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VIEWPOINT

Why are the inner and outer sides of many flower petals differently coloured?

X.-Q. Fan[1,2], J. Trunschke[1]*, Z.-X. Ren[1], H. Wang[1], G. H. Pyke[1,3], C. J. van der Kooi[4] & K. Lunau[1,5]

1 CAS Key Laboratory of Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China
2 College of Life Sciences, Shaanxi Normal University, Xi'an, China
3 School of Natural Sciences, Macquarie University, Ryde, NSW, Australia
4 Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, the Netherlands
5 Faculty of Mathematics and Natural Sciences, Institute of Sensory Ecology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

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Correspondence
K. Lunau, CAS Key Laboratory of Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China.
E-mail: lunau@uni-duesseldorf.de

***Present address**
J. Trunschke, Chair of Nature Conservation and Landscape Ecology, Albert-Ludwigs-University of Freiburg, Freiburg, 79106, Germany

Casper J. van der Kooi and Klaus Lunau shared senior authorship.

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ABSTRACT

The flower perianth has various, non-mutually exclusive functions, such as visual signalling to pollinators and protecting the reproductive organs from the elements and from florivores, but how different perianth structures and their different sides play a role in these functions is unclear. Intriguingly, in many species there is a clear colour difference between the different sides of the perianth, with colour patterns or pigmentation present on only one side. Any adaptive benefit from such colour asymmetry is unclear, as is how the asymmetry evolved. In this viewpoint paper, we address the phenomenon of flowers with differently coloured inner and outer perianth sides, focusing on petals of erect flowers. Guided by existing literature and our own observations, we delineate three non-mutually exclusive evolutionary hypotheses that may explain the factors underlying differently coloured perianth sides. The pollen-protection hypothesis predicts that the outer side of petals contributes to protect pollen against UV radiation, especially during the bud stage. The herbivore-avoidance hypothesis predicts that the outer side of petals reduces the flower's visibility to herbivores. The signalling-to-pollinators hypothesis predicts that flower colours evolve to increase conspicuousness to pollinators. The pollen-protection hypothesis, the herbivore-avoidance hypothesis, and the signalling-to-pollinators hypothesis generate largely but not entirely overlapping predictions about the colour of the inner and outer side of the petals. Field and laboratory research is necessary to disentangle the main drivers and adaptive significance of inner–outer petal side colour asymmetry.

INTRODUCTION

The flower perianth, particularly the colour and shape of petals and sepals, is essential for plant reproductive fitness, because it aids in attracting pollinators and/or deterring potential floral herbivores (van der Kooi *et al.* 2019). The perianth can also protect the reproductive organs, particularly the pollen in bud stage and during pollen presentation, against the elements (e.g. wind, rain, radiation, extreme temperature; Galen 1999; Burns 2015; Pacini & Dolferus 2019; Yu *et al.* 2021).

A flower's shape plays a well-known role in mediating how pollen is placed on the pollinator's body and limiting nectar and pollen robbing (Benson *et al.* 1975; Krupnick *et al.* 1999; Strauss *et al.* 2004; Caruso *et al.* 2010; Koch *et al.* 2017; van der Kooi *et al.* 2021a), but it also determines how the reproductive organs are exposed to the outside world and what parts of the flower constitute the visual signal. For example, for erect flowers and inflorescences (e.g. buttercups, poppies, roses, composites), the inner (adaxial) side of their petals or ligules is oriented upwards and visible to approaching visitors. Conversely, nodding flowers are typically bent downwards (e.g. snowdrops, bluebells, fritillaries), and present the outer (abaxial) perianth side to the outside world. For zygomorphic flowers (e.g. aconites, orchids, papilionaceous flowers) the functional relevance of both flower sides varies, because both sides could, in principle, be visible to the outside world depending on the specific flower morphology and the pollinator's angle of approach.

The colours of flowers are caused by wavelength-selective absorption by floral pigments and scattering of incident light by floral structures (van der Kooi *et al.* 2016). The perception of flower colours depends on the observer's visual system; for example, many insects can perceive ultraviolet (UV) patterns, whereas humans cannot. Remarkably, the flowers of many species exhibit a clear colour difference between the inner and outer petal sides. This asymmetry in colouration can be prominently visible to humans, particularly in species with open, erect flowers, where the outer, generally downward-facing (abaxial) side is commonly paler to the human eye than the inner, generally upward-facing (adaxial) side (Figures 1–3). Floral colour patterns can also differ between the inner and outer sides. Colour patterns mostly occur on the inner side of flowers, which is typically viewed by pollinators (Figures 1–3; Lunau 2000; Gronquist *et al.* 2001).

