1   **Type of article:** Original article

2

3   **Pollinators show flower colour preferences but flowers with similar colours do not**

4   **attract similar pollinators.**

5   Sara Reverté[1*], Javier Retana[1,2], José M. Gómez[3,4], Jordi Bosch[1]

6   [1] CREAF, Cerdanyola del Vallès, 08193 Barcelona, Spain

7   [2] Univ. Autònoma Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain

8   [3] CSIC, Estación Experimental de Zonas Áridas, Almería, Spain

9   [4] Universidad de Granada, Granada, Spain

10

11   **Running title:** Flower colour and pollinator composition

12   **Author for correspondence:** Sara Reverté. **Email:** s.reverte@creaf.uab.cat

13

14

1 **Abstract**

2

3 *Background and aims.* Colour is one of the main floral traits used by pollinators to locate

4 flowers. Although pollinators show innate colour preferences, the view that the colour of a

5 flower may be considered an important predictor of its main pollinators is highly

6 controversial because flower choice is highly context-dependent, and initial innate preferences

7 may be overridden by subsequent associative learning. Our objective is to establish whether

8 there is a relationship between flower colour and pollinator composition in natural

9 communities.

10

11 *Methods.* We measured the flower reflectance spectrum and pollinator composition in four

12 plant communities (85 plant species represented by 109 populations, and 32305 plant-

13 pollinator interactions in total). Pollinators were divided into six taxonomic groups: bees,

14 ants, wasps, coleopterans, dipterans and lepidopterans.

15

16 *Key results.* We found consistent associations between pollinator groups and certain colours.

17 These associations matched innate preferences experimentally established for several

18 pollinators and predictions of the pollination syndrome theory. However, flowers with similar

19 colours did not attract similar pollinator assemblages.

20

21 *Conclusions.* The explanation for this paradoxical result is that most flower species are

22 pollination generalists. We conclude that although pollinator colour preferences seem to

23 condition plant-pollinator interactions, the selective force behind these preferences has not

24 been strong enough to mediate the appearance and maintenance of tight colour-based plant-

25 pollinator associations.

INTRODUCTION

Pollinators use flower traits, such as odour, shape, size and colour, as cues to locate pollen-nectar sources and discriminate between different flower species (Chittka and Raine 2006). Although ultimate flower choice undoubtedly depends on a combination of stimuli, various studies have demonstrated that some pollinators rely strongly on colour to make their foraging decisions (Dafni et al. 1990; Heiling et al. 2003; Ômura and Honda 2005; Dötterl et al. 2014).

Diurnal 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 developed for different groups (Chittka 1992; Troje 1993). The best studied species are the western honey bee, *Apis mellifera*, and the bumblebee *Bombus terrestris*, which share a similar trichromatic system. The visual system of the honey bee, which is usually used as a model for all bees, ranges between 300 and 700 nm, with three types of photoreceptors peaking in the UV, blue and green regions of the spectrum (corresponding to 344, 438 and 560 nm, respectively). Most other insects studied so far also have a trichromatic system, but there are also species with dichromatic (certain flies and coleopterans) and tetrachromatic (mostly butterflies) systems (Briscoe and Chittka 2001). The trichromatic state is supposed to be ancestral, and the loss or gain of photoreceptor types would be secondary (Briscoe and Chittka 2001). Insect colour

vision appeared long before the emergence of the angiosperms (Chittka 1997). For this reason, it is believed that flowers tuned their visual signals to the sensory system of pollinators, thus becoming as conspicuous and attractive as possible to them. In support of this view, some pollinator species have been found to show innate preferences for certain colours (Lunau and Maier 1995; Raine et al. 2006; Raine and Chittka 2007; Willmer 2011).

To this extent, colour constitutes one of the main traits used in pollination syndrome theory (Faegri and Van der Pijl 1979). According to this theory, unrelated plant species adapted to the same pollinators should show convergence of floral traits, including colour. For example, bee-visited flowers are expected to be blue or violet, and beetle-visited flowers are expected to be white or cream (Faegri and Van der Pijl 1979). Accordingly, some studies have shown that colour changes among related plant species or between populations within a same species are sometimes accompanied by changes in pollinator composition (Bradshaw and Schemske 2003; Rodríguez-Gironés and Santamaría 2004; Wolfe and Sowell 2006). Other studies have shown pollinator colour preferences through experimental manipulations of flower colour (Campbell et al. 2010). Ultimately, flower colour could be considered an important predictor of pollinator group (Fenster et al. 2004). However, other studies do not support this view. Colour preferences predicted by pollination syndromes are not always matched by innate colour preferences (Lunau and Maier 1995), which in many cases are displayed at a finer taxonomic level, such as genus or species, rather than functional group (Raine et al. 2006). Other studies report that changes in flower colour are not always associated with distinct pollinator assemblages (Cooley et al. 2008).

