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Flowering phenology of species with similar flower colours in species-rich communities[☆]

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ABSTRACT

Within a community, co-occurring plant species are expected to diverge in floral display or flowering phenology to decrease interspecific competition and thus increase intraspecific pollination. However, co-occurring species can also benefit from floral signal standardisation (similar colour signals among flowers of different species) because it facilitates pollinator attraction. Considering the interaction of flower colour display and flowering phenology, we investigated the visual similarity of rewarding flowers among species from highly diverse tropical and temperate vegetation types. For six groups of co-occurring, closely related bee-pollinated species with similar floral displays from Brazilian campo rupestre (51 species) and Spanish Mediterranean vegetation (30 species), we first investigated whether flower colours can be discriminated by bees based on colour locus distance in the bee vision hexagon. We then tested whether flowering phenology overlapped or was segregated. We found that within both vegetation regions, flower colour was generally not distinguishable within groups by bees. The small perceptual distance of colour loci in the bee visual space did not enable discrimination. The flowering periods of the Mediterranean species overlapped, while the Brazilian campo rupestre species tended to have segregated phenologies. Mediterranean species may benefit from the increased standardisation of signals displayed during the short flowering season, while the sequential flowering phenology of campo rupestre species may decrease interspecific competition and help maintain a recognizable signal for bees over time, favouring flower constancy. We concluded that the standardisation of the floral colour signal within these two species-rich plant communities is advantageous for most of the species studied, despite having different flowering phenologies.

1. Introduction

Bees are important pollinators and often show floral constancy — the tendency to sequentially visit flowers of the same species. Floral constancy depends on the cognitive ability of bees to memorise and discriminate floral traits and on the association between these traits and floral rewards (Grant 1950; Chittka et al., 1999; Grüter and Ratnieks 2011). Floral constancy benefits bees by reducing the time spent finding and manipulating flowers, and benefits plants as well by improving the visitation of conspecific flowers, favouring intraspecific pollen flow and decreasing heterospecific pollen deposition (Chittka et al. 1999; Dyer and Chittka 2004).

Flower colour is one of the most important traits used by bees to select flowers (Wilson and Stine 1996; Chittka et al., 1997, 1999), and controlled experiments indicate that bees strongly discriminate among colours (Chittka 1992; Garcia et al. 2017). However, under natural conditions, subtle differences in flower colour do not suffice to ensure significant levels of floral constancy, with consequences for pollination success (Chittka et al. 1997; Spaethe et al. 2001; Dyer and Chittka 2004) (but see Jimenez-Lopez et al. 2019). Flower colour is an evolutionarily labile trait that shows low phylogenetic inertia (Arnold et al., 2009; McEwen & Vamosi, 2010; Shrestha et al., 2014; Ortiz et al. 2021), and colour changes are frequent within flowering plant clades (e.g., Beardsley et al., 2003; McEwen & Vamosi, 2010; van der Kooi and Stavenga, 2019). Thus, co-occurring species may be under selective pressure for flower colour divergence because strong flower colour differences facilitate floral constancy and thus enable heterospecific pollen transfer to be avoided (Chittka et al. 1999; Dyer and Chittka, 2004, van der Kooi et al. 2016). However, some plants may benefit from displaying flower signals that pollinators are not capable of discriminating from those of other plant species (Roy and Widmer 1999; Lunau and Wester 2017, Coetzee et al. 2021). The display of similar colour signals among flowers of different species is known as colour signal standardisation, a possible ecological advantage of reproducing a signal recognizable by potential pollinators as an honest signal of resource availability. Moreover, colour signal standardisation could benefit each of the plant species involved, especially rare species (Roy and Widmer 1999; Moeller 2004, Benitez-Vieyra et al. 2007, Lunau and Wester 2017, Bergamo et al. 2020).

Pollinator preference and interspecific pollen transfer may determine competition or facilitation among co-flowering species and the evolution of flower traits such as colour (Morales and Traveset, 2008). Heterospecific flower visits and pollen transfer appear to have particularly strong negative effects among closely related species (Streher et al. 2020), which are expected to be under stronger selective pressure to avoid these negative effects. Differences in the timing of flowering of cooccurring plant species may prevent interspecific competition and favour intraspecific pollination (Genini et al. 2021). By flowering at different times, species with similar flowers prevent interspecific competition while maintaining resource

availability for pollinators over extended periods (Mosquin 1971; Waser and Real 1979; Moeller 2004, Jensen et al. 2019, Albor et al. 2022). However, the timing of flowering may be constrained to periods of favourable climatic conditions (Smith-Ramirez and Armesto 1994; Staggemeier et al. 2010), limiting opportunities for temporal segregation, especially in seasonal environments with restricted flowering and growth seasons (Staggemeier et al. 2015, 2020). Therefore, colour differentiation between closely related taxa may occur as a way to alleviate interspecific pollen transfer by shared pollinators in regions with short flowering seasons. Conversely, when climatic conditions allow for long blooming seasons, pressures for floral differentiation may be weaker, as species may segregate flowering phenologies over the course of the year. In species-rich plant communities such as the tropical campo rupestre and temperate Mediterranean vegetation, species frequently have similar flowers, a phenomenon that is frequently observed among some of the most diverse genera and families (Cueto et al. 2018, Camargo et al. 2019). However, these two plant communities differ strongly in their seasonality. In the campo rupestre, species flower year-round, with no defined flowering peak (Rocha et al. 2016, Le Stradic et al. 2018), while in the Mediterranean vegetation, the flowering season is restricted to a 4- to 5-month period (Arroyo 1990; Flo et al. 2018). Thus, the plant communities of these two regional vegetation groups are expected to follow different strategies to optimise their interactions with pollinators. In the Mediterranean, the short flowering

season limits species' ability to stagger their flowering periods; hence, species are expected to avoid heterospecific pollen transfer by differentiating their flower colour during overlapping blooming periods. In contrast, in the campo rupestre, species are able to segregate their flowering temporally; thus, they are expected to prioritise colour signal standardisation to reliably attract shared pollinators among sequentially flowering species.

Here, we investigated patterns of flower colour and phenology among co-occurring species in these highly diverse tropical and temperate vegetation types. Based on the interaction of flower colour and flowering phenology, we considered three hypothetical scenarios (Fig. 1). In the first scenario, species have flowers that can be discriminated by bees based on their colour (Fig. 1A). In the second scenario, flowers cannot be discriminated by bees, and species have overlapping flowering phenologies; under this scenario, the advantages of a standardised signal (pollination facilitation) could offset the cost associated with interspecific competition (Fig. 1B). Last, in the third scenario, flowers cannot be discriminated by bees, but species show a segregated flowering phenology; thus, species could maintain a standardised signal for easy recognition by bees while avoiding competition (Fig. 1C).