The inner–outer side colour asymmetry is interesting in several ways, because it hints at an evolutionary trade-off. On the one hand, the pale colour of the outer side is due to low amounts of floral pigments in the outer epidermal layer. Low pigment deposition at the flower side that is hardly ever visible to pollinators seems expected from a plant's economic point of view (Stavenga & van der Kooi 2016). On the other hand, despite its pale appearance in the visible spectrum, the outer sides often contain a large amount of UV-absorbing pigments (Eisner *et al.* 1973), which suggests that there still is an adaptive function of having pigments at that flower side. That is, even for flowers, of which one side is pale, this flower side is not entirely released from selection on pigmentation.

Studies documenting the extent and diversity of this outer–inner side asymmetry in petal colouration are lacking, and so are explanations for colour asymmetry, which appears to be widespread. What ecological factors could explain the seemingly widespread phenomenon of differently coloured flower sides?

The aim of this viewpoint article is to present an overview of our current knowledge and formulate testable hypotheses to explain the phenomenon of differently coloured flower sides. Focusing on petals we first discuss physiological, ecological and evolutionary factors that contribute to this widespread asymmetry in the colouration of petal sides. Drawing on our observations in different geographic regions, we present an overview of the phenomenon of asymmetrically coloured petal sides using UV-imaging cameras and spectroscopy. We further use existing literature on the optical properties of flowers as well as the ecological significance of flower pigmentation for pollen protection to delineate three hypotheses that provide evolutionary/functional/adaptive explanations for petal side colour variation. We furthermore discuss the importance of the flower bud stage in determining colour asymmetry.

FLOWERS WITH ASYMMETRICALLY COLOURED SIDES ARE FOUND IN MANY PLANT SPECIES

To expand on the very limited literature on flower petal asymmetry and to illustrate the diversity of species, shapes and colours that occur in species with asymmetrically coloured flower sides, we photographed the flowers of more than 100 species with a UV-sensitive camera setup. A selection of 30 species is presented in Figures 1–3 that covers the entire spectrum of the phenomenon. The plants were photographed from 2019 to 2023 in the botanical garden of the Heinrich-Heine University, Germany, in the field in alpine areas of Switzerland (Natural Park Beverin), and on Yulong Snow Mountain and Baima Snow Mountain in Yunnan Province, China, a global biodiversity hotspot (Liang *et al.* 2018). The photography method is explained in detail by Verhoeven *et al.* (2018) and Lunau *et al.* (2020), and was found to be efficient to process large numbers of flowers in the field (Lunau *et al.* 2021). The reproducibility of photos was ensured by white balance for both colour and UV photos (Verhoeven *et al.* 2018). More sophisticated (camera) equipment is needed to quantify reflectance in more detail (e.g. Garcia *et al.* 2014).

Although our sampling was neither exhaustive nor systematic, our sample of plant species reveals that in numerous species, flower colour differs between the inner and outer side of the perianth, with some patterns apparent. For example, we found many species with clear colour differences between inner and outer perianth sides (Fig. 1A, B, E–H, L, M, O and Fig. 2A–C, E, H, I, K, L). Furthermore, we observed that outer flower sides more commonly absorb UV than inner flower sides (Fig. 1A–C, E–H, L, M, O, and Fig. 2A–C, E, H, I, K, L), which is in line with previous observations in the literature (Eisner *et al.* 1973; Gronquist *et al.* 2001; Stavenga & van der Kooi 2016). We found uniform UV absorption on outer flower sides is typical for most species, while inner flower sides typically exhibit a UV pattern. Notable exceptions are *Gentiana verna* (Fig. 2A) and *Hypericum calycinum* (Fig. 3 displaying a UV pattern on the outer side).

In addition, the appearance in either human-visible colour and/or the ultraviolet wavelength range between the inner and outer flower side differs in many species with disc- and bowl-shaped flowers (Fig. 1). Examples include the ray florets of *Calendula arvensis* (Fig. 1G), *Rudbeckia fulgida* (Fig. 1E), *Bellis perennis* (Fig. 1O), *Senecio jacobaea* and *S. vulgaris* (Fig. 1F). Also, differences in human-visible colour between the flower sides are found in *Convolvulus tricolour* (Fig. 1L, M), stronger UV absorption of outer sides are found in *Ficaria verna* (Fig. 1A) and *Potentilla anserina* (Fig. 1B), as well as between outer sides of buds and open flowers of *Verbascum nigrum* (Fig. 1C) and *Chelidonium majus* (Fig. 1D). In contrast, the nodding inflorescence of *Cremanthodium nobile* has ray florets reflecting UV light at both sides (Fig. 1I). *Papaver rhoeas* (Fig. 1J) and *Geranium pratense* (Fig. 1K) display similar colours on both sides, excluding the floral guide. In *Stellera chamaejasme* (Fig. 1H) the buds are reddish and UV-absorbing, whereas in *Platycodon grandiflora* (Fig. 1N) the buds are greenish and UV-reflecting and undergo a colour change when the flowers open.

