Relationship between floral colour and pollinator composition in four plant communities

Sara Reverté Saiz

Màster en Ecologia Terrestre i Gestió de la Biodiversitat; especialitat en Ecologia Terrestre

Jordi Bosch[1] i Javier Retana[1, 2]

[1] CREAF, Centre de Recerca Ecològica i Aplicacions Forestals, Universitat Autònoma de Barcelona, Bellaterra E-08193, Espanya

[2] Unitat d'Ecologia, Universitat Autònoma de Barcelona, Bellaterra E-08193, Espanya

16 de Setembre de 2014

The present study started on February 2014. I was provided with the database of pollinator censuses I needed for the study. My contribution to this work has been: 1) Conducting fieldwork along the flowering season to obtain the flower samples and also performing censuses to expand the database; 2) conducting flower colour measurements with the spectrometer; 3) definition of the objectives and methodology (with supervisors' advice); 4) analysis and interpretation of results (with supervisors' advice); 5) writing of the document (with supervisors' advice).

The manuscript has been formatted attending to the guidelines provided by the journal *Proceedings of the Royal Society B- Biological Sciences.*

Title: Relationship between floral colour and pollinator composition in four plant communities

Abstract

Pollinators use a variety of floral cues to locate resources, but the relative importance of these different cues on pollinator foraging decisions is unclear. However, floral colour is undoubtedly one of the most important, as evidenced in previous works revealing the importance of floral colour on pollinator choices and determining flower visitor composition. Our purpose is to establish whether there is a relationship between flower colour and pollinator composition in natural communities. We measured the floral reflectance spectrum of 109 plant populations, from 300 to 700 nm, and divided it in four broad bands of 100 nm each. We found a phylogenetic signal on floral colour variables, and considered this in our analyses. Our results show a lack of concordance between colour spectra and pollinator assemblages. Nevertheless, this work proves that colour determines plant-pollinator relationships in some degree. Thus, particular pollinator groups display preferences for certain bands of the colour spectrum. These preferences match those expected from the pollination syndromes theory. Future work on this issue should combine floral colour along with other floral traits, to assess the importance of the combination of the main floral features for determining pollinator attraction and behaviour together.

Keywords: colour preferences, flower colour, phylogenetic signal, pollinator composition, pollination syndromes.

Introduction

Pollinators use floral cues such as odour, shape and colour to locate pollen-nectar sources [1]. These same cues allow pollinators to discriminate between different flower species and to maintain flower constancy (or fidelity) within and between foraging bouts [2–4]. Although the relative importance of these different cues on pollinator foraging decisions is unclear, some studies have demonstrated that certain pollinators rely more strongly on colour than fragrance cues [5–7]. Pollinators may show innate preferences for certain colours [8,9]. In addition, colours may be used by pollinators as signals of floral rewards, so that initial innate preferences may be modulated by subsequent associative learning [10,11]. Thus, the role of colour in determining ultimate flower choice will depend on the interaction between innate and learned colour preferences.

Pollinators have well-developed colour vision, which in most cases covers a wider range of the spectrum than human vision. The vast majority of pollinators in temperate regions are insects, and different visual models have been found for different groups [12,13]. The best studied species is the honeybee (*Apis mellifera*), with a trichromatic visual system. Honeybee vision ranges between 300 and 700 nm with its three receptors peaking at the UV, blue and green parts of the spectrum (corresponding to 344, 438 and 560 nm respectively). Most other studied insect groups share a trichromatic visual system, but there are also known di-chromatic (certain flies and beetles) and tetrachromatic models (butterflies) [14]. The trichromatic state is supposed to be ancestral, and the loss or gain of photoreceptor types is secondary [14]. Insect colour vision appeared long before the emergence of Angiosperms [15]. Therefore, it is believed that flowers tuned their visual signals to the sensory system of pollinators, thus becoming as conspicuous as possible to them.

Traditionally, colour has been considered an essential cue in determining flower choice by pollinators. To this extent, colour constitutes one of the main traits used in pollination syndrome theory [16], according to which flowers have adopted different suites of traits as an adaptation to attract different pollinator functional groups. Such a view implies that unrelated plant species adapted to the same pollinators show convergence of floral traits, including colour. For example, bee-visted flowers are expected to be blue or violet and moth flowers are expected to be light-coloured [16]. In agreement with this view, flower colour is considered by some authors to be an important predictor of pollinator group [17]. However, this view is highly controversial. Although some pollinators are known to present innate floral colour preferences, these preferences do not always match the colour expected from pollination syndromes [8]. In most cases, colour preferences are displayed at a finer taxonomic level, such as genus or species, rather than at the functional group level. A number of studies have analyzed pollinator choices among differently coloured flower species within a genus or between colour morphs within a species. Some of these studies have found evidence of the importance of flower colours in determining flower visitor composition [18–21], but others have not [22,23]).

Even if pollinators show preferences for certain colours, and even if colours reflect reward levels, the role of colour as a driver of ultimate flower choice in natural communities may be difficult to establish for several reasons. First, most plants are pollinator generalists, attracting a wide array of pollinators from different functional groups [24]. Second, in a natural setting, flower choice may be influenced by the presence and abundance of other pollinators potentially competing for flower resources [25]. Pollinators show plasticity at the individual level, and are known to switch plants in response to changes in pollen-nectar levels irrespective of colour [26]. In sum, ultimate flower choice may be highly context-dependent [27]. A handful of studies have analysed the influence of floral colour on pollinator partitioning at the community level [20,28–31], and most of them conclude that floral colour is an important cue. However, colours in these studies were categorized from a human point of view. Waser et al. [24] analyzed pollinator partitioning in a community context and characterized colour based on wavelength measures. In their analysis, the association between floral colour and pollinator group composition narrowly failed significance. Clearly, further research on this topic is badly needed.

The aim of this study is to establish whether there is a relationship between flower colour and pollinator composition in natural communities. To do this, we studied four plant communities (85 species, 109 populations). The four communities were close to one another, and were exposed to similar climatic conditions. Therefore, they shared the same regional pool of pollinator species and pollinator groups. For each flower population, we measured the floral reflectance spectrum and conducted pollinator surveys. The association between flower colour and pollinator composition may be affected by phylogenetic constraints. Closely-related plant species may have similar colours based on shared evolutionary history (e.g., due to similar floral pigments). Therefore, our analyses account for phylogenetic relatedness. We ask the following questions: 1) Is flower colour in our sample of flower species phylogenetically constrained? 2) Are plant species with similar colours visited by similar arrays of pollinators? 3) Do different pollinator functional groups show preferences for certain colours?