We selected groups of bee-pollinated, co-occurring, and phylogenetically closely related species with similar floral display to the human eye from the Brazilian campo rupestre and from Spanish Mediterranean shrubland to evaluate whether (i) bees can discriminate among flower colours (using distance between colour loci in the bee-colour space as a proxy) and (ii) species have overlapping or segregated flowering times. We expected a larger number of species whose flowers can be distinguished by bees in the Mediterranean shrubland than in the campo rupestre since the Mediterranean short growing season constrains the possibility of temporally segregated flowering (Fig. 1A). In contrast, in the Brazilian campo rupestre, we expected a lower degree of flowering season overlap, allowing the coexistence of flower colours that are indistinguishable among species in the bee visual system (Fig. 1C).

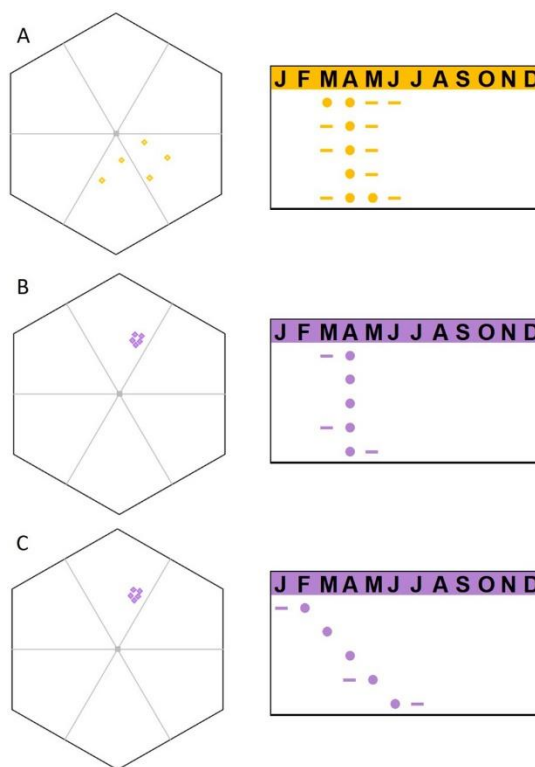


Fig. 1. Hypothetical framework illustrating, on the left, the distribution of flower colours (colour loci) of species whose flower colour is similar to the human eye in the colour space of bees (bee-hexagon) and, on the right, their corresponding flowering phenology patterns, with circles indicating the month of peak flowering. Each point in the hexagon and each line in the flowering phenology represent one species. (A) flower colours of the species are discriminable by bees, preventing heterospecific pollen deposition even with temporal overlap in flowering. (B) and (C) represent species whose flowers that cannot be discriminated by bees: (B) aggregated, overlapping flowering species facilitate each other's pollination by signal standardisation among the co-flowering species, such that the advantages of an increased standardized signal outweigh the costs of possible interspecific pollination interferences; (C) species with temporally staggered flowering maintain a standardised signal that is recognized by bees over time in the shared space, and consequently have a decreased risk of heterospecific pollination interference.

2. Material and methods

The study was carried out in two seasonally dry regional vegetations that are both considered global biodiversity hotspots (Silveira et al. 2016, Cueto et al. 2018, Myers et al. 2000). The first area was in Serra do Cipó National Park and its buffer zone (19°13'55" S, 43°34'35" W), south of the Espinhaço Mountain Range, in Minas Gerais state, southeastern Brazil. This area is characterised by campo rupestre vegetation. The other study sites comprised Mediterranean vegetation in three natural parks in Andalusia, Spain: Sierra de Grazalema (36°46'47" N, 5°26'21" W), Doñana (37°12'54.0" N 6°25'40.6" W) and Sierra de Aracena y Picos de Aroche (37°47'44.4" N 6°44'33.6" W) (Supplementary material Appendix 1, Fig. A1). The campo rupestre is a species-rich tropical grassland restricted to mountain tops above 900 m above sea level, mainly across the Espinhaço range (Silveira et al. 2016, Mattos et al. 2019). Many species groups in the campo rupestre have floral displays that appear similar to the human eye, including species-rich taxa such as Melastomataceae and the genera *Vellozia* and *Xyris* (Camargo et al. 2019, Mattos et al. 2019), all of which were represented in this study. The campo rupestre has a seasonal climate, with dry winters (April to September) and wet summers (October to March), and species flower year-round (Rocha et al. 2016, Le Stradic et al. 2018). The Mediterranean study sites occur in one of the main biodiversity hotspots of the Mediterranean Basin (Médail and Quézel 1997). Here too, there are related species whose flowers are similar in appearance to the human eye, including diverse taxa such as Ranunculaceae, Cistaceae and Genisteae (Cueto et al. 2018; pers. obs.), all included in this study. The climate is markedly seasonal, with rainy, cold winters and hot-dry summers (Cueto et al. 2018), and most species flower between April and July during the spring and early summer (Arroyo 1990; Flo et al. 2018).

We collected data and analysed 81 species grouped according to the taxa to which they belong (i.e., accounting for phylogenetic relatedness). In both regional vegetation groups, we selected species from important taxa in terms of species richness and abundance and defined 12 groups of closely related species that share a similar floral display: six groups from the campo rupestre (51 species total) and six from the Mediterranean vegetation (30 species total) (Fig. 2; Supplementary material Appendix 1, Tab. A1). These groups have four to 14 species that are pollinated primarily by bees, as determined based on direct observation (Mediterranean scrubland: Herrera 1988; Ortiz, 1991; Aparicio and García-Martin, 1996; Fiz et al. 2008; Polidori et al. 2010; Aygören, 2021; Arista and Ortiz pers obs; campo rupestre: Carstensen et al. 2014, 2016; Monteiro et al. 2021; Morellato and Camargo pers. obs.). Flowers of the selected species within each group are similar in shape, size, and colour to the human eye and co-occur at the study sites. Both reflectance and phenological data from field observations were collected over at least one year (Supplementary material Appendix 1, Tab. A1).

The main pollinators of the plant species in the campo rupestre investigated in this study are Apidae and Halictidae bees, mainly the *Lasioglossum*, *Augochloropsis*, *Ceratina*, and *Bombus* genera, as well as *Apis mellifera*, which is invasive in the region (Monteiro et al. 2021). For the Mediterranean vegetation, eusocial bees such as *Apis* and *Bombus* (Apidae), as well as a large number of solitary bee species, are the most important pollinators (Herrera 1988; Magrath et al., 2021). All the campo rupestre plant species were collected at the Serra do Cipó. In the Mediterranean region, four of the six groups were collected in the Sierra de Grazalema Natural Park, and the other two, the *Cistus* and Genisteae groups, were collected in the Doñana Natural Park and the Sierra de Aracena y Picos de Aroche Natural Park, respectively. The species within each group coexist in the same communities and were collected only a few metres from each other. To ensure that flower sizes and shapes were similar among species, flower size data were obtained in the field during data collection or from the literature (Supplementary material Appendix 1, Tab. A1).

