1982; Talavera, 1999; Peñas and Morales Torres, 2009; Dantas and Silva, 2013). Although the yellow and pink flowers surveyed were similar for the human eye, they differed in their UV reflectance, thus perceived differently by bees. These community patterns of floral signals result in differences in colour diversity as perceived by bees, since bees rely on distinct colour parameters that affects the foraging behaviour of flower visitors (Papiorek *et al.*, 2016). For example, yellow flowers, which are UV-reflecting, are uvgreen for bees while UV-absorbing flowers are bee-green. According to Papiorek *et al,* 2016, bee-pollinated flowers display a pattern with UV-reflecting peripheries and UV-absorbing centres while bird-pollinated yellow flowers are entirely UV-absorbing. Based on the visual system of bees, a high proportion of UV-reflecting flowers were surveyed at the *campo rupestre* and at the Mediterranean shrubland. Such pattern could be considered as a protection against UV radiation, which is more intense in open vegetation such as those of *campo rupestre* and of the Mediterranean shrublands. Therefore, UV reflection is not only an important signal for bees, but is also related to environmental selective pressures acting upon flower colours (Arnold *et al.*, 2009; Shrestha *et al.*, 2014; Koski and Ashman 2015; Papiorek *et al.*, 2016; Bergamo *et al.*, 2018).

The distance of flower colour loci, from which we can infer the ability of bees to discriminate flower colours, was higher than 0.1 hexagon units in at least 70% of flowers in all studied plant communities. Thus, our results suggest that there is a selective pressure promoting flower discrimination and favouring effective pollination, but more tests are necessary to infer whether the discrimination pattern is caused to avoid competition. Species with distinct flower colours are expected to be easily discriminated by floral visitors, which increases the likelihood of floral constancy and intraspecific pollen deposition (Chittka *et al.*, 1999; Bukovac *et al.*, 2017). However, it is important to assume that bee species and their foraging behaviour are key components that affect discrimination values in plant communities (Garcia *et al.*, 2017; 2018). Even when flowers were in the same hexagon colour category, indistinguishable to the human eye, they can be discriminated by bees, suggesting that bees perceive a greater diversity of flower colours among Fabaceae flowers than the colour categories analysed in this study (Kevan 1983). The lowest values of colour discrimination were observed for cerrado flowers and the highest values for the Mediterranean shrubland, where flowers were distributed in all bee-hexagon categories. This result, along with the frequency of bee-colours, shows that Fabaceae species of the Mediterranean shrubland have the highest flower colour diversity among the studied communities. Differences in sample size among study sites could be responsible for the lower colour diversity found in tropical Fabaceae, however, when the two tropical communities are grouped, thus increasing sample size, colour diversity is still lower. Based on the proportion of flowers in each human-colour category, the different communities showed similar flower colour diversity, which highlights the importance of analysing flower colours according to the pollinator's visual system. Contrary to our expectations, the higher species diversity in the tropics did not reflect a higher colour diversity in Fabaceae flowers. The shorter and much more restricted reproductive season in the Mediterranean may act as an important selective pressure to increase flower colour diversity and avoid competition for pollinators during the short flowering season (Mitchell *et al.*, 2009). Moreover, flower colour is a labile trait and changes occur frequently within plant clades (McEwen & Vamosi, 2010).

On the other hand, most of the tropical Fabaceae flowers showed colour patterns with a more complex display than temperate Fabaceae flowers. Colour pattern, which is a common signal in bee-pollinated species, can interfere in pollinators' behaviours and interactions (Fenster *et al.*, 2004; Arnold *et al.*, 2009; Shrestha *et al.*, 2014, Camargo *et al,*. 2019). Colour patterns act as floral guides, reducing flower damage and increasing flower detectability and discrimination (Koski and Ashman, 2015). Therefore, colour patterns can be advantageous as it reduces pollen interference among plants sharing pollinators in highly diverse communities, such as tropical ecosystems (Koski and Ashman, 2015).

As expected, along with the observed differences in leaf-background colouration, we also found differences in colour signals among the three studied communities. Chromatic signals (colour contrast and purity) were higher among Mediterranean shrubland species, while achromatic signals (green contrast and brightness) were higher in tropical species of seasonally dry environments. The absence of these differences using a standard background, reinforced the hypothesis that colour signals are promoted by each vegetation own background. This result also highlights the importance of considering the vegetation colour background in comparative studies. High colour contrasts increase flower contrast against the background and the ability of flower colour discrimination by bees (Dyer *et al.*, 2008, Hempel de Ibarra *et al.*, 2001). In temperate communities, where species face a stronger seasonality and different species flower at the same time (Petanidou & Lamborn 2005), high values of colour contrast may be important to maximize flower discrimination among flowering species (McEwen and Vamosi 2010, Rohde et al. 2013, van der Kooi *et al.*, 2019). Considering the achromatic signals, the habitat type influences the green contrast (Menzel *et al.*, 1997, van der Kooi *et al.*, 2019), and the observed differences in green contrast among the studied communities may be related to differences in species composition and in seasonal changes inducing the leaf fall and leaf bud (Reys *et al.*, 2013; Rundel *et al.*, 2016; Silveira *et al.*, 2016). Menzel *et al.* (1997).

Similar to the pattern observed for Fabaceae species in this study, Menzel *et al.* (1997) found that the desert background promoted higher green contrasts of flowers than the background of a Mediterranean vegetation (Menzel *et al.*, 1997). Thus, we would expect that flowers from closed vegetation would be more contrasting to increase its detection. However, flowers from cerrado, which is the densest vegetation among our study sites, showed an intermediate green contrast and the lowest colour contrast among the studied communities. Binkenstein and Schaefer (2015) also found no difference in flower colour or green contrast between closed forests and open grasslands, suggesting that differences in light conditions may be compensated by the colour constancy mechanism of bees with no consequence for flower detection. Although the role of brightness in pollinator attraction is not clear, evidence suggests that floral brightness is important for colour detection in nocturnal pollinators (van der Kooi *et al.*, 2019). In tropical communities, crepuscular and nocturnal bees (see Soares and Morellato, 2018) can use brightness to locate flowers. Under dim light, flower detectability may increase if high brightness is combined to other colourful parameters (Neumeyer, 1981; Dyer, 1998; Kinoshita and Arikawa, 2000; van der Kooi *et al.*, 2016). In contrast, nocturnal behaviour is absent in Mediterranean bees and thus, selective pressures acting on brightness are likely to be higher in the Tropics.

We found that differences in the background colouration and in the vegetation structure led to differences in colour signals that probably affect the way bees perceive and select flowers (Menzel *et al.*, 1997; Camargo *et al.*, 2014; Bukovac *et al.*, 2017). For example, the background colouration is one of the drivers of flower constancy, and flower contrast against the background is essential for bees to discriminate flowers (Lunau *et al.*, 1996; Bukovac *et al.*, 2017). However, there is a lack of studies on how changes in the natural background may affect the way bees perceive specific colour signals. Thus, it is difficult to foresee the biological consequences of such differences and the importance of different signals as drivers of flower colour selection in different plant communities. Variations in colour signals between communities may also be related to the perception of bee species in each community as different species may rely on different colour signals (Dyer *et al.*, 2016, Koethe *et al.*, 2018). Considering that Brazil has a high diversity of bees and pollination systems, and that bee preferences have not been fully understood, tropical bee species can show distinct preferences and a combination of colour parameters (Koethe *et al.*, 2018). Besides local pollinators, the background colouration promoted by the vegetation structure, composition, and seasonality are important drivers of flower visual signals, influencing the diversity of flower colours found in plant communities. Therefore, colour signals also face local environmental selective pressures ensuring flower conspicuousness for pollinators.

SUPPLEMENTARY INFORMATION

Fabaceae species surveyed in the studied sites and the corresponding habit, bee-colour, human-colour, and presence or absence of colour patterns. It also includes a comparison of colour parameters calculated according to the bee visual system for the tropical cerrado and *campo rupestre* communities and the temperate Mediterranean shrubland community.

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Figures

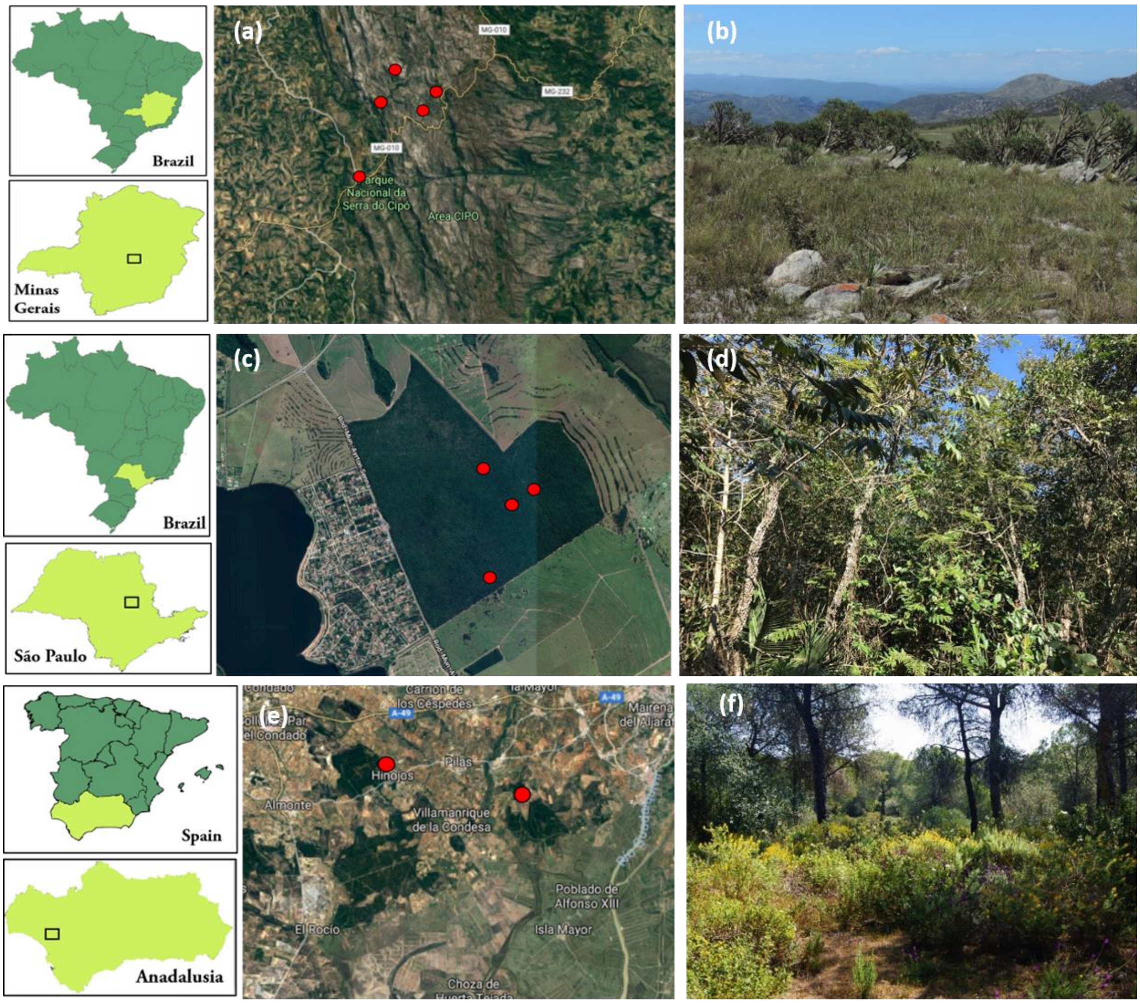

Figure 1. Study sites and overview of the vegetation structure. *Campo rupestre* at the Serra do Cipó, Minas Gerais State, southeast Brazil (a) and a detail of the vegetation structure (b); cerrado at Itirapina, São Paulo, southeast Brazil (c) and a detail of the vegetation structure (d); and the Mediterranean shrubland at Doñana National Park, Huelva, Spain (e) and a detail of the vegetation structure (f). Red dots represent the locations where data was collected. Photos by: Maria Gabriela Gutierrez de Camargo (b), Amanda Eburneo Martins (d) and Pilar Fernandez Diaz (f).

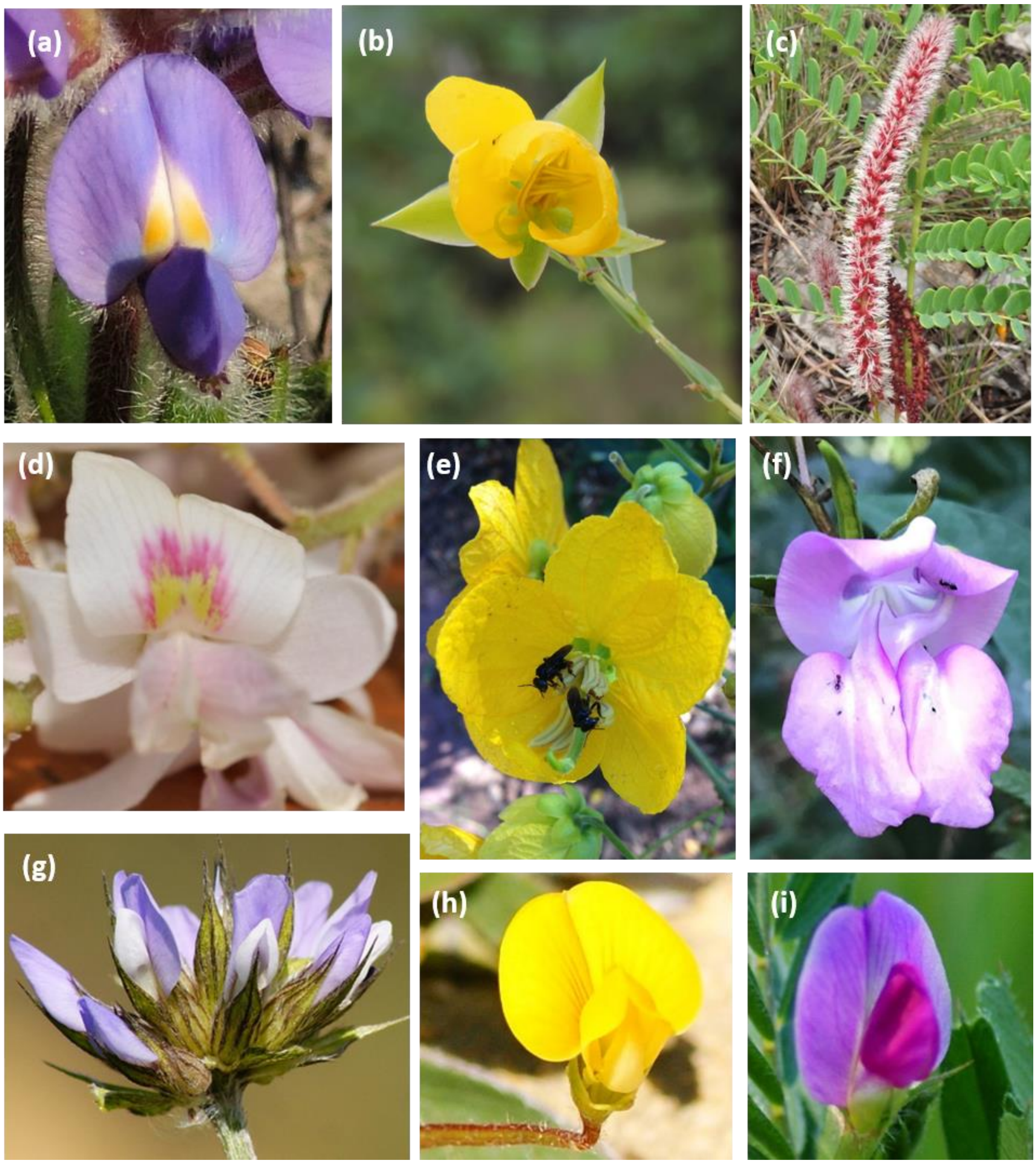

Figure 2. Fabaceae bee-flowers sampled in the *campo rupestre* (a-c), cerrado (d-f) and Mediterranean shrubland (g-i). (a) *Lupinus coriaceus*; (b) *Chamaecrista ramosa*; (c) *Stryphnodendron sp*; (d) *Pterodon pubescens*; (e) *Senna rugosa*; (f) *Vigna peduncularis*; (g) *Psoralea bituminosa*; (h) *Scorpiurus muricatus*; (i) *Vicia sativa*. Photos by: Maria Gabriela Gutierrez de Camargo (a-d), Amanda Eburneo Martins (e,f) and Pilar Fernandez Diaz (g-i).

