Color-matching between pollen and corolla: hiding pollen via visual crypsis?

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Summary

• Visual signals attractive to friends may also attract enemies. The bright colors of anthers and pollen have generally been thought to attract pollinators. We hypothesize that visual crypsis of anthers, and particularly pollen, should be favored in flowering plants because protection from pollen collectors reduces the loss of male gametes.

• To understand adaptive strategies relating to the color of pollen, we measured the color of pollen, undehisced anther sacs, and their background, the corolla, with a spectrometer for 104 insect-pollinated flowering species from a natural community in Hengduan Mountains, southwest China.

• The colors of anthers, pollen and corollas were diverse in these species. The color diversity of exposed pollen was significantly higher than that of concealed pollen (i.e. where anthers are enclosed or shielded by corollas). The color contrast between pollen and corolla was significantly smaller in species with exposed pollen than in those with concealed pollen. Unlike anther color, exposed pollen color tended to match its background corolla color.

• Our phylogenetic comparative analysis showed contrasting effects of pollen color patterns between flowers with exposed pollen and those with concealed pollen, revealing a strategy of hiding pollen from pollen thieves via visual crypsis.

Introduction

Organisms on Earth display a wide variety of color variation. Colorful visual signals that are attractive to friends may also attract enemies. The coloration of some species is cryptic, meaning their colors match those of their habitat (e.g. peppered moths and sand rats), making it more difficult for predators to see them; others display bright colors or conspicuous patterns that are easily distinguished (e.g. poisonous butterflies and male birds), deterring predators and/or attracting partners. Compared to plants that are pollinated by wind or water, animal-pollinated plants usually have bright, colorful flowers and employ a diversity of visitors to transfer pollen. Flower coloration is often thought to be a visual signal for pollinator attraction (van der Kooi *et al.*, 2019).

Evolutionary transitions in flower color are generally associated with shifts in functional pollinator groups in diverse plant lineages (Rausher, 2008). For example, the shift from pink or purple to red flowers with the transition from bee- to hummingbird-pollinated plants appears repeatedly in *Aquilegia* (Whittall & Hodges, 2007), *Iochroma* (Smith *et al.*, 2008), *Ipomoea* (Des Marais & Rausher, 2010), *Mimulus* (Bradshaw & Schemske, 2003), *Penstemon* (Wilson *et al.*, 2006, 2007), *Silene* (Reynolds *et al.*, 2009) and others. Color convergence or divergence among species within a community could also be driven by pollinator preference for a given color (McEwen & Vamosi, 2010; Shrestha *et al.*, 2016, 2019; Kemp *et al.*, 2019). A bee-vision perspective of

bird-pollinated flowers seems to confirm that these plants favor some pollinators over others, and bird flowers usually present a larger dose of pollen for vectors than bee flowers (Castellanos *et al.*, 2003; Thomson, 2006). Birds can see red flowers (Raven, 1972), and the red coloration of bird-pollinated flowers could be favored due its inconspicuousness to bees (Chittka, 1992). Given that bees generally serve as ineffective pollinators for bird-pollinated flowers, a reduction in attractiveness to bees, who are wasteful pollen consumers for such species, could facilitate pollination by birds (Castellanos *et al.*, 2003; Thomson, 2006).

Pollen in many plants appears intense yellow (Lunau, 1995), owing to the presence in the pollenkitt of carotenoids such as ficarotene, lutein and antheraxanthine (Dobson, 1988; Wiermann & Gubatz, 1992); but in some species pollen can be other colors, such as blue, green, orange, red or white (Wang *et al.*, 2018). Colorful pollen (or anthers) might act as an additional visual signal, helping a foraging pollinator to locate the flower over short distances; this is especially true if the pollen and the corolla differ in spectral reflectance, resulting in a stronger visual contrast (Lunau, 1995, 2000). A recent study of a pollen-color polymorphic herb, *Campanula americana*, showed that its specialist *Megachile* pollinators strongly and consistently preferred purple over light purple or white pollen in natural populations (Ison *et al.*, 2018).

There is a conflict of interest between plants and pollen collectors with respect to pollen production (Westerkamp, 1997; Thorp, 2000; Hargreaves *et al.*, 2009; Vallejo-Marín *et al.*,

2010). Pollen collected as bee food and consumed by other animals is lost in terms of plant sexual reproduction. In a natural community, < 5% of the pollen produced by each species actually reaches the conspecific stigmas, indicating a huge cost of producing pollen that is not used for reproduction (Gong & Huang, 2014). One might expect that animal-pollinated flowers would exhibit pollen signals that are visible to pollinators but inconspicuous to pollen thieves, to protect pollen from overexploitation by flower visitors (see also Thomson, 2006). Theft-mediated pollen loss may have driven plants to evolve pollen crypsis or concealment to escape pollen thieves (Hargreaves et al., 2009). Given that plants usually confront conflicting selection from pollinators and florivores (Armbruster, 1997) as well as parasites (Johnson et al., 2015) – particularly pollen thieves – we hypothesize that visual crypsis might be a common strategy for pollen protection, especially for species with exposed pollen.

