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Functional significance of the optical properties of flowers for visual signalling

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• **Background** Flower coloration is a key enabler for pollinator attraction. Floral visual signals comprise several components that are generated by specific anatomical structures and pigmentation, and often have different functions in pollinator attraction. Anatomical studies have advanced our understanding of the optical properties of flowers, and evidence from behavioural experiments has elucidated the biological relevance of different components of floral visual signals, but these two lines of research are often considered independently.
• **Scope** Here, we review current knowledge about different aspects of the floral visual signals, their anatomical and optical properties, and their functional significance in plant–pollinator visual signalling. We discuss common aspects, such as chromatic and achromatic contrast, hue, saturation and brightness, as well as less common types of visual signals, including gloss, fluorescence, polarization and iridescence in the context of salience of floral colour signals and their evolution, and highlight promising avenues for future research.

Keywords: Absorbance, colour preference, colour vision, evolution, flower colour, pigment, pollination, reflectance, structure, iridescence, gloss.

INTRODUCTION

Many plants attract pollinators by displaying coloured flowers. The diversity of floral colours is wide, which can partly be explained by the fact that featuring distinct colours increases dissimilarity of flowers from their neighbourhood (Kevan, 1972; Levin, 1985; McEwen and Vamosi, 2010; Hopkins and Rausher, 2012). Flower colour is often described by the key elemental factors that influence human perception (Wyszecki and Stiles, 1982; Kelber and Osorio, 2010), i.e. hue (the spectral descriptor of colour), saturation (the purity of a colour) and brightness (the intensity of a signal). In addition, colour contrast and green contrast (the contrast between a stimulus and its background mediated solely by the green photoreceptor) are also considered to be important factors enabling the visual perception of flowers by pollinators (Table 1).

A challenge for modern studies of plant–pollinator interactions is understanding which perceptual factors are biologically relevant for flower evolution, and how such factors interact with other types of visual signals, such as gloss, fluorescence or iridescence effects (Table 1). Different components of the flower's visual signals are attributable to specific structural features and/or pigmentation. By collecting high-quality data on the physical properties of flowers it may be possible to untangle major traits that influenced evolutionary processes.

The coloration of flowers is typically due to the combined effect of light scattering by floral structures and wavelength-selective absorption by pigments (Kay *et al.*, 1981; Kevan and Backhaus, 1998; van der Kooi *et al.*, 2016*a*). Backscattering of light occurs at boundaries of media with different refractive indices, such as air/cell wall or water/cell wall interfaces and cellular inhomogeneities such as (pigment) granules (Fig. 1) (van der Kooi *et al.*, 2016*a*). Flower interiors are commonly stratified, with different layers having specific scattering and pigmentation properties. For example, floral interior layers can vary in shape, size and type (e.g. mesophyll or starch cells), and pigments can be distributed throughout the flower or in specific layers (e.g. Kay *et al.*, 1981; Koes *et al.*, 1994; Kevan and Backhaus, 1998; Vignolini *et al.*, 2012; van der Kooi *et al.*, 2016*a*). Light that is not backscattered by the flower's interior or surface is transmitted through the flower (Fig. 1); transmitted light may under specific circumstances contribute to the visual signal (van der Kooi *et al.*, 2016*a*).

Floral pigments selectively filter the backscattered and transmitted light. For example, flowers with blue–green-absorbing anthocyanins are purple, and flowers with blue-absorbing carotenoids are yellow (reviewed by Mol *et al.*, 1998; Grotewold, 2006). For some pigments, in particular anthocyanins, the absorption of light depends on the vacuolar pH (Mol *et al.*, 1998). The degree of filtering by pigments strongly determines the strength of a flower's visual signal (Figs 2 and 3) (van der Kooi *et al.*, 2016*a*) and whether a pollinator may view such information as salient or cryptic (Dyer *et al.*, 2007).

Detailed measurements suggest a wide variance in floral reflectance spectra between species, although in pollinator

TABLE 1. *Glossary of terms for different aspects of the visual signals of flowers*

Visual signal	Perceptual counterpart	Measurement	Optical characteristics	Current evidence for functional significance for visual signalling in nature
Dominant wavelength	Hue	Reflectance spectrum measured with bifurcated probe or integrating sphere and a colour space model.	Wavelength-selective absorption by pigments.	Important; many pollinators have innate and learned preferences for specific hues. Effects of dominant wavelength cannot always be disentangled from colour contrast.
Spectral purity	Saturation	Reflectance spectrum measured with bifurcated probe or integrating sphere and a colour space model.	Colourfulness of the stimulus as opposed to greyness; characterized by the slope and amplitude at the inflection points of the reflectance curve.	Has been shown to be important for a few bee species. Effects of spectral purity cannot always be disentangled from colour contrast and dominant wavelength.
Intensity	Brightness/luminance	Integrating sphere or other technique that allows measuring the absolute amount of reflectance.	Amplitude of the reflectance curve.	Not known to be important for diurnal pollinators.
Colour contrast	Perceptual contrast between two colours as detected by all photoreceptors. Depending on the species, this may be affected by dominant wavelength and/or spectral purity.	Reflectance spectrum and colour vision model.	Difference in the amplitude of the stimuli's reflectance spectra in the visible range of wavelengths.	Important – many species use colour contrast to distinguish and/or detect flowers from close and large distances. Increased colour contrast has been shown in several bee species to increase the probability of correct target flower identification. The relative importance of hue versus saturation can be difficult to discern.
Green contrast	Perceptual contrast between two colours as detected by the green photoreceptor.	Reflectance spectrum measured with bifurcated probe or integrating sphere and excitation values of green photoreceptor for background versus stimulus.	Difference in the amplitude of the stimulus' and background's spectra in the green wavelength range.	In honey bees important for long-distance detection. In many insects vital for motion processing, and the processing of complex patterns, shapes or sizes of objects such as flowers.
Specular reflection	Gloss	Angle-dependent reflectance measurements	Specular (mirror-like) reflection. Gloss requires very smooth and flat flower surfaces (Fig. 4).	Unclear. No evidence supporting or rejecting gloss as a signalling function.
Iridescence	Angle-dependent coloration	Angle-dependent reflectance measurements	Physical interactions of light waves with periodically ordered nanostructures that differ in refractive index.	Very little or no functional significance in natural conditions.
Polarization	Gloss	Angle-dependent reflectance measurements or high-quality photo camera with polarization-sensitive filter.	Geometrical orientation of the oscillation of light waves. Polarization effects generally occur at smooth and flat surfaces and often co-occur with gloss (Fig. 4).	Very little or no functional significance in natural conditions.
Fluorescence	Fluorescence	Fluorescence microscopy.	Emission of light by pigments.	Very little or no functional significance in natural conditions.