Material and Methods

Study area

We conducted our study in four Mediterranean communities near Barcelona (NE Spain), whose coordinates are given in Table 1. The first community (CA) was a herbaceous grassland, dominated by *Hyparrhenia hirta* and *Brachypodium retusum,* located in Canet de Mar. The second community (CO) was a grassland dominated by herbaceous (*Hyparrhenia hirta, Foeniculum vulgare*) along with some shrubby species (*Cistus monspeliensis, Spartium junceum*), located in Collserola Natural Park. The third community (GA) was a Mediterranean scrubland dominated by *Quercus coccifera*, *Rosmarinus officinalis* and *Pistacia lentiscus*, located in the Garraf Natural Park. The last community (PA) was another Mediterranean scrubland dominated by *Quercus coccifera, Ulex parviflorus* and *Pistacea lentiscus*, located in Roques Blanques near El Papiol. Mean distance between the four sites is 36 km (range: 10-66 km). The climate of the area is Mediterranean, with a strong sea influence. Summers are dry and most precipitation occurs in spring and autumn. Weather conditions are very similar across the four sites (Table 1).

Pollinator assemblages

Pollinator data were obtained throughout the main general flowering period of the area (February-July). Very few species are in bloom during the summer drought. Each species was surveyed on several days covering its entire flowering period. In CA and CO, pollinator surveys were carried out by slowly walking through 25- to 50-m-long x 1-m-wide vegetation transects. This was done several times, from early morning to late evening on every sampling day. In GA and PA, selected individuals of each plant species in bloom were observed during 4-5-minute periods every 1-2 h. All insects observed foraging on the flowers were identified by sight and recorded. Pollinators were assigned to one of six taxonomic groups: bees, ants, wasps, coleopterans, dipterans and lepidopterans. From these surveys, we characterized the pollinator assemblage (proportion of visits from each group) of 17 plant species in CA, 46 in CO, 25 in GA and 21 in PA (total: 109 populations, 85 species; some species were present in more than one community).

Flower colour measurement

Flowers of each population were collected and transported with a portable cooler to the laboratory, where they were temporarily placed in a cold room at 4ºC. Flower reflectance spectra were obtained using an USB4000 spectrometer with a USB-DT deuterium tungsten halogen source (Ocean Optics Inc., Dunedin, FL, USA) connected to a computer running SpectraSuite (Ocean Optics). The light spectrum analyzed ranged from 300 to 700 nm divided into 0.22 nm intervals, and the spectrometer sensor was fixed at an angle of 45º from the measuring area. Petals were mounted on an adhesive tape to obtain a relatively flat surface, thus minimizing reflectance variability due to uneven distances between the petals and the sensor. For small flowers, we had to use several petals from different flowers to cover the entire measuring area. Some flowers had corollas with parts displaying two or more clearly different colours. In these cases, we took colour measures of the different parts separately. To obtain a single colour measure for these flowers, we then calculated a weighted mean (according to the surface occupied by each part in the corolla). Most measures were taken on the day of flower collection, but a few were taken the day after.

Spectrometer outputs showed a certain amount of noise. To minimize noise and obtain smoother reflectance spectra we used the software Avicol [32] to clean negative values and correct by triangular smoothing. The latter correction is based on a floating mean with weights, with a window size of 15 nm. Sample size was 4-5 measures per population, except for three populations for which we could only obtain one measure. Different measures of a population were averaged to obtain a single measure per population.

Colour characterization

Different pollinator groups are known to have different visual systems (different numbers of photoreceptors and peak sensitivities). Using the reflectance spectrum we avoid biasing our results towards any visual system. We characterized flower colour based on three different sets of variables (I) First, we used the nine colour categories in Chittka et al.[33], representing the main types of floral reflectance spectra in a natural reserve in Germany. Although they did not provide a name for each category, we named floral colours as perceived by humans with the addition of UV where necessary (Appendix 1). (II) Second, we obtained three commonly used [34] colour properties: brightness, chroma and hue. Brightness, calculated as the sum of the reflectance values over the entire spectrum, represents an achromatic value of reflection of the sample. Chroma, calculated as the difference between the maximum and the minimum values of reflectance between the average reflectance of the spectrum, is a measure of spectral purity. Hue, defined as the wavelength with the highest value of reflectance, represents the degree to which a stimulus can be described as similar to, or different from, stimuli that are described as red, green, blue, or yellow. (III) Third, since the majority of the inflection points in floral reflectance spectra are located near 400, 500 and 600 nm [35], we divided the floral reflection spectra in 4 broad bands of 100 nm each (300-400, 400-500, 500-600, 600-700 nm) following Chittka et al. [33]. We then calculated the proportion of the reflectance spectra that was contained within each band (henceforth colour composition), obtained by dividing the brightness of each of the four bands by the total brightness of the sample. By using the proportion, instead of the raw values of brightness, of each band we avoid differences between chromatically equivalent spectra, i.e., spectra with the same shape, but differences in brightness. Each flower colour can then be characterized by the proportion of each of the four bands (henceforth colour band variables %350, %450, %550 and %650). Roughly, the first band corresponds to the UV part of the spectrum, the second to the blue-violet, the third to the green-yellow and the last one to the orange-red. It is important to bear in mind that some colours as perceived by humans may result from the combination of two or more bands. For example, white flowers, reflect from 400 to 700 nm; yellow flowers, reflect from 500 to 700 nm; and pink flowers, usually reflect in the blue and the red parts of the spectrum with a variable proportion in the yellow part (Appendix 1).

Data analysis

Phylogenetic signal of colour variables

To know whether colour was phylogenetically constrained, we constructed a phylogenetic tree of the 85 species with Phylocom [36] with family names following the Angiosperm Phylogeny Group classification [37]. We used the “bladj” function in Phylocom to achieve an ultrametric rooted tree. Polytomies generated by the program were hand-resolved. All distances between families (assessed as millions of years of divergence) and some distances between genera were obtained from the database www.timetree.org [38]. Additional distances between genera and distances between species were extracted from the literature [36,37(Fabaceae), 38(Cardueae), 39(Ericales)]. The resulting tree (henceforth regional tree) is shown in Appendix 2.

