How flower colour signals allure bees and hummingbirds: a community-level test of the bee avoidance hypothesis

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Summary

- Colour signals are the main floral trait for plant-pollinator communication. Due to visual specificities, flower visitors exert different selective pressures on flower colour signals of plant communities. Although it evolved to attract pollinators, matching their visual sensitivity and colour preferences, floral signals may also evolve to avoid less efficient pollinators and antagonistic flower visitors.
- We evaluated evidence for the bee avoidance hypothesis in a Neotropical community pollinated mainly by bees and hummingbirds, the campo rupestre. We analysed flower reflectance spectra, compared colour variables of bee- (bee-flowers; 244 species) and hummingbird-pollinated flowers (hummingbird-flowers; 39 species), and looked for evidence of bee sensorial exclusion in hummingbird-flowers.
- Flowers were equally contrasting for hummingbirds. Hummingbird-flowers were less conspicuous to bees, reflecting mainly long wavelengths and avoiding red-blind visitors. Bee-flowers reflected more short wavelengths, were more conspicuous to bees (higher contrasts and spectral purity) than hummingbird-flowers and displayed floral guides more frequently, favouring flower attractiveness, discrimination and handling by bees.
- Along with no phylogenetic signal, the differences in signal strategies between bee- and hummingbird-flowers are the first evidence of the bee avoidance hypothesis at a community level and reinforce the role of pollinators as a selective pressure driving flower colour diversity.

Key-words: campo rupestre, colour contrast, floral guides, grassland, marker points, pollen mimicry, pollination, savanna.

Introduction

Flower colour is a major communication trait between plants and their pollinators that promotes flower detection and signals the availability of resources to potential pollinators (Shrestha et al., 2013; Lunau et al., 2017). Due to the diversity of colour vision systems caused by sensitivity or insensitivity to ultraviolet and red light, members of different groups of pollinators perceive and select flowers differently (Briscoe & Chittka, 2001; Wester & Lunau, 2017). Therefore, pollinators’ visual capability and preferences may act as selective
pressures on flower colour signals within a community, selecting floral displays that are most efficiently pollinated (Fenster et al., 2004; Rosas-Guerrero et al., 2014).

Two important groups of pollinators of Neotropical plant species, bees and hummingbirds, have different visual systems: while bees have trichromatic colour vision and are sensitive to ultraviolet, blue, and green wavelengths, hummingbirds have a tetrachromatic visual system and are sensitive to ultraviolet, blue, green, and red wavelengths (Menzel & Backhaus, 1991; Vorobyev et al., 1998). Considering the hypothesis that flower colour signals evolved to match the visual systems of pollinators (Chittka & Menzel, 1992; Dyer et al., 2012), it is expected that flowers present colours and signals that correspond to their pollinators’ visual capability and preferences, favouring attraction.

A key signal for bees and birds’ attraction is the flower contrast against the background perceived as colour saturation or colour contrast (Spaethe et al., 2001; Schmidt et al., 2004; van der Kooi et al., 2018). The colour contrast depends on the visual system and, therefore, flowers are probably more contrasting to their main pollinators than to other potential flower visitors. For bees, flowers displaying more saturated and high-contrasting colours are easily detected and more frequently visited than less conspicuous flowers (Spaethe et al., 2001; Rohde et al., 2013). In addition, floral displays composed by colourful structures associated to the flower, such as bracts and old flowers that remain attached to the inflorescence, may also improve the overall size, contrast, and detection of the flower at distance (Spaethe et al., 2001; Brito et al., 2015).