For each aspect, we list the perceptual and physical counterpart, how it is measured, its optical characteristics and a conclusion as to its currently believed importance for visual signalling to pollinators in natural conditions. For details and calculations see Supplementary Data File S1.

visual space this diversity can be different (e.g. Daumer, 1958; Kevan, 1972; Menzel and Shmida, 1993; Chittka *et al.*, 1994). A large variety of animals, particularly numerous insect species, with often very different visual capabilities, is involved in plant–pollinator interactions. It is nevertheless convenient to consider some key pollinators such as bees for appropriate
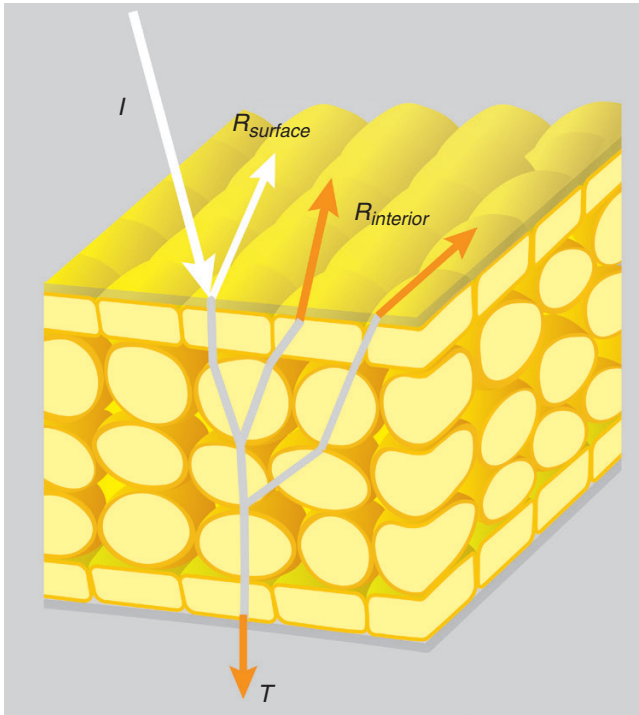
FIG. 1. Diagram of light reflection in a flower. Part of the incident light (I) is reflected by the surface ($R_{surface}$) or by the interior ($R_{interior}$), part of the light is transmitted (T) through the flower, and light of a specific wavelength range is absorbed by pigments. The light that is reflected by the surface is largely unmodulated by pigments, whereas light that is reflected by the petal interior or transmitted through the flower will be modulated by pigments inside the flower (for visualization purposes the light rays inside the flower are shown in grey).
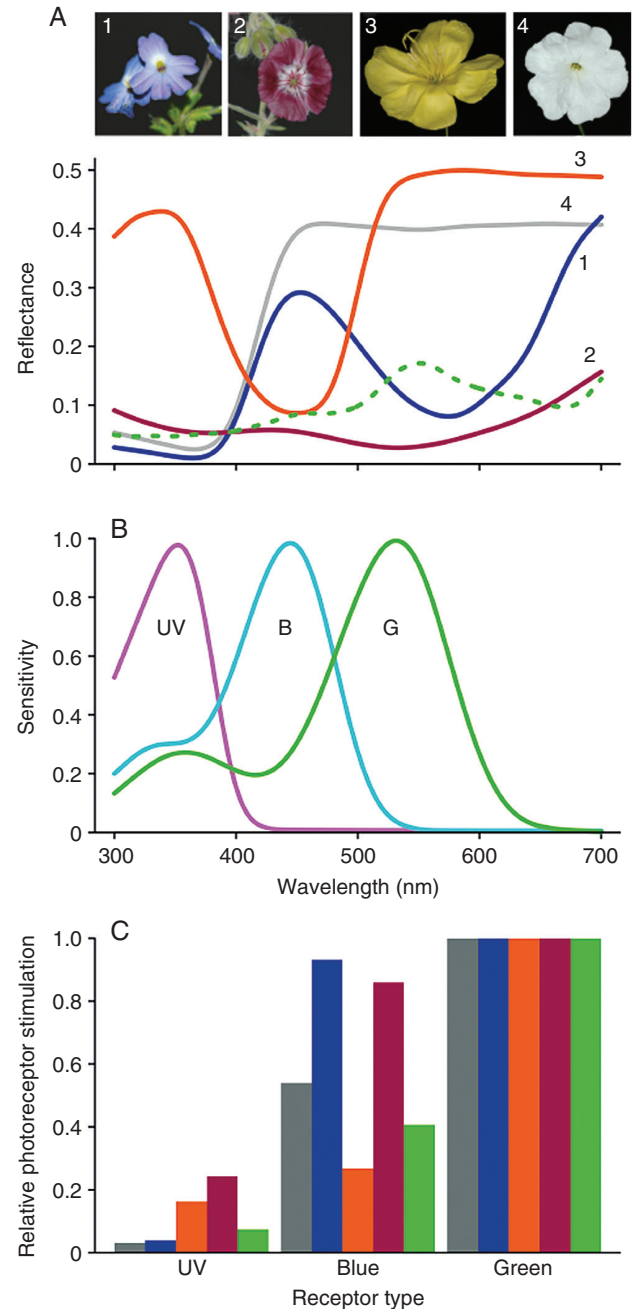