We tested for the presence of phylogenetic signal in all colour variables (brightness, chroma, hue, %350, %450, %550 and %650) with the Blomberg's K test [43]. This test compares the actual distribution of traits on the obtained phylogenetic tree with those on trees generated under a Brownian-motion model, and calculates significance through permutations. Blomberg's K performs better than other indices due to its sensitivity to small changes in the distribution of the analyzed traits [44]. K values lower than 1 suggest lack of phylogenetic signal of the trait, while values around 1 suggest an evolution of the trait fitting the Brownian motion model and thus occurrence of phylogenetic signal. This test was done for each community separately and for the 85 species lumped together. These analyses were performed with the "phylosig" function of the package "phytools" developed by Liam Revell [45] for the R Statistical Software [46].

Relationship between flower colour descriptors and pollinator composition

To determine whether plants with similar colour properties attract similar pollinator assemblages, we conducted partial Mantel tests between distance matrices of colour traits and of pollinator assemblages, with a phylogenetic distance matrix as covariable. We conducted four partial Mantel tests, one with colour composition (proportions of the four spectrum bands), one with brightness, one with chroma, and one with hue. We used Bray-Curtis distances between pairs of species for pollinator composition and colour composition, and Euclidean distances between species for brightness, chroma, and hue. Tests were performed with the function "mantel.partial" in the package "vegan" for R [47]. This analysis was done for each community separately and for the 109 populations lumped together. In the latter case, we included all populations in the regional tree and assigned a low value of divergence (1000 years) to populations of the same species.

Relationship between colour bands and pollinator groups

Irrespective of a potential association between flower colour composition and pollinator composition, certain pollinator groups could show a preference for certain colour bands. This would not be detected in the Mantel test described above, which uses composite colour and pollinator variables. To explore potential pollinator group – colour band associations, we performed Canonical Correspondence Analyses (CCA) with the pollinator assemblage of each population and the four colour bands. CCA is a multivariate analysis technique that detects the patterns of variation in a dataset that can be best explained by combinations of the explanatory variables [48]. Again, this was done for the four communities separately and together. CCAs were performed using the function “CCorA” in the package “vegan”.

Results

The total amount of plant-pollinator interactions recorded was 32,315. Most of the pollinators recorded were bees, accounting for nearly half of the overall interactions. The second most frequent group were coleopterans, with 21% of the interactions, followed by ants (15%) and dipterans (9%). The least important groups in terms of number of interactions were lepidopterans and wasps (3.5% and 2.5%, respectively).

The four communities presented roughly similar patterns of floral colours (Appendix 1). The most common floral colour in the four communities was pink (30 to 50% of the species), followed by white (16-29%). UV-yellow flowers were also well represented (14-24%), although they were lacking in GA. Yellow (12- 15%), purple (4- 9%) and green (4- 6%) flowers were less frequent.

Phylogenetic signal in colour variables

Blomberg's tests revealed significant phylogenetic signal in many of the colour traits tested (Table 2), that is, phylogeny appears to impose certain constraints on flower colour. All communities showed phylogenetic signal in two or more colour variables. Variables showing phylogenetic signal were similar in CO, GA, PA (Hue, %350, %450), but, surprisingly, CA showed a completely opposite pattern. When analyzing the four communities together, all colour variables showed a strong phylogenetic signal.

Relationship between flower colour descriptors and pollinator composition

Plants with similar flower colour characteristics did not attract similar pollinator assemblages in three of the four communities or overall (Table 3). Only in one of the four communities, CA, there was a significant association between pollinator composition and two colour properties (brightness and hue).

Relationship between colour bands and pollinator groups

CCAs revealed clear associations between certain pollinator groups and certain colour bands (Fig. 1). Visual inspection of the resulting biplots revealed that some of these patterns were relatively consistent across the four communities (Table 4). Bees were positively linked to the UV-blue bands (350-450) of the spectrum, except in CA where they were not clearly related to any band. Ants, wasps and dipterans showed similar colour preferences. In general, they were positively associated with the yellow band (550). In some communities these groups were also associated to the UV-blue bands (350-450), but this relationship was sometimes positive and sometimes negative. Coleopterans and lepidopterans were associated to various colour bands, but these associations were not consistent across communities. When analyzing the four communities together, colour axes were well-defined and the four colour bands were distinctly separated (Fig. 1). The first axis was defined by yellow (550, negative values) and blue (450, positive values). The second axis was defined by the two extreme bands in the spectrum, UV (350) and red (650). Bees were positively associated with the blue and UV bands, ants, wasps and dipterans with the yellow band, coleopterans with the red band, and lepidopterans with the blue and red bands (Fig. 1; Table 4).

Discussion

Our results contrast with other studies analysing phylogenetical constraints on floral colour. Most of previous studies have found floral colour to be a highly labile trait, with low phylogenetic signal. Smith et al. (2008) analyzed variation in brightness, chroma and hue within a plant clade and found no phylogenetic signal. Other studies have analysed phylogenetic clustering on floral colour at the community level and have also found lack of phylogenetic signal [50–52]. In our study, phylogenetic signal at the community level was weak but consistent in three of the four communities studied. Phylogenetic constraints became clearly apparent in all the colour properties considered when plants of all four communities were lumped together. These results indicate that colour changes fit a Brownian-motion evolution model, implying that expected phenotypic differences between species increase with phylogenetic distance. Some studies show that the capacity to produce particular flower pigments has been lost in entire lineages [53], thus providing a biochemical mechanism for the phylogenetic signal found in our study.

Notwithstanding phylogenetic constraints, the evolution of floral colour may be driven by selective forces exerted by various factors. Traditionally, pollinators have been considered to be the main drivers of floral colour evolution. This belief gained momentum when it became clear that insect pollinator colour vision predated angiosperm radiation, implying that flowers evolved flower colours so as to become as conspicuous as possible to pre-existing pollinators [14]. Later studies suggest that many flower lineages have converged to display floral colours in areas of the spectrum maximizing discrimination by Hymenoptera, both in the northern [33,35] and the southern hemispheres [54]. Surprisingly, however, there is still very little evidence on the potential selective pressures exerted by pollinators on floral colour [55].

In addition to pollinators, flower predators may also be important drivers of colour evolution. Then, plants would face a trade-off between becoming more conspicuous to pollinators, while reducing conspicuousness to herbivores and parasites, which usually are insects and therefore have similar visual capabilities to pollinators [56]. In such a scenario, flower colour might be under opposing selective pressures. A situation in which opposing selective forces have seemingly resulted in a neutral balance has been described in *Raphanus* [57]. White and yellow *Raphanus* morphs were the most visited by pollinators and were found to be under positive selection. However, these same morphs were under negative selection by herbivores because they were the morphs with lowest concentrations of chemical defences, thus counterbalancing pollinator selective pressure. Floral colour may also evolve in response to abiotic factors, such as solar radiation and drought. Light colours may be advantageous in dry environments, where dark colours may absorb radiation in excess and raise petal temperatures, thereby increasing water loss [58].