Even if pollinators show innate preferences for certain colours, actual flower choice in natural communities may not reflect these preferences for two reasons. First, in a natural setting,

flower choice may be influenced by the presence and abundance of other co-pollinators potentially competing for flower resources (Waser 1983; Lázaro et al. 2009). Second, colours may also be used by pollinators as signals of floral rewards, so that initial innate preferences may be modulated by subsequent associative learning (Gumbert 2000; Goyret et al. 2008). Individual pollinators show remarkable plasticity and are known to switch plants in response to changes in pollen or nectar levels (Heinrich 1979). Thus, the role of colour in determining ultimate flower choice will depend on the interaction between innate and learned preferences. A handful of studies have analysed the influence of floral colour on pollinator partitioning in plant communities, and most of them conclude that floral colour is an important cue (McCall and Primack 1992; Bosch et al. 1997; Hingston and McQuillan 2000; Wolfe and Sowell 2006; Lázaro et al. 2008). However, colours in these studies were categorized as perceived by humans. Waser et al. (1996) characterized colour based on wavelength measures that were incorporated into the visual model of the honeybee to generate colour categories. In their study, the association between floral colour category and pollinator composition narrowly failed significance. Clearly, further research on this topic is needed.

The aim of this study is to establish whether there is a relationship between flower colour and pollinator visitation in natural communities. To do this, we studied the flower reflectance spectrum and flower visitor assemblages in four plant communities comprising 85 species and 109 plant populations. The four communities are located in the same geographic region, and therefore have similar climatic conditions and share the same regional pool of pollinators. Because the association between flower colour and pollinator composition may be affected by phylogeny, our analyses account for phylogenetic relatedness. We ask two questions: 1) Do different pollinator groups show preferences for certain flower colours? 2) Do plant species with similar colours attract similar pollinator assemblages? Although apparently similar, these

two questions address colour-pollinator relationships from two different and complementary perspectives. The first question addresses plant-pollinator relationships from the pollinators' point of view, and the second from the plants' perspective. The existence of colour preferences by different pollinator groups does not necessarily imply that plants with the same colour attract similar pollinator assemblages. For example, dipterans may preferentially visit flowers with high levels of reflectance in the yellow region of the spectrum, but different yellow flowers may be visited by different pollinator assemblages. The distinction is important because pollination syndrome theory relies on the assumption that plants with similar traits have evolved to attract similar pollinators.

MATERIALS AND METHODS

Study area

We conducted our study in four Mediterranean communities near Barcelona (NE Spain), whose geographic coordinates are given in Table 1. Distance between sites ranged from 10 to 66 km. The climate 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). We studied the vast majority of the entomophilous species in each community, without selecting species based on their flower traits or pollinator visitation patterns (Table 1). Therefore our plant sample was unbiased and representative of the flora of the region. Some species were present in more than one community. In these cases, we sampled flower colour and pollinator composition of each population separately (total: 85 species, 109 populations).

Pollinator assemblages

Pollinator data were obtained throughout the main general flowering period in the study area (February-July). Very few species bloom in August. Each species was surveyed on several days covering its entire flowering period. In two communities (CA and CO), pollinator surveys were carried out by slowly walking along 25- or 50-m-long x 1-m-wide vegetation transects. This was done several times throughout the day, from early morning to evening. In the other two communities (GA and PA), selected individuals of each plant species in bloom were observed during 4- or 5-minute periods every 1-2 h. All insects observed contacting the reproductive organs of 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 of each plant species in each of the four communities as the proportion of visits from each pollinator group.

Flower colour measurement

Flowers of each species 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 measured 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 analysed ranges 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 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 species had corollas displaying two or more clearly different colours.

When these different colours were largely separated (for example, some Fabaceae in which the keel is clearly differently coloured than the wings and the standard), we took colour measures of the different parts separately and calculated a weighted mean (according to the surface occupied by each part in the corolla), thus obtaining a single colour value. In corollas showing only small colour markings such as nectar guides or small dots, a single measure was taken.

Spectrometer readings incorporate a certain degree of noise. We used Avicol (Gomez 2006) to clean this noise, thus smoothening the reflectance spectra. This correction (triangular smoothing) is based on a floating mean with weights and a window size of 15 nm. We measured 4-5 flowers per species except for three species for which we could only obtain one measure. Different measures were averaged to obtain a single value per species and community.