FIG. 2. Reflectance spectra and their visual signals for four example flowers. (A) Reflectance spectra of four differently coloured flowers and an average green background spectrum: 1, *Browallia americana*; 2, *Geranium phaeum*; 3, *Oenothera glazioviana*; 4, *Petunia nyctaginiflora*; dashed curve, average green background. (B) Honey bee photoreceptor spectral sensitivities (after Peitsch *et al.*, 1992). Relative photon stimulation for the different reflectance spectra of panel A for the different photoreceptors, using the photoreceptor spectral sensitivity shown in panel B. See Supplementary Data Table S1 for specific aspects of the spectra and interpretation with vision models.

ways to investigate how flower colour may be perceived. Behavioural assays with pollinators using tailored stimuli can help to elucidate the relative importance of different aspects of floral signals. Data from behavioural assays can subsequently be compared with modelling results that enable interpretation of a flower's reflectance spectrum with a 'pollinator-subjective view'. Bees have a phylogenetically conserved trichromatic visual system with peak photoreceptor sensitivities at about 350, 440 and 540 nm (Fig. 2) (Peitsch *et al.*, 1992; Briscoe and Chittka, 2001), and by using colour vision models such as the colour hexagon (Chittka, 1992) or the receptor noise-limited model (Vorobyev and Osorio, 1998) perceptual contrast values can be calculated. Hence, data from behavioural and modelling studies provide insight into which spectral receptors govern the decisions of pollinators and whether the spectral characteristics of the flowers and the pollinators' vision are tuned.

Recent flower studies using anatomy and spectroscopy have elucidated the anatomical features that can cause various optical effects, and behavioural experiments with insect pollinators have tested the relative importance of different types of visual signals. However, knowledge on the behaviour and visual systems of pollinators is often considered independently from current knowledge on the optical properties of flowers.

In this review, we aim to link the studies on the optical properties of flowers with experimental studies on pollinator visual systems and behaviour. We discuss how the flower's anatomy and pigments generate different components of the visual signal, and we discuss the current scientific evidence supporting each type of visual signal. We examine what is currently known about the achromatic aspects of bee pollinator perception, i.e. green contrast and brightness, as well as the chromatic aspects
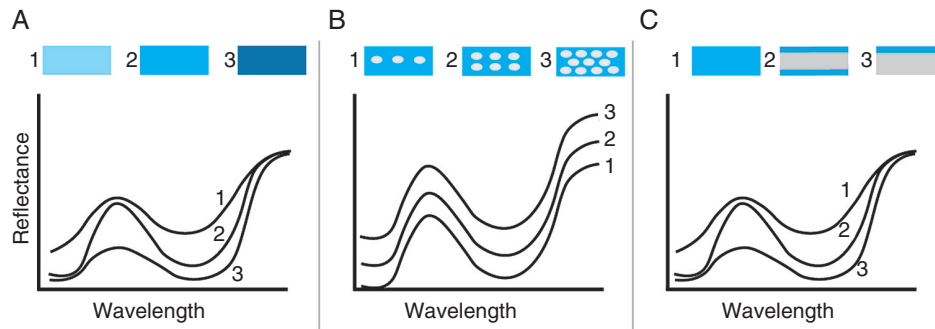
FIG. 3. Diagram showing how different optical properties determine a flower's reflectance spectrum. In each panel only one optical property is varied to illustrate the different mechanisms; at the top of each panel three model petals are shown (illumination and observation are from above). (A) Changes in the amount of pigment will alter the modulation of the reflected light; little pigment yields a pale colour, and very much pigment yields a dull colour. (B) The intensity of the reflected light increases with the number of backscattering structures, which are illustrated by white ovals. When the reflectance increases, the transmittance will decrease. For the sake of simplicity, the relative modulation of the spectra was kept constant, although in real flowers the filtering by pigments will change with floral anatomy. (C) When the total amount of pigment remains constant but its localization changes, this will alter the modulation of the reflectance spectrum. Pigment deposition at the side of viewing yields the strongest modulation. For details, see van der Kooi *et al.* (2016*a*) and Stavenga and van der Kooi (2016).

of floral colours, i.e. hue and saturation. Finally, we discuss several types of more uncommon visual cues that are due to specific anatomical properties, namely fluorescence, iridescence, gloss and polarization effects. For each visual cue we first consider the optical mechanism and subsequently its contribution to the overall visual signal of flowers and the current knowledge on its biological significance in natural conditions. Throughout the text we highlight promising avenues for future research. We will not discuss the principles of different vision models, as this subject has recently been reviewed elsewhere (Kemp *et al.*, 2015; Renoult *et al.*, 2017).