2.1. Flower colour reflectance and discrimination

To analyse flower colour according to the trichromatic colour vision of bees (see details below), we collected reflectance data between 300 and 700 nm for petals of five to 10 flowers from at least three different individuals per species with a spectrophotometer (Jaz Modular Optical Sensing Suite; Ocean Optics, USA) following Camargo et al. (2019). For each of the 12 studied plant groups, using the mean reflectance spectrum for each species, we plotted the flower colour position in the bee colour space—that is, the flower colour locus in the bee-hexagon (Chittka 1992). We used the mean reflectance spectrum of leaves collected at each study site as the green background (see Martins et al. 2021), the standard D65 daylight as the ambient light, and the photoreceptor sensitivity of *Apis mellifera* and *Bombus terrestris* as parameters for the bee-hexagon function (Peitsch et al. 1992). We utilised *Apis mellifera* and *Bombus terrestris* parameters for visual modelling because they are either themselves important pollinators or they are closely related to important pollinators of the plant species studied, and their colour discrimination capability is well known under controlled and natural conditions (Spaethe et al. 2001; Dyer and Chittka 2004, Garcia et al. 2017). The colour vision systems (photoreceptor number and sensitivity) are relatively well conserved among Hymenoptera species for which such data are available (van der Kooij 2021), but there are few or no experimental studies on colour discrimination and flower constancy in tropical bees, including the main pollinator species of the campo rupestre. Therefore, for tropical groups, we highlighted the results found for *B. terrestris* due to the importance of bumblebees as pollinators in the campo rupestre (see Monteiro et al. 2021 and references therein).

To verify whether bees discriminate the colour of flowers between species within the same group, we calculated the distance between the colour loci of each pair of species. We considered the flower colours to be discriminable by bees if the distance between their loci was above 0.2 hexagon units (HU) (according to Spaethe et al. 2001, Dyer and Chittka 2004). Discrimination by bees at a minimum of 0.2 HU is evidenced by a 60% probability of flower constancy in *Apis mellifera* and six species of bumblebees (Spaethe et al. 2001), and 80% constancy is reached at a value of 0.4 HU. We also determined whether the flowers displayed UV patterns generated because parts of the perianth reflected UV light while other parts of the perianth absorbed it. This differential reflectance of UV light across the perianth results in patterns that can be used by bees to discriminate among flowers but are invisible to the human eye. To determine the presence of UV patterns, we scanned the petals using a spectrophotometer and complemented those data with UV photographs when available. We considered UV-stimulus reflectance values to be significant if there was > 5% variation in reflectance values between 300 and 400 nm across the petal surface (Camargo et al. 2019).

2.2. Phenological data and flowering overlap

To verify the flowering overlap of the studied species from the campo rupestre, we used direct observation of phenological data at the same sites where flower reflectance data were collected at Serra do Cipó. These data correspond to four years of direct observations, from August 2012 to December 2014 and from November 2009 to November 2011 (Rocha et al. 2016, Le Stradic et al. 2018). We compiled flowering records from photos, author field notes, and vouchers of the flowering species, including their respective locations and dates, that were collected during field surveys conducted at the Serra do Cipó site (Rocha et al. 2016, Camargo et al. 2019, Mattos et al. 2019, Monteiro et al. 2021) (Supplementary material Appendix 1, Tab. A2). We filled in any missing phenological data by supplementing direct data collection with information from herbarium vouchers of specimens collected at our campo rupestre area from all Brazilian herbaria with available digitised information (Brasil, 2020). For the Mediterranean shrubland vegetation, we applied the same procedure using phenological data from direct observations of the same communities and complemented with data acquired from herbarium vouchers of specimens collected in Andalusia, Spain (HER SEV) (Supplementary material Appendix 1, Tab. A2). To ensure representativeness, we considered flowering to occur in a given month when that month contained at least 20% or 30% of the maximum number of records (herbarium samples) for a given species in Mediterranean vegetation and in the campo rupestre, respectively. The herbarium records are largely from the same areas where we conducted the present work and reflect with good accuracy the overall phenological patterns of a plant species and community at a specific location (Proença et al. 2012). Furthermore, phenological information either wholly based on or supplemented by herbarium records is widely used in the literature (Miller-Rushing et al., 2006, Willis et al., 2017). We determined the month in which the flowering period started (flowering onset) and the month with the highest number of flowering records (flowering peak), adding herbarium phenology only as needed to fill in gaps in direct phenological information (Supplementary material Appendix 1, Tab. A2).

2.3. Data analysis

We used a null model to test whether flowering patterns were segregated, random, or aggregated among species from the same group (Pleasants 1990; Staggemeier et al., 2010, 2015). We first estimated the flowering overlap among species within each group using the index of niche overlap calculated by the Schoener equation (Schoener 1970), according to Staggemeier et al. (2010). The index of niche overlap varies from zero to one, with zero indicating no overlap and one indicating total overlap among species (Schoener 1970; Staggemeier et al. 2010). We then assessed temporal niche overlaps among species within each group following the mean pairwise overlap proposed by Pleasants (1990). Finally, we tested the mean overlap indices between all possible pairs of species within a group, that is, the observed mean overlap, against the distribution of 1000 Monte Carlo-simulated overlap values for the same group of species. Therefore, for each pairwise combination of species, an overlap index was calculated, and the mean observed value of all species was compared with 1000 simulated values. If the observed mean overlap is higher than the simulated overlap, this indicates a significant tendency for aggregated or overlapping flowering patterns, while if the simulated overlap value is higher, this indicates a trend towards a segregated or staggered flowering pattern. We used the binomial test to verify the probability of each pair of species of a given group presenting flowering overlap equal to or higher than 0.5, that is, intermediate phenological overlap (Pleasants 1990; Staggemeier et al. 2010), and a distance among their colour loci in the bee hexagon equal pattern arises when the observed flowering overlap is smaller (or greater) than the overlap of 950 simulated values (see details and graphic interpretation in Staggemeier et al. 2010). We calculated the mean pairwise overlap for the flowering onsets and peaks; however, since the results were similar, we presented only the results for the flowering peaks. We carried out all of the analyses in R (R Core Team, 2018) and used the default version of the 'PAVO' package (Maia et al., 2013) for the analyses of flower colour reflectance.