To test the pollen crypsis hypothesis, we randomly sampled 104 summer-flowering species from a subalpine community in southwest China. These species have variously colored corollas. Some species hide their anthers and pollen in corollas, and others expose them without any structure to conceal them. To protect pollen from pollen consumers, if a plant species has exposed pollen without a physical defense against pollen theft (i.e. pollen concealment), pollen visual crypsis would be favored. One would expect that the color contrast between pollen and its corolla background should be smaller in 'exposed' species than 'concealed' species – that is, the anthers or pollen may closely resemble the corolla in color, making them less conspicuous to pollen collectors. Our phylogenetic comparative analysis showed that the color contrast between pollen and corollas (but not between anthers and corollas) was smaller in exposed species than concealed species, supporting the pollen crypsis hypothesis.

Materials and Methods

Study species

Flowers of a total of 104 species from 80 genera and 30 families (Supporting Information Table S1) were randomly sampled in summer 2018 at a field station at Shangri-La Alpine Botanical Garden (lat 27°54′5″N, long 99°38′17″E; 3300–3350 m asl) in Yunnan Province, southwest China. The plant community is located southeast of the Hengduan Mountains, in one of the world's biodiversity hotspots. Our previous investigations of plant–pollinator interactions in the subalpine meadows over several years indicated that these native flowers are pollinated by a diversity of insects, including the dominant floral visitors: bumblebees and solitary bees (see Gong & Huang, 2009; Fang & Huang, 2012, 2013). The summer flowering season from late June to early September covered the most active period for insect pollinators in that mountainous region.

Color measurements

To measure the color of anthers, pollen and corolla, flower buds from one to three individuals of each species (Table S1) were randomly selected and bagged to prevent pollen removal by pollinators. At least three newly open flowers or inflorescences were collected and temporarily placed in a cold box at 4°C. The flower reflectance spectrum of each floral organ was measured in the 300–700 nm range using a spectrometer (Ocean Optics JAZ-EL200; Ocean Optics Inc., Dunedin, FL, USA) connected to a computer running SPECTRASUITE (Ocean Optics), with a fiber optic reflection probe (QR400-7-SR; Ocean Optics) held at a 45-degree angle relative to the surface of the measuring material (Johnson & Andersson, 2002). To measure anther background (i.e. corolla) color, the part of the inner surface of the corolla closest to where the anthers are located was mounted under the holder of the probe, presenting a flat surface and thus minimizing reflectance variability due to uneven distances between the corolla and the sensor. To measure the outer surface color of anther sacs, undehisced and newly dehisced anthers were used. For small flowers and anthers, we had to use corollas and anther sacs from multiple inflorescences to cover the entire measuring area. Pollen was vibrated from the anthers and gathered into a visible pollen mass for color measurement. one species yielded three color values for each of the corolla, anthers and pollen. Values were averaged from repeated measurements to obtain a single reflectance spectrum for each species.

Color characterization

To evaluate color difference between floral organs (anther, pollen and corolla), we used color composition to characterize their color, following Reverté et al. (2016). The color spectrum (300–700 nm) was divided into 40 wavebands of 10 nm each (Tables S2–S4). To calculate the proportion of the reflectance spectrum, the intensity (brightness) of reflected light for each band was divided by the total intensity of light reflected by the sample. Instead of the raw values of reflected brightness, its proportion was used here to exclude the difference between chromatically equivalent spectra (spectra with the same curve shape but different brightness values; Endler, 1990). To remove the effect of variation in the amount of sunlight across these 40 wavebands, the raw intensity of the light was adjusted using a D65 illumination spectrum (Chittka, 1992) before color composition calculation.

Bumblebees and solitary bees were the main pollen collectors observed on these flowers (Gong & Huang, 2009; Fang & Huang, 2012, 2013; Fig. 1). To investigate whether bee pollinators can distinguish the colors of the pollen/anthers from the corollas, we characterized floral organ color according to a bee-subjective view using the color hexagon model developed by Chittka (1992), which takes account of the background color, pollinator photoreceptor sensitivities and ambient illumination (Tables S2–S4). This was performed using the *vismodel* function of the package PAVO (Maia et al., 2019) in R v.3.5.0 (R Core Team, 2018) with the reflectance spectrum of green foliage as background and the D65 illumination spectrum as ambient illumination.