ACHROMATIC ASPECTS OF FLOWER COLORATION

Brightness

Brightness refers to the perceived intensity of a stimulus, independent of hue and saturation. The perceived brightness depends on adaptation processes in the visual system, and it is often considered to be a relative percept, because a stimulus is judged relative to its surroundings. Hence, a piece of white paper is perceived as white in both open daylight and indoors, even though there may be a couple of orders of intensity differences reflected from that same page in the different environments. It is therefore important to consider the perception of a pollinator when considering the light intensity reflected by a flower as its visual signal.

Brightness is a product of information processing by the brain, whereas intensity is a physical property of a stimulus (Table 1). In humans, brightness is a dimension of our colour sense because the primate brain has multiple pathways for chromatic and intensity signals, and the brain combines such signals to enable a combined precept (Clery *et al.*, 2013). However, given the complex nature of visual information processing, we may not readily assume that insect pollinators such as bees also perceive brightness in the context of colour signalling. Stimulus brightness as potentially perceived by bees has been modelled as the sum of all photoreceptor excitations (Spaethe *et al.*,

2001). The brightness contrast between a stimulus and its background is the difference between stimulus brightness and background brightness (Hempel de Ibarra *et al.*, 2014). Brightness contrast can be positive or negative, i.e. a flower can have a higher or lower brightness than its background.

A flower's reflectance (the fraction of incident light that is reflected) is determined by the type and amount of structures inside the petals. The absolute amount of backscattering by a flower can be measured with an integrating sphere (Vukusic and Stavenga, 2009; van der Kooi *et al.*, 2016*a*). Apart from pigments that selectively absorb light at specific wavelength ranges, three main characteristics determine a flower's reflectance: the refractive index difference between floral structures, the flower's interior inhomogeneity and the flower's thickness. Firstly, the refractive index difference between two structures determines the reflectance at their boundaries; an increase in refractive index difference causes a higher reflectance. In contrast to animals, where the tissue's refractive indices have been studied for various species, such as bird feathers and butterfly wing scales (Leertouwer *et al.*, 2011), there have been virtually no studies on the refractive indices of floral tissue. Although the refractive indices of media commonly found in flowers, e.g. water and cell walls (Stavenga and van der Kooi, 2016), are expected to be similar between species, we are not aware of any comparative studies on this subject. Secondly, a flower's reflectance will increase when the amount of scattering structures per unit thickness increases (Fig. 3B). For example, flower areas with relatively uncompressed cells scatter less light than veined areas, where many cells are packed together (Kevan and Backhaus, 1998; Stavenga and van der Kooi, 2016). Thirdly, when the flower's total thickness increases, the amount of scattering structures a propagating light wave encounters also increases, resulting in higher reflectance.

A comparative study covering 39 species from 23 plant families showed that the interior inhomogeneity and thickness of flowers varies greatly, but that the overall reflectance is rather similar among species (van der Kooi *et al.*, 2016*a*). In the long-wavelength range, i.e. where modulation by pigments is negligible, the reflectance value – and thus the amount of backscattering – can be estimated. Flowers reflect between 20 % and

50 % of the incident light, which suggests that a reflectance in this range is sufficient for strong visual signalling to pollinators (van der Kooi *et al.*, 2016*a*). In other words, a 20 % light reflectance allows for a sufficient overall contrast with the average leaf background. Given the great variation in interior inhomogeneity and thickness of flowers, it seems unlikely that physiological restrictions would thwart reflectance values above 50 %; rather, this suggests that further increases in the amount of light reflected will not significantly enhance the flower's visibility for pollinators (van der Kooi *et al.*, 2016*a*).

Evidence suggests that overall floral brightness is not likely to be a major cue for detection of colour signals from flowers by insect pollinators. Multiple behavioural experiments with bees, flies and wasps showed that these insects do not use brightness for object detection, at least in the presence of colour signals (Daumer, 1956; Backhaus *et al.*, 1987; Chittka *et al.*, 1992; Spaethe *et al.*, 2001; Reser *et al.*, 2012; Papiorek *et al.*, 2013; Rohde *et al.*, 2013). Early as well as more recent studies suggest that bees ignore intensity differences when foraging (Daumer, 1956; Backhaus *et al.*, 1987; Chittka *et al.*, 1992; reviewed by Kevan *et al.*, 1996). Indeed, bees have great difficulty in detecting and learning to recognize targets that differ from their background only in brightness (Ng *et al.*, 2018), and also flies and the diurnal hummingbird hawkmoth, *Macroglossum stellatarum*, (largely) ignore intensity differences and are more triggered by specific hues and/or overall chromatic contrast (Goyret and Kelber, 2012; Woodcock *et al.*, 2014). To date, the number of species studied in this respect is fairly small, however, so this claim cannot be simply extrapolated to all insect pollinators.

Brightness can generally be considered an unlikely trait that drove flower signal evolution in habitats where bees are the main pollinators. Previously reported effects of pollinators selecting for flower brightness in the cornflower *Centauria cyanus* (Renoult *et al.*, 2013) and the deceptive orchid *Anacamptis morio* (Sletvold *et al.*, 2016) may be a result of the fact that the spectral signal intensity is often confounded with other components of the visual signal. Brightness differences are often difficult to disentangle from differences in green contrast, because flowers with a high overall reflectance reflect more light at long wavelength ranges, and may thus exhibit a higher green contrast to the background. Additional evidence suggesting brightness is not very important comes from the fact that intensity differences may be unreliable in nature. Brightness *per se* can be considered as an unreliable signal (Kelber *et al.*, 2003), because it is determined both by the object and by illumination conditions, such as shadows, weather and time of day, and greatly differs between and during days.