If pollinators are an important selective force behind the evolution of floral colour, we would expect a close association between flower colour properties and pollinators. Some studies have shown that achromatic colour properties are not perceived by many insect groups [59]. That is, insects can easily discriminate objects differing only slightly in reflectance spectrum, but cannot discriminate between objects with similar reflectance spectrum differing only in brightness [59]. Therefore, we would expect the association between pollinator composition and colour to be weaker for achromatic than chromatic variables. In agreement to these expectations, our results indicate lack of correspondence between pollinator composition and brightness (achromatic variable). As for chromatic variables, we obtained different results when we analysed colour composition (lack of correspondence when considering all colour bands together) versus each colour band separately (consistent relationships).

Several factors may contribute to explain the lack of correspondence between pollinator composition and colour composition in a field situation. First, most of the plant species in our study communities are pollinator generalists, and therefore are visited by various pollinator groups [29,60]. This seems to be a general trend in temperate plant-pollinator communities [24,61]. Second, in a natural setting, pollinator innate colour preferences may be overridden by learned preferences. It is believed that innate preferences may initially drive flower choice, but pollinators do not restrict foraging to a unique colour pattern and may subsequently switch to other flower colours if these are associated to greater floral rewards [26,62]. In other words, pollinator foraging behaviour is flexible and preferences acquired through associative learning may override innate preferences. This is not to say that innate preferences are not important. Pollinators may still have a tendency to explore flowers with colour properties that can be easily detected by their visual system. Of especial importance is the ability to detect flowers against the background (usually green). Some studies have shown that pollinators forage more efficiently (are able to visit more flowers per unit time) when they forage on flowers of certain colours [63].

Due to the lack of specialisation by both plants and pollinators, establishing pollinator-colour associations may be easier when considering pollinator groups separately (instead of pollinator assemblages) and specific colour bands (rather than the entire spectrum). This possibility was explored with Canonical Correspondence Analysis (CCA), which yielded some notable coincidences among communities. The overall CCA plot generated four quadrants clearly defined by the four colour bands. The upper-left quadrant included UV-yellow flowers. Ants, dipterans and wasps were linked to the yellow band, with dipterans also showing a slight affinity to the UV band. The upper-right quadrant included purple flowers reflecting in the UV. Bees were strongly linked to this quadrant. Interestingly, closer exploration of the bee data revealed differences between small (< 12 mm) and large (> 12 mm) bees (results not shown here). Small bees showed a stronger affinity to the UV band, while large bees were mostly related to the blue band. The lower-right quadrant included blue-red reflecting flowers (pink to human vision). Lepidopterans were tightly related to this colour pattern. Finally, the lower-left quadrant included yellow flowers not reflecting in the UV part of the spectrum. White flowers are related to negative values of the second axis of the biplot, halfway between the yellow and the blue part of the spectrum along with reflection in the red part. Coleopterans were linked to white, slightly tending to the yellow quadrant. Our ability to detect a correspondence between certain pollinator groups and certain colour bands but not between pollinator composition and overall colour composition is congruent with results found on colour preferences by specific pollinators. Working with *Eristalis tenax*, Lunau [64] found that inexperienced flies land only on human-yellow stimuli, in the small range of wavelengths from 520 to 600 nm.

Interestingly, the above-mentioned pollinator-colour associations are quite consistent with colour preferences described in the pollination syndromes, according to which bees favour blue flowers, flies yellow and white, lepidopterans pink and red, beetles white and cream and wasps favour brown and yellow [9,16]. Other studies have also found similar consistencies. Waser *et al*. [24] explored the association between pollinator groups and bands of the colour spectrum in a natural community in Germany. Their results narrowly failed significance, but the observed trends were consistent with pollination syndromes. Other community studies have also found associations consistent with pollination syndromes [20,28,31]. The pollination syndrome has been highly contested and their ability to predict plant-pollinator associations has been strongly questioned [24,61,65]. However, a recent meta-analysis provides support for the existence of pollinator groups being associated to suites of floral traits [66], and pollination syndromes are still considered by many authors to be a valid framework for the evolution on plant-pollinator interactions [17,67].

Our study provides some evidence of pollinator-colour associations in natural communities, and highlights the fact that these associations are based on particular colour bands, rather than on entire colour patterns. However, flowers are not only coloured spots against a dull background. To fully understand mutualistic relationships between plants and pollinators, flowers have to be assessed as a whole. Future work should include other floral traits potentially influencing pollinator attraction and behaviour (fragrance, shape, size, corolla depth), as well as floral rewards (pollen and nectar). Such an integrative approach may reveal association between floral traits and certain pollinator groups, and provide a more complete answer to the long-asked question of how do pollinators choose flowers.

Acknowledgements

I want to thank Marta Escolà, Anselm Rodrigo, Sergio Osorio, Anna Torné, Helena Barril, Guillem Bagaria and Mercè Galbany for support in any of the phases. I am also grateful to Víctor Flo for valuable discussions and technical help. I am very grateful to José Maria Gómez for his constant help and statistical advice, and also for kindly providing the spectrometer, without which this work would not have been possible. Finally, I want to thank my directors, Jordi Bosch and Javier Retana, for providing me full support and enthusiasm, and for patiently teaching me much about ecological processes and pollination.

References

1. Chittka, L. & Raine, N. E. 2006 Recognition of flowers by pollinators. *Curr. Opin. Plant Biol.* **9**, 428–435. (doi:10.1016/j.pbi.2006.05.002)

2. Heinrich, B. 1976 The foraging specializations of individual bumblebees. *Ecol. Monogr.* **46**, 105–128.

3. Goulson, D., Stout, J. C. & Hawson, S. A. 1997 Can flower constancy in nectaring butterflies be explained by Darwin's interference hypothesis? *Oecologia* **112**, 225–231.

4. Goulson, D. & Wright, N. P. 1998 Flower constancy in the hoverflies Episyrphus balteatus (Degeer) and Syrphus ribesii (L .)(Syrphidae). *Behav. Ecol.* **9**, 215–219.

5. Dafni, A., Bernhardt, P., Shmida, A., Ivri, B. Y., Greenbaum, S., O'Toole, C. & Losito, L. 1990 Red bowl-shaped flowers : convergence for beetle pollination in the Mediterranean region. *Isr. J. Bot.* **39**, 81–92.