Fig. 2. Examples of flowers of some species belonging to the 12 phylogenetically closely related groups of co-occurring species with similar floral display that were included in the study. Campo rupestre, Brazil: 1. Melastomataceae (a- *Chaetostoma armatum*; b- *Microlicia juniperina*; c- *M. hirticalyx*; d- *M. cardiophora*); 2. *Chamaecrista* (a- *C. papillata*; b- *C. desvauxii* var. *graminea*; c- *C. cathartica*; d- *C. ramosa*); 3. *Vellozia* (a- *V. caruncularis*; b- *V. nivea*; c- *V. variabilis*; d- *V. compacta*); 4. *Vochysia* (a- *V. thyrsoidea*; b- *V. cinnamomea*; c- *V. pygmaea*; d- *V. elliptica*); 5. Asteraceae (a- *Lychnophora* sp.; b- *Prestelia eriopus*; c- *Lessingianthus warmingianus*; d- *Chrysolaena desertorum*); 6. *Xyris* (a- *X. pilosa*; b- *X. glaucescens*; c- *X. asperula*; d- *X. trachyphylla*). Mediterranean shrubland vegetation, Spain: 7. *Hypericum* (a- *H. undulatum*; b- *H. perforatum*; c- *H. tomentosum*; d- *H. perforatum*); 8. *Cistus* (a- *C. salvifolius*; b- *C. libanotis*; c- *C. populifolius*; d- *C. monspeliensis*); 9. Geraniaceae (a- *Erodium moschatum*; b- *E. malacoides*; c- *Geranium rotundifolium*; d- *G. dissectum*); 10. *Ranunculus* (a- *R. ficaria*; b- *R. muricatus*; c- *R. macrophyllus*; d- *R. paludosus*); 11. Genisteae (a- *Genista tridentata*; b- *G. cinerea*; c- *G. hirsuta*; d- *Stauracanthus genistoides*); 12. yellow-Cistaceae (a- *Fumana lacidulemiensis*; b- *Helianthemum syriacum*; c- *F. ericoides*; d- *Halimium atriplicifolium*). Photos by the authors, except: 11b. [public domain]; 11d. [CC BY-SA 4.0]; 12c. [CC BY-SA 3.0]; and 12d. [CC-BY-2.0], reproduced from <http://commons.wikimedia.org>, under the referred licenses.

3. Results

3.1. Flower colour discrimination by bees

The flowers within all studied groups presented similar reflectance spectra, with most variations observed in the UV band (from 300 to 400 nm) of UV-reflecting flowers (Figs. 3 and 4). For the campo rupestre, considering the spectral sensitivity of *B. terrestris*, no species pairs within the same species group had colour locus distances above 0.2 HU (Table. 1, Fig. 3). Considering the spectral sensitivity of *A. mellifera*, only eight out of 223 species pairs within the same species group had colour locus distances above 0.2 HU (Table. 1)—two pairs were from the Asteraceae and six were from the *Xyris* group. Within Asteraceae, one species (out of 11 total) was involved at distances above 0.2 HU (*Chrysolea desertorum*), while in the *Xyris* group, two species (of a total of 14) accounted for the discriminable differences (*Xyris piranii* and *Xyris pilosa*). Of the Mediterranean species, seven of the 67 species pairs presented colour locus differences above 0.2 HU—one from Geraniaceae and six from the *Ranunculus* group (Table. 1, Fig. 4). The discriminable difference in Geraniaceae was between *Erodium laciniatum* and *Geranium lucidum*. In the *Ranunculus* group, two species were involved in the discriminable differences; *Ranunculus macrophyllus* differed from four other species of *Ranunculus*, and *Ranunculus paludosus* differed from two other species of the genus. In the campo rupestre, the binomial test indicated that distances greater than 0.2 HU were not expected within groups when considering the spectral sensitivities of *Bombus*, with probabilities of 4% for Asteraceae and 7% for *Xyris* when considering the spectral sensitivities of *A. mellifera* (Fig. 5A; Supplementary material Appendix 1, Table. A3 and Fig. A2). In Mediterranean vegetation, these probabilities were 4% for Geraniaceae and 13% for *Ranunculus* in the case of *Bombus* and 4% and 40%, respectively, in that of *Apis* (Fig. 5B; Supplementary material Appendix 1, Table. A3 and Fig. A2). The largest mean colour locus distances were observed for *Ranunculus* species, the group with the highest number of pairs of species in which flowers are potentially discriminated by bees (Fig. 5B). The UV patterns differed among species in three Mediterranean groups: Genisteae, *Ranunculus*, and *Hypericum* (Supplementary material Appendix 1, Fig. A3). Three of the four species of Genisteae presented a UV pattern consisting of a UV-reflecting banner petal and UV-absorbing wing and keel petals; only *Genista hirsuta* was entirely UV-reflecting. Almost all the *Ranunculus* species displayed a bull's-eye pattern, consisting of a UV-reflecting petal apex and a UV-absorbing petal base; only *Ranunculus macrophyllus* was entirely UV-absorbing. The *Hypericum* species also displayed a bull's-eye pattern, with the exception of *H. perforatum*, in which the petals are entirely UV-reflecting; we could not collect reflectance data from the *H. tomentosum* petal base. We considered all species in the yellow-flowered Cistaceae group (hereafter named yellow-Cistaceae) to display a bull's-eye pattern, although we were unable to collect reflectance data from the petal base of *Fumana lacidulemiensis*. All *Cistus* species presented a colour pattern with a yellow spot at the petal base. In the campo rupestre, all *Vochysia* species had UV-reflecting petals and UV-absorbing sepals. There was no colour or UV pattern of the perianth in any of the other studied species from the campo rupestre.

3.2. Flowering patterns and overlap

The flowering patterns differed between the campo rupestre and Mediterranean vegetation plant groups. The campo rupestre groups had a more segregated flowering pattern (Fig. 3); the mean flowering overlap between species within the same group was always below 0.5 (Fig. 5A). The probability of a mean overlap between species within the same group higher than 0.5 was 7% for *Chamaecrista*, 18% for *Melastomataceae*, and approximately 35% for the other four groups (Supplementary material Appendix 1, Table. A3). According to the null model test, *Chamaecrista* and *Melastomataceae* presented a higher probability of staggered flowering than would be expected by chance, while Asteraceae, *Vellozia* and *Xyris* had a higher probability of overlapping than expected by chance, and *Vochysia* presented a random flowering pattern (Table. 2). The flowering patterns of the Mediterranean vegetation overlapped within all groups of plants (Table. 2; Fig. 4). The probability of mean overlap greater than 0.5 between species within the same group was 83% for yellow Cistaceae, 67% for *Ranunculus*, and near 100% for the other groups (Fig. 5B; Supplementary material Appendix 1, Table. A3). Therefore, the observed flowering overlap was higher than expected by chance for all groups (Tab. 2).