Although brightness may not be very important for the detection of visual signals in most insect pollinators, at least some species can learn to use it as a stimulus. Behavioural experiments have shown that two diurnal Lepidoptera species are able to distinguish stimuli solely based on brightness contrast with the background, namely the hummingbird hawkmoth (Kelber, 2005) and a swallowtail butterfly (Kinoshita *et al.*, 2012). In addition, the nocturnal moth *Deilephila elpenor* can more successfully distinguish bright than non-bright stimuli at low illumination conditions (Kelber *et al.*, 2002), suggesting that for nocturnal insects brightness may be important. For nocturnally pollinated flowers, strong brightness between the flower and background may enhance conspicuousness, as colour vision becomes less reliable when illumination intensities are low (Endler, 1993; Cronin *et al.*, 2014). In line with this hypothesis is the observation that many nocturnally pollinated flowers seem to be bright to the human eye (White *et al.*, 1994), although this observation would benefit from further studies. Hence, under nocturnal conditions, plants with brighter flowers could be more conspicuous than their co-flowering conspecific neighbours.

In summary, evidence suggests that brightness is not a major visual signal for detection by diurnal pollinators, especially bees, but this may differ between species and habitats. We emphasize that there is a need for systematic comparative studies on the functional significance of brightness perception.

Green contrast

In addition to brightness contrast, many insects detect flowers from large distances by means of their green-receptor contrast. Green contrast is the contrast between a stimulus and its background mediated solely by the green photoreceptor (reviewed by Osorio and Vorobyev, 2005). Green contrast can be positive or negative, i.e. a flower can have a higher or a lower green reflectance than the background vegetation. For many insects, the sensitivity of the long-wavelength photoreceptor extends from the ultraviolet to long wavelengths (Fig. 2). The bimodal nature of the bees' long-wavelength photoreceptor is due to the secondary sensitivity in the ultraviolet of nearly all photoreceptors (Fig. 2B) (Peitsch *et al.*, 1992). The green photoreceptor provides a high signal-to-noise ratio compared to the other photoreceptors, so this receptor may have a particular role in signal detection (Vasas *et al.*, 2017). Green contrast is an achromatic signal and thus partly similar to brightness contrast (i.e. high reflectance difference between flower and background yields a strong contrast), but for green contrast, modulation by floral pigments is important.

Green contrast can vary over a wide range between wild flowers (Papiorek *et al.*, 2014; Vasas *et al.*, 2017), but whether the variation is linked to the pollination system or plant life-history traits is unclear. Two life-history traits have been suggested to influence green contrast of flowers: floral display size and the plant's habitat. Spaethe *et al.* (2001) showed that bumble bees rely more on green contrast when searching for small flowers, but more on colour contrast when searching for large flowers. This suggests that plants with small floral displays need to compensate for their reduced conspicuousness via stronger green contrast. Indeed, a comparative analysis of floral patterns and green contrast by Hempel de Ibarra and Vorobyev (2009) suggested that smaller flowers exhibit stronger green contrast. However, given these preliminary results are based on photographed flowers and not on spectral and anatomical measurements, this hypothesis needs further testing. In a comparative study on Israeli flora, it was found that desert plants exhibited weaker green contrast than plants growing in the Mediterranean region (Menzel *et al.*, 1997). Interestingly, this was not due to a change in the desert flowers' visual signal, but due to a relatively strongly coloured background of desert plants. Whether floral green contrast is less important for desert plants than for Mediterranean plants remains unknown. Finally, Ohashi *et al.*

(2015) found that flowers that undergo a colour change (e.g. following pollination) generally retain their (high) green contrast. This presumably increases long-distance detection of the floral display as a whole, so as to increase visitation of flowers that have not yet lost their colour and require pollination.

Green contrast of flowers seems mostly important for long-distance detection by pollinators, and its relative importance may depend on the type of pollinator and habitat. Bees have difficulty in detecting flowers based solely on green contrast. In experiments with honey bees and bumble bees, green contrast as a sole factor results in rather poor flower detection, but combined green and chromatic contrast leads to very reliable flower detection, especially at greater distances (Giurfa *et al.*, 1996; Dyer *et al.*, 2008). There is a clear need for large-scale comparative studies on the degree of variation of green contrast between species, and to what extent this is determined by pollinator-mediated selection, phylogenetic ancestry or habitat.

CHROMATIC ASPECTS OF FLOWER COLORATION

Colour Contrast

Whereas green contrast involves only one photoreceptor class, colour contrast is the overall contrast between two visual stimuli mediated by all photoreceptor types involved in colour vision. Colour contrast and detection thresholds are often inferred using vision models for bee colour perception (Chittka, 1992; Vorobyev and Osorio, 1998). Colour contrast can represent the perceptual difference between the flower and background, between flowers or within flowers in the case of colour patterns. Differences in either hue or saturation can contribute to the colour contrast between two stimuli, but such factors may also be considered separately for what are the main driving factors for the evolution of specific flower spectral properties (see section on Hue and saturation). Floral pigments play a key role in the colour contrast of flowers, but because of the complex interplay of pigments and light-scattering structures, understanding the relative importance of the key elements may be difficult.