6. Heiling, A. M., Herberstein, M. E. & Chittka, L. 2003 Crab-spiders manipulate flower signals. *Nature* **421**, 334.

7. Omura, H. & Honda, K. 2005 Priority of color over scent during flower visitation by adult Vanessa indica butterflies. *Oecologia* **142**, 588–96. (doi:10.1007/s00442-004-1761-6)

8. Lunau, K. & Maier, E. J. 1995 Innate colour preferences of flower visitors. *J. Comp. Physiol. A* **177**, 1–19.

9. Willmer, P. 2011 *Pollination and floral ecology*. New Jersey: Princeton University Press.

10. Goyret, J., Pfaff, M., Raguso, R. a & Kelber, A. 2008 Why do Manduca sexta feed from white flowers? Innate and learnt colour preferences in a hawkmoth. *Naturwissenschaften* **95**, 569–76. (doi:10.1007/s00114-008-0350-7)

11. Gumbert, A. 2000 Color choices by bumble bees (Bombus terrestris): innate preferences and generalization after learning. *Behav. Ecol. Sociobiol.* **48**, 36–43.

12. Troje, N. 1993 Spectral categories in the learning behaviour of blowflies. *Zeitschrift für Naturforsch.* **48**, 96–104.

13. Chittka, L. 1992 The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *J. Comp. Physiol. A* **170**, 533–543.

14. Briscoe, A. D. & Chittka, L. 2001 The evolution of color vision in insects. *Annu. Rev. Entomol.* **46**, 471–510.

15. Chittka, L. 1997 Bee color vision is optimal for coding flower color, but flower colors are optimal for being coded-why? *Isr. J. Plant Sci.* **45**, 115–127.

16. Faegri, K. & Van der Pijl, L. 1979 *The principles of pollination ecology.* 3rd edn. Oxford; New York: Pergamon Press.

17. Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. & Thomson, J. D. 2004 Pollination Syndromes and Floral Specialization. *Annu. Rev. Ecol. Evol. Syst.* **35**, 375–403. (doi:10.1146/annurev.ecolsys.34.011802.132347)

18. Campbell, D. R., Bischoff, M., Lord, J. M. & Robertson, A. W. 2010 Flower color influences insect visitation in alpine New Zealand. *Ecology* **91**, 2638–2649.

19. Bradshaw Jr, H. D. & Schemske, D. W. 2003 Allele substitution at flower colour locus produces a pollinator shift in monkeyflowers. *Nature* **426**, 176–178. (doi:10.1007/s00294-003-0432-1)

20. Wolfe, L. M. & Sowell, D. R. 2006 Do pollination syndromes partition the pollinator community? A test using four sympatric morning glory species. *Int. J. Plant Sci.* **167**, 1169–1175.

21. Rodríguez-Gironés, M. a & Santamaría, L. 2004 Why are so many bird flowers red? *PLoS Biol.* **2**, e350. (doi:10.1371/journal.pbio.0020350)

22. Armbruster, W. S. 1996 Evolution of floral morphology and function: an integrative approach to adaptation, constraint, and compromise in Dalechampia (Euphorbiaceae). In *Floral biology: Studies on floral evolution in animal-pollinated plants* (eds D. G. Lloyd & S. C. H. Barrett), pp. 241–272. New York: Chapman & Hall.

23. Cooley, a M., Carvallo, G. & Willis, J. H. 2008 Is floral diversification associated with pollinator divergence? Flower shape, flower colour and pollinator preference in Chilean Mimulus. *Ann. Bot.* **101**, 641–50. (doi:10.1093/aob/mcn014)

24. Waser, N. M., Chittka, L., Price, M. V., Williams, N. M. & Ollerton, J. 1996 Generalization in pollination systems , and why it matters. *Ecology* **77**, 1043–1060.

25. Waser, N. M. 1983 Competition for pollination and floral character differences among sympatric plant species: a review of evidence. In *Handbook of experimental pollination biology.* (eds C. E. Jones & R. J. Little), pp. 277–293. New York: Van Nostrand Reinhold New York.

26. Heinrich, B. 1979 Majoring and minoring by foraging bumblebees, Bombus vagans: an experimental analysis. *Ecology* **60**, 245–255.

27. Mitchell, R. J., Flanagan, R. J., Brown, B. J., Waser, N. M. & Karron, J. D. 2009 New frontiers in competition for pollination. *Ann. Bot.* **103**, 1403–13. (doi:10.1093/aob/mcp062)

28. McCall, C. & Primack, R. B. 1992 Influence of flower characteristics, weather, time of day, and season on insect visitation rates in three plant communities. *Am. J. Bot.* **79**, 434–442.

29. Bosch, J., Retana, J. & Cerda, X. 1997 Flowering phenology , floral traits and pollinator composition in a herbaceous Mediterranean plant community. *Oecologia* **109**, 583–591.

30. Hingston, A. B. & McQuillan, P. B. 2000 Are pollination syndromes useful predictors of floral visitors in Tasmania ? *Austral Ecol.* **25**, 600–609.

31. Lázaro, A., Hegland, S. J. & Totland, O. 2008 The relationships between floral traits and specificity of pollination systems in three Scandinavian plant communities. *Oecologia* **157**, 249–57. (doi:10.1007/s00442-008-1066-2)

32. Gomez, D. 2006 AVICOL, a program to analyse spectrometric data.

33. Chittka, L., Shmida, A., Troje, N. & Menzel, R. 1994 Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. *Vision Res.* **34**, 1489–1508.

34. Andersson, S. & Prager, M. 2006 Quantifying colors. In *Bird colorations, vol. I, Mechanisms and measurements* (eds G. E. Hill & K. J. McGraw), pp. 41–89. Cambridge, Massachusetts, USA: Harvard University Press.

35. Chittka, L. & Menzel, R. 1992 The evolutionary adaptation of flower colours and insect pollinators' colour vision. *J. Comp. Physiol. A* **171**, 171–181.

36. Webb, C. O., Ackerly, D. D. & Kembel, S. W. 2008 Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**, 2098–2100.

37. Group, T. angiosperm phylogeny 2009 An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants : APG III. *Bot. J. Linn. Soc.* **161**, 105–121.