According to bees’ preferences, flowers primarily pollinated by bees (hereafter bee-flowers) reflect mainly in one or two of the three relevant ranges of wavelengths (ultraviolet, blue, green) and commonly present more than one colour (colour pattern) (Chittka et al., 1994; Lunau et al., 1996; Dyer et al., 2016; Papiorek et al., 2016). These colour patterns are generally composed by a predominant colour located peripherally and small colourful structures found in the central part of the flower that act as floral guides, improving flower detection and resource location (Lunau et al., 1996, 2017; de Ibarra et al., 2015). A recurrent floral signal is a yellow UV-absorbing area in the centre of the flower, which corresponds to the colour of anthers and pollen or to pollen/anther mimicking structures (Heuschen et al., 2005; Lunau et al., 2017). Such yellow UV-absorbing colour plays a major role in attracting bees as they innately respond to this stimulus (Lunau et al., 2017).
Flowers reflecting red wavelengths have been considered as favouring ornithophily (Grant & Grant, 1968; Cronk & Ojeda, 2008). Although red targets are conspicuous for birds, flower-visiting birds do not exhibit an innate preference for red colours (Lunau & Maier, 1995; Cronk & Ojeda, 2008). Some authors have suggested that colours of flowers primarily pollinated by hummingbirds (hereafter hummingbird-flowers) evolved not to improve attraction, but to avoid less efficient pollinators and nectar or pollen robbers by sensorial exclusion, acquiring colours that are less conspicuous to trichromatic insects (Lunau et al., 2011; Bergamo et al., 2016). For example, red and white hummingbird-flowers are less attractive to bees compared to bee-flowers with the same colours due to their specific ultraviolet reflectance properties. That is, red bee-flowers reflect more UV and white bee-flowers absorb more UV than red and white hummingbird-flowers (Lunau et al., 2011; Bergamo et al., 2016). The same phenomenon occurs in yellow flowers. While yellow bee-flowers possess a UV-pattern with a yellow UV-reflecting periphery and a yellow UV-absorbing centre, yellow hummingbird-flowers completely absorb UV-light and, consequently, are less attractive to bees (Papiorek et al., 2016). Therefore, besides attraction, flower colour diversity may also be a result of floral visitor avoidance based on visual system bias (Lunau et al., 2011; Bergamo et al., 2016).

Lunau et al. (2011) presented evidence for the bee avoidance hypothesis based on colour preference tested with Euglossine bees and Trochiline hummingbirds and spectral reflectance properties of Neotropical red and white bee- and hummingbird-flowers mainly from botanical gardens. This hypothesis has been corroborated at the population level by Bergamo et al. (2016), by showing distinct colour preferences of bees and hummingbird for two colour morphs of Costus arabicus. The rationale of our study was to evaluate evidence for the bee avoidance hypothesis in a Neotropical community where bees and hummingbirds are the main pollinators based on an overall description of flower colour signal strategies. We tested the bee avoidance hypothesis addressing the following questions: Do hummingbird-pollinated flowers display colours less conspicuous to bees than bee-flowers? Do spectral reflectance properties provide evidence that colours are adapted for detection and discrimination by bird or bee pollinators? More specifically, to estimate bee- and bird-specific conspicuousness we analysed flower reflectance spectra and categorical and quantitative colour variables influencing flower detection and discrimination by pollinators. Additionally, based on these variables, we evaluated the sensorial exclusion of bees by hummingbird-flowers.
Material and Methods

Study area and species survey

The study site (hereafter ‘Serra do Cipó’) comprises the Serra do Cipó National Park and its buffer zone, Morro da Pedreira – an environmentally protected area, located in Minas Gerais, southeastern Brazil. The Serra do Cipó is dominated by *campo rupestre*, or rupestrian grassland, a Neotropical mountain vegetation that occurs across the Espinhaço mountain range above 900 m altitude and is immersed in the cerrado domain (Silveira et al., 2016; Morellato & Silveira, 2018). The *campo rupestre* is a mosaic of vegetation physiognomies composed by typical rocky outcrops, covered by herbs, shrubs and small trees, immersed in a matrix patches of sandy, stony, and wet grasslands, which are dominated by Poaceae and Cyperaceae, but also with the occurrence of herbs (mainly Asteraceae, Xyridaceae, and Velloziaceae) and small shrubs (Le Stradic et al., 2015; Silveira et al., 2016). The *campo rupestre* holds a high species diversity and endemism, with plant populations constrained to small areas or patches (Silveira et al., 2016). Plants are mainly self- and wind-dispersed, with a limited seed dispersal distance (Guerra et al., 2016; Silveira et al., 2016). Therefore, cross pollination is the main process favouring genetic diversity among populations, and bees and hummingbirds are the most important pollen vectors in this ecosystem, assuring long-distance pollen transfer (Carstensen et al., 2014, 2016; Guerra et al., 2016; Silveira et al., 2016).