Colour characterization

We characterized flower colour using two sets of variables: colour descriptors and "colour composition". Colour descriptors (brightness, chroma and hue) are physical properties of colours extracted directly from the reflectance spectrum (Endler 1990; Smith 2014). Brightness is a measure of the total intensity of light reflected by a surface; chroma is a measure of purity or saturation of a colour, and it is a function of how rapidly intensity changes with wavelength; hue, represents the usual meaning of colour (such as red, pink, yellow, etc.), and it is a function of the shape of the spectrum. These three colour descriptors were calculated following Smith (2014). To obtain "colour composition", we divided the reflectance spectrum into 4 broad bands of 100 nm each (300-400, 400-500, 500-600, 600-

700 nm; Chittka *et al.* 1994). Breakpoints delimiting bands were chosen assuming that the majority of the inflection points in floral reflectance spectra are located near 400, 500 and 600 nm (Chittka and Menzel 1992). 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 fourth to the orange-red (henceforth U, B, Y and R bands). We calculated the proportion of the reflectance spectrum attributable to each band, obtained by dividing the brightness of each band 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 different brightness (Endler 1990). It is important to note that flower colours result from the combination of reflectance levels across the spectrum. For example, white flowers reflect from 400 to 700 nm and yellow flowers from 500 to 700 nm. Lilac-pink flowers reflect in the blue and red regions with a variable proportion in the yellow region, and purple flowers reflect in the UV, blue and red regions of the spectrum (see Table S1 [**Supplementary Information**]).

Data analysis

Phylogenetic signal of colour variables

To establish whether colour was phylogenetically constrained, we built a phylogenetic tree of the 85 species using Phylocom (Webb et al. 2008), with family names following the Angiosperm Phylogeny Group classification (The Angiosperm Phylogeny Group 2009). 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 (Hedges et al. 2006). Additional distances between genera and distances between species were extracted from the literature [Allan and Porter, 2000; Lavin *et al.*, 2005 (Fabaceae); Barres *et al.*, 2013 (Cardueae); Bremer, 2009 (Ericales); Guzmán and Vargas, 2009 (Cistaceae); Mansion *et al.*, 2009 (Boraginaceae); Couvreur *et al.*, 2010 (Brassicaceae); Bendiksby *et al.*, 2011; Drew and Sytsma, 2012 (Lamiaceae); Koopman and Baum, 2008 (Malvaceae); Ruiz-Sanchez *et al.*, 2012 (Papaveraceae); Riina et al. 2013 (Euphorbiaceae); Banasiak et al. 2013 (Apiaceae)]. The resulting tree is shown in Figure S1 [**Supplementary Information**].

We tested for the presence of phylogenetic signal in brightness, chroma and hue with the Blomberg's K test (Blomberg et al. 2003) using the "phylosig" function of the R package "phytools" (Revell 2012). This was done for each community separately and for the 85 species together. We also tested for the presence of phylogenetic signal in flower colour composition. To do this, we divided the colour spectrum into 40 bands of 10 nm each and obtained the mean reflectance value of each band. We used 40 bands instead of the 4 bands used to define colour composition to increase the resolution of this analysis. We then used the generalized K statistic described by Adams (2014), K_{mult}, specifically developed for high-dimensional multivariate data. This analysis was performed with the function "physignal" of the package "geomorph" for R (Adams and Otarola-Castillo 2013). Again, we conducted this analysis for each community separately and for the 85 species together.

Association between pollinator groups and regions of the colour spectrum

We explored possible preferences of the different pollinators groups for certain regions of the colour spectrum by means of Canonical Correspondence Analyses (CCA) including the

pollinator assemblage of each population and relative brightness of the four above-mentioned colour bands (U, B, Y and R). This was again done for the four communities separately and for all species together. CCAs were performed using the function "CCorA" in the R package "vegan" (Oksanen et al. 2015).

Relationship between flower colour and pollinator assemblages

To test whether flowers with similar colours had similar pollinator assemblages, we conducted a partial Mantel test between distance matrices of colour composition (combination of the proportion of the four colour bands) and pollinator assemblages, including the phylogenetic distance matrix as covariable. We then followed the same approach to test the association between pollinator assemblages and the other colour variables (brightness, chroma, 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. These analyses were done for each community separately and for the four communities lumped together. In the latter case, because the same species may attract different pollinators in different communities, we maintained all 109 populations. For plant species present in more than one community, we assigned a very low value of divergence (1000 years) between conspecific populations. These analyses were performed with the function "mantel.partial" in the R package "vegan".

RESULTS

We recorded 32,315 plant-pollinator interactions. The number of interactions recorded in each community ranged from 3505 (CA) to 13673 (CO) (Table S2 [**Supplementary**

Information]). Mean number of interactions per population was 206 in CA (range: 43 to 1454); 297 in CO (range: 42 to 1911); 364 in GA (range: 26 to 1730) and 307 in PA (range: 31 to 1359). Overall, 88.5 % of the populations surveyed had more than 50 recorded interactions. Most of the pollinators recorded were bees, accounting for 48.9% of the flower visits. The second most frequent group was coleopterans (21.5% of the interactions), followed by ants (14.7%), dipterans (8.8%), lepidopterans (3.5%) and wasps (2.5%). Bees and coleopterans were the two most abundant groups in all four communities, except CA, which was largely dominated by ants (Table S2 [**Supplementary Information**]).