Pollen visibility

To compare pollen visibility to insect visitors between plants, we recorded whether the male organs (anthers and pollen) are
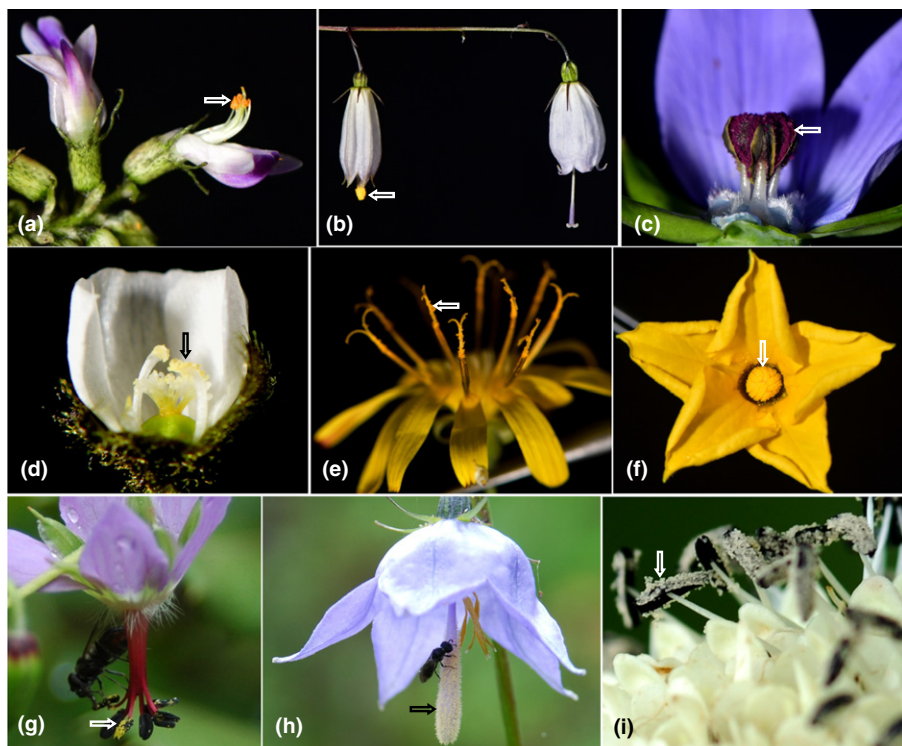
Fig. 1 Examples of species with concealed (a, b) or exposed (c–i) pollen, illustrating corolla and pollen color. (a) Concealed orange pollen in *Astragalus pullus*, with keel petals removed; (b) yellow pollen presenting in the evening in a moth-pollinated white flower *Adenophora capillaris*; (c) secondary presentation of purple pollen in *Codonopsis convolvulacea*; (d) light yellowish-white pollen in the open-shaped flower of *Drosera peltata*; (e) yellow pollen presented in yellow-flowered *Ixeris chinensis*; (f) yellow anthers and pollen in yellow-flowered *Herpetospermum pedunculosum*; (g) yellow pollen in pink-flowered *Geranium delavayi*, with a fly as a pollen theif; (h) light-colored pollen presented on white stigmatic lobes in *Adenophora jasionifolia*, with a solitary bee feeding on pollen; and (i) white pollen grains exposed in white-flowered *Dipsacus asper*. Arrows point to pollen presentation in each species.

exposed or concealed in each species. Flowers were categorized as the exposed type if their anthers and pollen are visible to potential floral visitors, or as concealed if they are hidden in the corolla tube or blocked by the corolla. For example, flowers were considered to be exposed in *Ixeris chinensis* (Asteraceae), *Codonopsis convolvulacea* (Campanulaceae) and *Herpetospermum pedunculosum* (Cucurbitaceae) because their anthers and pollen are not shielded by the corolla or located at the very entrance of the flower opening (Fig. 1). Anthers and pollen enclosed by the corolla in Fabaceae flowers (Fig. 1a) or hidden by sepals in the bell-shaped flower of *Clematis akebioides* (Ranunculaceae) were considered to be concealed.

Data analysis

To compare the color diversity of floral organs in the concealed and exposed species, we performed a principal component analysis (PCA) to integrate across the reflectance spectrum (40 wavebands of 10 nm each from 300–700 nm), and explored color variance within pollen visibility categories with the function *prcomp* in R v.3.5.0 (R Core Team, 2018).