38. Hedges, S. B., Dudley, J. & Kumar, S. 2006 TimeTree: a public knowledge-base of divergence times among organisms. *Bioinformatics* **22**, 2971–2972. (doi:10.1093/bioinformatics/btl505)

39. Lavin, M., Herendeen, P. S. & Wojciechowski, M. F. 2005 Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. *Syst. Biol.* **54**, 575–94. (doi:10.1080/10635150590947131)

40. Allan, G. J. & Porter, J. M. 2000 Tribal delimitation and phylogenetic relationships of Loteae and Coronilleae (Faboideae: Fabaceae) with special reference to Lotus: evidence from nuclear ribosomal ITS sequences. *Am. J. Bot.* **87**, 1871–1881.

41. Barres, L., Sanmartín, I., Anderson, C. L., Susanna, A., Buerki, S., Galbany-Casals, M. & Vilatersana, R. 2013 Reconstructing the evolution and biogeographic history of tribe Cardueae (Compositae). *Am. J. Bot.* **100**, 867–82. (doi:10.3732/ajb.1200058)

42. Bremer, B. 2009 Asterids. In *The timetree of life* (eds S. B. Kumar & S. Hedges), pp. 177–187. Oxford University Press.

43. Blomberg, S. P., Garland, T. & Ives, A. R. 2003 Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**, 717–45.

44. Münkemüller, T., Lavergne, S., Bzeznik, B., Dray, S., Jombart, T., Schiffers, K. & Thuiller, W. 2012 How to measure and test phylogenetic signal. *Methods Ecol. Evol.* **3**, 743–756. (doi:10.1111/j.2041-210X.2012.00196.x)

45. Revell, M. L. J. 2014 Package " phytools ."

46. Core Team, R. 2014 R: A language and environment for statistical computing.

47. Oksanen, A. J., Blanchet, F. G., Kindt, R., Legen-, P., Minchin, P. R., Hara, R. B. O., Simpson, G. L., Solymos, P. & Stevens, M. H. H. 2014 Package " vegan ."

48. Ter Braak, C. J. F. 1986 Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* **67**, 1167–1179.

49. Smith, S. D., Ané, C. & Baum, D. a 2008 The role of pollinator shifts in the floral diversification of Iochroma (Solanaceae). *Evolution (N. Y).* **62**, 793–806. (doi:10.1111/j.1558-5646.2008.00327.x)

50. Beardsley, P. M., Yen, A. & Olmstead, R. G. 2003 AFLP phylogeny of Mimulus section Erythranthe and the evolution of hummingbird pollination. *Evolution (N. Y).* **57**, 1397–1410.

51. McEwen, J. R. & Vamosi, J. C. 2010 Floral colour versus phylogeny in structuring subalpine flowering communities. *Proc. R. Soc.* **277**, 2957–2965. (doi:10.1098/rspb.2010.0501)

52. Arnold, S. E. J., Savolainen, V. & Chittka, L. 2009 Flower colours along an alpine altitude gradient, seen through the eyes of fly and bee pollinators. *Arthropod. Plant. Interact.* **3**, 27–43. (doi:10.1007/s11829-009-9056-9)

53. Tanaka, Y., Sasaki, N. & Ohmiya, A. 2008 Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *Plant J.* **54**, 733–749. (doi:10.1111/j.1365-313X.2008.03447.x)

54. Dyer, A. G., Boyd-Gerny, S., McLoughlin, S., Rosa, M. G. P., Simonov, V. & Wong, B. B. M. 2012 Parallel evolution of angiosperm colour signals: common evolutionary pressures linked to hymenopteran vision. *Proc. Biol. Sci.* **279**, 3606–15. (doi:10.1098/rspb.2012.0827)

55. Rausher, M. D. 2008 Evolutionary transitions in floral color. *Int. J. Plant Sci.* **169**, 7–21.

56. Goldsmith, T. H. & Bernard, G. D. 1974 The visual system of insects. In *The physiology of insecta Vol II* (ed M. Rockstein), New York: Academic Press.

57. Irwin, R. E. & Strauss, S. Y. 2005 Flower color microevolution in wild radish : evolutionary response to pollinator-mediated selection. *Am. Nat.* **165**, 225–237.

58. Stanko, S. A. 1957 Characteristics of the passing of sun energy by plant leaves, depending on the coloration of their flowers. *Tr Sect. Astrobot Akad Nauk Kazssr* , 162–173.

59. Kevan, P., Giurfa, M. & Chittka, L. 1996 Why are there so many and so few white flowers. *Trends Plant Sci.* **1**, 280–284.

60. Bosch, J., González, A. M. M., Rodrigo, A. & Navarro, D. 2009 Plant-pollinator networks: adding the pollinator's perspective. *Ecol. Lett.* **12**, 409–19. (doi:10.1111/j.1461-0248.2009.01296.x)

61. Herrera, C. M. 1996 Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In *Floral biology: Studies on floral evolution in animal-pollinated plants* (eds D. G. Lloyd & S. C. H. Barrett), pp. 65–87. New York: Chapman & Hall.

62. Schiestl, F. P. & Johnson, S. D. 2013 Pollinator-mediated evolution of floral signals. *Trends Ecol. Evol.* **28**, 307–15. (doi:10.1016/j.tree.2013.01.019)

63. Raine, N. E. & Chittka, L. 2007 The adaptive significance of sensory bias in a foraging context: floral colour preferences in the bumblebee Bombus terrestris. *PLoS One* **2**, e556. (doi:10.1371/journal.pone.0000556)

64. Lunau, K. 1988 Innate and learned behaviour of flower-visiting hoverflies flower-dummy experiments with Eristalis pertinax (SCOPOLI) (Diptera, Syrphidae). *Zool Jb Physiol* **92**, 487–499.

65. Ollerton, J., Alarcón, R., Waser, N. M., Price, M. V, Watts, S., Cranmer, L., Hingston, A., Peter, C. I. & Rotenberry, J. 2009 A global test of the pollination syndrome hypothesis. *Ann. Bot.* **103**, 1471–80. (doi:10.1093/aob/mcp031)

66. Rosas-Guerrero, V., Aguilar, R., Martén-Rodríguez, S., Ashworth, L., Lopezaraiza-Mikel, M., Bastida, J. M. & Quesada, M. 2014 A quantitative review of pollination syndromes: do floral traits predict effective pollinators? *Ecol. Lett.* **17**, 388–400. (doi:10.1111/ele.12224)

67. Johnson, S. D. & Steiner, K. E. 2000 Generalization versus specialization in plant pollination systems. *Trends Ecol. Evol.* **15**, 140–143.

Table 1. Community descriptors including location, elevation, mean annual precipitation, and mean annual temperature.