We collected and analysed reflectance data of flowers, leaves, floral guides, and accessory structures of 283 species pollinated mainly by bees (244 species) and hummingbirds (39 species) (Supporting Information Table S1 and Table S2). Flowers were opportunistically collected in six sites along an altitudinal gradient of Serra do Cipó ranging from 824 m to 1420 m high (see Rocha et al. 2016 for a detailed description). These sites encompass the diversity of *campo rupestre* vegetation types: open to woody cerrado in the low-altitude sites; *campo rupestre* stricto sensu (rupestrian grassland) in the intermediate sites; and altitudinal grasslands in the high-altitude sites. We identified the species surveyed with taxonomists, by comparison with specialized bibliography and herbaria collections, and based on previous studies developed at the same study site (Carstensen et al., 2014; Le Stradic et al., 2015; Carstensen et al., 2016; Rocha et al., 2016). Voucher specimens were lodged in the Herbarium Rioclaricense (HRCB) of the São Paulo State University.
We determined the main pollinator based on the frequency of interactions registered for a given plant species according to the plant-pollinator interaction database developed by Carstensen et al. (2014, 2016) for the Serra do Cipó. We also determined the main pollinator based on personal observations of floral traits (size, shape, colour, scent and reward) and of flower visitors during field work. Such observations were complemented with information from studies that carried out direct observations of plant-pollinator interactions in the field for the same species or genera (for example, Oliveira & Gibbs, 2000; Freitas & Sazima, 2006; Gottsberger & Silberbauer-Gottsberger, 2006).

**Colour reflectance measurements**

We measured the flower reflectance spectrum between 300 nm and 700 nm with a spectrometer (Ocean Optics - Jaz Modular Optical Sensing Suite), thus covering the visual range of all pollinators, including those sensitive to ultraviolet light (UV). For each species, we collected reflectance data of 10 to 20 flowers, 10 leaves, and 10 samples of all structures associated or inside the flowers (accessory structures, floral guides, and exposed anthers or stigmas) (Dalrymple et al., 2015). For some species, it was not possible to measure all small structures found inside the flower due to the diameter of the spectrometer’s light spot. For all species, we considered the flower colour as the predominant colour in its display, which usually corresponds to the petals.

**Visual models of bees and birds**

To test possible differences in flower colours according to the pollinators’ visual systems, we plotted flower colours (colour loci) in diagrams representing the pollinators’ perceptual colour space: a hexagon for trichromatic bees (Menzel & Backhaus, 1991; Chittka, 1992; Lunau et al., 2011) and a tetrahedron for tetrachromatic birds (Endler & Mielke Jr, 2005; Kantsa et al., 2017). Each vertex of the diagram corresponds to a photoreceptor present in the pollinators’ retina according to its sensitivity: ultraviolet (uv), blue (s), and green (m) wavelengths for bees and ultraviolet (uv), blue (s), green (m), and red (l) wavelengths for birds. The position of each colour locus in the colour space depends on the quantum catches captured by each photoreceptor from the light reflected by a given flower. To calculate bee quantum catches, we used the model proposed for *Apis mellifera* (Menzel & Backhaus, 1991). We calculated bird quantum catches based on the UVS visual system according to the model proposed by Vorobyev et al. (1998) for *Leiothrix lutea*, a species whose UV sensitivity is around 365 nm, similar to hummingbirds (~370 nm; Herrera

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et al., 2008). For both models, we used the mean reflectance spectra calculated from leaves of all species sampled as the green background (Camargo et al., 2014) and the D65 standard daylight as the ambient light (Wyszecki & Stiles, 1982) (Supporting Information Fig. S1).

**Colour variables**

We surveyed categorical and quantitative variables previously described as important visual signals for target detection and discrimination by bees and birds (Spaethe et al., 2001; Cazetta et al., 2009; Dyer et al., 2016; Bukovac et al., 2017; Lunau et al., 2017; van der Kooi et al., 2018). We considered as categorical variables the presence of colour patterns, floral guides, and UV-patterns (Fig. 1) based on the mean reflectance spectra of each structure; except when structures were too small that measurements were unattainable – in such cases we used only the human colour vision. We also observed the presence of accessory structures such as bracts, old flowers, flower buds, and pedicels with colours (reflectance spectra) different from the main flower colour. We considered as floral guides lines and spots displaying different colours from the main flower colour, including pollen/anther mimicry (Lunau et al., 1996, 2017). We considered as UV-pattern the combination of UV-reflecting structures, generally displayed in the flower periphery, associated to UV-absorbing structures displayed in the centre of the flower, such as floral guides and exposed anthers, stamens, and stigmas (Papiorek et al., 2016; Lunau et al., 2017).