To establish whether the colors of the corolla, anthers and pollen are phylogenetically constrained and to examine the effect of pollen exposure on the color contrast, we built a phylogenetic tree of the 104 species with three outgroup species based on nuclear Internal Transcribed Spacer (*nr*ITS) and plastid marker *mat*K and *rbc*L regions. All GenBank accession nos. are shown in Table S1. Preliminary alignments were produced using MUSCLE v.3.8 (Edgar, 2004), then adjusted manually in GENEIOUS v.11.0 (www.geneious.com). Aligned matrices of three DNA regions were combined using SEQUENCEMATRIX v.1.8 (Vaidya *et al.*,

2011). Sequences of the *nr*ITS, concatenated *mat*K, and *rbc*L regions were analyzed separately and in combination. No sequences were excluded from the analyses.

Bayesian Inference (BI) methods were used for phylogenetic reconstruction. Partitioned BI analyses were performed using MRBAYES v.3.2.6 (Ronquist & Huelsenbeck, 2003), with DNA substitution models selected for each gene partition using the Bayesian information criterion (BIC) in JMODELTEST v.2.0 (Darriba *et al.*, 2012). Markov Chain Monte Carlo (MCMC) analyses were run in MRBAYES for 100 000 000 generations for each dataset, with each run comprising four incrementally heated chains. The BI analyses were started with a random tree and sampled every 1000 generations. The first 25% of the trees were discarded as burn-in, and the remaining trees were used to generate a majority-rule consensus tree. BI analyses, as well as JMODELTEST, were performed at the CIPRES Science Gateway (http://www.phylo.org).

To see whether the color contrast between anthers and pollen and their background corollas differs between concealed and exposed species, we calculated two color distance parameters. For color composition, Euclidean distances (Théry & Casas, 2002) based on the proportion of the reflectance spectrum for 40 wavebands were calculated between the anthers/pollen and the corolla. For the bee-subjective view, we first mapped the reflectance spectrum of anthers and pollen into Chittka's hexagon model with the corresponding corolla as background (Chittka, 1992) and then calculated the Euclidean distances between the sample dot and center of the hexagon as the color distance between the anthers/pollen and the corolla. We produced a phylogenetic linear model using maximum likelihood with Pagel's lambda model (Pagel, 1999), with color distance as the dependent variable and

pollen visibility as a fixed factor, to examine whether pollen visibility affects color contrasts between anthers/pollen and the corolla. This analysis was performed with the function *phylolm* in the package PHYLOLM (Ho & Ane, 2014) in R v.3.5.0 (R Core Team, 2018) separately.

To examine the effect of phylogenetic constraints on the evolution of the color of floral organs in the 104 species, the presence of phylogenetic signal in color composition and color in a bee-subjective view on the phylogenetic tree, Kmult values (the generalized *K* statistic; Adams, 2014) specifically developed for high-dimensional multivariate data were calculated using GEOMORPH's *physignal* function (Adams & Otarola-Castillo, 2013) in R v.3.5.0 (R Core Team, 2018). Color difference of pollen vs corolla and anther vs corolla with Blomberg's *K* test (Blomberg *et al.*, 2003) were calculated with the *phylosig* function of the PHY-TOOLS package (Revell, 2012) in R v.3.5.0.
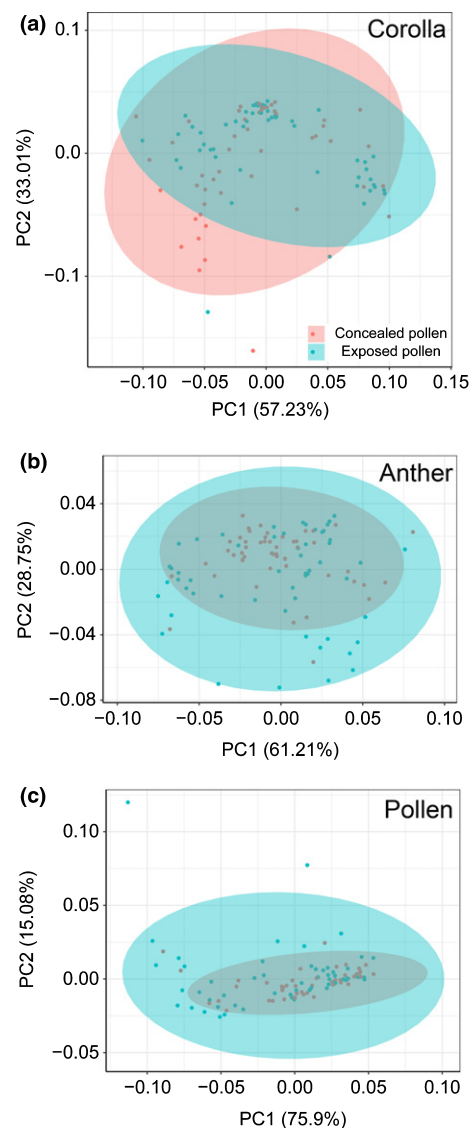
Results