The four communities showed a high degree of similarity in flower colours. The most common floral colour in the four communities was lilac-pink (30-50% of the species), followed by white (16-29%) (Table S1 [**Supplementary Information**]). 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. The association between colour categories and pollinator composition is shown in Table S3 [**Supplementary Information**].

Phylogenetic signal of colour variables

All colour descriptors considered showed significant phylogenetic signal when the four communities were pooled together (Table 2). In most cases, however, significance was lost when the communities were analysed separately, possibly due to small sample sizes. In all cases, K and K_{mult} values were lower than 1, indicating that related species were less similar than expected under the Brownian motion evolution model.

Association between pollinator groups and regions of the colour spectrum

1

2 CCAs revealed clear associations between certain pollinator groups and certain colours (Figs.

3 1 and 2). Visual inspection of the resulting biplots revealed that some of these patterns were

4 relatively consistent across the four communities (Fig. 1, Table 4).

5

6 Overall, bees were associated with purple flowers, and ants with UV-Yellow and green

7 flowers. Wasps and dipterans were mostly associated with UV-yellow flowers. Coleopterans

8 were associated with white and yellow flowers, and lepidopterans with pink flowers (Table 4,

9 Fig. 2).

10

11 *Relationship between flower colour and pollinator assemblages*

12

13 Results from the partial Mantel test showed no significant association between flower colour

14 and pollinator assemblages (Table 3). Plants with similar colour descriptors, including colour

15 composition, did not attract similar pollinator assemblages in any of the communities, and

16 similar results were obtained when data from the four communities were lumped together.

17

18 **DISCUSSION**

19

20 We traced the phylogenetic signal of floral colour under a neutral evolution model (Brownian

21 motion). In this model, changes occur slowly and gradually along phylogenetic branches, and

22 character similarity between species is related to phylogenetic proximity (Blomberg et al.

23 2003). Phylogenetic signal for flower colour was mostly lacking when we analysed the four

24 communities separately, but when all species were lumped together all colour properties

25 measured showed significant phylogenetic signal (related species were more similar in colour

than expected from a random association between species and colours). Notwithstanding this influence of phylogeny on flower colour, the fact that all K values were lower than 1 indicates that similarity among related species tends to be lower than expected under Brownian motion. Most previous studies have found lack of phylogenetic signal in flower colour (Smith et al. 2008; Arnold et al. 2009; McEwen and Vamosi 2010). However, one recent study on four Himalayan plant communities (Shrestha et al. 2014) obtained results similar to ours (significant phylogenetic signal with K values < 1). A corollary of our phylogenetic analysis is that the different colour categories considered are widely spread across the phylogenetic tree. This outcome is in agreement with reports showing important changes in flower colour as a result of relatively simple genetic changes (Rausher 2008).

We analysed the colour preferences of the pollinator groups present in our communities. Results of the CCAs showed notable coincidences among communities and provided compelling evidence for the association between pollinator groups and certain colours. These associations were less clear in the communities with lower number of plant species, but were very apparent when the results of all 109 populations were lumped together (Fig. 2). Importantly, these associations are notably congruent with innate colour preferences found for specific pollinators (Lunau and Maier 1995; Willmer 2011). For example, inexperienced *Eristalis tenax* hover flies were found to land only on artificial flowers with yellow stimuli in the range of 520 to 600 nm (Lunau and Maier 1995). Naive honey bees showed preference for stimuli reflecting between 410 and 530 nm (Giurfa et al. 1995), and bumblebees preferred artificial flowers reflecting in one or two of the three colour bands corresponding to their photoreceptor types (ultraviolet, blue, green) (Lunau 1992). *Papilio demoleus* butterflies selected blue, green, and red colours while neglecting yellow (Ilse and Vaidya 1956) and *Pieris brassicae* preferred artificial flowers reflecting at 450 and 600 nm (Scherer and Kolb

1987). The hawkmoth *Macroglossum stellatarum* was shown to have a strong preference for wavelengths around 440 nm and a weaker preference for wavelengths of 540 nm (Kelber 1997; Telles et al. 2014).

Importantly, the above-mentioned pollinator-colour associations found in our study are consistent with colour preferences described in pollination syndromes, according to which bees favour blue, flies yellow and white, lepidopterans pink and red, coleopterans white and cream and wasps favour brown and yellow flowers (Faegri and Van der Pijl 1979; Willmer 2011). Waser *et al*. (1996) 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 also consistent with associations predicted by pollination syndromes. Other community studies using more subjective (human-perceived) colour measures also found associations that were more or less consistent with pollination syndromes (McCall and Primack 1992; Wolfe and Sowell 2006; Lázaro et al. 2008).