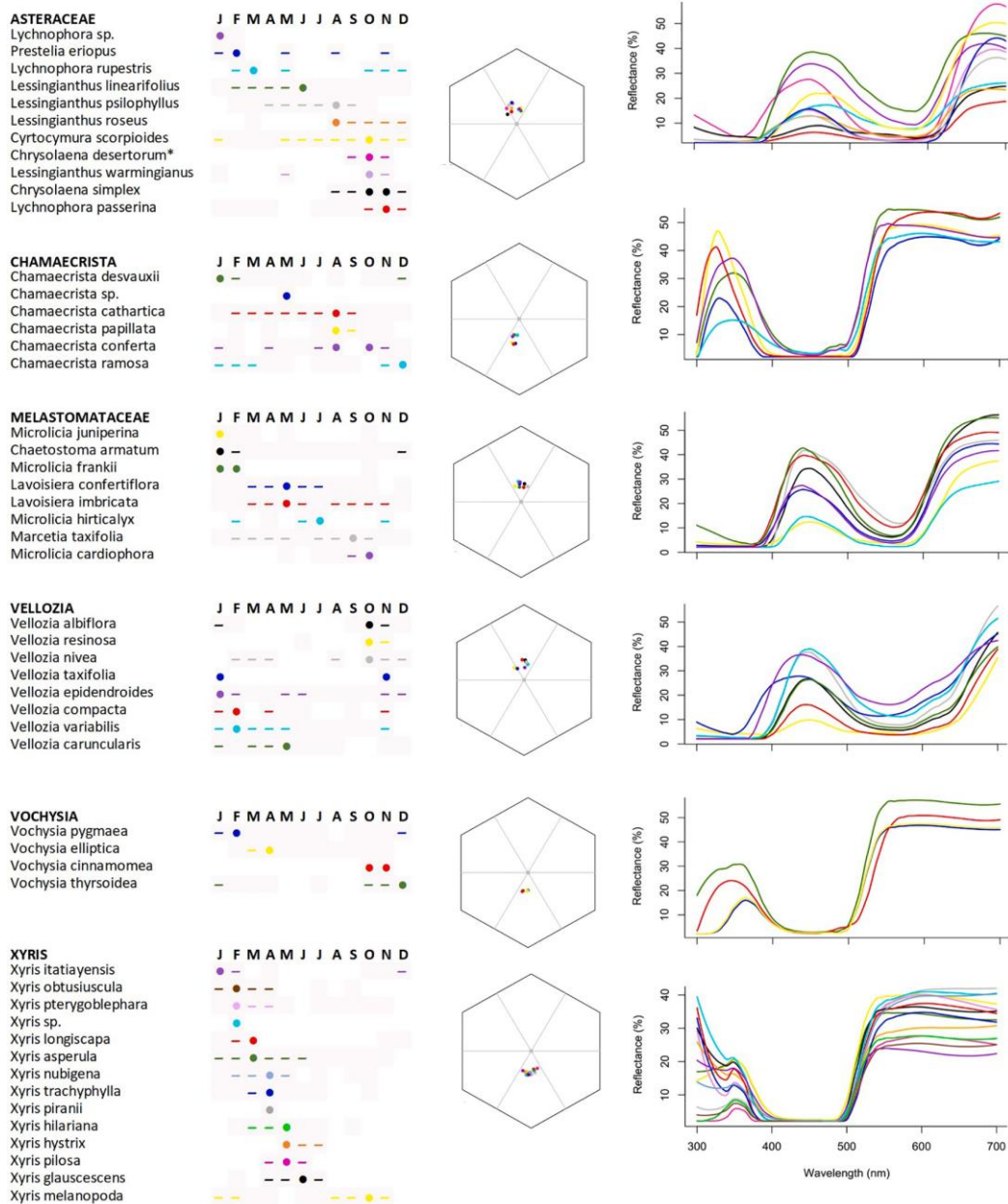


Fig. 3. Flowering patterns (left) of the 51 species from the campo rupestre (Serra do Cipo^o, Southeastern Brazil) studied, organised by groups of flowers with similar display and their respective colour loci in the bee-hexagon (centre) and reflectance spectrum (right). Colour loci were calculated considering the photoreceptor sensitivity of *Bombus terrestris*. Within each group, the same colour indicates the same species in the three graphs. In the phenological pattern the grey are a represents flowering activity, dashes the period of higher activity and the dots the peak of activity.