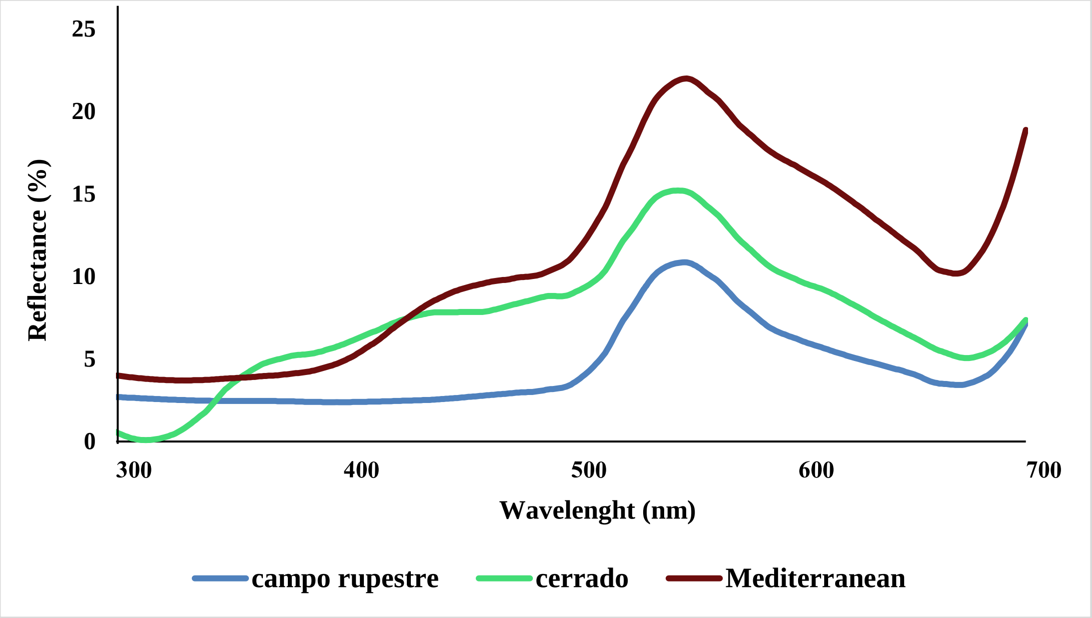

Figure 3. Predominant leaf-background colour represented by the mean reflectance spectra of leaves of different species collected in each study site: *campo rupestre* (n=316 species), cerrado (n = 80 species), Mediterranean shrubland (n = 22 species).

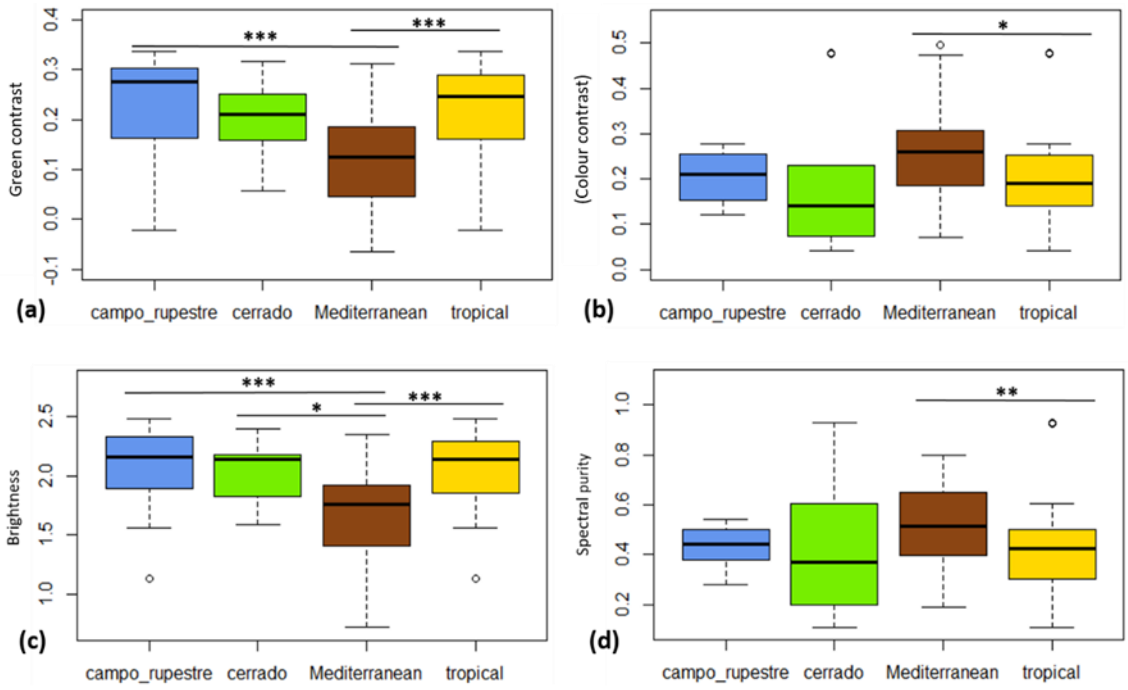

Figure 4. Colour variables for flowers of Fabaceae species collected in the tropical *campo rupestre* and cerrado communities (southeast Brazil), the temperate Mediterranean shrubland (southern Spain), and both tropical communities joined together, considering the natural background of each

community. (a) Green contrast; (b) Colour contrast; (c) Brightness; and (d) Spectral purity. Asterisks indicate significant differences between vegetation types (*p < 0.05, **p < 0.01, ***p < 0.001).

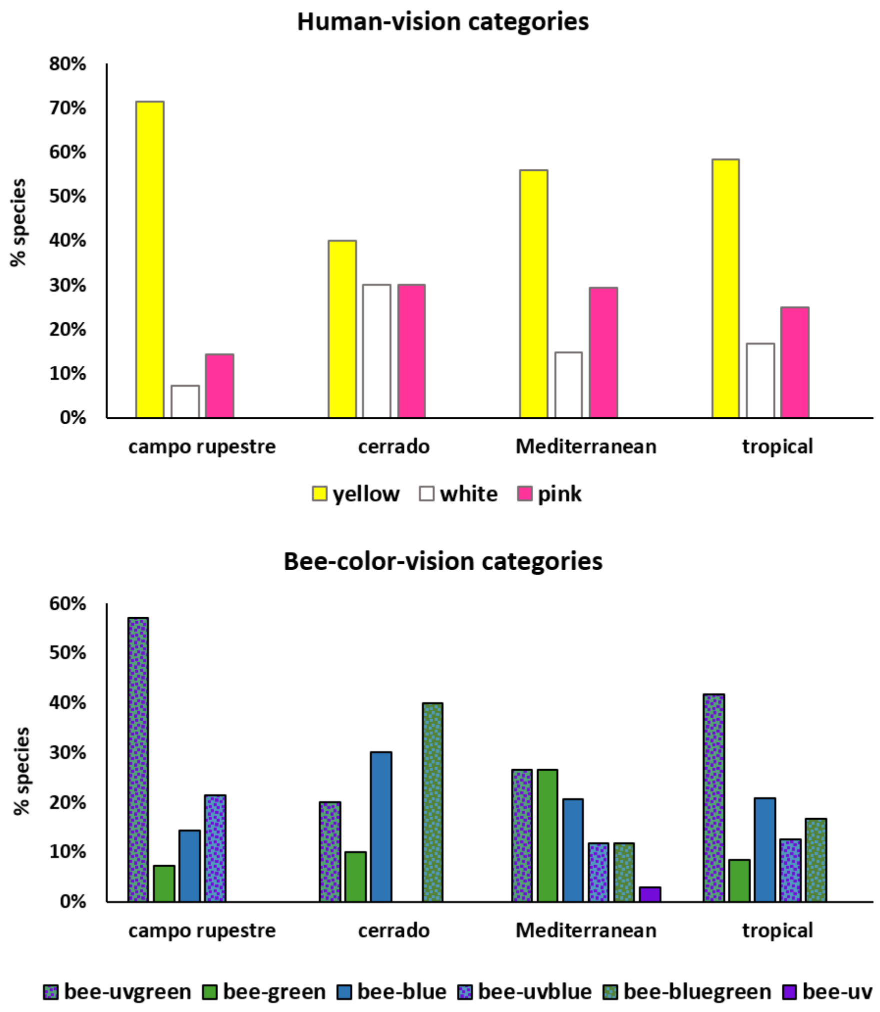

Figure 5: Flower colour of 58 bee-pollinated Fabaceae species according to human colours vision (a) and bee-colours (b) from the tropical *campo rupestre* (n = 14 species) and cerrado (n = 10 species) communities (southeast Brazil), in the temperate Mediterranean shrubland (southern Spain) (n = 34 species), and both tropical communities joined together (n = 24 species).

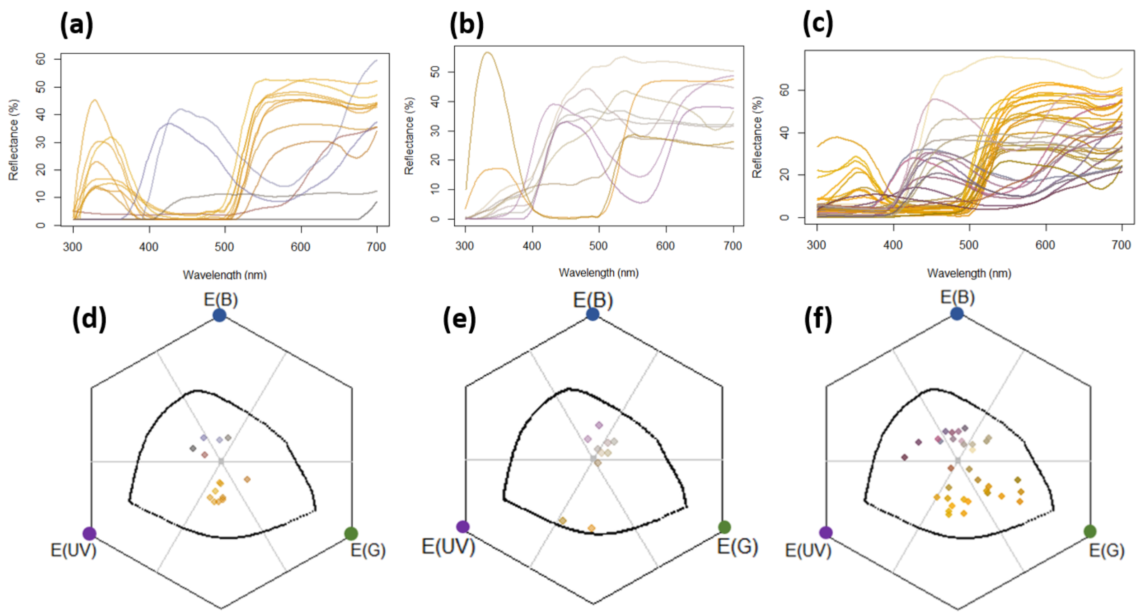

Figure 6. Reflectance spectra (a-c) and respective flower-colour loci in the bee visual space represented by the bee-hexagon (d-f) of bee-pollinated flowers of Fabaceae species collected in the tropical *campo rupestre* (a,d) (n = 14 species) and cerrado (b,e) (n = 10 species) communities from southeast Brazil, and in the temperate Mediterranean shrubland (c,f) (n = 34 species) from southern Spain. The gray point in the bee-hexagon represents the achromatic centre (leaf-background) and the colourful points in the vertices represent the following bee-photoreceptor: blue, green, and ultraviolet. The black line inside the hexagon represents the maximum sensitivity of each photoreceptor to a monochromatic light and each of the six parts of the hexagon (limited by a grey line) represents a bee-colour.

SUPPLEMENTARY MATERIAL

Figure 1. Colour variables for flowers of Fabaceae species collected in temperate and tropical regions for different backgrounds: standard background (*Pavo* package) and natural background of each region. (a) Colour contrast; (b) Green contrast; (c) Spectral purity; and (d) Brightness.