Colour contrast has been proven to be important at the plant community level, between flowers and their background as well as between different areas within single flowers. Several hypotheses predict the evolution of flower colours at the plant community level, with different predictions depending on the hypothesis. First, floral signals may have converged to some degree, for example when they are serviced by the same (generalist) pollinator (Schiestl and Johnson, 2013). This was found in an island community for plants pollinated by flies (Shrestha *et al.*, 2016), and on the European mainland for plants that were rare in their community (Gumbert *et al.*, 1999). Second, plants may maximize floral colour contrast in respect to other species blooming at the same time, to distinguish themselves from their co-flowering neighbours (Levin, 1985; McEwen and Vamosi, 2010; van der Kooi *et al.*, 2016*b*). This was convincingly shown in *Phlox drummondii* that produces light blue flowers in most of its range, but in areas where it co-occurs with the light blue-flowered *P. cuspidata* it produces dark-red flowers (Levin, 1985), in order to reduce interspecies hybridization (Hopkins and Rausher, 2012).

In addition to contrasting with neighbouring flowers, colour contrast to the background and within-flower contrast improves detection and navigation by pollinators. Colour patterns are generally due to differences in pigmentation between flower areas, although in some cases the anatomy of differently coloured flower areas varies (Kay *et al.*, 1981; van der Kooi *et al.*, 2017). Behavioural choice tests confirmed the importance of colour contrast against the background for visual detection by bees, for both artificial stimuli (Giurfa *et al.*, 1996; Spaethe *et al.*, 2001; Morawetz *et al.*, 2013) and real flowers (Dyer *et al.*, 2007). A bumble bee's decision to visit a flower depends both on the colour contrast between the central and peripheral part of the colour pattern, and on the direction of the colour contrast, i.e. on the colour of the centre versus that of the periphery. Many flowers have ultraviolet-absorbing centres and ultraviolet-reflecting peripheries (sometimes referred to as floral 'bulls-eyes'). Such a pattern increases both the contrast with the background and that within the flower (Kevan and Backhaus, 1998). Ultraviolet-reflecting petal tips have high contrast with the green background (which has low ultraviolet reflectance; Fig. 2A), and at short range the bee will perceive a strong contrast between the ultraviolet-absorbing centre and the ultraviolet-reflecting periphery. In addition to providing high contrast, such colour patterns – in both the ultraviolet and the visible wavelength ranges – can constitute a gradient of increasing spectral purity towards the centre, which bumble bees prefer (Lunau, 1992). Nonetheless, as discussed above, green contrast also appears to be important for flower detection at a distance, and there seems to be a complex way in which these visual descriptors interact to influence bee target detection (Giurfa *et al.*, 1996; Dyer *et al.*, 2016*b*).

We conclude that colour contrast is important at different levels and in different contexts. For honey bees green contrast of the flower against the background is important for detection from longer distances and colour contrast is important from shorter distances, but other important pollinators such as bumble bees and stingless bees use colour and green contrast at similar distances (Table 2; Dyer *et al.*, 2008; Wertlen *et al.*, 2008). It will thus be of high value to evaluate the importance of chromatic and achromatic contrast in other important pollinators. We will now discuss the different aspects of colour contrast.

Hue and saturation

Colour contrast is determined by differences in hue and saturation between a stimulus and the background. Hue is generally described as a categorization of what humans consider 'colour', e.g. blue, yellow or red (Wyszecki and Stiles, 1982); however, for pollinators hue is typically quantified as an angle in a colour space, expressed in dominant wavelength (Chittka, 1992) (Table 1, Supplementary Data File S1). The saturation of a colour refers to its 'pureness', e.g. for human perception red is more saturated than pink. The saturation of a colour is determined by the slope of the reflectance curve at the inflection point(s), i.e. the wavelength range where the slope is maximal (Fig. 2). Very steep changes in the reflectance curve yield visual signals of high saturation. A decrease in slope yields a more uniform reflectance at different wavelengths, resulting in a greyer visual signal, i.e. a visual signal of lower saturation.

TABLE 2. *Behavioural studies with free flying bee species on the relative importance of hue and saturation, in conjunction with other aspects of the visual signal*

Species	Tested parameters	Stimuli	Main finding	Reference
Apis mellifera	Dominant wavelength	Interference filters	Strong preference for dominant wavelength parameter of stimuli	(Menzel, 1967)
Apis mellifera	Dominant wavelength, colour contrast, green contrast	Artificial coloured paper stimuli	Strong preference for dominant wavelength parameter of stimuli	(Giurfa *et al.*, 1995)
Apis mellifera	Dominant wavelength, spectral purity, intensity, green contrast, colour contrast	Artificial printed colours with very small perceptual differences	Bees approached stimuli according to spectral purity difference as compared to the background	(Rohde *et al.*, 2013)
Bombus terrestris	Dominant wavelength, spectral purity, intensity, colour contrast	Artificial stimuli were presented simultaneously and the choices by bees were recorded	Bees approached stimuli according to spectral purity difference as compared to the background	(Lunau, 1990)
Bombus terrestris	Dominant wavelength, spectral purity, intensity, green contrast, colour contrast	Artificial coloured paper stimuli	Choices by bees were principally mediated by hue	(Gumbert, 2000)
Bombus terrestris	Hue	Artificial colour stimuli and field-based testing with real flowers	Preference for hue observed in lab-based studies results in improved foraging efficiency on flowers at field sites	(Raine and Chittka, 2007)
Bombus terrestris	Dominant wavelength, spectral purity, intensity, green contrast, colour contrast	Artificial coloured paper with very small perceptual differences	Bees approached stimuli according to spectral purity difference as compared to the background	(Rohde *et al.*, 2013)
Bombus terrestris	Hue, saturation, intensity, colour contrast, green contrast	Snapdragon (*Antirrhinum majus*) flowers with genetic modification of colour and wild type	Bees ignored intensity cues and preferred flowers of higher chromatic contrast	(Dyer *et al.*, 2007)
Melipona mondury	Dominant wavelength, spectral purity, intensity, green contrast	Artificial mixtures of pigments	Strong preference for dominant wavelength parameter of stimuli	(Koethe *et al.*, 2016)
Melipona quadrifasciata	Dominant wavelength, spectral purity, intensity, green contrast	Artificial mixtures of pigments	Strong preference for dominant wavelength parameter of stimuli	(Koethe *et al.*, 2016)
Tetragonala carbonaria	Dominant wavelength, spectral purity, intensity, green contrast, colour contrast	Artificial coloured papers	Preference for spectral purity, but only when in interaction with green contrast. Preference also observed for dominant wavelength	(Dyer *et al.*, 2016a)