Community	**Location**	**Elevation (m.a.s.l.)**	**Mean annual precipitation (mm)**	**Mean annual temperature (ºC)**
CA	41º 35’ N, 2º 34’ E	50	590	16.1
CO	41º 24’ N, 2º 6’ E	280	630	14.8
GA	41º 16’ N, 1º 55’ E	330	580	15.7
PA	41º 27’ N, 2º 0’ E	150	628	15.4

Table 2. Results of Blomberg's K tests for the presence of phylogenetic signal in colour descriptors in the four study communities and overall (data of the four communities lumped together). Significant results (p < 0.05) in bold. Marginally significant results (0.05< p <0.1) in italics.

Community	**Brightness**		**Chroma**		**Hue**		**UV band (350%)**		**Blue band (450%)**		**Yellow band (550%)**		**Red band (650%)**	
	K	**P**	**K**	**P**	**K**	**P**	**K**	**P**	**K**	**P**	**K**	**P**	**K**	**P**
CA	0.73	*0.07*	0.96	**0.01**	0.66	0.20	0.44	0.50	0.51	0.31	0.77	**0.05**	1.14	**0.00**
CO	0.35	0.62	0.43	0.37	0.71	**0.03**	0.45	0.26	0.58	**0.02**	0.39	0.45	0.43	0.26
GA	0.26	0.33	0.21	0.53	0.74	*0.06*	0.90	**0.02**	0.60	**0.04**	0.20	0.45	0.17	0.65
PA	0.34	0.22	0.33	0.27	0.88	**0.01**	0.73	**0.03**	0.73	**0.02**	0.28	0.31	0.19	0.59
CA+CO+GA+PA	0.79	**0.00**	0.57	**0.00**	0.81	**0.00**	0.65	**0.00**	0.63	**0.00**	0.89	**0.00**	0.72	**0.00**

Table 3. P-values of phylogenetically-controlled Mantel tests between colour descriptors and pollinator composition in the four communities and overall (data of the four communities lumped together). Significant results ($p < 0.05$) in bold.

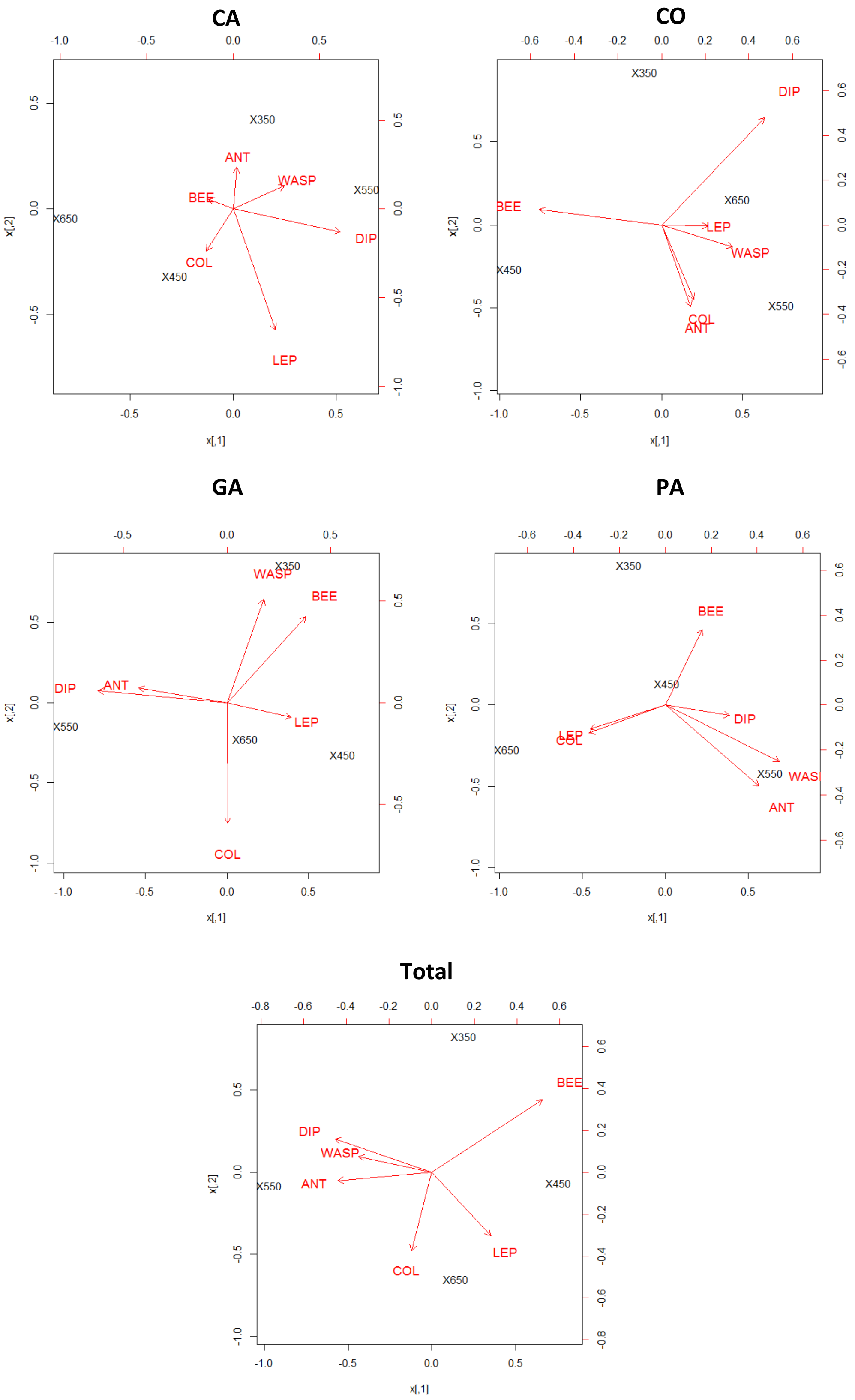
Community	Brightness	Chroma	Hue	Colour composition*
CA	**0.016**	0.130	**0.038**	0.303
CO	0.558	0.952	0.188	0.378
GA	0.605	0.655	0.560	0.152
PA	0.186	0.537	0.751	0.200
CA+CO+GA+PA	0.113	0.896	0.553	0.308

* Proportion of UV, blue, yellow, and red bands.

Table 4. Positive and negative relationships between pollinator groups and the four bands of the colour spectrum (350, 450, 550, 650 nm; UV, blue, yellow, and red, respectively) in the four study communities and overall (data of the four communities lumped together), estimated visually from the CCA biplots of Fig. 1.