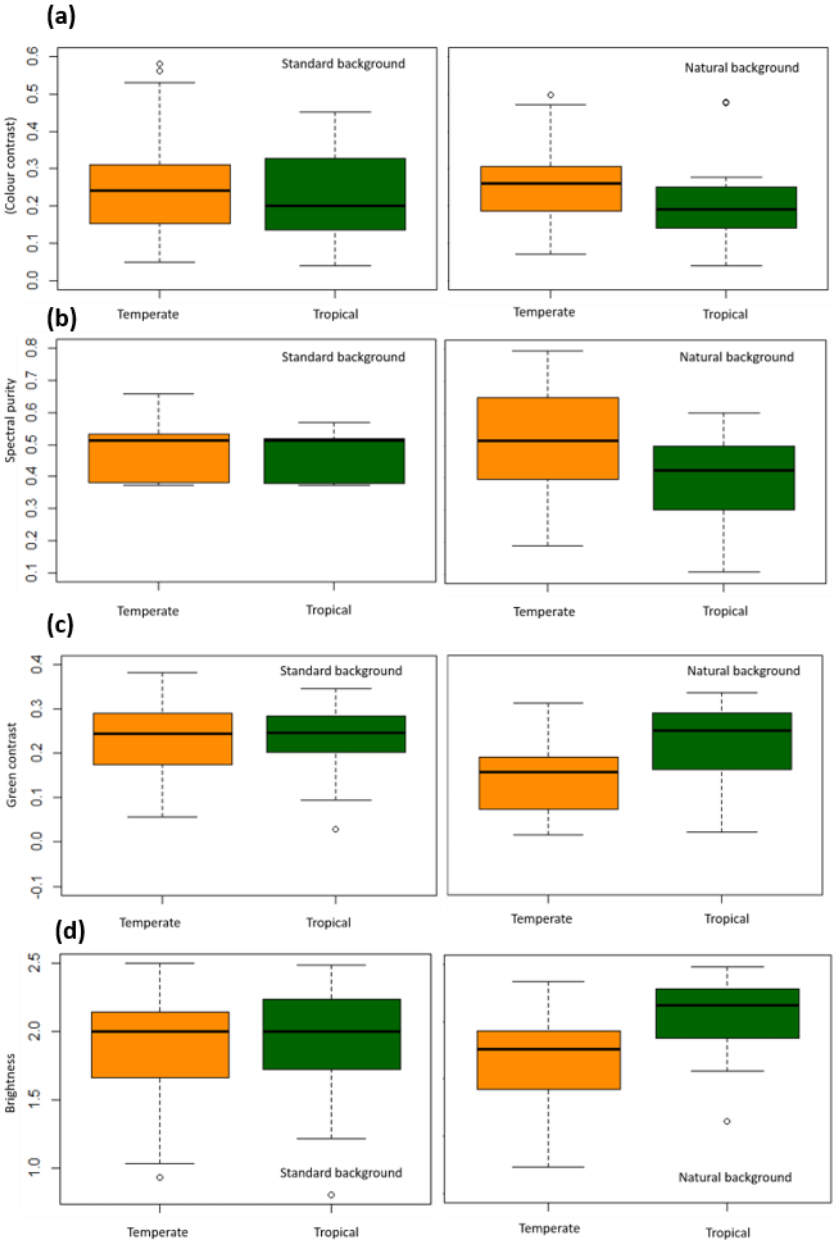

Table 1. Bee-pollinated Fabaceae species surveyed in the cerrado, *campo rupestre*, and Mediterranean shrubland communities and the correspondent habit, bee-colour, human-colour, and presence (Y) or absence (N) of colour patterns.

Study site	Species	Habit	Bee colour	Human colour	Colour-pattern
campo rupestre	*Chamaecrista cathartica*	herb	uvgreen	yellow	Y
campo rupestre	*Chamaecrista conferta*	woody	uvgreen	yellow	Y

campo rupestre	*Chamaecrista desvauxii var. graminea*	herb	uvgreen	yellow	Y
campo rupestre	*Chamaecrista ochnacea*	woody	uvblue	red	N
campo rupestre	*Chamaecrista papillata*	woody	uvgreen	yellow	Y
campo rupestre	*Chamaecrista ramosa*	woody	uvgreen	yellow	Y
campo rupestre	*Chamaecrista sp.1*	woody	uvgreen	yellow	Y
campo rupestre	*Chamaecrista sp.4*	woody	uvgreen	yellow	Y
campo rupestre	*Clitoria sp.*	woody	blue	blue	Y
campo rupestre	*Dalbergia miscolobium*	woody	uvblue	pink	Y
campo rupestre	*Lupinus coriaceus*	herb	uvblue	violet	Y
campo rupestre	*Senna cf. rugosa*	woody	uvgreen	yellow	Y
campo rupestre	*Stryphnodendron sp.*	woody	blue	whitish	N
campo rupestre	*Zornia latifolia*	herb	green	yellow	Y
cerrado	*Anadenanthera peregrina var. falcata*	woody	bluegreen	white	Y
cerrado	*Chamaecrista flexuosa*	herb	green	yellow	Y
cerrado	*Chamaecrista ramosa*	herb	uvgreen	yellow	Y
cerrado	*Mimosa cf. alleniana*	herb	blue	pink	N
cerrado	*Mimosa debilis*	herb	bluegreen	purple	N
cerrado	*Pterodon pubescens*	woody	blue	white	Y
cerrado	*Senna rugosa*	woody	uvgreen	yellow	Y
cerrado	*Strypnodendrum obovatum*	woody	bluegreen	white	Y
cerrado	*Tachigali pilgeriana*	woody	blue	yellow	N
cerrado	*Vigna peduncularis*	woody	bluegreen	purple	Y
Mediterranean	*Anthyllis cytisoides*	woody	uvgreen	yellow	Y
Mediterranean	*Anthyllis tetraphylla*	herb	bluegreen	white	Y
Mediterranean	*Astragalus hamosus*	herb	bluegreen	white	N
Mediterranean	*Astragalus lusitanicus*	herb	bluegreen	white	N
Mediterranean	*Coronilla juncea*	woody	uvgreen	yellow	Y
Mediterranean	*Coronilla scorpioides*	herb	uvgreen	yellow	Y
Mediterranean	*Cytisus grandiflorus*	woody	uvgreen	yellow	Y
Mediterranean	*Genista hirsuta*	woody	uvgreen	yellow	Y
Mediterranean	*Genista triacanthos*	woody	uvgreen	yellow	Y
Mediterranean	*Lathyrus annuus*	herb	uvgreen	yellow	Y
Mediterranean	*Lathyrus aphaca*	herb	green	yellow	N
Mediterranean	*Lathyrus clymenum*	herb	uvblue	pink	Y

Mediterranean	*Lotus creticus*	herb	green	yellow	Indet.
Mediterranean	*Lupinus angustifolius*	herb	uvblue	blue	N
Mediterranean	*Lupinus hispanicus*	herb	blue	violet	N
Mediterranean	*Lupinus luteus*	herb	green	yellow	N
Mediterranean	*Melilotus indicus*	herb	green	yellow	Indet.
Mediterranean	*Ononis baetica*	herb	blue	pink	Y
Mediterranean	*Ononis pinnata*	herb	uvblue	pink	Y
Mediterranean	*Ononis pubescens*	herb	uvgreen	yellow	Y
Mediterranean	*Ononis repens*	herb	blue	pink	Y
Mediterranean	*Ononis viscosa*	herb	uvgreen	yellow	N
Mediterranean	*Ornithopus sativus*	herb	blue	pink	N
Mediterranean	*Psoralea bituminosa*	herb	blue	violet	N
Mediterranean	*Retama sphaerocarpa*	woody	green	yellow	N
Mediterranean	*Scorpiurus muricatus*	herb	green	yellow	N
Mediterranean	*Scorpiurus sulcatus*	herb	green	yellow	N
Mediterranean	*Scorpiurus vermiculatus*	herb	uv	yellow	N
Mediterranean	*Trifolium campestre*	herb	green	yellow	N
Mediterranean	*Ulex ericocladus*	woody	green	yellow	Y
Mediterranean	*Vicia benghalensis*	herb	uvblue	violet	N
Mediterranean	*Vicia faba*	herb	blue	white	Y
Mediterranean	*Vicia lutea*	herb	bluegreen	white	N
Mediterranean	*Vicia sativa*	herb	blue	violet	Y

Table 2. Comparison of colour parameters calculated according to the bee visual system for the tropical cerrado and *campo rupestre* communities and the temperate Mediterranean shrubland community.

Colour parameters		cerrado vs campo rupestre	cerrado vs Mediterranean	campo rupestre vs Mediterranean	Tropical vs Temperate
Colour contrast	Kruskal-Wallis chi-squared	5.46	5.46	5.46	4.74
	df	2	2	2	1
	P-value	0.06	0.06	0.06	0.03
	Adjusted *P*-value	1.00	0.08	0.51	0.02*
Green contrast	Kruskal-Wallis chi-squared	13.83	13.83	13.83	12.39
	df	2	2	2	1
	P-value	0	0	0	0
	Adjusted *P*-value	0.69	0.21	<0.001*	<0.001*
Brightness	Kruskal-Wallis chi-squared	14.64	14.64	14.64	14.59
	df	2	2	2	1
	P-value	<0.001*	<0.001*	<0.001*	<0.001*
	Adjusted *P*-value	1.00	0.02*	<0.001*	<0.001*
Spectral purity	Kruskal-Wallis chi-squared	5.61	5.61	5.61	5.60
	df	2	2	2	1
	P-value	0.06	0.06	0.06	0.06
	Adjusted *P*-value	1.00	0.25	0.12	0.01*
Dominant wavelength	Kruskal-Wallis chi-squared	1.08	1.08	1.08	0.20
	df	2	2	2	1
	P-value	0.58	0.58	0.58	0.58
	Adjusted *P*-value	1.00	1.00	1.00	0.65

CHAPTER 2.

Flower colours phenology and the seasonality of visual signals in a tropical savanna

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Abstract

Background and Aims: Flowering patterns are crucial to understand the ecological dynamics of plant communities such as temporal changes in plant reproduction and resource availability for pollinators. For tropical seasonally dry vegetations, climate may constraint flower and leaf phenology, influencing flower conspicuousness and plant-pollinator synchronicity. Vision is the main sense used by floral visitors to search for resource and most flowering plants present conspicuous colours to attract these potential pollinators. Changes on colouration of the leaves between seasons alter the predominant background, probably interfering in the flower colour perception by pollinators. For a seasonal woody cerrado (Brazilian savanna), we described the pollination syndromes and the flower colour diversity based on the visual system of humans and bees, and compared the flower contrasts against the background in the dry and wet seasons, in a seasonal woody cerrado (Brazilian savanna). We then verify: (i) does the seasonality influence the diversity of flowers' colours over time? (ii) are the flower colours related to pollination syndromes? (iii) do the bee flower colour signals differ between dry and wet seasons consistent with the changes in the background colouration?

Methods: The community flowering pattern of 106 species was described based on 11-year of phenological monitoring. We classified the pollination syndromes of 222 species based on: an extensive bibliographic search by species, genera, and on papers performing pollinators' survey, on flower morphology and field observations. To describe the diversity of flower colours we classified the flower colours by human (222) and bee vision using the flower spectral reflectance. For 33 flower species that were both, in the long-term phenological observations and in the colour reflectance analyses, we compared the flower colour diversity and colour variables of contrasts, brightness, spectral purity and dominant wavelength between seasons.

Key Results: The cerrado flowering pattern were markedly seasonal, peaking in the transition between dry and rainy seasons. The diversity of flower colours changed over the year. The highest diversity of flower colours was observed in September, in the transition between dry and wet seasons. Only cream flowers presented their main peak out of this transition, in December, the middle of the wet season. More than a half of the cerrado species are pollinated by bees and colours related to melittophily (human white, pink, yellow and bee-bluegreen) predominated in the community. Flowers produced during the dry season presented higher values of colour contrast and spectral purity, while in the wet season flowers presented higher values of brightness. Regardless the flowering season, the yellowish background of the dry season promoted higher colour contrast while the green background of the wet season higher green contrast.

Conclusions: The cerrado flowering were seasonal and peak dates differed according to the flower colours. Nevertheless, a high colour diversity was observed year-round in the cerrado, even during the dry season, when fewer species were flowering. Changes in the background colouration influenced colour signals, ensuring flower conspicuousness to bees across the seasons. Therefore, seasonality was the main driver of flowering peak pattern, flower colour diversity and signals in the cerrado community, likely also influenced by pollinators' selective pressures. We recommended the adoption of flower colours by according to the pollination view using spectral measurements to study plant-pollination interactions.

Key-words: seasonality, flowering patterns, background colouration, pollination syndromes, cerrado *sensu stricto*, flower colour diversity, floral signals, bee visual system.

Introduction

Flowering patterns are strongly related to the ecological dynamics of plant communities, defining the temporal changes on plant reproduction, the resources availability for flower visitors, the maintenance of plant and pollinators diversity and the plant reproductive successes (Gentry 1974, Lieth 1974, Ramirez 2006, Morellato et al. 2016). In the tropics, flowering time is constrained primarily by seasonal changes in rainfall, temperature, and day length (Abrahamczyk et al. 2011, Morellato et al. 2000, Cortéz-flores et al. 2017) but it is also shaped by to biotic factor, mainly plant-pollinator interactions. Pollinators exerts a key selective pressure on flowering patterns, affecting the intensity (productivity) and length of reproductive season and the species` synchronicity (Frankie et al. 1974, Gentry 1974). In tropical seasonal vegetations, flowering is concentrated in the favourable period of the year, increasing when pollinators' diversity increases and are likely to diverge according to the pollination syndromes (Newstron et al. 1994, Frankie et al. 1974, Ramirez et al. 2006). Also, in seasonally dry tropical vegetations most species are deciduous or lose most of their leaves and the leaf aging process changes the vegetation colours, a process that may facilitate the visualization of flowers by pollinators (Gentry 1974, Camargo et al. 2018).

Vision is the main sense used by floral visitors to find and select flowers, and the flower colours the key feature of pollinators attraction (Fenster et al. 2004, Warren & Billington 2005, Arnold et al. 2009a, Arnold et al. 2009b, Shrestha et al. 2014). Yet, the current Angiosperms flower colour diversity is mainly a consequence of the selective pressure exerted by the pollinators according to their visual sensitivity and colour preferences (Chittka et al., 1999, Arnold 2012, Brito et al. 2014, Camargo et al. 2019). Despite some innate preferences for certain colours, flower visitors select and visit more frequently flowers that are easily detected, that is, more contrasting against the background (Spaethe et al. 2001, Arnold 2012, Telles et al. 2017). The climatic seasonality alters the background colouration of flowers and fruits which is composed mainly by leaves. The leaves lose chlorophyll and become more yellowish, brown or reddish in the dry season, changing the predominant green colour of the young and mature leaves during the rainy season. Therefore, differences in the background colour may interfere the colour perception by animals (Endler 1993, Binkenstein & Schaefer 2015, Camargo et al. 2013, 2014) and, consequently, the flower contrast, detection and visitation by pollinators.

Cerrado, the Neotropical savanna, holds a complex of vegetation physiognomies, with high ecological and landscape diversity (Coutinho 2006, Klink & Machado 2005). The cerrado *sensu stricto* is the predominant cerrado physiognomy, an open woody savanna dominated by shrub and tree species mostly pollinated by animals, and more than 50% of the flowering species are bee pollinated (Coutinho 2002, 2006, Oliveira & Gibbs 2000, Oliveira & Gibbs 2002, Gottsberger & Silberbauer-Gottsberger 2006,). The Cerrado, as other savannas around the world, is subject to a seasonal climate, alternating dry and rainy seasons (Coutinho 2002, 2006, Klink & Machado 2005, Reys et al. al., 2016, Camargo et al. 2018). Climate seasonality is the factor conditioning the vegetative and reproductive patterns of cerrado, with a high senescence in the dry season and leaf flushing and flowering of most species in the transition between dry and rainy seasons (Batalha et al., 1997, Batalha & Mantovani 2000, Batalha & Martins 2004, Gottsberger & Silberbauer-Gottsberger 2006, Alberton et al., 2014, Camargo et al., 2018). Therefore, considering the climatic seasonality and the importance of flower colours for plant-pollinators interactions, we may expect that in the cerrado vegetation the diversity of flower colours over the year, as well as their perception by the pollinators is influenced by the seasonal changes in the background colouration (Alberton et al. 2014, Camargo et al. 2013, 2014, 2018).