The different appearance in either human-visible colour and/or the ultraviolet wavelength range between the inner and outer flower side is more complex in zygomorphic and tubular flowers, since their inner side is visible to approaching flower visitors in some but not all species (Fig. 2). A UV pattern is present on the outside of the petals of *Gentiana verna* with only the UV-absorbing part being visible during the floral bud stage (Fig. 2A). The beak of *Pedicularis* flowers covering the stamens is UV-absorbing in *P. superba* (Fig. 2C), *P. gruina* (Fig. 2H) and *P. alopecuros* (Fig. 2I). In *Pedicularis gruina* (Fig. 2H) the petal sides differ in human-visible colour as well as in UV reflection. Examples for the diversity in the colour of both flower sides include the papilionaceous flowers of

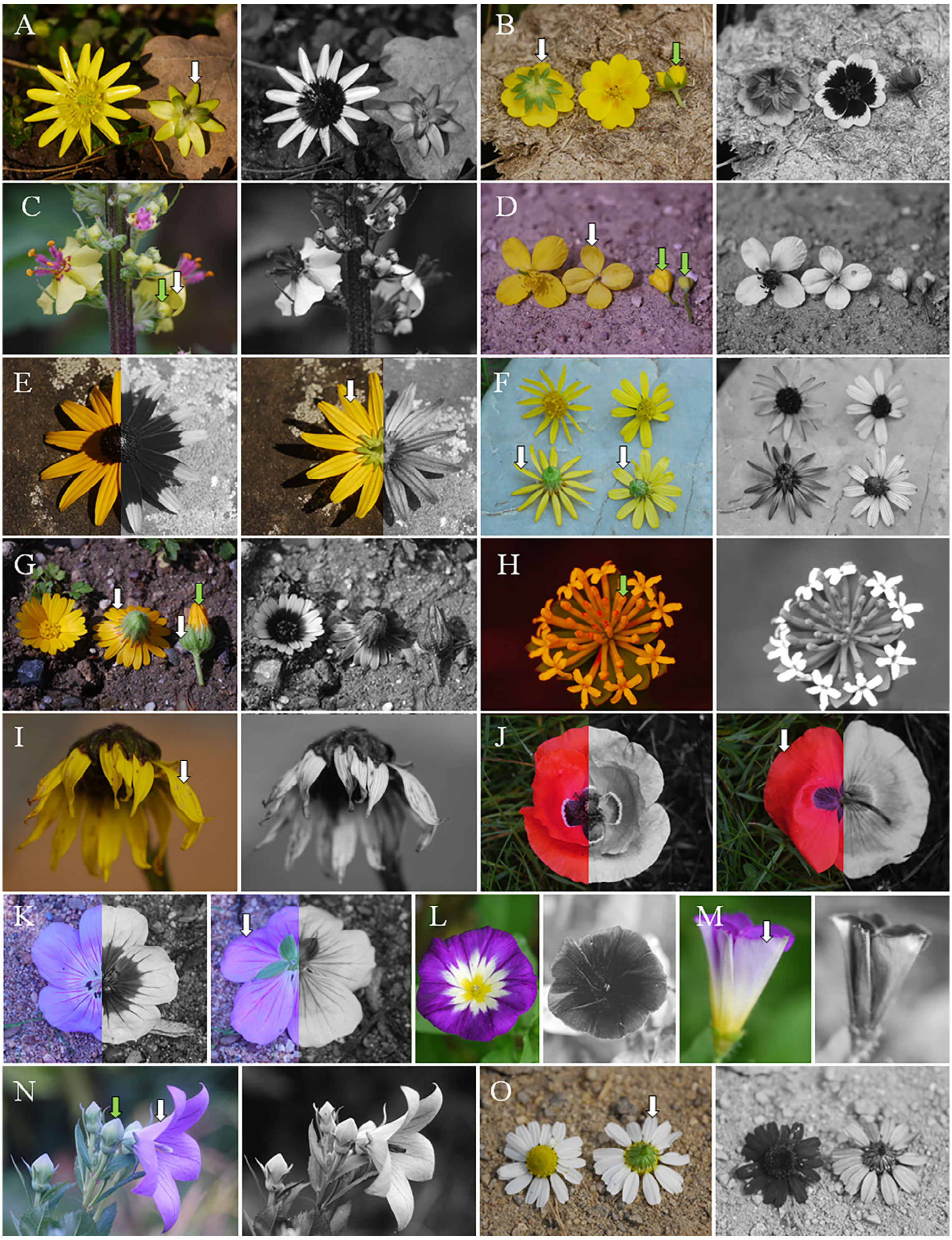

Fig. 1. Diversity of disc- and bowl-shaped flowers exhibiting flower colour asymmetry. Outer sides are indicated by a white arrow; buds displaying outer sides are indicated by a green arrow. Colour photo (left) and UV photo (right) of (A) *Ranunculus ficaria*, (B) *Potentilla anserina*, (C) *Verbascum nigrum*, (D) *Chelidonium majus*, (E) *Rudbeckia fulgida*, (F) *Senecio jacobaea* (left) and *S. vulgaris* (right), (G) *Calendula arvensis*, (H) *Stellera chamaejasme*, (I) *Cremanthodium nobile*, (J) *Papaver rhoeas*, (K) *Geranium pratense*, (L) *Convolvulus tricolour*, (M) *Convolvulus tricolour*, (N) *Platycodon grandiflora*, (O) *Bellis perennis*.

Indigofera heterantha (Fig. 2J), *Cytisus scoparius* (Fig. 2K) and *Colutea orientalis* (Fig. 2L). Different UV reflection between buds and outer sides of petals is present in *Primula bulleyana* (Fig. 2B) and *Myosotis palustris* (Fig. 2E). Colour differences between outer and inner sides are small in *Salvia lutea* (Fig. 2F), *Viola delavayi* (Fig. 2G) and *Iris reticulata* (Fig. 2D)