We classified hummingbird- and bee-pollinated species according to flower absorbance (represented by ‘-’) and reflectance (represented by ‘+’) into categories within the 300-700 nm spectrum: ultraviolet band (‘uv’; 300-400 nm), blue band (‘b’; 401-500 nm), green band (‘g’; 501-600 nm) and red band (‘r’; 601-700 nm), as proposed by Chittka et al. (1994). Based on our mean reflectance data (Supporting Information Fig. S2), we considered as reflectance (‘+’) values above 5% in the UV band and above 10% in the other bands. Additionally, flowers that reflect within the green band (‘g+’) but with a difference ≥ 10% in relation to the reflectance peak observed for the blue and red bands were considered as ‘g-’. This way, we were able to differentiate white, yellow and whitish flowers from pink, purple and blue flowers.
Based on the visual system of bees, on the colour loci in the bee colour hexagon and on the assumed perceived colour parameters, we calculated the following quantitative variables: dominant wavelength, spectral purity or colour purity (correspondent wavelength to the closest colour locus point in the monochromatic limit), and colour intensity (sum of photoreceptor excitations) (Lunau et al., 1996; Spaethe et al., 2001; Rohde et al., 2013). Based on the colour loci in the bird tetrahedron visual space, we calculated the spectral purity, which is the distance from the tetrahedron achromatic centre to the colour locus (Stoddard & Prum, 2011). For both bees and birds, we also calculated the frequency and position of marker points in the flower reflectance spectra and the chromatic and achromatic contrasts between the flower colour and the leaf background (see details below).

Considering the bee colour space, the chromatic contrast is the distance between a given colour locus and the hexagon centre (background colour locus) or the distance between two colour loci of different floral structures (Chittka, 1992; Spaethe et al., 2001; Rohde et al., 2013). Based on controlled experiments, bees can chromatically discriminate two colours or a colour target from the background when the distance of their colour loci are equal or greater than 0.1 hexagon units (Chittka et al., 1993). Therefore, the capacity of discrimination can be reduced to at least 0.2 hexagon units in natural conditions (Spaethe et al. 2001; Dyer & Chittka 2004). The bee-achromatic contrast against the background, also known as green contrast, corresponds to the green photoreceptor excitation adapted to the background (Chittka, 1992; Spaethe et al., 2001). The green contrast is important for flower detection at distance, whereas the chromatic vision is activated from short distances depending on the minimum visual angle (Spaethe et al., 2001; Dyer et al., 2008). Considering the bird tetrachromatic visual system, we calculated achromatic and chromatic contrasts according to the model proposed by Vorobyev & Osorio (1998). Bird achromatic contrast is related to differences in brightness of any two structures. Bird chromatic contrast is calculated based on the quantum catches resulting from the stimulus of the photoreceptors by two structure and is related to the distance in just noticeable differences (JNDs) between the structures, in which higher values of JNDs indicate increasing discrimination.

Finally, to test whether flowers were better discriminated by their main pollinator, we examined the number of marker points and correspondent wavelengths of the flowers’ main colour. The marker points are inflection points that correspond to rapid changes (increases or decreases) in the reflectance spectra and tend to match the wavelengths that are best

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discriminated by the pollinator’s photoreceptors (Dyer et al., 2012; Shrestha et al., 2013; Bukovac et al., 2017). We extracted the marker points using the ‘peakshape’ function of the ‘pavo’ package in R (detailed methodology and script in the Supporting Information Methods S1, Fig. S2, Fig. S3). To be considered a marker point with a significant stimulus, we determined a minimum reflectance change based on the mean reflectance spectra of all flower colours sampled (Chittka et al., 1994): 5% in the UV band (300-400 nm) and 10% in the blue (401-500 nm), green (501-600 nm), and red (601-700 nm) bands (Supporting Information Fig. S2). The marker points were graphically related to the wavelengths of the best colour discrimination of Apis (400 nm and 500 nm; von Helversen, 1972) and UVS birds (416, 489, and 557 nm; Goldsmith & Butler, 2003). We then calculated the mean absolute deviation (MAD) between the marker points and the closest wavelengths of the best colour discrimination, and the minimum absolute deviation (minAD) of each wavelength of the best discrimination according to Shrestha et al. (2013). MAD and minAD variables allow us to quantify the match between marker points and a given visual system (Shrestha et al., 2013).