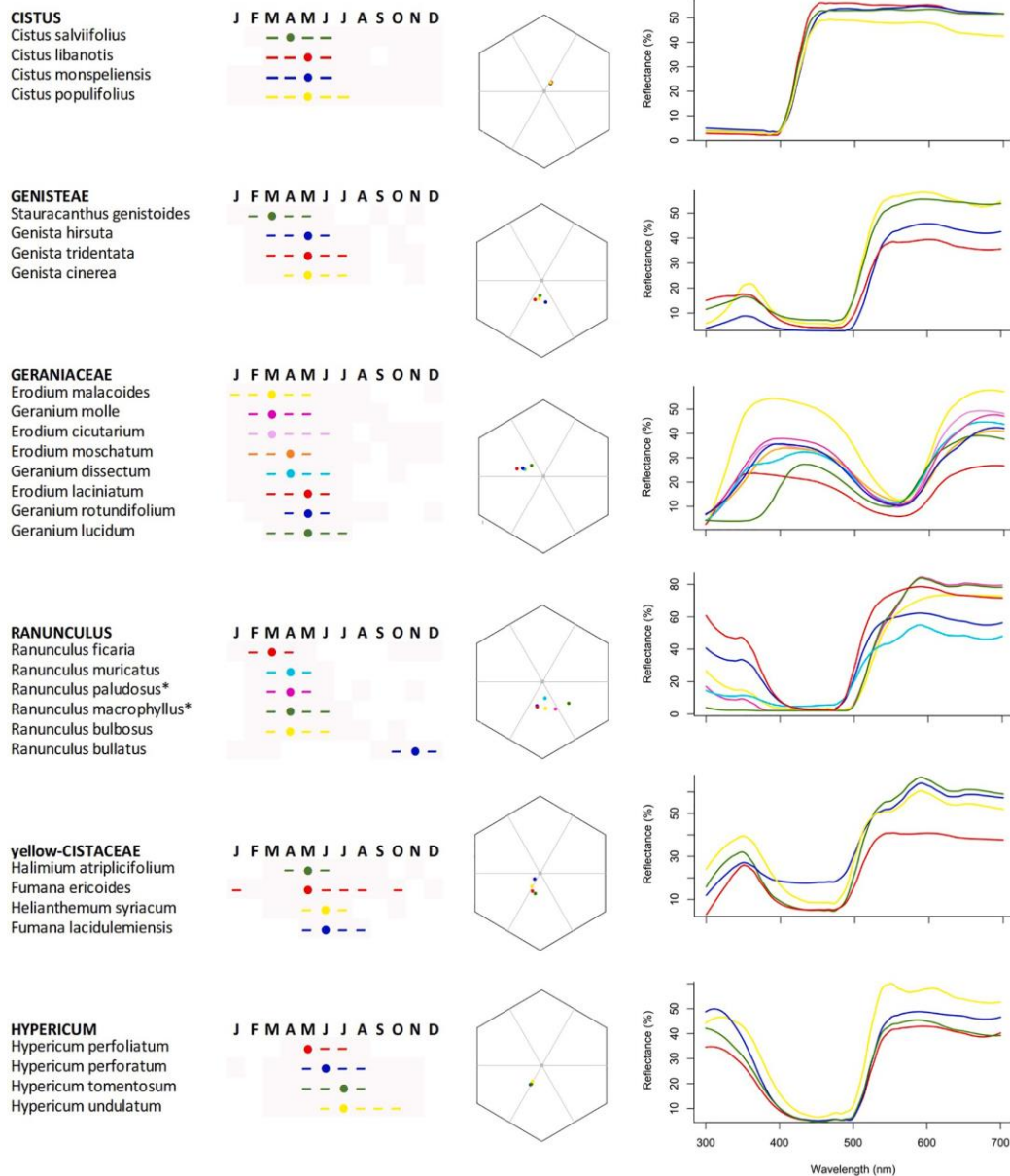


Fig. 4. Flowering pattern (left) of the 30 species studied in the Mediterranean shrubland vegetation (Sierra de Grazalema, Don˜ana, and Sierra de Aracena y Picos de Aroche natural parks, Andalusia, Spain), organised by groups of flowers with similar display and their respective colour loci in the be-hexagon (centre) and reflectance spectrum (right). Colour loci were calculated considering the photoreceptor sensitivity of *Apis mellifera*. Within each group, the same colour indicates the same species in the three graphs. In the phenological pattern (A) the grey area represents flowering activity, dashes the period of higher activity and the dots the peak of activity. *species with a colour distance of more than 0.2 hexagon units from more than two other species from the same group.

Table 1 Colour locus distances in the bee-colour hexagon between pairs of species (sp1 and sp2) that can be discriminated by bees promoting flower constancy (>0.2 HU). The expected probability of floral constancy is around 60% for pairs of species above 0.2 hexagon units (HU) distant from each other, and higher than 80% for distances over 0.4 HU (*) (Spaethe et al. 2001). Colour loci and distances were calculated using the spectral sensitivity of *Apis mellifera* and *Bombus terrestris*. We considered a total of 223 species pairs in the campo rupestre and 67 species pairs in the Mediterranean shrubland vegetation.

| Group | sp1 | sp2 | Distance (HU) | |
|---------------------------------|---|-------------------------------------|---------------|---------------|
| | | | <i>Apis</i> | <i>Bombus</i> |
| Campo rupestre | | | | |
| Asteraceae | <i>Chrysolaina desertorum</i> | <i>Lessingianthus linearifolius</i> | 0.21 | <0.2 |
| | | <i>Cyrtocymura scorpioides</i> | 0.21 | <0.2 |
| Xyris | <i>Xyris piranii</i> <i>Xyris Pilosa</i> | <i>Xyris itatiayensis</i> | 0.21 | <0.2 |
| | | <i>Xyris itatiayensis</i> | 0.25 | <0.2 |
| | | <i>Xyris asperula</i> | 0.21 | <0.2 |
| | | <i>Xyris</i> sp. | 0.21 | <0.2 |
| | | <i>Xyris hystrix</i> | 0.21 | <0.2 |
| | | <i>Xyris glauscescens</i> | 0.21 | <0.2 |
| Mediterranean vegetation | | | | |
| Geraniaceae | <i>Erodium laciniatum</i> | <i>Geranium lucidum</i> | 0.20 | 0.22 |
| Ranunculus | <i>Ranunculus macrophyllus</i> | <i>Ranunculus bullatus</i> | 0.43* | 0.23 |
| | | <i>Ranunculus ficaria</i> | 0.43* | 0.23 |
| | | <i>Ranunculus muricatus</i> | 0.33 | <0.2 |
| | <i>Ranunculus paludosus</i> | <i>Ranunculus bulbosus</i> | 0.32 | <0.2 |
| | | <i>Ranunculus bullatus</i> | 0.25 | <0.2 |
| | | <i>Ranunculus ficaria</i> | 0.25 | <0.2 |

4. Discussion

Our results showed a low probability of within-group flower colour discrimination in bee visual systems in both the Brazilian campo rupestre and the Spanish Mediterranean vegetation. Species of these two regional vegetation groups differed in their phenological strategies, only partially fitting our initial hypotheses, which are discussed in detail below. We demonstrated that flowering overlap was higher under the limited flowering season in the Mediterranean vegetation and with similar flower colour displays, while the tropical grassland campo rupestre with a long flowering season had large displacement in blooming times of species with similar flowers.

The fact that related co-occurring species maintain a similar colour may be due to a pollination advantage or phylogenetic inertia. However, in other communities, including the Mediterranean shrubland studied here, a lack of phylogenetic signal in flower colour has previously been reported (Arnold et al. 2009; McEwen & Vamosi, 2010; Shrestha et al. 2014; Ortiz et al. 2021). Flower colour is evolutionarily labile, and changes commonly occur due to mutations in the anthocyanin or carotenoid biosynthetic pathways; thus, the maintenance of new colour phenotypes is usually attributed to selection mediated by pollinators (e. g., Hopkins & Rausher, 2012; Irwin & Strauss, 2005; Narbona et al. 2018). The colour similarity within the studied groups strongly suggests an advantage for sharing a standardised visual signal, despite the possible negative effect of heterospecific pollen flow (Streher et al. 2020). Thus, similarity in flower colour within closely related coexisting groups could be maintained by selection. A test of the phylogenetic constraint of campo rupestre flower colour is underway, and preliminary analysis indicates similar results to those of Mediterranean vegetation (Camargo, and Morellato, unpubl.).