In 49 of 104 sampled species, the anthers as well as the pollen were concealed (Table S1) in three main ways. Anthers and/or pollen were concealed completely in the flowers in species such as Fabaceae, *Pedicularis*, *Salvia przewalskii* and *Nepeta stewartiana*; or they were covered by the corolla tubes in completely or partly bell-shaped flowers; or pollen was released in the evening in moth-pollinated *Adenophora capillaris* (Fig. 1), which escaped temporally from pollen collection by bee activity in the day.

The colors of anthers, pollen and corollas showed great diversity across the 104 species (Fig. 2). PCA of the color composition indicated that the concealed and exposed species shared similar corolla color diversity (Fig. 2a, similar area of 95% confidence ellipse in PCA color space), varying from purple in 'concealed' *Tibetia himalaica* and 'exposed' *Geranium delavayi* and to red in 'concealed' *Onosma confertum* and 'exposed' *Lilium tigrinum* (Fig. S1). The anthers and pollen in the concealed species were mainly white and yellow (Fig. S1), and color diversity was much lower in these plants than in the remaining 55 species with exposed pollen (Fig. 2b,c, larger area of 95% confidence ellipse in PCA color space of exposed species). Anther color varied greatly in the exposed species, from dark in *Dipsacus asper* (Fig. 1i) to white in *Silene gracilicaulis*, with yellow seen in some Rosaceae species and pink in *Lamium amplexicaule*. Pollen color varied greatly from purple in *Codonopsis convolvulacea* (Fig. 1c) to bronze in *Lilium tigrinum* (Fig. S1).

The colors of anthers and pollen were not similar in some species. For example, anthers of *Dipsacus asper* (Fig. 1i), *Dipsacus chinensis*, *Pterocephalus hookeri*, *Aconitum piepunense* and *Geranium delavayi* (Fig. 1g) were dark (black), while their pollen grains were white or yellow. The former three species had white flowers, and their exposed pollen appeared the same color as their corollas, but this consistency was not observed in the latter two species, in which black anthers contrasting with white or yellow pollen grains were exposed within differently colored corollas.

The phylogenetic linear model analysis based on the color compositions indicated that the color difference between anther and corolla of species with exposed pollen did not differ

significantly from that of species with concealed pollen; this was found to be the case for all phylogenetic linear model analyses (Table 1, Fig. 3a). However, the color difference between pollen and corolla was significantly smaller in species with exposed pollen than in species with concealed pollen (Table 1, Fig. 3). A similar color difference pattern was observed in the phylogenetic linear model analysis when considering a bee visual system (Table 1, Fig. 3b). In a bee vision hexagon with corollas as background, anthers (26 of 55 = 47.3%) and pollen (20 of 55 = 36.4%) were more likely to be inconspicuous to bees in the exposed than in concealed species (12 of 49 = 24.5% and 8 of 49 = 16.3% for anthers and pollen, respectively, Fig. 4). Anther and pollen color distances from the background were smaller in the exposed than in concealed species (Fig. 4, smaller area of 95% confidence ellipse in color space of exposed species), indicating that in a bee-subjective view, color matching between pollen and corollas was

Fig. 2 Principal component analysis (PCA) plots of corolla (a), anther (b) and pollen color composition of species (c) with concealed (red) or exposed (blue) pollen with 95% confidence ellipses.

Table 1 Phylogenetic linear model results of color difference of pollen/anthers vs corolla between flowers with exposed and concealed anthers.

Color difference source	Color characterization	Dependent variable	Factor	Estimate	SE	T	P
Anther vs Corolla	Color composition	Color difference	Pollen exposure	−0.010	0.007	−1.445	0.151
	Color in bee-subjective view			−0.030	0.018	−1.694	0.093
Pollen vs Corolla	Color composition			−0.024	0.007	−3.414	**0.000**
	Color in bee-subjective view			−0.041	0.017	−2.375	**0.019**

The color contrast between pollen and corolla differed significantly (*P* values in bold) in both comparisons of color composition and color in a bee-subjective view between species with exposed and concealed anthers and pollen.

significantly higher in species with exposed pollen than species with concealed pollen.