Fig. 2. Diversity of tubular and zygomorphic flowers exhibiting flower colour asymmetry. On colour photos, outer sides are indicated by a white arrow; buds displaying outer sides are indicated by a green arrow. Colour photo (left) and UV photo (right) of (A) *Gentiana verna*, (B) *Primula bulleyana*, (C) *Pedicularis superba*, (D) *Iris reticulata*, (E) *Myosotis palustris*, (F) *Salvia lutea*, (G) *Viola delavayi*, (H) *Pedicularis gruina*, (I) *Pedicularis alopecuros*, (J) *Indigofera heterantha*, (K) *Cytisus scoparius*, (L) *Colutea orientalis*, (M) *Gentiana acaulis*, (N) *Corydalis cava* (inset with some flower parts removed).

but exclude floral guides. A UV pattern is present on the outer side of *Gentiana acaulis* (Fig. 2M). The lateral petals covering the anthers of *Corydalis cava* (Fig. 2N) strongly absorb UV light on both sides, exhibiting different human-visible colours.

Perianth parts or sides can vary in their importance for visibility from a distance and at close range. For example, inner sides of petals are generally viewed en face from short distances, whereas the flower's outer sides of petals can be more

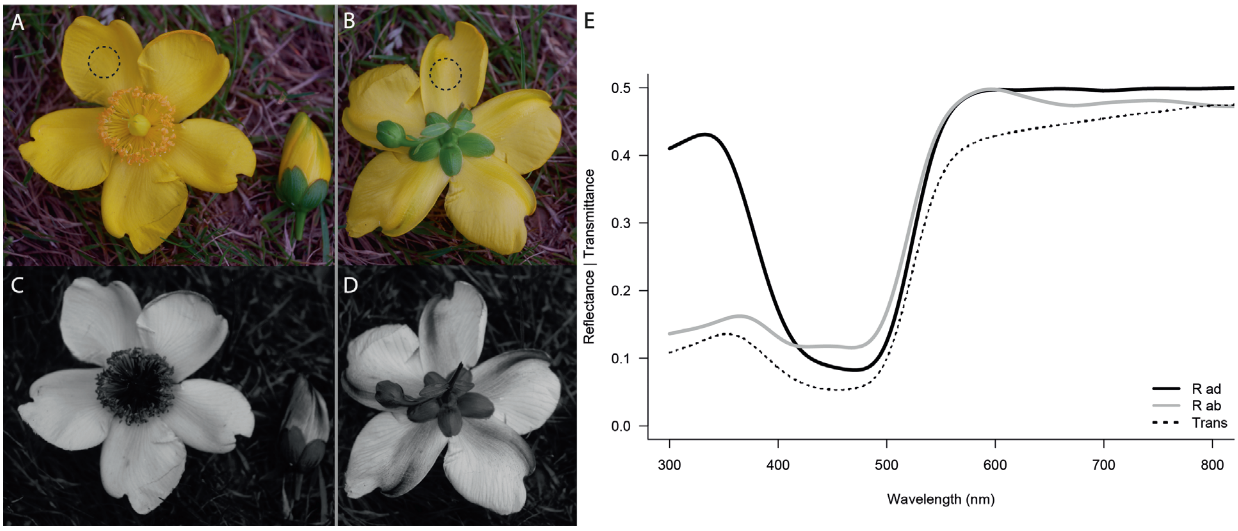

Fig. 3. Spectral differences between flower sides of *Hypericum calycinum*. (A) Colour image of the inner side of the petals and a flower bud. (B) Colour image of the outer side of the petals. (C, D) Corresponding UV images. (E) Reflectance and transmittance spectra of the inner (R ad) and UV-absorbing area of the outer (R ab) side. The measurement area is indicated in A and B (dashed circle). The transmittance (Trans) spectrum is identical for both sides, because of the reversibility of light rays.