Our tests using *Bombus terrestris* visual modelling showed that those bees are unlikely to discriminate among species based on flower colour in the campo rupestre groups, while for the Mediterranean species, flower colour could be discriminated among a few Geraniaceae and *Ranunculus* species. When considering the *Apis mellifera* spectral sensitivity, only three campo rupestre species and three from the Mediterranean had colour distances greater than 0.2 HU relative to other species from their groups. Distances above 0.4 HU, which may permit flower constancy probabilities above 80% (Spaethe et al. 2001; Dyer and Chittka 2004), were observed only within the Mediterranean *Ranunculus* group (six out of 15 pairs of species). *Ranunculus* spp. is an exceptional group, as flower colour is due to a rare combination of petal structure and pigmentary colouration that provides a strong visual signal to pollinators (van der Kooi et al. 2017). Therefore, *Ranunculus* may have a higher potential for floral colour shift than the other genera studied.

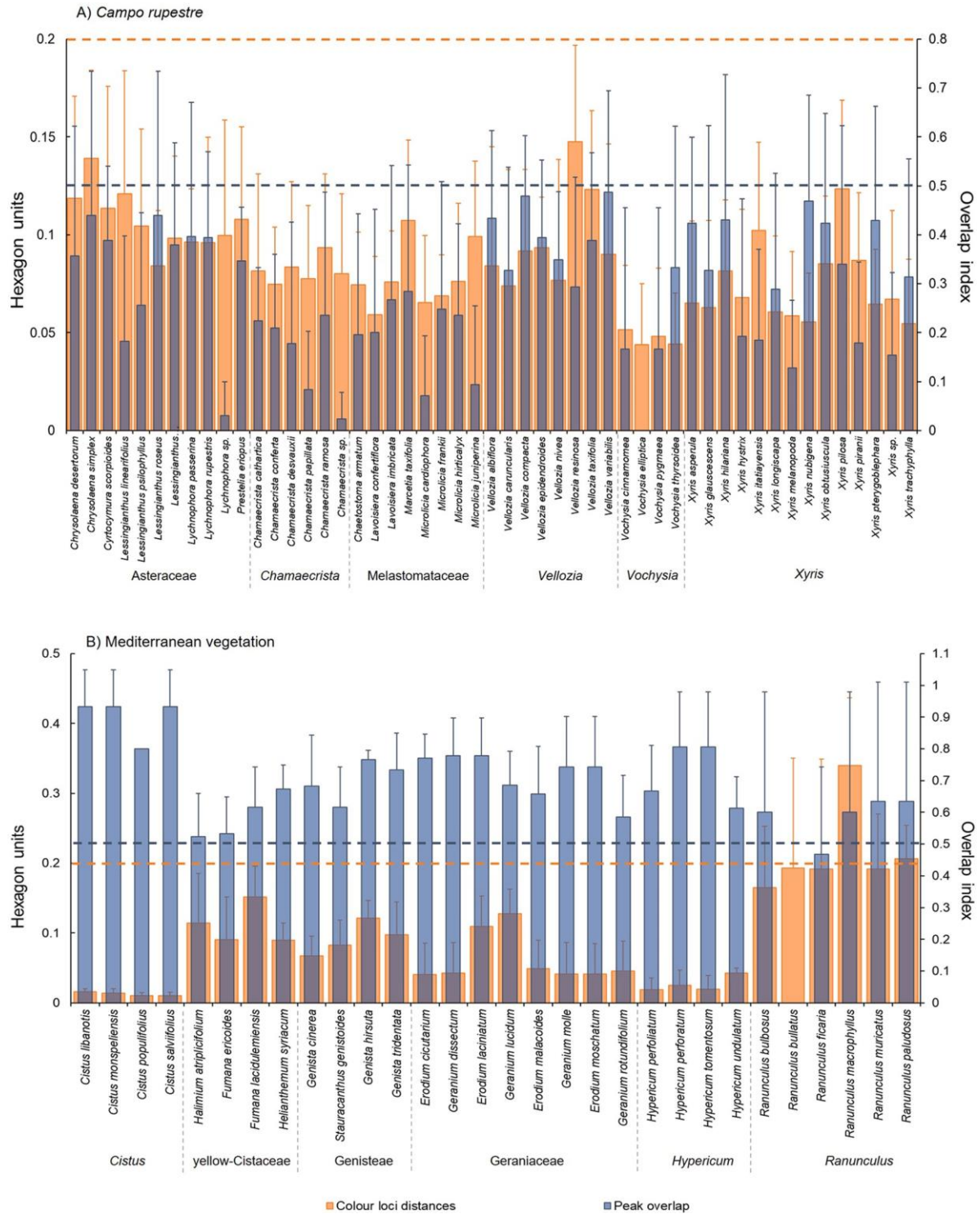


Fig. 5. Mean colour loci distances (+ standard error) in the bee hexagon and mean index of phenological overlap in the flowering peak between a given species and all of the other species from the same group in the campo rupestre, Brazil (A), according to the photoreceptor sensitivity of *Bombus terrestris*, and in the Mediterranean vegetation, Spain (B), according to the photoreceptor sensitivity of *Apis mellifera*. Note that the scales of the Y-axis differ between (A) and (B). Orange dashed lines indicate the threshold of 0.2 hexagon units above which two colours can be discriminated by bees to promote flower constancy. Blue dashed lines indicate a flowering overlap of 0.5, that is, an intermediate phenological overlap. An overlap index value of 0 indicates no overlap and 1 indicates total overlap between pairs of species. The mean colour locus distances for campo rupestre and Mediterranean plant species calculated according to the photoreceptor sensitivity of *A. mellifera* and *B. terrestris*, respectively, are provided in the Supplementary material Appendix 1, Fig. A2.