Testing sensorial exclusion in specific colours

We investigated whether red, white, and yellow flowers presented bee sensorial exclusion based on previous evidence of sensorial exclusion in these flower colours (Lunau et al., 2011; Papiorek et al., 2016). We selected the flowers based on human colours, on the percentage of reflectance in the blue, green, and red bands, and on the position of marker points. We considered as ‘red’, flowers that reached 10% of reflectance in some point within the red band (601-700 nm), less than 10% of reflectance in the blue and green bands, and marker points located in wavelengths above 590 nm. We considered ‘yellow’, flowers with at least 10% of reflectance in the green and red bands, less than 10% of reflectance in the blue band, and marker points between 500-560 nm. Flowers with at least 10% of reflectance in the blue, green, and red bands and marker points below 500 nm were considered ‘white’.

Data analyses

We used a one-way MANOVA (Wilks’ Lambda) to test for differences in the quantitative colour parameters of bee and hummingbird flowers, including bee-UV and bird-red photoreceptors excitation. We used post-hoc one-way ANOVAs to find the significantly different variables and adjusted the p-values of each variable using the Bonferroni method.
We used Blomberg’s K (Blomberg et al., 2003) to test for phylogenetic signals and restrictions in the quantitative colour variables. K values closer to 1 indicate no phylogenetic signal; K values higher than 1 indicate a positive phylogenetic signal; and K values lower than 1, a negative phylogenetic signal (Ackerly, 2009). To build the phylogenetic tree and estimate the branch-lengths, we used the ‘S.PhyloMaker’ function implemented in R by Qian & Jin (2016). The phylogenetic tree was based on the ‘PhytoPhylo’ megaphylogeny proposed by Qian & Jin (2016) according to the third scenario, that is adding the absent genera or species to their closest taxa as polytomies.

We carried out all the analyses in R (R Development Core Team 2009). We used the ‘pavo’ package (Maia et al., 2017) for analyses related to the flower colour reflectance spectra and contrasts, and the ‘phytools’ (Revell, 2012) and ‘picante’ (Kembel et al., 2010) packages for the phylogenetic analyses.

**Results**

Most flowers displayed colour patterns (74% of bee- and 67% of hummingbird-flowers). Floral guides were present in 52% of bee-flowers and in 26% of hummingbird-flowers. Anther or pollen mimicry was the most common type of floral guide, followed by white and pink/purple lines, centre, or spots. We observed mimicking floral guides in 41% of bee-flowers and in 20% of hummingbird-flowers. It should be noted that a given flower can present both floral guides and mimicking structures. UV-patterns (see Fig. 1) occurred in 25% of bee-pollinated flowers and in 15% of hummingbird-flowers. Accessory structures, mainly represented by bracts, were present in few species and were more frequent in hummingbird-flowers (26%) than in bee-flowers (6%).

According to flower colour loci in the bee colour space, most hummingbird-flowers were located closer to the hexagon achromatic centre (30% of hummingbird-flowers were up to 0.1 distant from the achromatic centre and 48% were up to 0.2 distant from the achromatic centre) (Fig. 2a). In addition, the quantitative colour variables calculated based on the bee’s visual system differed between bee- and hummingbird-flowers (Wilks’ lambda = 0.79; $F = 8.16; df = 1; P = < 0.001$). Bee-flowers presented higher chromatic and green contrasts, colour intensity and purity, and higher values of UV photoreceptor excitation than hummingbird-flowers, with no difference for the dominant wavelength values (Table 1).
Considering the bird colour space, hummingbird-flowers were located closer to the tetrahedron vertex related to the red photoreceptor (Fig. 2b). The quantitative colour variables calculated based on the visual system of birds differed between bee- and hummingbird-flowers (Wilks’ lambda = 0.77; \( F = 10.02; df = 1; P = < 0.001 \)). Hummingbird-flowers presented higher excitation of bird’s red photoreceptor and higher spectral purity than bee-flowers (Table 1). There were no differences between bee- and hummingbird-flowers regarding contrasts against the background considering the bird visual system (Table 1).

There was a change in the predominance of hummingbird- and bee-pollinated species among the reflectance/absorbance categories (Fig. 3). Flowers reflecting only in long and medium wavelengths (>500 nm) where predominantly hummingbird-pollinated, whereas flowers of bee-pollinated species also reflected in the blue and UV bands (Fig. 3). The only exception were flowers reflecting in all bands, which were mainly pollinated by hummingbirds.