These quantitative parameters can be determined using a colour space (Menzel and Shmida, 1993; Chittka *et al.*, 1994; Vorobyev and Brandt, 1997; Kevan and Backhaus, 1998) (see File S1).

Floral pigments determine a flower's colour and thus its hue and saturation. The type of pigment determines the hue, and the degree of filtering by pigments determines the flower's saturation. The degree of absorption by pigments is due to both the amount and localization of pigment. Generally, increases in pigment concentration will lead to stronger spectral modulation of the reflected light (Fig. 3A). Deposition of pigment in specific floral layers can also dramatically change the absorption by pigments (Fig. 3C). When pigments occur in the outer (epidermal) layers of the flower, the light that is backscattered by the interior traverses the pigmented layer twice and thus is more modulated than when pigments are located throughout the flower (Stavenga and van der Kooi, 2016; van der Kooi *et al.*, 2016a). Pigment deposition is probably phylogenetically constrained; anthocyanins generally occur in epidermal layers, whereas carotenoids are often more evenly distributed through the flower (reviewed by Mol *et al.*, 1998; Grotewold, 2006).

Evidence from studies on the optical properties of flowers and behaviour of bees suggests that intermediate amounts of floral pigments yield the highest salience to pollinators. For flowers with a low amount of pigment, an increase in pigment concentration will increase the flower's saturation. However, extreme increases in pigment concentration will yield a dull appearance and reduce the flower's conspicuousness, because floral pigments absorb in a broad wavelength range (see figure 3 in van der Kooi *et al.*, 2016a). This is in accordance with behavioural experiments with bees, which showed that stimuli with intermediate amounts of pigment yielded the strongest visual signal and were most easily detected by bees (Papiorek *et al.*, 2013). In other words, there will be a certain amount of pigment necessary to have a sufficiently strong visual signal, but having more pigments is not always better.

Hue may be important because many insect species from different orders have innate or learned preferences for specific hues. Menzel (1967) showed that honey bees exhibit innate preferences for colours of short wavelengths, which was also found in Australian native stingless bees (Dyer *et al.*, 2016a). It has been suggested that in honey bees, innate preferences for particular hues are more important than flower brightness, colour

contrast and green contrast (Giurfa *et al.*, 1995), although this probably depends on the experimental setup. Innate preferences for specific hues were also documented for flower-visiting flies, beetles and butterflies (Lunau and Maier, 1995; Johnson and Midgley, 2001; Woodcock *et al.*, 2014; Kinoshita *et al.*, 2017). For example, the diurnal hummingbird hawkmoth *M. stellatarum* has increased spectral sensitivity and innate preferences for blue light (Telles *et al.*, 2014), and various species of hoverflies, including frequent flower-visitors such as *Eristalis tenax* and *Episyrphus balteatus*, have innate preferences for yellow (reviewed by Lunau, 2014). In an island environment where flies were the sole pollinators their visual systems appeared to impose strong selection on flower colours (Shrestha *et al.*, 2016). In contrast to the wide-held belief that birds innately prefer red hues, experimental and field studies have found no results supporting that idea; preferential visits of birds to red flowers are probably the result of learning behaviour (reviewed by Lunau and Maier, 1995). At least bees and various species of Lepidoptera can learn to associate specific colours with a reward and so overcome their innate preferences (Lunau and Maier, 1995; Gumbert, 2000; Chittka and Raine, 2006; Goyret *et al.*, 2008; Rohde *et al.*, 2013). Flies are less likely to overcome their innate colour preferences due to limited learning capability. Whether a species strictly follows its innate colour preference during foraging can be relevant when certain plant species are rewarding at a specific moment and the pollinator can learn to associate a specific hue with a reward.

The importance of hue versus saturation differs strongly between pollinators. For five flower-visiting insect species (the Western honey bee, one bumble bee and three stingless bee species) the relative importance of hue versus saturation – sometimes in combination with other aspects of the visual signal – has been experimentally investigated in laboratory and field settings (Table 2). In these experiments, bees were presented with artificially coloured stimuli that systematically differed in one or a few aspects of the visual stimuli. By combining different types of hues with various degrees of saturation, the relative importance of both could be determined. Whereas for honey bees and bumble bees saturation seems (slightly) more important than hue, for two stingless bee species, *Melipona mondura* and *M. quadrifasciata*, innate preferences for a particular hue are more important than high saturation (Table 2). In these four species, the (short-distance) detection was unrelated to the degree of green contrast. Interestingly, *Tetragonula carbonaria* stingless bee individuals preferred increased saturation, but only when in combination with high green contrast (Dyer *et al.*, 2016a). These bees also demonstrated a preference for hue (Table 2). Depending on the stimuli used, different aspects of a visual signal can be correlated. For example, saturation and colour contrast may be correlated (Gumbert, 2000), rendering it difficult to test the relevance of these different aspects in isolation. In addition, a pollinator's perceptions and preferences may be driven by multiple factors to enable a decision, meaning that testing potential signals in isolation could yield incomplete answers. For example, the blue hue preference of honey bees can mask other information present in complex colour signals (Morawetz *et al.*, 2013).