However, our results cannot be interpreted as supporting pollination syndrome theory because flowers with similar colours did not attract similar pollinators (Table 3). That is, pollinator preferences are a necessary but not sufficient condition for the establishment of colour-based pollination syndromes. The lack of relationship between flower colour and pollinator composition can be explained by the influence of other traits, or trait combinations, on flower choices. Some studies have found phenology (Herrera 1988), pollen/nectar rewards (Bosch et al. 1997), or corolla depth (Stang et al. 2007) to be the main drivers of pollinator partitioning in flower communities. A second factor contributing to the lack of relationship between flower colour and pollinator composition is pollinator generalisation. As in most temperate systems (Herrera 1996; Waser et al. 1996), the majority of plant species in our study are

1   pollinator generalists (with two or more pollinator groups each accounting for more than 10%

2   of the observed visits), and therefore cannot be readily assigned to a given pollination

3   syndrome. Even then, we could have found a correspondence between certain flower colours

4   and pollinator assemblages (rather than single pollinator groups), but this was clearly not the

5   case.

6

7   Our results are in agreement with the findings of Rezende et al. (2007), who found that plant-

8   pollinator networks are more strongly associated to animal than to plant phylogeny. That is,

9   related animal species are more likely to share host plants than related plant species are to

10   share pollinator visitors. This result is attributed to pollinator mobility (Bascompte and

11   Jordano 2007), which allows pollinators to play a more active role in the definition of

12   interaction identity.

13

14   Recently, Renoult et al. (2014) have used a novel approach to analyze the association between

15   flower colour and generalization in pollinators. They argue that pollinators should

16   preferentially visit flowers that are most conspicuous to them, and that colour perception

17   should be analyzed as a measure of colour conspicuousness between emitters (flowers) and

18   receivers (insects). They measure flower and background colour, and use evidence from

19   various sources to assign one of eleven described visual systems to each pollinator species.

20   Then they calculate 'colour matching' for each plant-pollinator pair as a measure of how a

21   floral colour stands out from the environment for each visual system. They find that colour

22   matching influences generalization in one of three regions studied. However, they also find

23   that other factors (spatiotemporal overlap and co-abundance between plants and pollinators)

24   play a greater role as determinants of plant generalization. The results of Renoult et al. (2014)

25   in general, and the strong influence of these other factors in particular, support our conclusion

that even though pollinator colour preferences seem to be conditioning plant-pollinator interactions, the selective force behind these preferences may not been strong enough to mediate the appearance and maintenance of tight colour-based plant-pollinator associations.

ACKNOWLEDGEMENTS

LITERATURE CITED

Adams DC. **2014**. A generalized K statistic for estimating phylogenetic signal from shape and other high-dimentional multivariate data. *Systematic Biology* **63**: 685–697.

Adams DC, Otarola-Castillo E. **2013**. geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution* **4**: 393–399.

Allan GJ, Porter JM. **2000**. Tribal delimitation and phylogenetic relationships of Loteae and Coronilleae (Faboideae: Fabaceae) with special reference to Lotus: Evidence from nuclear ribosomal ITS sequences. *American Journal of Botany* **87**: 1871–1881.

Arnold SEJ, Savolainen V, Chittka L. **2009**. Flower colours along an alpine altitude gradient, seen through the eyes of fly and bee pollinators. *Arthropod-Plant Interactions* **3**: 27–43.

Banasiak Ł, Piwczyński M, Uliński T, Downie SR, Watson MF, Shakya B, Spalik K. **2013**. Dispersal patterns in space and time: a case study of Apiaceae subfamily Apioideae (M Carine, Ed.). *Journal of Biogeography* **40**: 1324–1335.

Barres L, Sanmartín I, Anderson CL, Susanna A, Buerki S, Galbany-Casals M, Vilatersana R. **2013**. Reconstructing the evolution and biogeographic history of tribe Cardueae (compositae). *American Journal of Botany* **100**: 867–882.

Bascompte J, Jordano P. **2007**. Plant-Animal Mutualistic Networks: The Architecture of Biodiversity. *Annual Review of Ecology, Evolution, and Systematics* **38**: 567–593.

Beardsley PM, Yen A, Olmstead RG. **2003**. AFLP phylogeny of Mimulus section Erythranthe and the evolution of hummingbird pollination. *Evolution* **57**: 1397–1410.

Bendiksby M, Thorbek L, Scheen AC, Lindqvist C, Ryding O. **2011**. An updated phylogeny and classification of Lamiaceae subfamily Lamioideae. *Taxon* **60**: 471–484.

Blomberg SP, Garland T, Ives AR. **2003**. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

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

Bradshaw HD, Schemske DW. **2003**. Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* **426**: 176–178.

Bremer B. **2009**. Asterids. *The Timetree of Life*: 177–187.

Briscoe AD, Chittka L. **2001**. The evolution of color vision in insects. *Annual Review of Entomology* **46**: 471–510.