Table 2

Observed and simulated mean pairwise overlap of the flowering peak among species within each group and the probabilities of competition (tendency of overlapped peaks) or facilitation (tendency of staggered peaks) for campo rupestre and Mediterranean shrubland vegetation. Competition: simulated overlap is lower than the observed; facilitation: simulated overlap is higher than the observed.

| Vegetation | Group | Mean overlap | | Probability | | Tendency |
|----------------|------------|--------------|-----------|--------------|-------------|------------|
| | | observed | simulated | facilitation | competition | |
| Campo rupestre | Asteraceae | 0.32 | 0.29 | 0.09 | 0.91 | overlapped |

| | | | | | | |
|--------------------------|---------------------|------|------|------|------|--------------|
| | <i>Chamaecrista</i> | 0.16 | 0.21 | 0.96 | 0.04 | staggered |
| | Melastomataceae | 0.20 | 0.23 | 0.82 | 0.18 | staggered |
| | <i>Vellozia</i> | 0.39 | 0.26 | 0.00 | 1.00 | overlapped |
| | <i>Vochysia</i> | 0.17 | 0.19 | 0.51 | 0.52 | inconclusive |
| | <i>Xyris</i> | 0.30 | 0.20 | 0.00 | 1.00 | overlapped |
| Mediterranean vegetation | <i>Cistus</i> | 0.90 | 0.33 | 0.00 | 1.00 | overlapped |
| | yellow-Cistaceae | 0.59 | 0.26 | 0.01 | 1.00 | overlapped |
| | Genisteae | 0.70 | 0.33 | 0.03 | 0.98 | overlapped |
| | Geraniaceae | 0.72 | 0.32 | 0.00 | 1.00 | overlapped |
| | <i>Hypericum</i> | 0.72 | 0.33 | 0.01 | 0.99 | overlapped |
| | <i>Ranunculus</i> | 0.49 | 0.26 | 0.02 | 0.98 | overlapped |

When considering flowers with UV-reflecting petals, we found more variations in the reflectance of the UV band than in the red, blue, or green bands. Differences in UV reflectance are not perceived by the human eye, but they are important to bees, and differences in UV patterns among flowers can be distinguished by bees (Narbona et al. 2021). Differences in UV patterns were found mainly in Mediterranean species. For example, *Genista hirsuta* within the Genisteae group or *Hypericum perforatum* within the *Hypericum* group may be distinguishable by bees based on UV patterns. However, although bees perceive variation in UV patterns (Horth et al. 2014; Koski and Ashman 2015), more studies are necessary to verify the influence of UV patterns on flower constancy (but see Rae and Vamosi 2013).

As expected, almost all of the Mediterranean species studied overlapped in flowering period, likely due to the short duration of the season that is favourable for flowering. Flowering overlap represents a strong selective pressure on flower colour diversification as a potential way to avoid competition for pollinators (Dyer and Chittka 2004, van der Kooi et al. 2016). However, contrary to our initial hypothesis, except for some species with distinct UV patterns or structural colour, almost no plant species showed flowers differing by more than 0.2 HU, making most of the flowers likely indistinguishable to bees. One explanation for the maintenance of this visual signal is that most Mediterranean species gain by facilitating each other through flower signal standardisation (Fig. 1B, and see Lunau & Wester 2017). The pollination benefits promoted by signal standardisation may outweigh the potential negative effects of interspecific competition (Moeller 2004, Hegland and Totland 2005; Benitez-Vieyra et al. 2007, Bergamo et al. 2020), a matter deserving further investigation. Moreover, there are other floral traits that are not considered in this study, such as odour, that also influence flower discrimination (Dötterl and Vereecken 2010) among species whose flowers are similar in appearance. If pollinators can differentiate flowers based on these other traits, less selective pressure is expected on flower colour differentiation.

In the campo rupestre, flowering patterns were more diversified and did not always segregate as expected; flowering was segregated for *Chamaecrista* and Melastomataceae species, inconclusive for *Vochysia*, and overlapped for Asteraceae, *Vellozia* and *Xyris*, although for all groups, the mean overlap probability was generally low. As expected, the flowers of the campo rupestre were generally not discriminable under bee visual systems (<0.2 HU). Pollinators could use this standardised visual signal to locate flowers efficiently, but if they cannot distinguish between species, they could perform interspecific flights, which is disadvantageous to plants. However, the species showed a tendency to segregate flowering, suggesting facilitation as the likely mechanism (Fig. 1C), enabling the avoidance of interspecific competition for pollinators (Mosquin 1971; Waser and Real 1979; Moeller 2004, Jensen et al. 2019).

We have found some exceptions to the general hypothetical scenarios described for the Mediterranean vegetation that most species with similar flowers probably facilitate each other by signal standardisation and overlapped flowering (Fig. 1B) and for the campo rupestre, where species may facilitate each other by signal standardisation and segregated flowering phenology (Fig. 1C). For example, in the Mediterranean shrubland, *Ranunculus macrophyllus* is likely discriminated by bees from other *Ranunculus* species, increasing the chances of flower colour constancy even if flowering overlaps, corresponding to the hypothetical scenario described in Fig. 1B. In the campo rupestre, congeneric pairs such as *Xyris longiscapa* and *X. asperula*, *Lavoisiera confertiflora* and *Limbricata*, and some Asteraceae species may facilitate each other's pollination by signal standardisation among co-flowering species (hypothetical scenario described in Fig. 1B). If we examine species frequency for each study site (Supplementary material Appendix 1, Table. A1), it is evident that some of these species appear at a low frequency in their communities. Rare species that offer few rewards for pollination may benefit from resembling common co-flowering species, since that similarity increases the pollinators' encounters with plants from the same group species (Gumbert et al. 1999; Moeller 2004, Lunau and Wester 2017; Coetzee et al. 2021). It would be valuable to confirm our results based on the spectral sensitivity of bees using direct observations in the communities.

Species whose flower colours are perceived similarly by bee vision were more frequent in the campo rupestre than in Mediterranean vegetation, and also belonged to the most common and highest-diversity taxa. Asteraceae, Melastomataceae, *Chamaecrista*, *Vellozia*, and *Xyris* are among the 10 most diverse families and genera of campo rupestre vegetation (Mattos et al. 2019). In addition, the centre of diversification of Velloziaceae, *Xyris*, *Chamaecrista*, and *Lavoisiera* (Rapini et al. 2008; Wanderley, 2011) is in the campo rupestre. We suggest that facilitation by signal standardisation, along with the general tendency of temporal flowering displacement, may explain the maintenance of similar flower displays in the most diverse taxa of the campo rupestre. Future experimental studies that evaluate bee foraging among species, facilitation and fitness of related species, along with phylogenetic constraints in flowering phenologies, are needed to confirm such assumptions.

5. Conclusions

Considering the low probabilities of flower colour discrimination found within the studied groups, we argue that facilitation by signal standardisation could benefit most species, contributing to the maintenance of species with similar flowers in the studied communities regardless of the temporal flowering strategies. Although Mediterranean species may benefit from rapid recognition by bees through an increased signal during the short flowering season, species from the Brazilian

campo rupestre may benefit from the staggered flowering phenology of species with similar flowers by (i) avoiding interspecific competition, (ii) maintaining resource and pollinator availability, and (iii) maintaining a recognised flower signal for bees over time. Therefore, despite the expected selective pressure for flower differentiation, standardised flower signals can be advantageous (Lunau et al. 2017, 2020) and should be considered in research on the evolution of plant species and communities.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.flora.2023.152312](https://doi.org/10.1016/j.flora.2023.152312).

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