Most marker points of bee-flowers were located between 310-470 nm (43%), whereas marker points of hummingbird-flowers predominated in the interval between 500 and 660 nm (67%) (Fig 4). More specifically, for both, bee- and hummingbird-flowers maker points distribution form three distinctive groups, as they were most frequent around 410-430, 500-530, and 620-640 nm for bee-flowers (respectively, 19%, 24%, and 12% of the total number of marker points of bee-flowers), and around 410-430, 500-530, and 600-630 nm for hummingbird-flowers (respectively, 14%, 27%, and 26% of the total number of marker points of hummingbird-flowers) (Fig 4). Considering the wavelengths in which discrimination by bees is optimal, MAD and minAD\(_{400}\) were significantly lower in bee- than in hummingbird-flowers; and minAD\(_{500}\) was not significantly different even though it was two times higher for hummingbird-flowers (Table 1). The distances between the wavelengths of optimal colour discrimination for birds and the marker points of bee- and hummingbird-flowers were similar. However, the minAD\(_{416}\) was significantly lower for bee-flowers (Table 1).

We classified 17 species as displaying ‘red’ flowers: four bee-flowers (two UV-reflecting and two UV-absorbing; Fig. 5a), and 13 hummingbird-flowers, all UV-absorbing (Fig. 5b). From the 76 species displaying ‘yellow’ flowers, 67 were bee-pollinated (46 UV-reflecting and 21 UV-absorbing; (Fig. 5c). Out of the 46 yellow UV-reflecting flowers
pollinated by bees, almost all (44 species) presented flowers with UV-pattern. Just nine yellow-flower species were hummingbird-pollinated, four UV-reflecting (three with UV-pattern) and five UV-absorbing (Fig. 5d). We classified 48 species as displaying ‘white’ flowers: 44 were mainly pollinated by bees (four UV-reflecting and 40 UV-absorbing; Fig. 5e) and only four by hummingbirds (one UV-reflecting and three UV-absorbing; Fig. 5f).

Most colour variables presented negative phylogenetic signals ($P<0.05; K<1$) (Supporting Information Table S3) indicating that these traits were phylogenetically structured in the studied community but with a phylogenetic dispersion. Some traits did not show phylogenetic signals ($P>0.05$): achromatic contrast (green contrast), colour intensity, and mean absolute deviation of marker points for bees and chromatic contrast and red photoreceptor excitation for birds (Supporting Information Table S3).

Discussion

In the studied community, flowers pollinated mainly by hummingbirds were less conspicuous for bees. However, these flowers were equally contrasting for birds, although more saturated than bee-flowers. Although it is known that birds prefer colours that are more contrasting against the background, they can detect any colour with almost no evident preference for a specific pattern (Schmidt et al., 2004; Lunau et al., 2011; Bergamo et al., 2016). In addition, resource amount and quality, flower height, and orientation on the flowering plant are also important traits that influence flower selection by hummingbirds (Cronk & Ojeda, 2008; Fenster et al., 2015). The presence of accessory structures was more common in hummingbird-pollinated flowers and these structures are expected to improve flower contrast and bird detection at distance, as also observed for ornitochoric fruits (Schaefer & Schaefer, 2007).

Bee-flowers surveyed in our community presented higher values of spectral purity and green and chromatic contrasts, favouring flower detection and discrimination by bees (Spaethe et al., 2001; Rohde et al., 2013, van der Kooi et al., 2018), and frequently present floral guides, which increase flower conspicuousness at short distances and interspecific discrimination by bees (Leonard & Papaj, 2011; Lunau et al., 2011; Hansen et al., 2012; Lunau & Wester, 2017). Additionally, even with some deceptive costs, by displaying floral guides represented by pollen/anther mimicking structures, flowers improve their bee attractiveness without exposing real pollen grains, which improves plant reproductive success.
The presence of UV patterns occurred mainly in bee-flowers and in some hummingbird-flowers, which could eventually be pollinated by bees as well (pers. observation, Carstensen pers. comm., Carstensen et al., 2014, 2016). The predominance of UV-patterns among bee-flowers, which favours flower contrast, corroborates the idea that UV-patterns are a signal strategy related to bee pollination (Papiorek et al., 2016; Lunau et al., 2017). Therefore, our results support the hypothesis that the innate preference for more spectrally pure colours in bees acts as a selective pressure on flower colour evolution (Rohde et al., 2013). Our results also suggest that chromatic and achromatic contrasts, floral guides, and UV-patterns are visual signals that can also be driven by pollinator selection.