important for long-distance detection (Dafni 1994). In these flowers, the anthers are generally not exposed to the outside world directly, but covered by floral structures. Examples include papilionaceous flowers as for example *Colutea orientalis*, *Cytisus scoparius* and *Corydalis cava* (Fig. 2). For bell-shaped flowers, the outer part constitutes the visible display. This is the case for both hanging bells, such as snowdrops, as well as for erect bells, such gentians. For erect bells, the reproductive organs are partly exposed to the elements.

SPECTRAL CHARACTERISTICS OF ASYMMETRICALLY COLOURED FLOWERS

The inner–outer side colour differences that we observed with our photography (Figs 1 and 2) are likely ecologically relevant for visual signalling to flower-visiting insects and protecting pollen from UV radiation. Incident light that reaches a flower can be reflected, transmitted or absorbed. All these three aspects are relevant for visual signalling and protecting pollen. Reflection of light occurs at the boundaries of cell walls, vacuoles and other scattering structures (Kay *et al*. 1981; van der Kooi *et al*. 2016). Absorption is caused by pigments, which are deposited in one or several flower layers (van der Kooi *et al*. 2016). Light that is neither absorbed nor reflected will be transmitted.

Colour asymmetry between different flower sides is primarily determined by differences in pigment deposition between floral layers. Floral pigments, such as chlorophylls, carotenoids, anthocyanins and flavonols (Narbona *et al*. 2021), can be distributed in the inner epidermal layer, the outer epidermal layer, both or throughout the flower (van der Kooi *et al*. 2016). When floral pigment is concentrated in the epidermal layer at the side of viewing, the visual signal is more modulated and hence the flower more conspicuous than when pigment is distributed throughout the flower or in the epidermal layer on the other side (see figure 4 in van der Kooi *et al*. 2016). In addition, the (surface) structure of the epidermal layer determines the degree of glossiness of a flower, with very flat and smooth surfaces creating a glossy (shiny) appearance, and cone-shaped epidermal cells a more matte appearance (van der Kooi *et al*. 2014; Stavenga *et al*. 2020; Wilmsen *et al*. 2021). A comparative study covering 46 species in nine plant families revealed that, in general, the adaxial side of flowers is significantly more structured than the abaxial side (Kraaij & van der Kooi 2020). It is likely that this structural difference between the two outer epidermal layers bears a function for visual signalling. The structural and pigmentary aspects of flowers are not only important for the visual signal, but may also be important for how light is reflected to the reproductive organs and how much heat is accumulated in flowers (see below).

Illustrate the spectral characteristics of the inner and outer flower side, as an example, we examined the flowers of *Hypericum calycinum* in detail (Fig. 3). The different flower sides of *H. calycinum* are not only visibly different to humans, but most likely also to insects and highlight notable differences in the reflectance of UV light. The reflectance and transmittance spectra were obtained as per van der Kooi *et al*. (2016). The reflectance and transmittance values are high for wavelengths above 500 nm, regardless of the side of viewing. In contrast, the reflectance in the UV wavelength range (i.e. <400 nm) is different between the flower sides (Fig. 3E). The inner side, which generally constitutes the display to pollinators, has a high reflectance in the UV, whereas the outer side features a stripe with UV-absorbing pigment along the edge (Fig. 3A, C). It appears that parts of the outer epidermal layer contain a UV-absorbing pigment, which also filters the transmitted light (Fig. 3B, D). The part with low reflectance in the UV wavelength range is what is visible during the floral bud stage, which does not reflect UV (see the bud right of the flower in Fig. 3A, C). In the blue wavelength-range (between 420 and 500 nm) there is also a small but noticeable difference between the flower sides, which is probably due to differences in the epidermal cell shape.

THREE EVOLUTIONARY HYPOTHESES TO EXPLAIN WHY FLOWERS HAVE DIFFERENTLY COLOURED SIDES

We propose three non-mutually exclusive hypotheses to explain the asymmetric colouration of the inner and outer sides of the perianth, focusing on petals of erect flowers. We refer to these as the pollen-protection, herbivore-avoidance and signalling-to-pollinators hypotheses.