In the present study, using a community-level approach and the cerrado as a model of tropical seasonal vegetation, we analysed the flowering patterns, determined the main pollinators' syndromes, and measured the flower colour diversity and respective flower colour signals according to the visual system of bees. More specifically, we aimed to answer the following questions: (i) is the cerrado flowering pattern seasonal and does the seasonality influence the diversity of flowers’ colours over time? (ii) Are the flower colours related to pollination syndromes? (iii) Do the bee flower colour signals differ between seasons according to changes in the background colouration? We expected seasonal flowering patterns, which the seasonality will influence the flowering pattern by flower colours, with highest flower colour diversity during the dry to wet seasons transition, period with large abundance of flowers and pollinators. Considering the differences in vision systems and colour perceptions among pollinators, we expected to find a relation between pollination syndromes and flower colours. Finally, we expected to find differences in the visual signals between the seasons, due to the seasonal changes in the background colouration.

Material and Methods

Study area

The study site is a private land located at Itirapina, São Paulo State, Southeastern Brazil (22º 10' 31.41"S; 47° 52' 26.1 3"W) and covered by cerrado *sensu stricto* (Reys et al. 2013), the typical and most widespread Brazilian woody savanna vegetation (Coutinho 2006)(Figure S1A). The studied cerrado is a semi-deciduous vegetation, marked by a discontinuous tree layer of around 6 to 7 meters high with some individuals reaching up to 12 meters, scattered shrubs and a continuous herbaceous layer (Camargo et al. 2018, Reys et al. 2013)(Figure S1B). The climate is marked by a rainy, warm season from October to March, and a dry and colder season from April to September (Camargo et al. 2018). The mean annual temperature is 20°C, and the annual total rainfall 1524 mm (Camargo et al. 2011, 2018). The cerrado area present high diversity of shrubs and trees, represented by around 120 woody species of 40 families, with the Myrtaceae, Fabaceae, Malpighiaceae and Vochysiaceae the most species rich families; the most abundant species are *Bauhinia rufa* (Bong.) Steudel, *Xylopia aromatica* (Lam.) Mart., *Miconia rubiginosa* (Bonpl.) A.D., *Virola sebifera* (Aubl) and *Myrcia guianensis* (Aubl.) DC. (Reys et al. 2013).

Flowering phenology

Phenological monitoring has been carried as part of a long-term phenological study since September 2004 by the Phenology Laboratory at UNESP-Rio Claro (Mariano et al. 2016, Escobar et al. 2018). A total of 36 transects of 25 x 2 m were established, 50 m apart from each other, where all woody plants with > 3 cm of circumference above the ground were sampled and identified to species level (Reys et al., 2013, Camargo et al. 2018).

Phenological observations were registered every month for 106 species and 2121 individuals, when data of presence or absence and intensity of reproductive (flower buds, flowering or anthesis, unripe fruit and ripen fruits) and leafing (leaf flush and leaf fall) phenophases were recorded, according to Morellato et al. (1989) and see Camargo et al. (2018), for details. The intensity of the phenophases was evaluated according to Opler et al. (1980) using three classes of intensity that corresponded to the absence of phenophase (class 0), presence at moderate intensity (class 1) and presence at high intensity (class 2). For the present study, to access the temporal pattern of flower colour diversity in the cerrado community, we analysed the flowering peak phenology data from January 2005 to December 2015, totalizing 11 years of observations.

Pollination syndromes

To define the pollination syndromes of the cerrado species we used the list of all species surveyed at the study site since 2004 by the Phenology Laboratory team, totalizing 222 woody plant species (Borgiani et al. unpubl.). The species vouchers were lodged in the Herbarium Rioclarense (HRCB) of the São Paulo State University (UNESP). The classification of pollination systems was made based on an extensive bibliographic search by species and genera and others pollinators' survey (e.g., Gottsberger & Silberbauer-Gottsberger 2006, Yanagizawa & Rodella 2007; Balestra et al., 2014). The literature information was applied to infer the pollination syndromes considering the method of observation described by the authors and accuracy is modified from Ollerton et al. (2019) and is presented in Table 1. We used the inferences to investigate the relationships between flowers colours and pollination syndromes in the cerrado community.

Table 1. Parameters adopted to classify plant species by pollination syndromes. The accuracy of the inference ranges from 1 high accuracy- to 3 – less accurate (Adapted from Ollerton et al. 2019).

	Method of pollinators survey by literature
1	Study focus on species; direct observation of the floral visitor in the field was reported, contacting the flower reproductive structures and identified as pollinator.
2	Community studies, general field observations of flower visitor with inference of pollination effectiveness.
3	Pollination syndrome inferred based on flower morphology and other floral attributes (colour, odour, resource, etc.), or based on the genera.

Flower colour and signals

We classified the flower colours according to the human view as white, yellow, pink (including violet and blue flowers), cream, red (including orange and brown flowers) and green (Gottsberger & Silberbauer-Gottsberger 2006). The approach is similar to that developed by Camargo et al. (2013), to analyse the fruiting patterns according to fruit colour in the same cerrado community.

We defined flower colours according to the visual systems of bees. We collected reflectance data monthly, between April 2017 and April 2018, to cover the diversity of flowers over time. The analyses of flower colour were based on the mean reflectance spectra calculated from the reflectance data of 10 flowers of each species (Dalrymple et al. 2015) measured with a spectrophotometer (Ocean Optics – Jaz Modular Optical Sensing Suite) between 300 and 700 mm, including the ultraviolet light (UV). We considered as the flower colour the reflectance spectra of the predominant colour in the floral display, generally correspondent to the colour of the petal. As the community leaf-background we used the mean reflectance data of 10 leaves per species, collected at the same moment that we collected the flowers according to Camargo et al. (2014). To calculate flower colours diversity and colour variables based on the visual systems of bees, we used diagrams representing the bee colour space, the bee-hexagon proposed by Chittka (1992). The vertices of the hexagon represent one of each photoreceptors present on the bees' retina: ultraviolet, blue and green. In the hexagon, each flower is represented by a point (colour loci) corresponding to the Euclidian distance between the light reflected from the flower against its background, according to a previously specified visual system (Chittka 1992). Here, we used the model proposed for the visual system of *Apis mellifera* by Chittka 1992. We used the community leaf-background to calculate and describe the bee-colour diversity of the community. According to the colour loci in the bee-hexagon, we classify flower colours as seen in six bee-colours categories: bee-blue, bee-green, bee-UV, bee-bluegreen, bee-UVgreen and bee-UVblue, as proposed by Chittka et al. (1994).

To analyze differences in the colour signals between dry and wet seasons we calculated the colour and green contrasts, the spectral purity, the dominant wavelength and the brightness of the sampled flowers. To do that we used the model proposed for the visual system of *Apis mellifera* and the respective background of each season calculated by Camargo et al. (2013) for the same and study site. We also calculated and compared the contrasts of flowers of dry and wet seasons against the background of their opposite season. The colour contrast in the bee-hexagon is represented by the distance between the center and a colour locus (Chittka et al. 1992), it is important for bees to detect flowers from the background and is activated only at short distances (~1m from the target) (Spaethe et al. 2001, van der Kooi et al. 2018). The green contrast is used for long-distance detection of colour, sizes and shapes of flowers, always activated during bee forage, calculated by the contrast among two colours detected by the green photoreceptor and adapted to the community background (Chittka 1992, Spaethe et al. 2001, van der Kooi et al. 2018). The spectral purity is represented by the relation between the colour contrast and the distance between the dominant wavelengths, which are featured by the correspondent wavelength to the closest colour locus point in the monochromatic limit, and the centre of the hexagon (Chittka 1992, Lunau et al.1996, Rohde et al. 2013, van der Kooi et al. 2018). Flower brightness is calculated by the sum of the photoreceptors excitations and corresponds to the amplitude of the reflectance curve (Lunau *et al.*, 1996; Spaethe et al., 2001; Rohde et al. 2013, van der Kooi et al. 2018).

Data analyses

We analyzed the flowering phenology according to the flowering peak, defined as the species most frequent recurring flower month throughout the 11 years of observations. To test for seasonality in the flowering peak of all the sampled community and per species flower colour we used circular statistics as suggested by Morellato et al. (2000, 2010). Each peak flowering date was converted to a correspondent angle or vector direction (15^{o} correspond to January, successively, see Morellato et al. 2010). We applied the Rayleigh test (Z) to test for the significance of the mean angle or the corresponding mean date, that is, the concentration of flowering species around an angle or a given date of the year (Morellato et al. 2010). When significant, we infer the degree of seasonality of each colour by the length of the mean vector (r); the r vector ranges from 0, no concentration or seasonality, to 1, the highest data concentration around the mean angle or the highest seasonality (Morellato et al., 2010).

We described the community flowering pattern grouping the species according to their flower colour by human view. For the species which we had both long term phenological data and reflectance spectra, we classified and described the flowering pattern according to the colours as viewed by bees (Table S1 [Supplementary Material]).

To compare colours and colour signals of flowers between dry (April to September) and wet (October to March) seasons we determined the flowering season of each species by the flowering peak date.

We used a Wilcoxon rank sum test with continuity correction to compare the quantitative variables of brightness, dominant wavelength, spectral purity, colour and green contrasts between the dry and wet seasons. To verify if seasonal changes in the background colouration interfere in the flower conspicuity we compare the flower contrasts against the background of the correspond flowering season and against the background of the opposite season using the Wilcoxon paired signed rank test. All the reflectance spectra and colour variables were analyzed using the Pavo package (Maia et al., 2016; Maia et al., 2017) for R (R Development Core Team).

Results

We classified the flowers colour according to human view and the pollination syndrome of 222 cerrado plant species (Table S1). Of these, 106 species were observed in the long-term phenology monitoring and 91 species flourished during the 11-year of monthly observations (Table S1). From these 106 observed species, we collected reflectance data of 33 species for which we analysed the flowering pattern according the bee-colours and compared the colour signals between the wet and dry seasons (Table S1). In addition to these 33 species, we collected the spectral reflectance of other 47 species (out of the long-term phenological observations), including trees, shrubs, herbs, lianas and epiphytes (Table S1), that were used to describe the bee-colour diversity in the community. Of these 80 species with reflectance data, 66 species were identified at species level, eight at gender level, four at family level and two in process to identification.

Pollination syndromes and flower colours

Of 222 cerrado community, bee pollination or melittophily was the dominant pollination syndrome of 161 species (72%), followed by flies or myophily (5%), moths or phalaenophily (4%), hummingbirds or ornithophily (4%), beetles or cantharophily (3%), butterflies or psychophily (2%), wind or anemophily (2%), bats or chiropterophily (1%) and (7%) in the additional category diverse insects (Table S1).

Considering the accuracy of our pollination syndromes (Table 1), for 71 species (32%) the determination accuracy was level 1 - direct observation of the floral visitor contacting the reproductive structures; for 75 species (35%) the accuracy was level 2 – pollinator inferred based general field observations of flower visitor in community surveys; and for the last 74 species (33%), we inferred the syndrome based on the genera, flower morphology or other floral attributes such as colour, odour and resource.

According to the human eye, white and pink were the most frequent flower colours among the 222 species analyzed (30% and 25%, respectively), followed by yellow (20%), cream (10%), red (4%) and green (3%) colours (Figure 1A). For the 80 flowers analysed by the bee visual system, bee-bluegreen represented half of the flower colours (50%), followed by bee-blue (19%), bee-green (15%), bee-uvgreen (9%) and bee-uvblue (8%) colours (Figure 1B, 2).

The distribution of flower colours based on human vision by pollination syndrome showed that bee-pollinated flowers were mainly white, pink and yellow (Figure 3). Fly pollination was represented by all human flower colours, but mainly red and green (Figure 3). Hummingbird' pollinated flowers were mainly red and pink (Figure 3). Among flowers pollinated by moths, bats, diverse insects and wind predominated the dull colours. Flowers pollinated by beetles and butterflies were green, white and yellow. (Figure 3).

Flowering phenology

Flowering was observed throughout the year in the community (91 species), peaking in September, in the transition between the dry and wet seasons (Figure S2A). The flowering peak pattern was significantly seasonal (Z=17.82; p<0.001; vector r=0.43; mean group: October). For the 33 species with phenological and reflectance data presented a flowering patterns similar to that observed for the community (91 species), also significantly seasonal (Z=5.80; p=0.003; vector r=0.41; mean group: October), peaking in the transition of the dry to wet season, (Figure S2B). From these 33 species, 20 species presented their peak of flower production during the rainy season, between October to March, while 13 species peaked during the dry season, from April to September (Figure S2B).

Flowering phenology according to the human vision, showed the white and green flowers presented a significant highly seasonal pattern, peaking in September, and with significant mean dates in October (Z=18.73; p<0.001; vector r=0.72) and September (Z=7.70; p=<0.001; r=0.95), respectively (Figure 4A, 4B). Only cream flowers were observed throughout the year, with most species peaking on December (Figure 4D), but no significant mean date (Z=0.26; p=0.77; vector r=0.13). Yellow flowers were also distributed along all the year but May and June (Figure 4C). Pink flowers were observed mainly in the wet season (from September to March) presenting a bimodal pattern with peaks in September and March, in the transitions between seasons (Figure 4E).

Flower colour, signals, and seasonality

According to the bee-colour space (Figure 5A, D), during the wet season the flowers colours loci were more concentrated in the center of the hexagon, while in the dry season the flowers colours loci were more spread to other hexagon parts (Figure 5E, F). The flowering pattern of these predominant bee-bluegreen species was significantly seasonal, peaking in the dry and wet seasons transition, in September, with mean date in October (Z=5.5; p=0.003; vector r=0.47; mean group: October) (Fig. 4F).

The colour contrast, spectral purity and brightness differed between seasons. Flowers displayed in the dry season presented higher values of colour contrast (W=47, *p*-value=0.003*) and spectral purity (W=45, *p*-value=0.002*) (Figure 6A, B), while brightness was higher in flowers produced during the rainy season (W=182.5, *p*-value=0.02*) (Figure 6C). There was no difference in the green contrast (W=169, *p*-value=0.07) (Figure 6D) and dominant wavelength (W=79, *p*-value=0.08) between seasons. Regardless of the flowering season, flowers presented higher values of chromatic contrast against the background of the dry season (dry season flowers: V=0, *p*-value=<0.001; wet season flowers: V=0, *p*-value=9.56) and higher values of achromatic contrast against the background of the wet season (dry season flowers: V=91, *p*-value=<0.001; wet season flowers: V=210, *p*-value=1.91) (see Figure 5E, F).

Discussion

The species were flowering all over the year in the studied cerrado community. The highest diversity of species flowering and of flower colours were observed in the transition from dry to wet seasons. Our results corroborate the bees as the main pollinators in the cerrado community, and the white and bee-bluegreen as the most common colours according to human and bee eyes, respectively. The floral signals differed among seasons due to the changes in the background colouration, with more conspicuous flowers (higher values of colour contrast and spectral purity) during the dry season.

Flower colours, flowering patterns and pollination syndromes

White, yellow and pink were the most common flower human colours in the cerrado community, following the pattern described for other cerrado areas (Oliveira & Gibbs 2000, Oliveira & Gibbs 2002, Gottsberger & Silberbauer-Gottsberger 2006).