Marker points of bee- and hummingbird-flowers were frequently closer to the wavelengths of maximal spectral discrimination of their main pollinators, similar to previous studies (Dyer et al., 2012; Shrestha et al., 2013). Hummingbird-flowers reflected more and presented more marker points at long wavelengths (red band), while bee-flowers reflect and presented more marker points in short and medium wavelengths (ultraviolet and blue bands) in accordance to bees’ visual sensitivity and colour preferences (Chittka et al., 1994; Dyer et al., 2016). We also found that marker points of bee-flowers were significantly closer to the optimal wavelength for colour discrimination in bees, mainly at 400 nm, confirming that colours of bee-flowers are better discriminated by bees than colours of hummingbird-flowers.

The differences detected in the reflectance spectra of bee- and hummingbird-flowers were also observed for most of the red, yellow, and white flowers. All red hummingbird-flowers were UV-absorbing, probably achromatic for bees (Lunau et al., 2011). One out of the four white hummingbird-flowers presented reflectance in the UV-band, while none of the 44-white bee-flowers did. These results agree with previous studies (Kevan et al., 1996) and with the sensory exclusion of bees by white UV-reflecting flowers (Lunau et al., 2011; Bergamo et al., 2016). Moreover, the finding that the UV-patterns in yellow flowers is more frequent in bee- than in hummingbird-flowers confirms the findings of Papiorek et al. (2016), in which hummingbird-flowers often completely absorb ultraviolet light and display no UV-bull’s eye, used by bees as floral guides.
Therefore, our results confirm the hypothesis of sensorial exclusion of bees in yellow, white, and, mainly in red hummingbird-flowers co-occurring at the community level. By the sensorial exclusion of bees, hummingbird-flowers avoid red-blind nectar or flower-eaten visitors, that may eventually affect their reproductive success (McCall & Irwin, 2006; Strauss & Whittall, 2006). However, it is important to highlight that, in addition to flower colour, hummingbird-flowers can also mechanically avoid antagonistic or less efficient pollinators due to floral morphology and resource location (Cronk & Ojeda, 2008), as we observed for most of our hummingbird-pollinated flowers. Considering the similarity of bird-pollinated flower colour described here and in other tropical communities (Altshuler, 2003; Geerts & Pauw, 2009; Shrestha et al., 2013; Burd et al., 2014), it would be interesting to verify if this private niche for hummingbirds can be generalized for flowers pollinated by other birds that evolved in the presence of red-blind flower visitors.

Some exceptions found in yellow and white flowers related to the hypothesis of sensorial exclusion can be related to flowers in which both bees and hummingbirds are potential pollinators. For example, three out of four yellow UV-reflecting hummingbird-flowers presented UV-patterns and have bees as secondary pollinators (*Barbacenia flava*, *Vochysia rufa* and *Vochysia thyrsoidea*; pers. observation, Carstensen et al., 2014, 2016). In the same way, two out of three white UV-absorbing flowers pollinated mainly by hummingbirds may have bees as secondary pollinators (Carstensen et al., 2014, 2016; Freitas & Sazima, 2006). According to Rosas-Guerreiro et al. (2014), although flowers present adaptations to the most effective pollinator, they may also show traits related to secondary pollinators, thus contributing to plant reproduction success. These authors suggested that, in general, secondary pollinators correspond to the ancestral pollinator of related species and may be linked to signals of different pollination syndromes in the same flower. Even though hummingbirds are always cited as a more efficient pollinator, they can also act as a secondary pollinator or even an opportunistic visitor of bee-pollinated flowers, as observed for *Aspilea jolyana* (Maruyama et al., 2018) and some Vochysiaceae species (Oliveira, 1998).

The observed differences in colour reflectance and signal strategies between bee- and hummingbird-flowers along with no or negative phylogenetic signal related to the colour variables indicate that pollinators exert an important selective pressure and influence the diversity of flower displays found in the campo rupestre community. Flowers pollinated mainly by bees presented signalling strategies more related to the visual sensitivity and
preferences of bees, which favours bee attraction and flower discrimination and reinforces the hypothesis that bee-flowers are adapted to hymenopteran colour vision (Chittka & Menzel, 1992; Dyer et al., 2012; Rohde et al., 2013). Flowers pollinated mainly by hummingbirds although reflecting more in the red band were equally conspicuous to birds but tend to be less conspicuous to bees. Therefore, whereas bee-flowers signal to attract bees, hummingbird-flowers invest in a private channel that avoids less efficient pollinators as well as antagonistic interactions, confirming the bee avoidance hypothesis as an important selective pressure in Neotropical plant communities.