Significant or marginal phylogenetic signals existed in the calculations of pollen, anther and corolla color composition and their color in the bee-subjective view (Table 2). In all cases, *K* values were < 1, indicating that the color of floral organs of related species was less similar than was expected under the Brownian motion evolution model. However, significant phylogenetic constraints were not observed in either the color distance of pollen vs corolla or anther vs corolla in color composition and the bee-subjective view (Table 2, Fig. 5), indicating that color difference between floral organs varied across species independent of their phylogenetic relationships.

Discussion

Our comparative survey showed that the color of pollen rather than anthers resembled the color of the corolla in species with exposed anthers and pollen, resulting in the significantly higher color diversity of exposed pollen. We found that the color contrast between pollen and corolla was smaller in species with exposed pollen than in species with concealed pollen in which the anthers are hidden by the corolla. Although colorful pollen (or anthers) has been thought to act as a visual signal for attracting flower visitors, this contrasting color pattern in flowers with exposed or concealed pollen indicates that the resemblance of exposed pollen to the corolla may be selected for as a protection against pollen thieves, hiding the pollen via visual crypsis.

Flower colors in natural plant communities may not be randomly assembled; their diversity could be driven by different pollinator taxa with different color preferences (Gumbert *et al.*, 1999; Arnold *et al.*, 2009; Shrestha *et al.*, 2013, 2016; Briscoe Runquist *et al.*, 2016; Kemp *et al.*, 2019). Flower color convergence was observed in an island community in which the flower species were pollinated by flies (Shrestha *et al.*, 2016) and in daisy communities in South Africa in which a non-random assemblage of complex flower color patterns was proposed to be maintained by the preference of the dominant local pollinators (Kemp *et al.*, 2019). The diversity of flower color in five subalpine meadows in Canada increased the dissimilarity between flowers in a neighborhood, potentially reducing interspecific pollinator moves between co-flowering species with similar colors (McEwen & Vamosi, 2010). Although flower color is a simple floral trait, its diversity and evolution may be influenced by multiple factors, including pollinators and florivores as well as phylogenetic relationships (Armbruster, 1997; Chittka, 1997; Strauss & Whittall, 2006; Rausher, 2008).

Pollen can be consumed by pollinators as well as by robbers and thieves. In natural communities, pollen consumers include beetles, social and solitary bees, and syrphid flies, as well as a few species of butterflies and wasps (Lunau, 2000; Thorp, 2000). Bee pollinators are usually the most frequent pollinators, but they also act as pollen thieves on various plant species (Hargreaves *et al.*, 2009), consuming pollen themselves and collecting pollen as provisions for their larvae (Thorp, 1979). Plants may evolve to escape pollen thieves in time by presenting their pollen at a time when pollen thieves are not active (i.e. *Adenophora capillaris*, Fig. 1b) or in space by presenting cryptic or inaccessible (usually concealed) pollen (Hargreaves *et al.*, 2009). Therefore, for species with exposed pollen, anther and pollen color are likely to be under conflicting selection from pollinators and pollen consumers. Such conflicting selection seems to be generated in heterantherous species with two types of anthers in which feeding anthers are usually conspicuous, contrasting in color with the corolla, but pollinating anthers are inconspicuous and similar in color to the corolla (Vogel, 1978; Vallejo-Marín *et al.*, 2009). For example, in blue-flowered *Monochoria korsakowii*, bee visits to flowers greatly decreased when the yellow anthers were removed, but did not when the dark purple anthers were
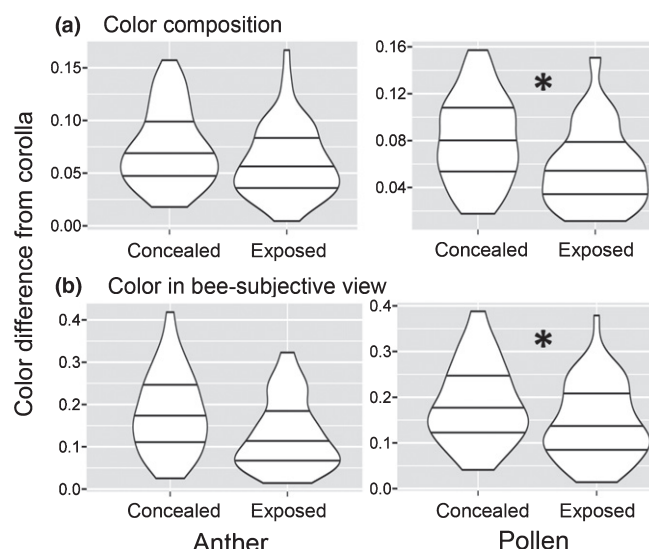
Fig. 3 Comparisons of color differences of anther vs corolla as well as pollen vs corolla between concealed pollen and exposed pollen species, estimated by phylogenetic linear model analysis (*, *P* < 0.05). The plot shows rotated kernel density on each side of the violin and the three horizontal lines inside the violin from the bottom to the top show the 25%, 50% and 75% quantiles of the density estimate.
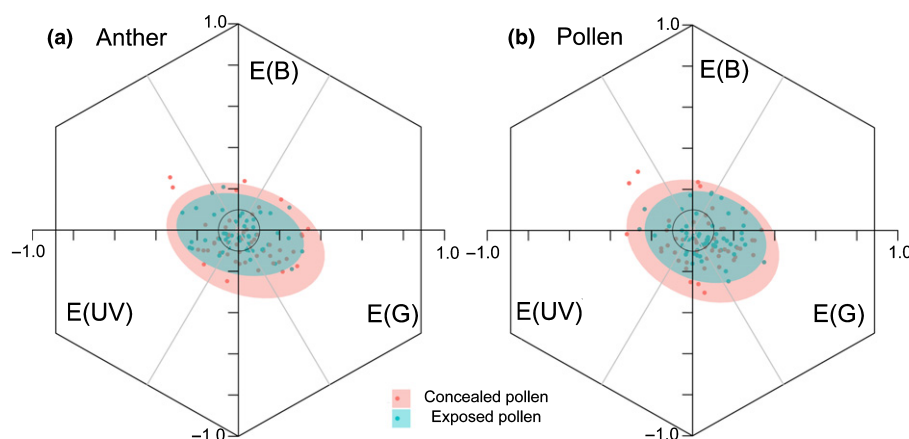
Fig. 4 Reconstruction of the phylogeny of 104 species with pollen exposure types (concealed pollen, open circles; exposed pollen, closed circles) and color contrast values (givendepicteon bar chart) between the pollen and the corolla mapped onto it. Numbers beside branches indicate Bayesian inference (BI) posterior probability (percent) support.