The highest diversity of flower colours and flower peaks were observed in the transition between dry and wet seasons (September), and only cream flowers peaking out of the transition, in the wet season (December). The high percentage of species flowering in the dry-rainy transition period followed the the pattern observed for other cerrado communities (Oliveira & Gibbs 2000), and is influenced by the cloud cover, day length, rainfall and temperature (Morellato et al. 2000, Cortéz-flores et al. 2017). During the wet season we observed mainly cream, white and pink flowers; in the dry season all colours but mainly cream and yellow flowers. The green flowers were restricted to the dry season, and yellow flower dominated year round cream. Flowering of yellow and pink flowers overlapped in the transition from rainy to dry seasons. The cream flowers flourished all year long, but peak did not overlap with the other colours peaks. Flowering during the dry season could decrease the interference with the high quantity of plants flourishing during the wet season, distributing the activity of pollinators all year long (Ramirez 2006), and be favoured by increasing light incidence, temperatures and reduced precipitation.

Bees preferred to pollinate saturated colours (Rohde et al. 2013), and their activity are correlated to the number of flowering species and resources diversity, being an active pollinator in the cerrado community all year long (Abrahamczyk et al. 2011). In the studied community, bees pollinated all flower colours, predominating white, pink and yellow, and are indeed the responsible for the pollination of more than a half of flowers in cerrado communities (Oliveira & Gibbs 2000, Oliveira & Gibbs 2002, Gottsberger & Silberbauer-Gottsberger 2006). The bees are in fact the dominant pollinator in distinct plant communities and biomes, thanks to their large morphologic variation, distinct foraging behaviours and the total dependency on floral resources, from larvae to adult stages (Ollerton 2017). Flies and diverse insects are featured as generalist pollinators (Pombal & Morellato 2000, Genini 2011), pollinated most flower colours in the community, including the time restricted green flowers. Among moth flowers we found dull colours: cream, white and green, which could be explained by the pollinator behaviour and visitation time during the night (Amorim et al. 2009, Duarte et al. 2012). Moths pollinated flowers peaked in the dry rainy transition and wet season. Hawkmoths are abundant almost all year long, decreasing their activity during the dry season (Amorim el at. 2014). In addition, Sphingidae moths have night-time activity being a specialist pollinator of cerrado native species, such as, *Tocoyena formosa*, *Amaioua guianensis* and *Qualea grandiflora* (Amorim et al. 2009). The bats flowers in the community, were white and cream, featured by less conspicuous colours being explained by the nocturnal behaviour of pollinators (Gottsberger & Silberbauer-Gottsberger 2006, Amorim et al. 2014).

Beetles and butterflies pollinated mainly green, white and yellow flowers. Lepidoptera populations in the tropics decrease their abundance and survival during the dry season due to the lack of water, nectar and new leaves (Bonebrake et al. 2010, Abrahamczyk et al. 2011). In addition, analysing the Psicophily, butterflies have a high developed visual system, choosing to pollinate vivid flower colour (Oliveira et al. 2004). Our results showed the opposite, with cerrado flowers pollinated by butterflies presenting dull, less conspicuous colours. Hummingbirds represent the most specialized species of bird visiting flowers, pollinating conspicuous flower colours, such as red, pink and yellow (Faegri & van der Pijl 1979, Fischer et al. 2014, Bergamo et al. 2016) as found in our cerrado community. The hummingbirds require high quality and quantity of resource, an important trait also influencing the flower selection (Fenster e al. 2015, Camargo et al. 2019). The low proportion of hummingbirds' pollination in the cerrado community could be related by the climatic factor and food seasonality. The proportion is similar to that described by Gottsberger & Silberbauer-Gottsberger 2006. The activity of hummingbirds during the dry months may decreased, due to the low food availability and diversity (Abrahamczyk et al. 2011, Genini 2011).

According to the "bee' eyes", the bee-bluegreen was the most common flower colour. The bee-bluegreen colour corresponded mainly to the white and cream flowers (see examples of flower colours that are bee-bluegreen flowers in Figure 2) (Kevan et al. (1996). Bee-bluegreen flowers phenology were concentrated in the dry to rainy season transition and early rainy season, following the community flowering pattern. The phenology confirmed the importance of the melittophily, influencing the observed flowering pattern in the studied community. The bee-UVgreen and bee-green is represented most for yellow flowers distributed throughout the year, and time-restricted green flowers. The bee-blue and bee-UVblue flowers are represented mainly by pink and red flowers in the human vision, which had flowering extended during all rainy season (Kevan et al. 1996).

Our results showed that majority of species and flower colours presented the flowering peak during the transition period, a distinctive feature of the cerrado community. Flowers were available for pollinators all year long, with the community developing a generalist pattern. Seasonality was the main driver in the flowering peak pattern, likely also influenced by pollinators' selective pressure. Moreover, we do not recommend the flower colour classification based in human visual system to classify and study the pollination syndromes and respective flowering patterns. We reinforce the importance of the analysis of flower colours based in the pollinators visual system to describe and understand the plant-pollinator interactions.

Seasons, flowers visual signals and leaf background contrasts

The flower colours were also influenced by abiotic conditions since the background colouration is significantly different between the dry and wet seasons (Camargo et al. 2013), acting as a selective pressure of the flower colour diversity and temporal patterns of different colours and colour signals.

Confirming our hypothesis that seasonality influences in flowers contrasts, during the wet season flowers were less contrasting against the green leaf-background of their flowering season. However, flowers observed mainly in the dry season were more conspicuous (high chromatic contrast and colour purity) against the yellowish background of their flowering season. According to Souza et al. 2018, during the dry season the plant-pollinator networks tends to be more specialized, due to the lower resource availability for pollinators. Therefore, it can be an adaptation to seasonality, with lower flower production during the cold and dry winters, being the visual signals a trait to maximize flower detection under non favorable conditions for some pollinators (van der Kooi et al. 2018).

The seasonality also influences the pattern of fruit colour between seasons in the same cerrado community. According to Camargo el al. 2013, the seasonal pattern of colour contrast of fruits was featured by a higher contrast during the wet season, when the fruit production is high, increasing the attractively and contrast to the background for seed dispersers. We found a similar result for chromatic contrast of flowers from the dry season and for achromatic contrast for flowers from the wet season. Therefore, we confirmed an influence of the seasonal changes in the background colouration for cerrado's flowers, with yellowish leaves promoting flowers chromatic contrast and green leaves promoting flowers achromatic contrast.

Besides that, most of flowers had reflected among 20% to 50% of the incident light, suggesting a strong visual signal to pollinators (van der Kooi et al. 2016, van der Kooi et al. 2018). The dominant wavelength values were consistent with the pollinator's importance, being the majority of flowers reflecting among 450 nm to 550 nm, considered an efficient wavelength for colour discrimination by bees and other hymenopteran (Chittka et al. 1993, Camargo et al. 2018,). Moreover, the lack of reflection in long wavelengths may also explain the low rate of hummingbirds' pollination in the cerrado community (Shrestha et al. 2013, Bergamo et al. 2016).

Our results showed that the seasonality influenced the flower colour diversity and colour signals in cerrado over time. Although with differences in the seasonality of flowering peaks according to flower colours, a high colour diversity was observed year-round in the cerrado, even during the dry season, when fewer species were flowering. The maintenance of this colour diversity over year and specificities in the peak time of some flower colours, favour the presence of pollinators along the year, the diversity of pollination modes in the community (Ramirez 2006, Genini 2011) and promotes the community pollination generalist pattern. Finally, we linked the contrasts to seasonality and found that the diversity and temporal patterns of flower colours are adapted to the cerrado seasonality and that changes in the background colouration influenced colour signals but ensuring flower conspicuousness to bees according to the season.

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Figures

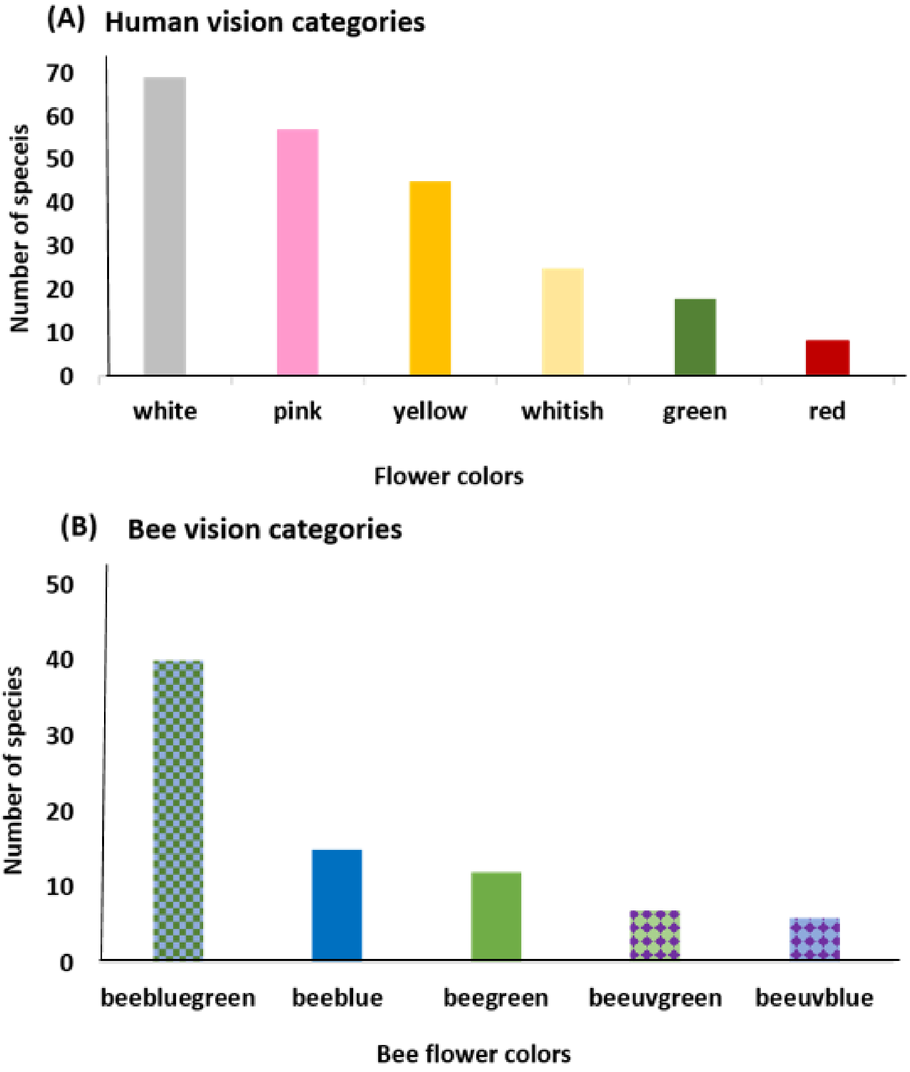


Figure 1. Proportion of flowers species in the cerrado according to the (A) human-colour (222 species) and (B) bee-colour (80 species) vision of flowers.

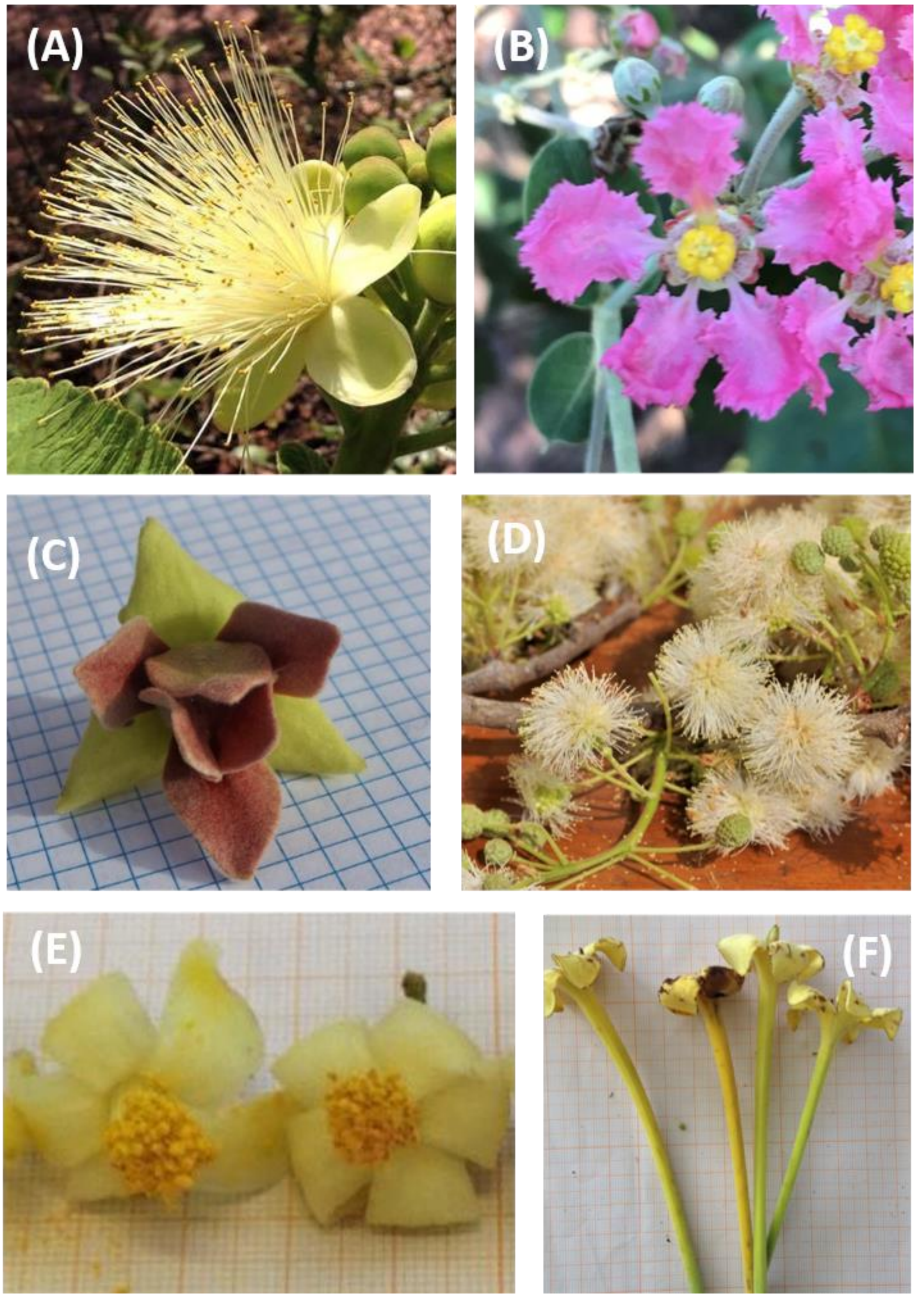


Figure 2. Examples of bee-bluegreen flowers sampled in the cerrado. (A) *Caryocar brasiliense*; (B) *Banisteriopsis campestris;* (C) *Duguetia furfuracea*; (D) *Anadenanthera peregrina var. falcata*; (E) *Eriotheca gracilipes*; (F) *Tocoyena formosa*. Photos by: Maria Gabriela Gutierrez de Camargo (D) and Amanda Eburneo Martins.