Pollen-protection hypothesis (PP hypothesis)

The pollen-protection hypothesis assumes that significant pollen damage can arise from exposure to UV radiation and that under some conditions, plants are selected for protecting their pollen from this radiation. The need for pollen protection against UV radiation is based on the fact that pollen is particularly vulnerable to UV radiation, causing damage to the DNA (Jansen *et al.* 1998; Peach *et al.* 2020). For example, Zhang *et al.* (2015) found that among 21 species at high altitude in the Hengduan Mountains of China with exposed pollen, only eight are sensitive to UV radiation during pollen germination, whereas among the 21 species with protected pollen, 17 species are sensitive to UV radiation during pollen germination.

Structures that protect against UV radiation are especially likely to be selected for in high-mountain species. UV radiation increases with altitude because of decreasing air density, surface albedo, clouds, aerosols and ozone (Pfeifer *et al.* 2006). Given the plant's fitness costs of damaged pollen, i.e. reduced viability, natural selection will select for pollen protection, especially in species that are exposed to high UV radiation.

The PP hypothesis also assumes that pollen damage by UV radiation is more likely to occur during the bud stage than when flowers are open. Pollen grains might be directly exposed to UV radiation during pollen presentation in open flowers, following anther dehiscence (Scott *et al.* 2004), but such open pollen presentation would usually last only a few hours or up to few days (Steinacher & Wagner 2010). In contrast, during the development of pollen grains (microsporogenesis), UV radiation transmitted through buds would usually last for days or even more than a week (Pacini & Dolferus 2016; Vanhaelewyn *et al.* 2020) if not protected through petals in the bud stage. In addition, during the flower bud stage, pollen will likely be especially vulnerable to UV, because the protective pollen walls are formed last during pollen development (Szalay 2006).

The PP hypothesis assumes that petals can protect pollen in flower buds from UV via either absorption or reflection of UV radiation. Protection of pollen from UV through UV-absorbing pigments is common, for example some floral pigments, such as flavonols, absorb UV light (Thompson *et al.* 1972; Iwashina *et al.* 2004). In the case of flower buds, UV radiation that is transmitted through the bud may reach the (developing) pollen. For transmission of light, the amount of UV-absorbing pigment is important but not its location in the petals. In our case, for example *H. calycinum* (Fig. 3), a part of the outer side contains a UV-absorbing pigment, which modulates the transmitted light (see also figure 5 in van der Kooi *et al.* 2016). Alternatively, scattering structures, such as vacuoles and air spaces inside petals and wax and hairs on petal surfaces, can reflect UV (van der Kooi *et al.* 2016). One of the rare cases is the flower buds of *Pedicularis gruina* that strongly reflect UV radiation (Fig. 2).

The PP hypothesis also assumes that, in open flowers, petals can protect exposed pollen from UV radiation through the colour of floral guides or other areas that surround the stamens. For example, in the case of erect bowl-shaped flowers, UV-absorbing floral guides (named after their guiding function towards floral resources for pollinators) may protect pollen against UV light that is reflected by the petals onto pollen-bearing anthers, with both the size of the UV-absorbing area, as well as the amount of the UV absorption being important (Kevan 1975; Koski & Ashman 2015). These UV-absorbing floral guides that protect pollen in open flowers could also protect pollen against UV radiation in the bud stage, provided the floral guides cover the anthers during bud stage.

The PP hypothesis also predicts that there is an overall correlation between vulnerability of pollen to UV radiation and protection against UV radiation. Vulnerability of pollen to UV radiation varies with altitude, mode of pollen presentation, exposure of pollen to sunlight, and pollen traits such as thickness of the exine, colour and amount of pollenkitt. Quantitative measures of UV protection for pollen, such as the amount of UV-absorbing pigment, location so as to provide such protection, and the number of (protective) layers are expected to be more prevalent in species that are exposed to high doses of UV radiation as compared to related species that are not.

In summary, the PP hypothesis predicts that:

1. At least one flower side contains UV-absorbing pigments. Exceptionally, the petals can possess UV-reflecting structures.
2. Differences in colour between flower sides are stronger in the UV as compared to any other wavelength range
3. At least one UV-absorbing layer covers the developing pollen grains in flower buds.
4. This UV-absorbing area is located on a structure that covers the anthers.
5. Vulnerability of pollen to UV radiation is correlated with the degree of protection against UV radiation among species.
6. Pollen fertility and/or survival is lower without the above-mentioned adaptations.

Herbivore-avoidance hypothesis (HA hypothesis)

The herbivore-avoidance hypothesis assumes that herbivory of flower buds has significant adverse impacts on plant reproductive fitness and that certain bud colours can reduce the visibility to herbivores. The outside colour of flower buds represents a visual cue for herbivores to find their host. Flower buds are particularly vulnerable to herbivores, which includes florivores that eat the floral tissue, and organisms that deposit eggs on the flowers (Ashman 2002; McCall & Irwin 2006; Rusman *et al.* 2018; Tagawa *et al.* 2022). Plants are thought to avoid, rather than resist, herbivore attacks on their buds by means of camouflage, masquerade, and aposematic colouration (Frey 2004; Lev-Yadun 2021).