Campbell DR, Bischoff M, Lord JM, Robertson AW. **2010**. Flower color influences insect visitation in alpine New Zealand. *Ecology* **91**: 2638–2649.

Chittka L. **1992**. The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *Journal of Comparative Physiology A* **170**: 533–543.

Chittka L. **1997**. Bee color vision is optimal for coding flower color, but flower colors are not optimal for being coded - why? *Israel Jornal of Plant Sciences* **45**: 115–127.

Chittka L, Menzel R. **1992**. The evolutionary adaptation of flower colours and insect

pollinators' colour vision. *Journal of Comparative Physiology A* **171**: 171–181.

Chittka L, Raine NE. **2006**. Recognition of flowers by pollinators. *Current Opinion in Plant Biology* **9**: 428–435.

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

Cooley AM, Carvallo G, Willis JH. **2008**. Is floral diversification associated with pollinator divergence? Flower shape, flower colour and pollinator preference in Chilean Mimulus. *Annals of Botany* **101**: 641–650.

Couvreur TLP, Franzke A, Al-Shehbaz I, Bakker FT, Koch M, Mummenhoff K. **2010**. Molecular phylogenetics, temporal diversification, and principles of evolution in the mustard family (Brassicaceae). *Molecular Biology and Evolution* **27**: 55–71.

Dafni A, Bernhardt P, Shmida A, Ivri Y, Greenbaum S, O'Toole C, Losito L. **1990**. Red bowl-shaped flowers: convergence for beetle pollination in the Mediterranean region. *Israel Journal of Botany* **39**: 81–92.

Dötterl S, Glück U, Jürgens A, Woodring J, Aas G. **2014**. Floral reward, advertisement and attractiveness to honey bees in dioecious Salix caprea. *PloS ONE* **9**: e93421.

Drew BT, Sytsma KJ. **2012**. Phylogenetics, biogeography, and staminal evolution in the tribe Mentheae (Lamiaceae). *American Journal of Botany* **99**: 933–953.

Endler JA. **1990**. On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society* **41**: 315–352.

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

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. **2004**. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* **35**: 375–403.

Giurfa M, Núñez J, Chittka L, Menzel R. **1995**. Colour preferences of flower-naive honeybees. *Journal of Comparative Physiology A* **177**: 247–259.

Gomez D. **2006**. AVICOL, a program to analyse spectrometric data. Last update october 2011. Free executable available at http://sites.google.com/site/avicolprogram/ or from the author at dodogomez@yahoo.fr.

Goyret J, Pfaff M, Raguso RA, Kelber A. **2008**. Why do Manduca sexta feed from white flowers? Innate and learnt colour preferences in a hawkmoth. *Naturwissenschaften* **95**: 569–576.

Gumbert A. **2000**. Color choices by bumble bees (Bombus terrestris): innate preferences and generalization after learning. *Behavioral Ecology Sociobiology* **48**: 36–43.

Guzmán B, Vargas P. **2009**. Historical biogeography and character evolution of Cistaceae (Malvales) based on analysis of plastid rbcL and trnL-trnF sequences. *Organisms Diversity and Evolution* **9**: 83–99.

Hedges SB, Dudley J, Kumar S. **2006**. TimeTree: A public knowledge-base of divergence times among organisms. *Bioinformatics* **22**: 2971–2972.

Heiling AM, Herberstein ME, Chittka L. **2003**. Pollinator attraction: Crab-spiders manipulate flower signals. *Nature* **421**: 334.

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

Herrera J. **1988**. Pollination relationships in southern Spanish Mediterranean shrublands. *Journal of Ecology* **76**: 274–287.

Herrera CM. **1996**. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In: Lloyd DG,, In: Barrett SCH, eds. *Floral biology: Studies on floral evolution in animal-pollinated plants*. New York: Chapman & Hall, 65–87.

Hingston AB, McQuillan PB. **2000**. Are pollination syndromes useful predictors of floral visitors in Tasmania ? *Austral Ecology* **25**: 600–609.

Ilse D, Vaidya V. **1956**. Spontaneous feeding response to colours in Papilio demoleus L. *Proceedings of the Indian Academy of Sciences, section B* **43**: 23–31.

Kelber A. **1997**. Innate preferences for flower features in the hawkmoth Macroglossum stellatarum. *Journal of Experimental Biology* **200**: 827–836.

Koopman MM, Baum DA. **2008**. Phylogeny and biogeography of tribe Hibisceae (Malvaceae) on Madagascar. *Systematic Botany* **33**: 364–374.

Lavin M, Herendeen PS, Wojciechowski MF. **2005**. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. *Systematic biology* **54**: 575–594.

Lázaro A, Hegland SJ, Totland Ø. **2008**. The relationships between floral traits and specificity of pollination systems in three Scandinavian plant communities. *Oecologia* **157**: 249–257.