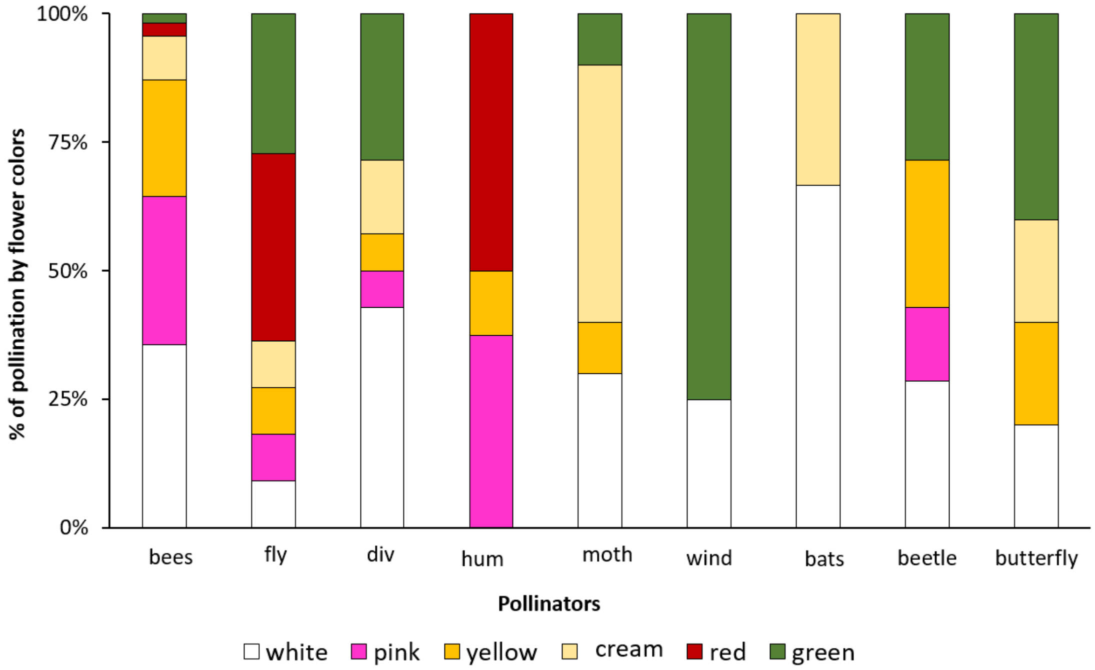
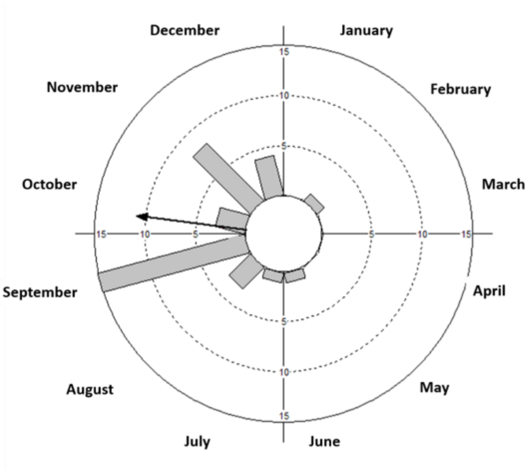
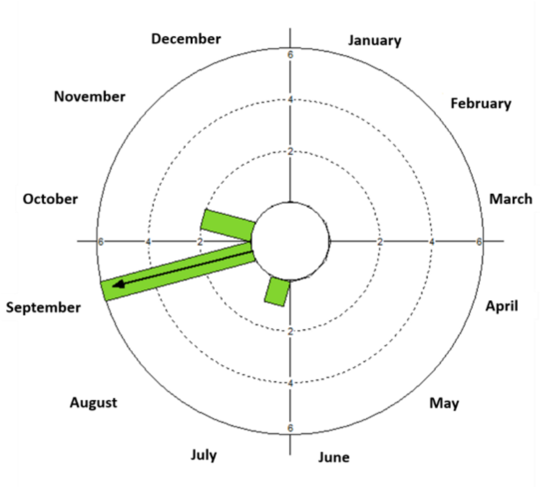


Figure 3. Proportion of flower species surveyed in a cerrado according to the human flower colour by pollination syndrome: bees or melittophily (163 plant species), flies or myophily (11), diverse insects (div) (14); hummingbirds (hum) or ornithophily (8), moths or phalaenophily (10) wind or anemophily (4); bats or chiropterophily (3); beetles or cantharophily (7); butterflies or psychophily (5).

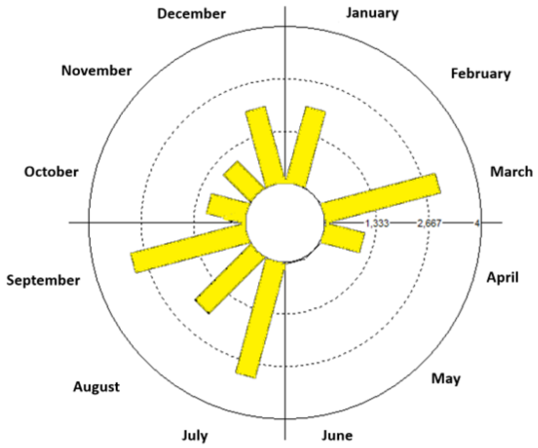
(A) White flowers (n=36)



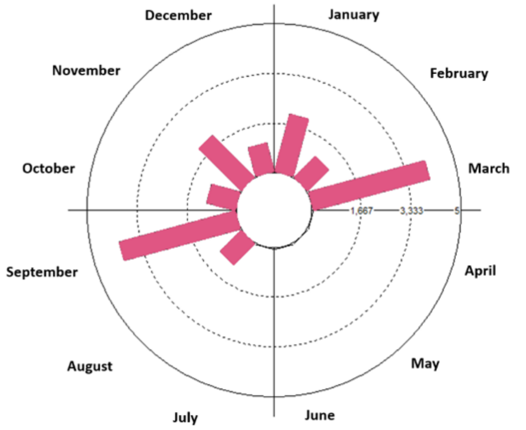
(B) Green flowers (n=9)



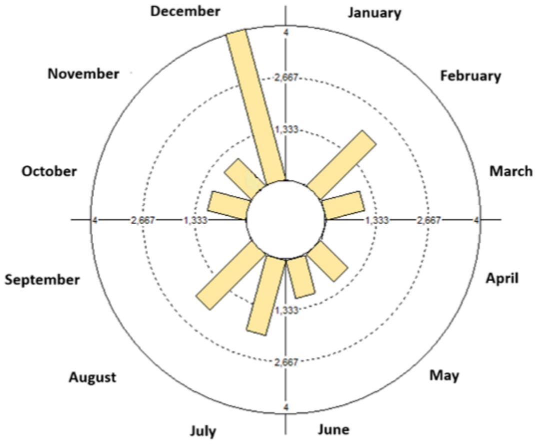
(C) Yellow flowers (n=18)



(D) Pink flowers (n=16)



(E) Cream flowers (n=15)



(F) Bluegreen (n=24)

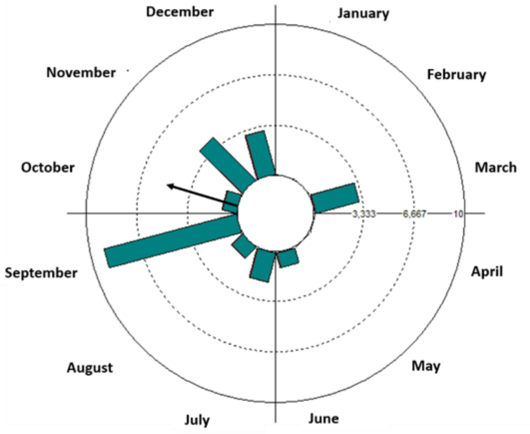


Figure 4. Flowering colours' pattern in the community according to the human vision (A-E) and for bee-bluegreen flowers (F), the most abundant bee-colour in the community according to the bee visual

system. The arrows point to the significant mean angles or dates, and the arrows' length the r-vector value (0 – 1).

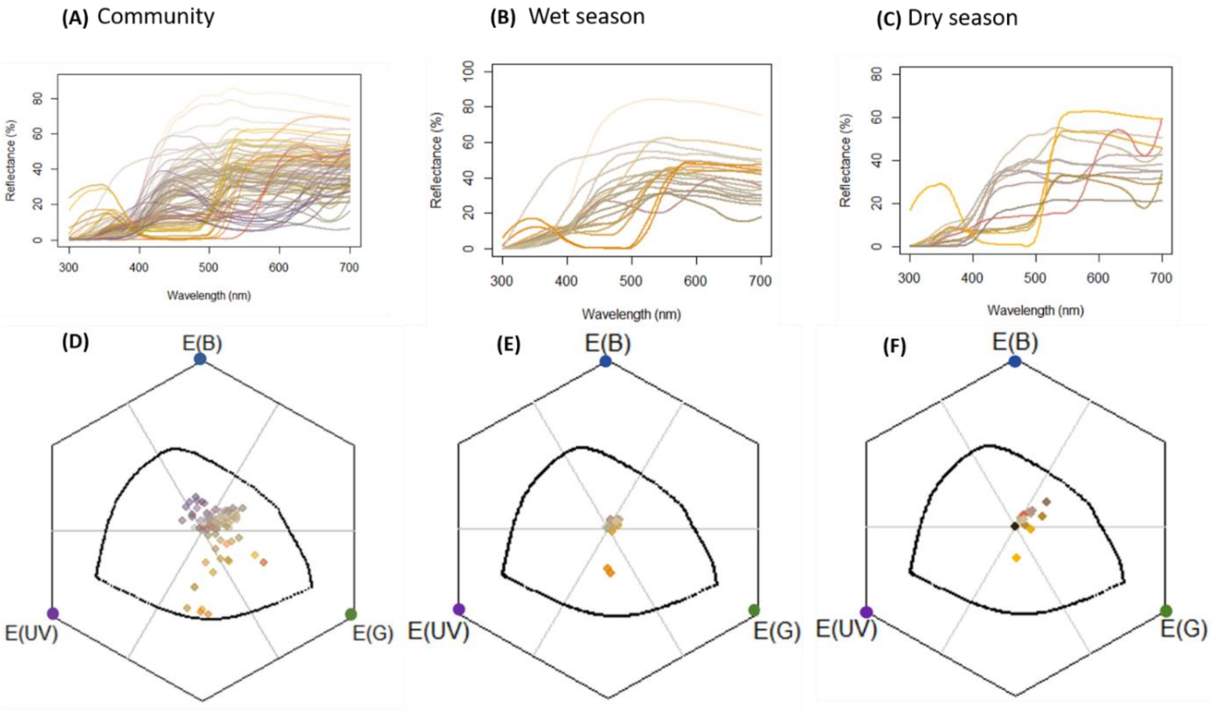


Figure 5. Reflectance spectra and colour loci in the bee visual space from flowers of 80 cerrado species (A and D), and from flowers of 20 species collected during the wet (B and E) and 13 species collected during the dry season (C and F). The bee visual space is represented by a hexagon, the gray point represents the achromatic center (leaf-background), and the colourful points in the vertices represents each bee-photoreceptor (blue, green and ultraviolet). The black line inside the hexagon delimits the maximum sensitivity of each photoreceptor to a monochromatic light. Each of the six parts of the hexagon, limited by a grey line, represents a bee-colour.

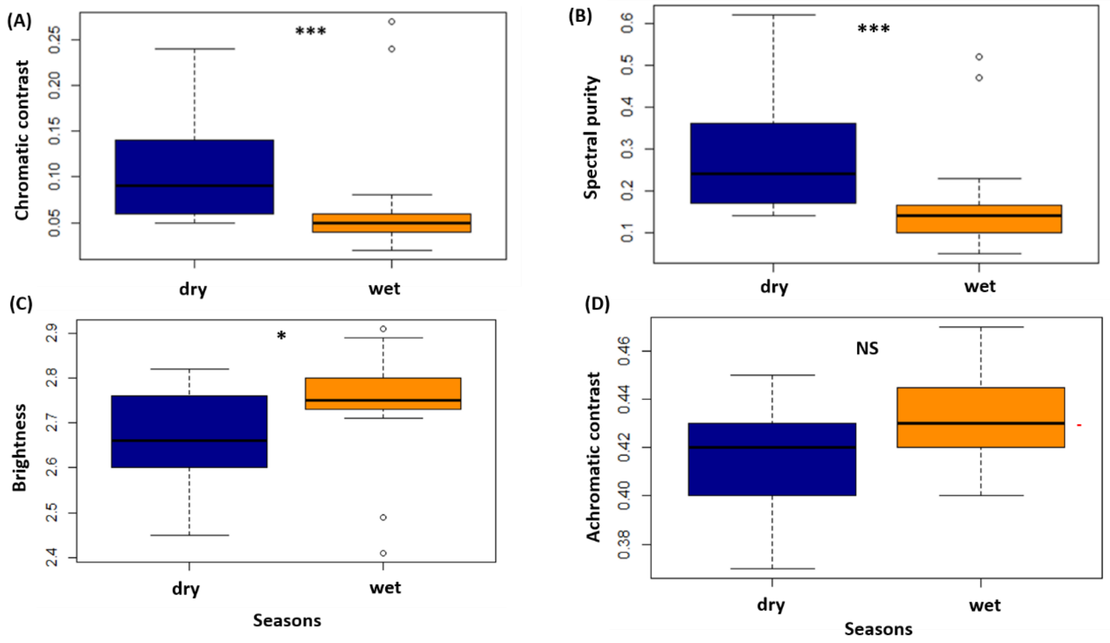


Figure 6 Colour parameters for cerrado flower species: (A) colour contrast against the leaf-background; (B) spectral purity; (C) brightness; (D) achromatic contrast against the leaf-background. Red line indicates the median value. Asterisks show significant differences between vegetations (*p < 0.05, ***p < 0.01).

SUPPLEMENTARY MATERIAL

Figure S1. Location of the study site at Itirapina, São Paulo State, Brazil (left) and the detail of study site (right). (A) yellow lines represent the area were phenology species were sampled and are observed for the long-term cerrado phenology; (B) view of the cerrado *sensu stricto* vegetation structure. Photo by: Amanda Eburneo Martins.