The HA hypothesis predicts that flowers will have inconspicuous colouration during the bud stage, because that should result in reduced visibility to and/or deterrence of herbivores. First, many insects have colour vision with sensitivity in the UV wavelength range (van der Kooi *et al.* 2021b), so UV is similarly meaningful for herbivores as it is for pollinators. Green or pale-coloured buds are thus expected to be relatively inconspicuous, because they contrast little against (green) foliage (Ruxton *et al.* 2004; van der Kooi 2021). Second, UV-absorbing buds may also repel herbivores, because UV-absorbing pigments, such as flavonoids, are distasteful (Gronquist *et al.* 2001; Treutter 2005; Roland *et al.* 2013). Thus, through reduced detection associated with a pale colour and/or increased deterrence associated with UV-absorbing pigments, there would be reduced herbivory, which would be advantageous to the plants.

In the case of temporal differences between pollinator and herbivory activity, the HA hypothesis predicts that flowers may exhibit nastic movements (opening/closure behaviour), with unpollinated flowers open when pollinators are active, but closed when herbivore pressure is high. For example, we expect a flower to be open during the day and closed at night when it is pollinated by diurnal insects such as bees or flies and when it suffers from herbivory by nocturnal animals such as snails or tortoises.

It is furthermore expected that species with nastic movements have more cryptic colours when flowers are closed and more conspicuous colours when they are open. Evidence supporting this prediction has been found in an empirical study on 77 Asteraceae species in South Africa by Kemp & Ellis (2019). Their study showed that flowers that close at night have larger colour differences between inner and outer flower sides than non-closing flowers, which is consistent with selection by herbivores for cryptic colouration of outer petal sides, and different colours between inner and outer petal sides in species with closing flowers (Kemp & Ellis 2019).

In summary, the HA hypothesis predicts that:

1. For flowers facing potential herbivore attacks in the bud stage, the inner and outer side of flowers will display different colours, with the outer side of the petals in flower buds being inconspicuous (green or pale), and/or containing UV-absorbing pigments that are distasteful to potential herbivores.
2. For plants that experience high herbivore pressure after anthesis, and herbivores are active at different moments than pollinators, flowers should exhibit daily opening/closure behaviour.
3. There is no specific colour for the inner sides of petals, as these will generally not be visible to herbivores during the bud stage.

Signalling-to-pollinators hypothesis (StP hypothesis)

The StP hypothesis assumes that flower colour is mainly determined by pollinator-mediated selection for high conspicuousness. Specifically, plants have evolved flower colours that (i) are attractive to pollinators when they are open (though not necessarily during the bud stage), and (ii) allow pollinators to discriminate between non-rewarding flowers/buds versus rewarding open flowers (Armbruster & Steiner 1992). Investigations of species that exhibit post-pollination flower colour change suggest that colour change enables pollinators to discriminate between rewarding unvisited (pre-change) and non-rewarding visited and pollinated (post-change) flower colours (Weiss 1991; Lunau 1996; Weiss & Lamont 1997; Ohashi *et al.* 2015). A similar effect may occur when buds and open flowers have different colours. This might enable pollinators to avoid visiting flower buds that are not yet accessible for reward.

The StP hypothesis also assumes that flower buds contribute to the overall floral display, and so increase visibility to pollinators from a large distance. The simultaneous display of many flowers in an inflorescence or any grouping of flowers increases a plant's attractiveness to pollinators (Harder *et al.* 2004), but requires open and potentially rewarding flowers to be visibly different from closed, unrewarding flowers.

The StP hypothesis further predicts that flower buds will have colours that are also visibly different from open flowers. This means that for erect flowers, the inner and outer sides of petals display different colours. Specifically, we predict that the outer side is less conspicuous to potential pollinators than the inner side, although it is still detectable. The colour contrast between flower buds and open flowers would then enable pollinators to avoid visiting non-rewarding buds, while buds might contribute to the overall colour display and long-distance attractiveness of plants (Gori 1989).

Simultaneous high flower visibility from long distances and low visibility at short distances could come about via differences in the chromatic and achromatic channel. Insects generally use achromatic contrast ("brightness" differences) at long distances, whereas chromatic contrast ("colour" difference) is important at short distances (Giurfa *et al.* 1996; reviewed by van der Kooi & Kelber (2022)). Following the StP hypothesis, buds should have high achromatic contrast to foliage but low chromatic contrast to other flowers, and flowers should have both high chromatic and high achromatic contrast against the foliage and buds.