Table 2 Analyses of phylogenetic signal for color composition and color in bee-subjective view (Kmult values) and color distance in color composition and bee-subjective view (Blomberg's K) values) for 104 species.

	Phylogenetic signal	P values
Color composition		
Corolla	0.285	0.086
Anther	**0.493**	**0.001**
Pollen	**0.564**	**0.001**
Color in bee-subjective view		
Corolla	0.293	0.081
Anther	**0.429**	**0.001**
Pollen	**0.417**	**0.001**
Color distance in color composition		
Anther vs corolla	0.238	0.574
Pollen vs corolla	0.271	0.240
Color distance in bee-subjective view		
Anther vs corolla	0.277	0.222
Pollen vs corolla	0.310	0.058

Significant results ($P < 0.05$) are in bold.

removed (Tang & Huang, 2007). The theoretical model predicts that heteranthery could evolve when bees consume excess pollen (Vallejo-Marín *et al.*, 2009). Visual crypsis of pollinating anthers could be a pollen protection strategy, hiding pollinating anthers from pollen collection but allowing the feeding anthers to attract pollinators.

In color polymorphic species, pollen color variation usually shows a geographical or environmental gradient pattern, and it has been suggested that this also may be the result of selection by local pollinators and pollen consumers. Interestingly, bees were observed to prefer certain colored pollen in three of the species which exhibit pollen color polymorphism: *Nigella degenii* (Jorgensen *et al.*, 2006), *Campanula americana* (Lau & Galloway, 2004; Ison *et al.*, 2018) and *Erythronium americanum* (Austen *et al.*, 2018). For example, in *N. degenii*, naive bee pollinators (including bumblebees and honey bees) preferred one color morph at one time, but their preferences changed between experiment

dates and locations (Jorgensen *et al.*, 2006). In *C. americana*, halictid bees preferred the male-phase flowers with pollen, especially the ones with tan-colored pollen (Lau & Galloway, 2004), while the *Megachile* (Hymenoptera) pollinators displayed a strong and consistent preference for purple pollen (Ison *et al.*, 2018). Pollen grains of *Epimedium pubescens* are completely exposed and easily accessible on two oblong valves of the anthers with valvular dehiscence, hanging in the green leaf background (Wang *et al.*, 2018). Although only one bee pollinator species was observed in *E. pubescens*, the high frequency of green anthers and pollen maintained in this species could be facilitated by visual crypsis providing protection from pollen collecting by other bees.