Lázaro A, Lundgren R, Totland Ø. **2009**. Co-flowering neighbors influence the diversity and identity of pollinator groups visiting plant species. *Oikos* **118**: 691–702.

Lunau K. **1992**. Innate recognition of flowers by bumble bees: orientation of antennae to visual stamen signals. *Canadian Journal of Zoology* **70**: 2139–2144.

Lunau K, Maier EJ. **1995**. Innate colour preferences of flower visitors. *Journal of Comparative Physiology A* **177**: 1–19.

Mansion G, Selvi F, Guggisberg A, Conti E. **2009**. Origin of Mediterranean insular endemics in the Boraginales: Integrative evidence from molecular dating and ancestral area reconstruction. *Journal of Biogeography* **36**: 1282–1296.

McCall C, Primack RB. **1992**. Influence of flower characteristics, weather, time of day, and season on insect visitation rates in three plant communities. *American Journal of Botany* **79**: 434–442.

McEwen JR, Vamosi JC. **2010**. Floral colour versus phylogeny in structuring subalpine flowering communities. *Proceedings of the Royal Society B* **277**: 2957–2965.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens HMH, Wagner H. **2015**. vegan: Community Ecology Package. R package version 2.3-0. http://CRAN.R-project.org/package=vegan.

Ômura H, Honda K. **2005**. Priority of color over scent during flower visitation by adult Vanessa indica butterflies. *Oecologia* **142**: 588–596.

Raine NE, Chittka L. **2007**. The adaptive significance of sensory bias in a foraging context: Floral colour preferences in the bumblebee Bombus terrestris. *PLoS ONE* **2**: 1–8.

Raine NE, Ings TC, Dornhaus A, Saleh N, Chittka L. **2006**. Adaptation, Genetic Drift, Pleiotropy, and History in the Evolution of Bee Foraging Behavior. *Advances in the Study of Behavior* **36**: 305–354.

Rausher MD. **2008**. Evolutionary Transitions in Floral Color. *International Journal of Plant*

Sciences **169**: 7–21.

Revell LJ. **2012**. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217–223.

Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J. **2007**. Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature* **448**: 925–929.

Riina R, Peirson J a., Geltman D V., Molero J, Frajman B, Pahlevani A, Barres L, Morawetz JJ, Salmaki Y, Zarre S, Kryukov A, Bruyns P V., Berry PE. **2013**. A worldwide molecular phylogeny and classification of the leafy spurges, Euphorbia subgenus Esula (Euphorbiaceae). *Taxon* **62**: 316–342.

Rodríguez-Gironés M a., Santamaría L. **2004**. Why are so many bird flowers red? *PLoS Biology* **2**.

Ruiz-Sanchez E, Rodriguez-Gomez F, Sosa V. **2012**. Refugia and geographic barriers of populations of the desert poppy, Hunnemannia fumariifolia (Papaveraceae). *Organisms Diversity & Evolution* **12**: 133–143.

Scherer C, Kolb G. **1987**. Behavioral experiments on the visual processing of color stimuli in Pieris brassicae L. (Lepidoptera). *Journal of Comparative Physiology A* **160**: 645–656.

Shrestha M, Dyer AG, Bhattarai P, Burd M. **2014**. Flower colour and phylogeny along an altitudinal gradient in the Himalayas of Nepal. *Journal of Ecology* **102**: 126–135.

Smith SD. **2014**. Quantifying Color Variation: Improved Formulas for Calculating Hue with Segment Classification. *Applications in Plant Sciences* **2**: 1300088.

Smith SD, Ané C, Baum D a. 2008. The role of pollinator shifts in the floral diversification of Iochroma (Solanaceae). *Evolution* **62**: 793–806.

Stang M, Klinkhamer PGL, van der Meijden E. **2007**. Asymmetric specialization and extinction risk in plant–flower visitor webs: a matter of morphology or abundance? *Oecologia* **151**: 442–453.

Telles FJ, Lind O, Henze JM, Rodríguez-Gironés MA, Goyret J, Kelber A. **2014**. Out of the blue: the spectral sensitivity of hummingbird hawkmoths. *Journal of Comparative Physiology A* **200**: 537–546.

The Angiosperm Phylogeny Group. **2009**. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**: 105–121.

Troje N. **1993**. Spectral Categories in the learning behaviour of blowflies. *Zeitschrift für Naturforschung* **48**: 96–104.

Waser NM. **1983**. Competition for pollination and floral character differences among sympatric plant species: a review of evidence. In: C. E. Jones,, In: Little RJ, eds. *Handbook of experimental pollination biology.* New York: Van Nostrand Reinhold New York, 277–293.

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

Webb CO, Ackerly DD, Kembel SW. **2008**. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**: 2098–2100.

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

Wolfe LM, Sowell DR. **2006**. Do pollination syndromes partition the pollinator community? A test using four sympatric morning glory species. *International Journal of Plant Sciences* **167**: 1169–1175.