	Type of relationship	**CA**	**CO**	**GA**	**PA**	**CA+CO+GA+PA**
BEES	(+)		**350, 450**	**350, 450**	**350**	**350, 450**
	(-)		**500**			
ANTS	(+)	**350**	**550**	**550**	**550**	**550**
	(-)		**350**	**450**	**350**	**450**
WASPS	(+)	**550**	**550**	**350**	**550**	**550**
	(-)		**450**			**450**
DIPTERANS	(+)	**550**	**350, 550**	**550**	**550**	**550**
	(-)			**450**		**450**
COLEOPTERANS	(+)	**450**	**550**		**650**	**650**
	(-)		**350**	**350**		**350**
LEPIDOPTERANS	(+)	**450, 550**		**450**	**650**	**450, 650**
	(-)	**350**	**450**	**550**	**550**	**550**

Figure 1. CCA biplots between pollinator groups and four bands of the colour spectrum (X350, X450, X550, X650; corresponding to UV, blue, yellow, and red, respectively) in each of the four communities separately (CA, CO, GA, PA) and for the four communities together (Total). Bee = bees, Ant = ants, Wasp = wasps, Dip = Dipterans, Col = coleopterans, Lep = lepidopterans.



CA

CO

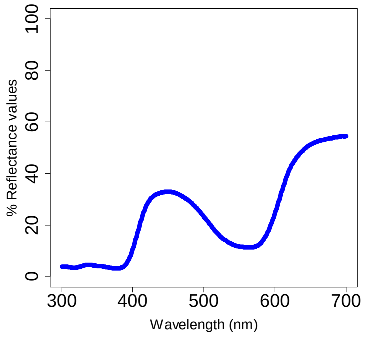
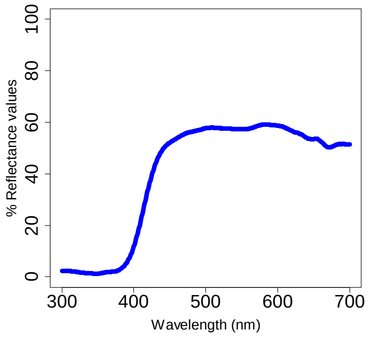
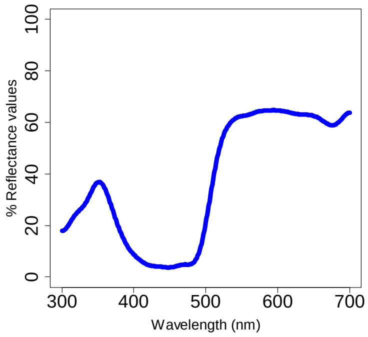
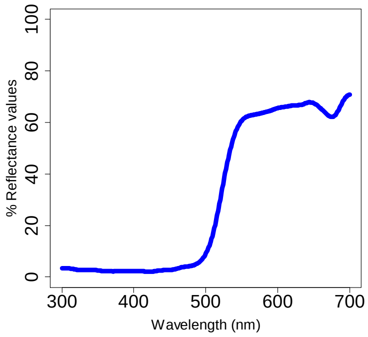
GA

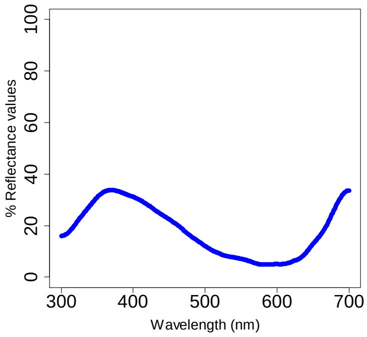
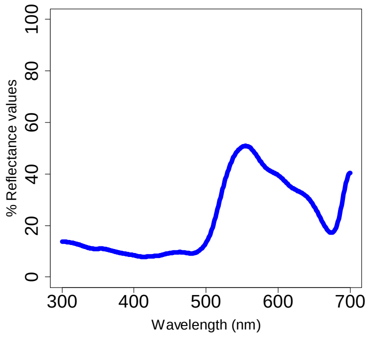
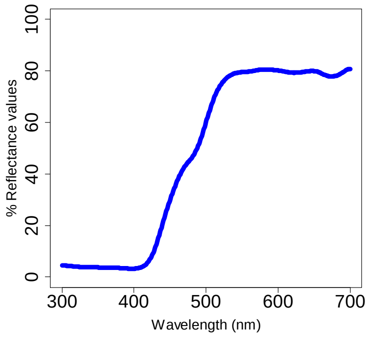
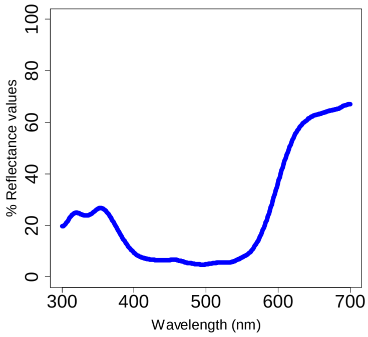
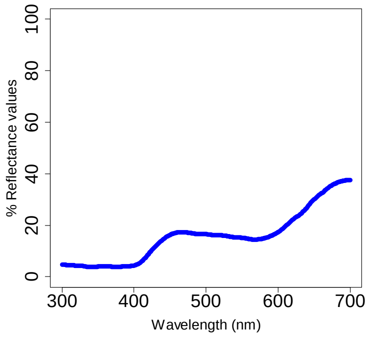
PA

Total

Appendix 1. Flower colour categories

Table S1. Flower colour categories (following Chittka et al. 1994), with an example species of each model and the proportion of each category in each of the four communities (CA, CO, GA, PA).

Colour category	Reflectance spectra	Species	CA	CO	GA	PA
Pink		*Cistus albidus* L. 	29%	30%	52%	38%
White		*Dorycnium pentaphyllum* L. 	18%	22%	16%	29%
UV-yellow		*Sonchus tenerrimus* L. 	24%	17%	0%	14%
Yellow		*Ranunculus gramineus* L. 	12%	15%	12%	14%

Purple		*Anagallis arvensis* L. 	6%	9%	4%	5%
Green		*Euphorbia flavicoma* DC. 	6%	4%	4%	0%
White-yellow		*Biscutella laevigata* L. 	0%	2%	8%	0%
UV-red		*Papaver rhoeas* L. 	6%	0%	0%	0%
Brown		*Orobanche latisquama* F.W. Schultz 	0%	0%	4%	0%

Appendix 2. Phylogeny

Figure S1. Phylogenetic tree of the 85 species surveyed.