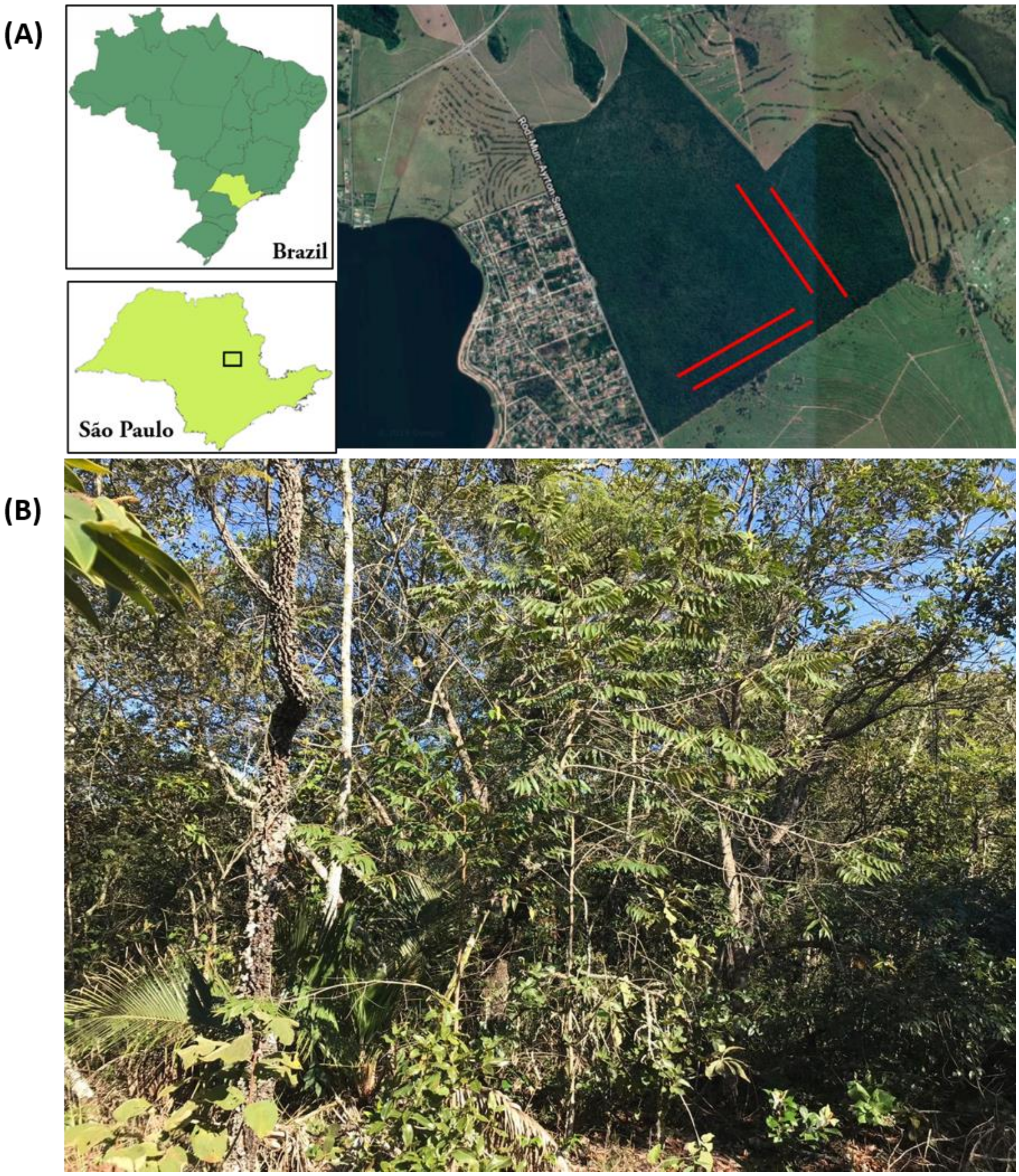


Figure S2. Flowering peak of the cerrado community (n=91) (A); and for the species (n=33) included in the phenological observations with reflectance data (B).

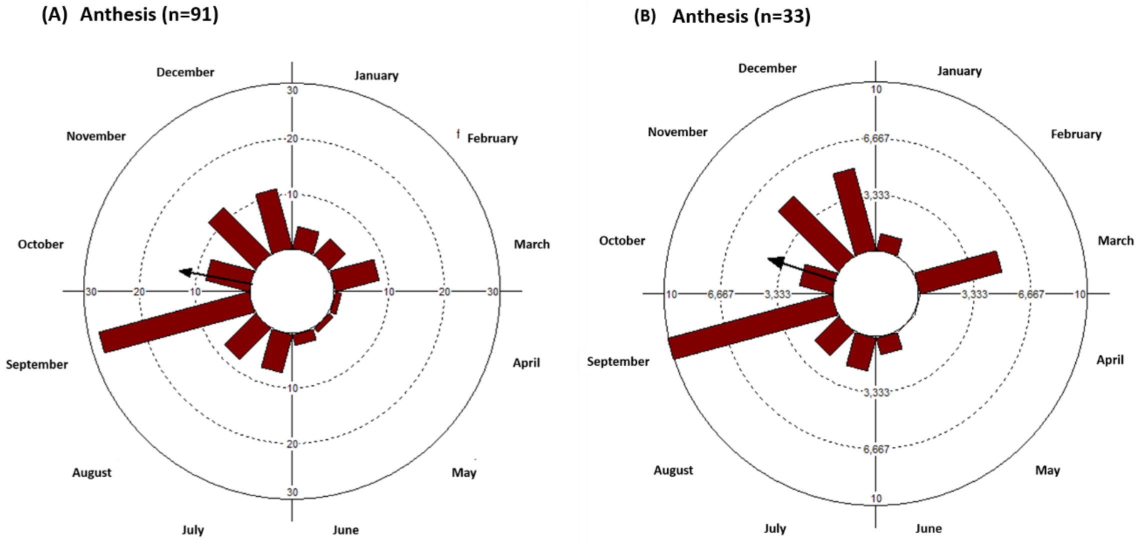


Figure S3. Leaf-green background represented by the mean reflectance spectrum of all the leaves in each season. Source: Camargo et al. 2013.

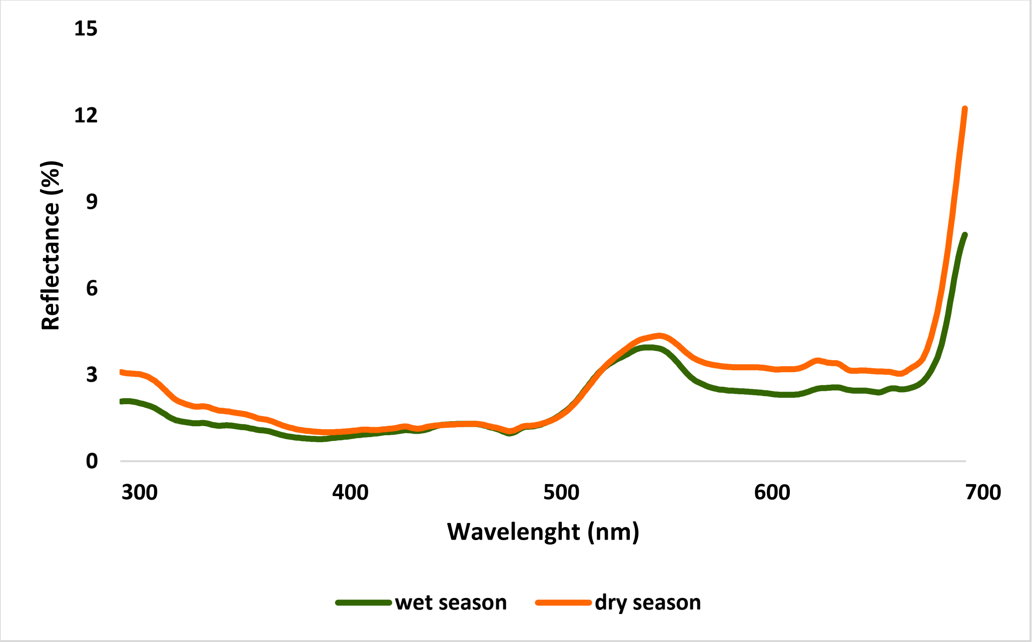


Table S1. Cerrado species survey and the correspondent flower colour, pollinator, the presence (Y) or absence (N) in the spectrum data, the presence (Y) or absence (N) in the phenology, month of flowering peak, bee colour and pollinator categorization.

Family	**Species**	**Flower colour**	**Pollinator**	**Spectrum**	**Phenology**	**Flowering peak**	**Bee colour**	**Categori-zation**
Fabaceae	*Acosmium dasycarpum*	white	bee	N	N flourished			1
Bignoniaceae	*Adenocalymma axillarum*	yellow	bee	Y	Y	December	uvgreen	2
Bignoniaceae	*Adenocalymma bracteatum*	yellow	bee	N	N			1
Bignoniaceae	*Adenocalymma peregrinum*	yellow	bee	N	N			1
Verbenaceae	*Aegiphila verticillata*	green	bee	N	Y	October		2
Rubiaceae	*Alibertia sp.1*	white	bee	N	Y	September		1
Rubiaceae	*Amaioua guianensis*	white	moth	Y	Y	September	bluegreen	1
Bignoniaceae	*Amphilophium elongatum*	white	bee	Y	N			2
Anacardiaceae	*Anacardium humile*	white	bee	N	N			2
Fabaceae	*Anadenanthera peregrina var. falcata*	white	bee	Y	Y	September	bluegreen	2
Bromeliaceae	*Ananas ananassoides*	violet	hum	Y	N			1
Bignoniaceae	*Anemopaegma arvense*	cream	bee	N	N			2
Bignoniaceae	*Anemopaegma cf. glaucum*	cream	bee	N	N			2
Annonaceae	*Annona coriacea*	green	beetle	N	N flourished			2
Aristolochiaceae	*Aristolochia esperanzae var. major*	brown	fly	Y	N			3
Aristolochiaceae	*Aristolochia labiata*	brown	fly	N	N			3
Apocynaceae	*Aspidosperma sp.1*	cream	moth	N	N flourished			2
Apocynaceae	*Aspidosperma tomentosum*	white	moth	N	Y	August		1
Asteraceae	*Asteraceae sp.1*	white	bee	Y	N			3

Arecaceae	*Attalea geraensis*	yellow	beetle	N	N			2
Fabaceae	*Stryphnodendron obovatum*	cream	bee	Y	Y	December	bluegreen	2
Asteraceae	*Baccharis trimera*	white	bee	N	N			2
Malpighiaceae	*Banisteriopsis campestris*	pink	bee	Y	Y	March	bluegreen	2
Malpighiaceae	*Banisteriopsis oxyclada*	pink	bee	N	N			3
Malpighiaceae	*Banisteriopsis stellaris*	yellow	bee	Y	Y	March	bluegreen	2
Fabaceae	*Bauhinia rufa*	white	bat	Y	Y	December	uvblue	1
Asteraceae	*Bidens gardneri*	pink	bee	Y	N			2
Myrtaceae	*Blepharocalyx salicifolia*	pink	bee	N	N flourished			1
Apocynaceae	*Blepharodon sp.1*	green	fly	N	N			3
Fabaceae	*Bowdichia virgilioides*	violet	bee	N	Y	August		1
Polygalaceae	*Bredemeyera floribunda*	white	bee	N	N			2
Bromeliaceae	*Bromelia cf. balansae*	red	hum	N	N			1
Moraceae	*Brosimum gaudichaudii*	green	wind	N	N flourished			3
Malpighiaceae	*Byrsonima basiloba*	yellow	bee	Y	Y	September	bluegreen	1
Malpighiaceae	*Byrsonima coccolobifolia*	pink	bee	Y	Y	November	blue	1
Malpighiaceae	*Byrsonima crassiflora*	pink	bee	N	Y	September		3
Malpighiaceae	*Byrsonima crassiflora*	pink	bee	N	N			1
Malpighiaceae	*Byrsonima intermedia*	yellow	bee	Y	Y	November	bluegreen	1
Malpighiaceae	*Byrsonima pachyphylla*	yellow	bee	N	N flourished			2
Malpighiaceae	*Byrsonima verbascifolia*	yellow	bee	N	Y	September		1
Asteraceae	*Calea cuneifolia*	yellow	butterfly	N	N			2
Myrtaceae	*Campomanesia pubescens*	white	bee	Y	Y	September	bluegreen	1
Rubiaceae	*Tocoyena formosa*	cream	sph	Y	Y	December	bluegreen	1

Salicaceae	*Casearia sylvestris*	white	fly	N	Y	September		2
Verbenaceae	*Casselia sp.1*	pink	bee	N	N			3
Solanaceae	*Cestrum sp.1*	white	sph	N	N			3
Fabaceae	*Chamaecrista campestris*	yellow	bee	N	N			1
Fabaceae	*Chamaecrista desvauxii*	yellow	bee	N	N			1
Fabaceae	*Chamaecrista flexuosa*	yellow	bee	Y	N			1
Fabaceae	*Chamaecrista ramosa*	yellow	bee	Y	N			1
Fabaceae	*Chamaecrista sp.1*	yellow	bee	Y	N			3
Fabaceae	*Fabaceae sp.1*	violet	bee	Y	N			3
Asteraceae	*Chresta sphaerocephala*	pink	bee	Y	N			1
Asteraceae	*Chromolaena laevigata*	violet	bee	N	Y	March		3
Vitaceae	*Cissus erosa*	red	fly	N	N			2
Rubiaceae	*Coccocypselum lanceolatum*	violet	small insects	N	N			3
Bixaceae	*Cochlospermum regium*	yellow	bee	N	N			2
Commelinaceae	*Commelina benghalensis*	blue	bee	N	N			2
Commelinaceae	*Commelina cf. erecta*	blue	bee	N	N			2
Connaraceae	*Connarus suberosus*	yellow	bee	Y	Y	September	bluegreen	2
Fabaceae	*Copaifera langsdorffii*	white	bee	N	Y	November		1
Rubiaceae	*Cordiera sessilis*	white	small insects	N	Y	July		3
Dilleniaceae	*Curatella americana*	white	bee	N	N			1
Bignoniaceae	*Cuspidaria pulchra*	pink	bee	Y	N			3
Cyperaceae	*Cyperus sp.1*	green	wind	N	N			3
Fabaceae	*Dalbergia miscolobium*	violet	bee	N	Y	January		1
Dilleniaceae	*Davilla elliptica*	yellow	bee	N	Y	April		2
Gentianaceae	*Deianira cf. chiquitana*	pink	fly	N	N			3
Fabaceae	*Dimorphandra mollis*	cream	bee	N	Y	December		1

Ebenaceae	*Diospyros hispida*	green	moth	N	Y	September		2
Bignoniaceae	*Distictella elongata*	white	bee	N	Y	August		1
Apocynaceae	*Ditassa cf. tomentosa*	white	beetle	N	N			3
Annonaceae	*Duguetia furfuracea*	pink	beetle	Y	Y	March	bluegreen	2
Bromeliaceae	*Dyckia cf. leptostachya*	orange	hum	N	N			1
Cactaceae	*Epiphyllum phyllanthus*	white	bat	N	N			3
Malvaceae	*Eriotheca gracilipes*	cream	bee	Y	Y	July	bluegreen	2
Erythroxylaceae	*Erythroxylum campestre*	white	wasp	N	N			1
Erythroxylaceae	*Erythroxylum cuneifolium*	white	wasp	N	Y	September		2
Erythroxylaceae	*Erythroxylum pelleterianum*	white	bee	N	N			2
Erythroxylaceae	*Erythroxylum suberosum*	white	wasp	N	Y	September		1
Erythroxylaceae	*Erythroxylum tortuosum*	white	wasp	N	Y	September		1
Myrtaceae	*Eugenia bimarginata*	white	bee	Y	Y	November	bluegreen	2
Myrtaceae	*Eugenia punicifolia*	white	bee	N	Y	September		1
Myrtaceae	*Eugenia pyriformis*	pink	bee	N	Y	October		2
Myrtaceae	*Eugenia sp.1*	pink	bee	N	Y	November		3
Asteraceae	*Eupatorium laevigatum*	violet	bee	N	Y	March		2
Moraceae	*Ficus citrifolia*	green	wasp	N	Y	September		1
Apocynaceae	*Forsteronia velloziana*	cream	bee	N	N			3
Bignoniaceae	*Fridericia formosa*	pink	bee	N	N			3
Bignoniaceae	*Fridericia platyphylla*	pink	bee	N	Y	February		2
Bignoniaceae	*Fridericia samydoides*	pink	bee	N	N			2
Bignoniaceae	*Fridericia speciosa*	pink	hum	Y	N			2