The flavonoid pigments in yellow pollen (Harborne & Grayer, 1993) are widespread in various families of angiosperms and wind-pollinated gymnosperms, such as Ginkgoaceae, Pinaceae, Taxaceae and Taxodiaceae (Stanley & Linskens, 1974), suggesting that the yellow color might be an ancestral feature (Lunau, 2000). In our study of 104 plant species, 50% of the species produced white pollen, and the majority of the colored pollen grains were yellow (33 of 104 = 31.7%, personal observation by eye), especially for the species with concealed pollen (19 of 49 = 38.8%, Fig. S1). In the 55 species with exposed pollen, pollen grains of some species have bright colors, such as yellow (14 of 55 = 25.5%), red and even purple (Fig. S1), but most of them were not conspicuous because they appear nearly the same color as the background corolla from the viewpoint of flying animals. An exception was *Geranium delavayi* in which exposed pollen grains were light yellow and conspicuous in the downward-facing pink flowers (Fig. 1g). In this herb, bumblebees were observed to be major pollinators (Fang & Huang, 2012), collecting nectar but not pollen, which was placed on the bumblebee body where it was not groomed and was effectively available for pollination (S-Q. Huang, pers. obs.).

Our phylogenetic linear model analysis of the 104 species detected smaller color differences between pollen and corolla in species with exposed pollen than in those with concealed pollen (Fig. 3a), indicating that the color of exposed pollen, relative to concealed pollen, more closely resembled that of its corolla, supporting the pollen crypsis hypothesis. From a bee-subjective

Fig. 5 Hexagon plot of anther (a) and pollen color (b) of concealed pollen (red) and exposed pollen (blue) species in a bee visual system, with the corresponding corolla as background. E(B), E(G) and E(UV) refer to the hyperbolic-transformed quantum catch in blue, green and UV photoreceptors of honeybees, respectively (Chittka, 1992). The 95% confidence ellipses are shown for the data. The distance between the center and each dot indicates the color contrast between anthers/pollen and corolla. The colors inside the central circle (0.1 hexagon units) appear achromatic to bees.

view, the color contrast between pollen and corollas was also significantly smaller in species with exposed pollen (Figs 3b, 4). In such cases, bee pollinators may not distinguish pollen from corollas because of the color similarity in many exposed species, so that pollen could be passively placed on the visitor's body and

delivered to other flowers. An early survey involving pollen color in Cactaceae, in which species are pollinated by diverse animals, showed that the pollen of 11% of species was red or brown. Such a pollen color, similar to a hummingbird pollinators' dark-colored beak, was considered as an adaptation to secure safe pollen

transfer (Rose & Barthlott, 1994). The red or brown color of the pollen, similar to the background red corollas, may perhaps serve as visual crypsis for pollen thieves.

Plant–pollinator interactions greatly affect the fate of pollen, and this may play an essential role in the evolution of floral traits (Hargreaves *et al.*, 2009; Minnaar *et al.*, 2019). To protect pollen, plants may evolve physical and/or chemical defenses against pollen overexploitation by diverse pollen collectors (Wang *et al.*, 2019). Considering conflicting selection on pollen from pollinators and consumers, we proposed the visual crypsis hypothesis for pollen color. Our phylogenetic comparative study found that pollen color generally matched the corolla background color in exposed, as compared to concealed, anthers in an insect-pollinated plant community. To our knowledge, a quantitative measurement of the male part (anther and/or pollen) color in natural communities remains unreported. It would be useful to further test the hypothesis for pollen color in other communities and particularly for plants serviced by other (non-insect) pollinators. This perspective of the color contrast between pollen and the background could provide insights into the evolution of flower color, a simple trait of the plant reproductive system which may have been driven by conflicting selection.

Author contributions

S-QH planned and designed the research. Y-ZX, L-BJ and CZ performed experiments and conducted fieldwork, Y-ZX and S-QH analyzed data and wrote the manuscript, and all authors commented.

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New
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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 The reconstruction of the phylogeny of 104 species with pollen exposure types and color of pollen, corollas and anthers mapped on.

Table S1 Accession nos. in GenBank (National Center for Biotechnology Information, NCBI) for three DNA markers (*nr*ITS, *mat*K and *rbc*L) for 104 plant species.

Tables S2–S4 Color compositions and color a in bee-subjective view (Chittka, 1992) of reflectance spectra for pollen (**Table S2**), anthers (**Table S3**) and corolla (**Table S4**) in 104 flowering species, generated by a spectrometer (Ocean Optics JAZ-EL200).

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