In summary, the StP hypothesis predicts that:

1. For erect flowers, the outer and inner sides of petals should display different colours.
2. The colour of the outer side should be less conspicuous to potential pollinators than that of the inner side, although both sides should be differently coloured to the foliage.
3. Flower buds are expected to be less colourful than open flowers, yet not green, so buds contribute to the overall visual display, but are visibly different from open, rewarding flowers at short distances.
4. The above differences are expected to play a major role in species, in which open flowers and buds are not easily discriminated by size or shape, e.g. in large inflorescences.

Comparing the hypotheses

The petals of many flowers exhibit flower colour asymmetry, which could have a function in the protection of pollen from UV radiation, in the deterrence of herbivores, and in the signalling to pollinators. Although these hypotheses arise in different contexts, they generate largely overlapping predictions.

Consequently, as the following examples illustrate, there are a few possible scenarios that would distinguish between the three hypotheses. Observing that petal sides generally have different colours would not favour any particular hypothesis, as this result would be consistent with all three hypotheses. However, the same colour for both sides would only be consistent with PP hypothesis. A conspicuous outside petal colour would be consistent with PP and StP hypotheses, but not with HA hypothesis. Only the PP hypothesis predicts that the differences in the colour between the two sides should be particularly prominent in the UV wavelength range. If both petal sides reflect UV, or there is no UV-absorbing area covering the pollen, or there is no correlation between UV vulnerability and UV protection, these observations would be inconsistent with the PP hypothesis. Finally, finding both petal sides UV-reflecting, as well as highly and identically colourful and conspicuous, would be inconsistent with all hypotheses.

CONCLUDING REMARKS

In this viewpoint we have made the case that flower side colour asymmetry is interesting and ecologically relevant for several reasons. We have delineated three specific hypotheses to explain this colour asymmetry, which we refer to as pollen-protection, herbivore-avoidance and signalling-to-pollinators hypotheses.

However, because these hypotheses are not mutually exclusive, it is hard to distinguish their effects in nature. It may nonetheless be possible to quantify the *relative* importance of different hypotheses in specific (model) systems by considering that only one of the hypotheses is the key driver of flower evolution. For example, if flower evolution is driven only by pollen protection, flower colour in the visible wavelength range would be unimportant, and only their UV reflection/absorption would be important. This would mean that, in the visible wavelength range, flower colour is not subject to natural selection and the flower petals should be dull. To protect the pollen from UV irradiance, the flower should exhibit high UV absorbance at the inner side. At the outer side (the exposed and visible side during the bud stage) UV light may also be reflected, to prevent it from being transmitted through the flower. In species, in which UV-absorbing floral guides on the basal parts of the inner sides of petals contribute to the protection of pollen from UV radiation, as for example in *Rudbeckia fulgida* (Fig. 1E), it seems questionable if the dull outer side additionally contributes to protection of pollen from UV radiation. Evaluation of the PP hypothesis will benefit from studies testing damage to pollen in buds.

If, on the other hand, flower colour is driven only by herbivore avoidance, flower colour is expected to evolve in such a way that the flowers are inconspicuous to herbivores. For this case it is more difficult to make testable assumptions, since the visual cues as well as the flowering stages that are relevant to herbivores (i.e. flower bud, open, or faded flowers) are poorly understood, and chemical cues seem to play a major role for herbivores in identification of host species, stage, and suitability for attack. Evaluation of the HA hypothesis will benefit from observations of colour choices in herbivores and knowledge about flowering stages that are susceptible to herbivory.

If flower colour is driven solely by signalling to pollinators, flower colours should be highly conspicuous to pollinators. However, there is no specific prediction as to UV reflection/absorption, as many types of pollinators can perceive UV, but UV reflectance is not a prerequisite for high conspicuousness (Kevan *et al.* 2001). In species, in which the outer side of the flower does not have a visual signalling role to pollinators, the outer side's colouration is not subject to natural selection, meaning it is expected to be inconspicuous. The validation of the StP hypothesis will benefit from behavioural tests.

We encourage more research on flower colour on different flower sides. Such research may gain from investigations on leaves with colour asymmetry, the function and evolution of which is also poorly understood (Hughes & Lev-Yadun 2023). Especially important is a better understanding of the anatomical properties of flowers, how different structures and pigments reflect/transmit/absorb light of different wavelengths, how visual signals elicit behavioural responses of pollinators and herbivores, and how vulnerable pollen is to UV light.

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AUTHOR CONTRIBUTIONS

Collecting flowers: XF, KL, Z-XR, HW, JT. UV photography: XF, KL. Spectroscopy: CJvdK. Plant identification: KL, XF, CJvdK, Z-XR, HW. Conceptualization: KL, CJvdK, GP, JT.

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