Orchidaceae	*Galeandra montana*	pink	bee	Y	N			3
Asteraceae	*Gochnatia barrosii*	cream	bee	N	Y	August		2
Asteraceae	*Gochnatia pulchra*	cream	bee	N	Y	May		1
Amaranthaceae	*Gomphrena cf. macrocephala*	pink	bee	N	N			3
Nyctaginaceae	*Guapira noxia*	green	small insects	N	Y	September		1
Nyctaginaceae	*Guapira opposita*	green	small insects	Y	Y	September	bluegreen	3
Annonaceae	*Guatteria australis*	green	beetle	N	N			2
Asteraceae	*Gyptis crassipes*	pink	bee	N	N			3
Bignoniaceae	*Handroanthus ochraceus*	yellow	bee	N	Y	July		1
Asteraceae	*Heterocondylus alatus*	pink	bee	N	N			3
Malpighiaceae	*Heteropterys byrsonimifolia*	yellow	bee	Y	N			3
Lamiaceae	*Hyptidendron sp.1*	violet	bee	Y	N			3
Bignoniaceae	*Jacaranda caroba*	violet	bee	N	Y	September		2
Bignoniaceae	*Jacaranda decurrens*	violet	bee	Y	N			1
Bignoniaceae	*Jacaranda rufa*	violet	bee	N	Y	November		2
Bignoniaceae	*Jacaranda sp.1*	violet	bee	N	N			3
Calophyllaceae	*Kielmeyera grandiflora*	white	bee	N	Y	December		3
Lacistemataceae	*Lacistema hasslerianum*	green	wind	N	N flourished			2
Verbenaceae	*Lantana camara*	pink	bee	Y	N			3
Melastomataceae	*Leandra solenifera*	pink	bee	N	Y	September		3
Fabaceae	*Leptolobium dasycarpum*	white	bee	N	Y	November		3
Asteraceae	*Lessingianthus grandiflorus*	pink	bee	Y	N			3
Chrysobalanaceae	*Licania sp.1*	white	bee	N	N flourished			1
Verbenaceae	*Lippia balansae*	white	bee	N	Y	February		3
Verbenaceae	*Lippia velutina*	white	butterfly	Y	N			3
Fabaceae	*Machaerium acutifolium*	white	bee	N	Y	November		2
Fabaceae	*Machaerium brasiliense*	white	bee	N	Y	November		3

Malpighiaceae	*Malpighiaceae sp.1*	yellow	bee	Y	N			3
Malpighiaceae	*Malpighiaceae sp.2*	white	bee	Y	N			3
Rubiaceae	*Manettia sp.1*	red	hum	Y	N			3
Melastomataceae	*Miconia albicans*	white	bee	Y	Y	September	bluegreen	2
Melastomataceae	*Miconia fallax*	white	bee	N	N			2
Melastomataceae	*Miconia langsdorffii*	white	bee	N	N			1
Melastomataceae	*Miconia ligustroides*	white	bee	Y	Y	August	bluegreen	1
Melastomataceae	*Miconia pepericarpa*	cream	bee	N	N			1
Melastomataceae	*Miconia rubiginosa*	white	bee	N	Y	November		1
Melastomataceae	*Miconia stenostachya*	white	bee	N	Y	September		3
Fabaceae	*Mimosa capillipes*	pink	bee	N	N			3
Fabaceae	*Mimosa cf. alleniana*	violet	bee	Y	N			3
Fabaceae	*Mimosa debilis*	pink	bee	Y	N			3
Fabaceae	*Mimosa nuda var. angusta*	pink	bee	N	N			2
Cucurbitaceae	*Momordica charantia*	yellow	beetle	Y	N			1
Myrtaceae	*Myrcia bella*	white	bee	Y	Y	October	blue	2
Myrtaceae	*Myrcia guianensis*	white	bee	Y	Y	September	bluegreen	1
Myrtaceae	*Myrcia splendens*	white	bee	N	Y	October		1
Myrtaceae	*Myrcia venulosa*	white	bee	N	N flourished			3
Primulaceae	*Myrsine guianensis*	cream	bee	N	Y	July		2
Primulaceae	*Myrsine umbellata*	white	small insects	N	Y	June		2
not. Determined	*not det.sp.1*	violet	bee	Y	N			3
not. Determined	*not det.sp.2*	cream	bee	Y	N			3
Lauraceae	*Ocotea corymbosa*	cream	small insects	N	Y	September		2
Asteraceae	*Baccharis dracunculifolia*	cream	bee	Y	N	March		2
Ochnaceae	*Ouratea spectabilis*	yellow	bee	Y	Y	August	uvgreen	1

Oxalidaceae	*Oxalis hirsutissima*	yellow	bee	Y	N			3
Asclepiadaceae	*Oxypetalum appendiculatum*	green	wasp	N	N			1
Rubiaceae	*Palicourea rigida*	yellow	hum	Y	Y	December	bluegreen	2
Passifloraceae	*Passiflora sp.1*	white	bee	N	N			3
Malpighiaceae	*Peixotoa reticulata*	yellow	bee	N	N			2
Orchidaceae	*Pelexia laminata*	white	bee	N	N			3
Malvaceae	*Peltaea polymorpha*	pink	bee	N	N			3
Celastraceae	*Peritassa campestris*	cream	bee	N	Y	August		3
Amaranthaceae	*Pfaffia sp.1*	white	bee	Y	N			3
Asteraceae	*Piptocarpha rotundifolia*	cream	butterfly	N	Y	February		2
Celastraceae	*Plenckia populnea*	yellow	bee	N	N flourished			2
Sapotaceae	*Pouteria ramiflora*	green	butterfly	N	Y	September		1
Sapotaceae	*Pouteria torta*	green	butterfly	N	Y	July		1
Sapotaceae	*Pradosia brevipes*	red	fly	N	N			2
Apocynaceae	*Prestonia coalita*	yellow	bee	Y	N			1
Burseraceae	*Protium heptaphyllum*	cream	small insects	N	Y	December		3
Myrtaceae	*Psidium cf. grandifolium*	white	bee	N	N			2
Myrtaceae	*Psidium cinereum*	white	bee	N	Y	December		3
Myrtaceae	*Psidium sp.1*	white	bee	N	N flourished			3
Rubiaceae	*Psychotria hoffmannseggiana*	white	bee	Y	N			3
Fabaceae	*Pterodon emarginatus*	violet	bee	N	N			1
Fabaceae	*Pterodon pubescens*	white	bee	Y	Y	September	blue	1
Bignoniaceae	*Pyrostegia venusta*	orange	hum	Y	N			2
Vochysiaceae	*Qualea dichotoma*	white	bee	N	Y	October		1
Vochysiaceae	*Qualea grandiflora*	yellow	sph	Y	Y	December	green	1
Vochysiaceae	*Qualea multiflora*	white	bee	N	Y	December		2
Vochysiaceae	*Qualea parviflora*	violet	bee	N	Y	January		3
Cyperaceae	*Rhynchospora sp.1*	white	wind	N	N			3
Rubiaceae	*Richardia grandiflora*	pink	bee	N	N			2

Orchidaceae	*Rodriguesia sp.1*	pink	bee	Y	N			3
Proteaceae	*Roupala montana*	cream	moth	N	N flourished			1
Connaraceae	*Rourea induta*	white	bee	Y	Y	September	bluegreen	2
Euphorbiaceae	*Sapium glandulosum*	yellow	small insects	N	N			1
Araliaceae	*Schefflera vinosa*	yellow	bee	Y	Y	July	bluegreen	2
Apocynaceae	*Schubertia grandiflora*	white	bee	Y	N			3
Fabaceae	*Senna rugosa*	yellow	bee	Y	Y	March	uvgreen	1
Sapindaceae	*Serjania caracasana*	white	bee	N	N			2
Sapindaceae	*Serjania lethalis*	white	bee	Y	N			1
Sapindaceae	*Serjania mansiana*	white	bee	N	N			2
Sapindaceae	*Serjania marginata*	white	bee	N	N			3
Sapindaceae	*Serjania meridionalis*	white	bee	N	N			3
Monimiaceae	*Siparuna guianensis*	yellow	fly	N	Y	October		3
Smilacaceae	*Smilax brasiliensis*	green	fly	Y	N			2
Solanaceae	*Solanum paniculatum*	violet	bee	Y	N			1
Solanaceae	*Solanum lycocarpum*	violet	bee	Y	N			1
Solanaceae	*Solanum sp.1*	violet	bee	Y	N			3
Solanaceae	*Solanum sp.2*	white	bee	Y	N			3
Loganiaceae	*Strychnos brasiliensis*	cream	moth	N	N flourished			3
Loganiaceae	*Strychnos pseudoquina*	cream	moth	N	Y	February		1
Fabaceae	*Stryphnodendron adstringens*	green	bee	N	Y	October		1
Lauraceae	*Ocotea pulchella*	cream	fly	Y	Y	November	bluegreen	2
Fabaceae	*Stylosanthes acuminata*	yellow	bee	N	N			2
Styracaceae	*Styrax ferrugineus*	white	bee	Y	Y	June	bluegreen	1
Arecaceae	*Syagrus flexuosa*	yellow	bee	N	Y	January		2
Arecaceae	*Syagrus petraea*	yellow	bee	N	N			1
Bignoniaceae	*Tabebuia aurea*	yellow	bee	N	Y	July		1

Fabaceae	*Tachigali pilgeriana*	cream	bee	Y	N			3
Talinaceae	*Talinum portulacifolium*	pink	bee	N	N			3
Apocynaceae	*Temnadenia violacea*	pink	hum	Y	N			2
Combretaceae	*Terminalia glabrescens*	cream	bee	N	N flourished			3
Melastomataceae	*Tibouchina stenocarpa*	violet	bee	N	N			1
Caryocaraceae	*Caryocar brasiliense*	cream	bat	Y	Y	October	bluegreen	1
Celastraceae	*Tontelea micrantha*	green	fly	N	N			3
Cannabaceae	*Trema micrantha*	green	bee	N	N			2
Iridaceae	*Trimezia juncifolia*	yellow	bee	N	N			3
Asteraceae	*Vernonanthura ferruginea*	white	bee	Y	N			3
Asteraceae	*Vernonia rubrimea*	white	bee	Y	N			2
Asteraceae	*Vernonia sp. 1*	violet	bee	Y	N			2
Fabaceae	*Vigna peduncularis*	violet	bee	Y	N			3
Myristicaceae	*Virola sebifera*	yellow	bee	N	Y	March		2
Vochysiaceae	*Vochysia cinnamomea*	yellow	bee	N	Y	August		1
Vochysiaceae	*Vochysia multiflora*	yellow	bee	N	N			3
Vochysiaceae	*Vochysia parviflora*	yellow	bee	N	N			3
Vochysiaceae	*Vochysia tucanorum*	yellow	bee	Y	Y	January	green	1
Asteraceae	*Wedelia sp.1*	yellow	bee	N	N			1
Annonaceae	*Xylopia aromatica*	white	beetle	Y	Y	November	bluegreen	2
Fabaceae	*Zornia sp.1*	yellow	bee	N	N			2

General Conclusion

In the first chapter, we confirmed that seasonal vegetations with different leaf-background colouration, also related to the vegetation structure and seasonality intensity, differ in flower colour diversity and colour signals as seen by bees. Although according the human eye yellow was the predominant colour and colour diversity was similar in the studied communities (Campo rupestre, Cerrado *sensu stricto* and Mediterranean shrubland), the diversity and predominance of bee-colours differed between vegetation. According to the bee-colours categories, the Mediterranean shrubland had the highest flower colour diversity among the studied vegetations. In addition, flower colour discrimination was higher at Mediterranean shrubland. The high proportion of yellow flowers UV-reflecting were surveyed at the open vegetations, Campo rupestre and Mediterranean, considered a mechanism of protection against the UV-radiation, which is more intense at those vegetations types. Flowers from tropical seasonal vegetations presented the higher values of achromatic signals (green contrast and brightness), while the temperate seasonal vegetation presented higher values of chromatic signals (colour contrast and spectral purity). At the Mediterranean shrubland, the presence of co-flowering species is common, and a higher colour contrast contribute to the flower colour contrast and discrimination. The higher achromatic signals in the tropics may be related to the high diversity of diurnal and nocturnal pollinators and the plants habitat. The role of brightness in flower detection be uncertain, but higher values combined to other colourful parameters may increase flower detection in the tropics, probably due to crepuscular and nocturnal bees. Therefore, along with different biotic interactions, colour signals are also under local environmental selective pressures and distinct vegetation structute all converting to ensure flower conspicuousness for pollinators.

We then chose the Cerrado *sensu stricto* to investigate possible effects of seasonality in the diversity of colours and colours signals displayed over the year or seasons. Flowering patterns were markedly seasonal, peaking in the dry-rainy transition period. Besides that, distinct flowers colours were available over the year to pollinators, but with differences in the seasonality of flowering peaks according to flower colours. Therefore, seasonality and pollinators selective pressures may influencing the flowering peaks in the cerrado.

We confirmed the importance of bees as pollinators of cerrado flowers, followed by flies, small insects, moths, beetles, hummingbirds and bats. Considering our results, we did not recommended the flower colour classification by human visual system to classify the pollination syndromes, reinforcing that is important the flower colours analyses be based in the pollinator visual system.

Linking the contrasts to seasonality, it shaped the community flowering patterns and also influenced the flower colour diversity and signals in cerrado over year, confirming our hypothesis. In the dry season, the yellowish leaves promoted flowers with more chromatic contrast, such as, more conspicuous for pollinators, and in the wet season, the green leaves promoted flowers with more achromatic contrast, increasing the contrast between leaves and flowers. The distinct signals over year may be considered an adaptation to seasonality to maximize flower detection by pollinators according to the background colouration and complexity. To conclude, the maintenance of flower colour diversity all year long favour the presence of pollinators, with a high diversity of pollination modes, featuring the tropical cerrado community with a generalist pattern of pollination. The flowers diversity, the temporal patterns of flower colours and the distinct flower signals according seasons were adapted to the cerrado seasonality ensuring the flower conspicuousness to bees.

Flowers with more saturated display and high-contrasting colours against the background are more easily detected by bees and frequently visited. We offered news perspectives about sensorial ecology, showing the importance among flower-background-pollinator relationships at a community level. The background colour also mediated the bee constancy in a flower, may varying the flower detection, discriminations and flower colour diversity. Hence, we reinforce the importance of consider the background colour when analysing flower signals and diversity in natural communities, supporting the idea that visual signals contribute to the pollination evolutionary pressures on floral colour.