1

2

1   **Figure 1.** CCA biplots of pollinator groups and bands of the colour spectrum (colour squares)

2   corresponding to UV, blue, yellow, and red, respectively, in each of the four communities

3   (CA, CO, GA, PA). Each dot represents a plant population and dot colour corresponds to

4   flower colour categories as shown in the legend (see Table S1 [**Supplementary Information**]

5   for example spectra of each category). Bee = bees, Ant = ants, Was = wasps, Dip = dipterans,

6   Col = coleopterans, Lep = lepidopterans.

7

Figure 2. CCA biplot of pollinator groups and bands of the colour spectrum (colour squares) corresponding to UV, blue, yellow, and red, respectively (data from the four communities lumped together). Each dot represents a plant population and dot colour corresponds to the flower colour categories as shown in the legend (see Table S1 [**Supplementary Information**] for example spectra of each category). Bee = bees, Ant = ants, Was = wasps, Dip = dipterans, Col = coleopterans, Lep = lepidopterans.

1   **Table 1.** Descriptors of the four communities studied, including vegetation type, dominant species, location, elevation, mean annual

2   precipitation, mean annual temperature, and number of species sampled.

3

Community	Vegetation	Dominant species	Location	Elevation (m.a.s.l.)	Mean annual precipitation (mm)	Mean annual temperature (ºC)	Plant species studied
CA	Grassland	*Hyparrhenia hirta, Brachypodium retusum*	Canet de Mar 41º 35' N, 2º 34' E	50	590	16.1	17
CO	Open scrubland	*Hyparrhenia hirta, Foeniculum vulgare, Cistus monspeliensis*	Collserola 41º 24' N, 2º 6' E	280	630	14.8	46
GA	Scrubland	*Quercus coccifera, Rosmarinus officinalis, Pistacia lentiscus*	Garraf 41º 16' N, 1º 55' E	330	580	15.7	25
PA	Scrubland	*Quercus coccifera, Ulex parviflorus, Pistacia lentiscus*	El Papiol 41º 27' N, 2º 0' E	150	628	15.4	21

4

1 **Table 2.** Analyses of phylogenetic signal for colour descriptors brightness, chroma, hue

2 (Blomberg's K values) and colour composition (K_{mult} values) in the four study communities

3 separately and lumped together. Significant results ($p < 0.05$) in bold.

4

Community	n species	Brightness	Chroma	Hue	Colour composition
CA	17	0.73	**0.78**	0.85	0.48
CO	46	0.35	**0.57**	**0.71**	0.33
GA	25	0.26	0.61	0.44	0.25
PA	21	0.34	**0.52**	0.76	0.26
CA+CO+GA+PA	85	**0.57**	**0.60**	**0.71**	**0.38**

5

Table 3. R values of phylogenetically-controlled partial Mantel tests between colour descriptors and pollinator composition in the four communities and overall (data of the four communities lumped together). All results are non-significant.

Community	Brightness	Chroma	Hue	Colour composition
CA	-0.089	0.096	0.199	0.158
CO	-0.002	-0.031	0.015	-0.080
GA	-0.025	0.115	-0.082	-0.038
PA	-0.103	-0.063	-0.035	0.051
Overall	0.021	-0.023	0.019	0.006

1   **Table 4.** Relationships between pollinator groups and the different floral colours in the four

2   communities and overall (data of the four communities lumped together) estimated visually

3   from the CCA biplots of Fig. 1 for the four communities and Fig. 2 for the overall tendencies.

4

Community	Bees	Ants	Wasps	Coleopterans	Dipterans	Lepidopterans
CA	Purple UV-yellow	Purple UV-yellow	UV-yellow	White Yellow Pink	White UV-yellow	White Pink
CO	Purple	White Yellow	Yellow	Yellow Green White	Purple UV-yellow	Yellow UV-yellow
GA	Purple Pink	Yellow Green	Purple	White Pink	Yellow Green	Pink
PA	Purple Pink	Yellow Green White	White	Pink	White Pink	Pink
Overall	Purple	UV-yellow Green	UV-yellow	White Yellow	UV-yellow	Pink

5

6

1   **Suplementary Information**

2   **Figure S1.** Phylogenetic tree of the 85 species surveyed. Colour dots represent the colour

3   category of each plant species (see colour legend in Fig. 1).

4

1   **Table S1.** Percentage of species in each of the four communities (CA, CO, GA, PA)

2   corresponding to the flower colour categories defined by Chittka *et al.* (1994) with an

3   example species of each category.

4

1   **Table S2.** Number (and percentage) of pollinators of each pollinator group surveyed in each

2   of the four communities (CA, CO, GA, PA).

3

1   **Table S3.** Mean percentage of visits of each pollinator group in each colour category (data of

2   the four communities lumped together).

3

4

5