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Authors Contributions: Conceived and designed the study: MGGC, LPCM, KL and MAPL; collected data: MGGC and LPCM; analysed data: MGGC, MAPL and SB; wrote and revised the manuscript: MGGC, LPCM, KL, MAPL, SB and VLGB.
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Supporting Information

**Table S1** Studied plant species with the respective main pollinator, predominant colour according to human vision, presence of floral guides, mimicking and accessory structures.

**Table S2** Reflectance data of the sampled flowers according to their main pollinator.

**Fig. S1** The green background and D65 standard daylight spectra used in the colour hexagon model.

**Methods S1** Method and R-script used to survey the marker points of each reflectance spectrum.

**Fig. S2.** The mean reflectance of the predominant flower colour of all species analysed.

**Fig. S3** An example showing the peaks, limits, and the three marker points of a flower spectrum.

**Table S3** Blomberg’s K values for the quantitative colour variables
Table 1 Comparison (post-hoc one-way ANOVA) and adjusted P-values (according to Bonferroni method) of quantitative colour variables of bee- and hummingbird-pollinated flowers calculated according to the visual system of bees and birds, and their respective mean and standard deviation (SD).

<table>
<thead>
<tr>
<th></th>
<th>F-value</th>
<th>df</th>
<th>P-value</th>
<th>adjusted P-value</th>
<th>bee-flowers</th>
<th>hummingbird-flowers</th>
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<td><strong>Bee’s visual system</strong></td>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
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<tr>
<td>spectral purity</td>
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<td>&lt;0.001</td>
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<td>0.14</td>
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<td>1.00</td>
<td>488.50</td>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
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MAD, mean absolute deviation; minAD, minimum absolute deviation for each wavelength of optimal colour discrimination for bees (400 nm and 500 nm) and UVS birds (416, 489 and 557 nm).
Figure legends

Figure 1. Examples of flowers pollinated mainly by bees (a–f) and hummingbirds (g–l) that show colour patterns (a, g), only one colour (b, h), floral guides (c, d, i, j), floral guides represented by anther or pollen mimicking structures (d, j), and UV-patterns (e, k, regular photos; f, l, UV-photos in which white corresponds to UV-reflecting parts and black to UV-absorbing parts), collected in the campo rupestre plant community of Serra do Cipó, Southeastern Brazil. Plant species: Kielmeyera rubriflora (a), Myrcia guianensis (b), Sisyrrinchium cf. vaginatum (c), Microlicia amplexicaulis (d), Heteropterys sp. (e, f), Siphocampylus nitidus (g), Camptosema isopetalum (h), Esterhazya splendida (i), Augusta longifolia (j), Vochysia thyrsoidea (k, l).

Figure 2. Colour loci of bee- (blue points) and hummingbird-flowers (red points) plotted in the bee (a) and bird visual space (b). The grey central point in the visual spaces represents the achromatic centre and the colourful points in the vertices represent each photoreceptor: green, blue, and UV for bees and red, green, blue, and UV for birds. In the bee-hexagon (a), the grey line represents the maximum sensitivity of each photoreceptor to a monochromatic light.

Figure 3. Relative frequency of species according to the spectral absorbance or reflectance of their flowers in the four bands established within the 300 - 700 nm spectrum: ‘uv’ (ultraviolet; 300–400 nm), ‘b’ (blue; 401–500 nm), ‘g’ (green; 501–600 nm) and ‘r’ (red; 601–700 nm). The total number of species per category is indicated in the label. The categories (‘uv-b-g-r’; ‘uv+b-g-r’; ‘uv-b+g+r’ and ‘uv+b+g+r’) showed no hummingbird-pollinated species or < 0.5% of bee-pollinated species.

Figure 4. Wavelength position and frequencies of marker points estimated for bee-flower (a) and hummingbird-flower (b) colours in the campo rupestre plant community of Serra do Cipó, Southeastern Brazil. The grey lines correspond to the spectral sensitivity of each photoreceptor and the best points of colour discrimination are indicated by the arrows.

Figure 5. Reflectance spectra of red (a, b), yellow (c, d), and white (e, f) flowers pollinated mainly by bees (left) and hummingbirds (right) surveyed in the campo rupestre plant community of Serra do Cipó, Southeastern Brazil. Each line represents one species. In detail, the number of flowers that reflect (UV+) or absorb (UV-) ultraviolet wavelengths (300–400 nm).