We conclude that chromatic contrast is important for plant–pollinator signalling, especially for short-distance discrimination. Hue and saturation are both potentially very important,

but their relative importance seems to be species-specific. The great variation in preference for hue versus saturation between flower-visiting species opens perspectives for comparative studies between species. It would be particularly interesting to investigate whether the variation in hue and saturation found in natural populations of flowering plants can be linked to preferences by local pollinators.

OTHER TYPES OF VISUAL PHENOMENA SUGGESTED TO PLAY A ROLE IN VISUAL SIGNALLING

The surface properties of flowers can contribute to the flower's reflection and/or modify the light that is backscattered by the interior. The shape of both the epidermal surface and the cuticle may contribute to the visual signal. For example, very flat epidermal surfaces can give flowers a glossy appearance (Parkin, 1928; Vignolini *et al.*, 2012; Papiorek *et al.*, 2014; van der Kooi *et al.*, 2017). In order for surface reflections to be visible to pollinators under normal (daylight) conditions, the salience of the visual signal needs to be comparable to that of the visual signal generated by the petal interior. Comparing the relative conspicuousness of particular visual signals to the overall visual signal requires detailed optical and behavioural studies.

Structural coloration and iridescence

Structural colours arise in regularly ordered, nano-sized structures composed of materials with different refractive indices (Srinivasarao, 1999; Kinoshita, 2008), and are fairly common among animals (reviewed by Vukusic and Sambles, 2003). Typical for structural coloration is that it is highly directional and angle-dependent (i.e. iridescent); thus, the hue changes with angle of observation or illumination (Fig. 4).

Some plant species have petals with (quasi-regular) cuticular striations (Kay *et al.*, 1981) that could potentially serve as a diffractive grating. Although several studies on communities of plants suggested the absence of any iridescence effects (Kay *et al.*, 1981; Menzel and Shmida, 1993; Lee, 2007), more recently several species have been suggested to be iridescent (Whitney *et al.*, 2009). However, detailed optical analyses narrowed the possible incidence of floral iridescence in wild species to only one variety of the flower-of-an-hour, *Hibiscus trionum* (Vignolini *et al.*, 2015; van der Kooi *et al.*, 2014, 2015). The virtual absence of iridescent wild flowers is due to the highly irregular periodicity of the petal's striations. In many species the irregularity of the striation's periodicity, combined with light backscattering by irregularities inside the petal, prevent iridescence effects being visible under natural conditions (van der Kooi *et al.*, 2014).

Despite advances in our understanding of its mechanistic basis, it remains unknown whether iridescence plays a role in plant–pollinator signalling. The contribution of surface structures to the flower's visual signal under natural conditions is virtually always very low, thereby limiting a role as signal to pollinators (van der Kooi *et al.*, 2014, 2015; Lunau, 2016). Laboratory experiments showed that bees could be trained to associate highly iridescent artificial objects with a reward (Whitney *et al.*, 2009, 2016; Moyroud *et al.*, 2017); however, it remains unclear whether the bees were attracted by the changes
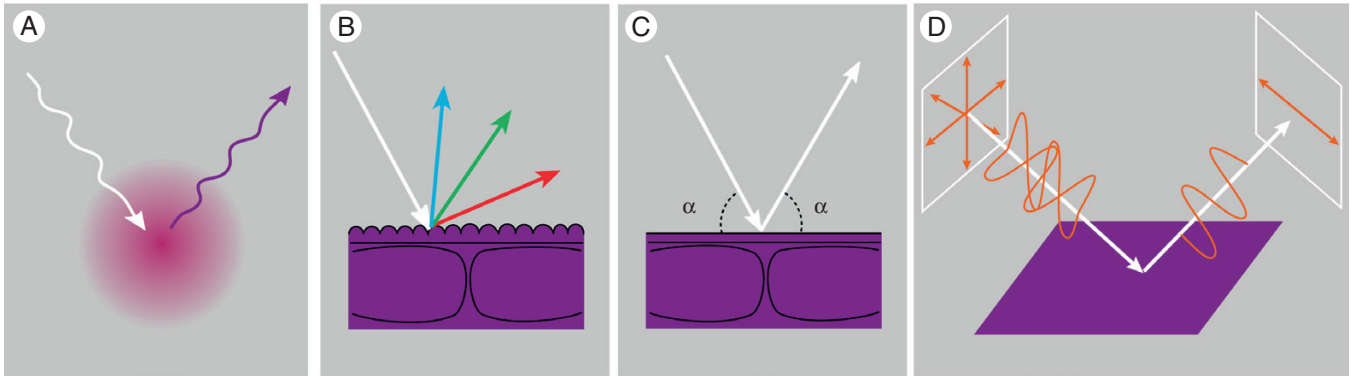
Fɪɢ. 4. Various types of visual signals are not due to backscattering by the flower's interior. (A) Fluorescence is the emission of light by pigments that absorbed light, for example absorption of ultraviolet light leads to emission of long-wavelength light such as blue. (B) Periodically structured striations of the flower's cuticle may cause incident light to be diffracted, yielding a visual signal that is dependent on the angle of illumination and observation, a phenomenon known as iridescence. (C) A very flat and smooth flower surface may cause specular reflection when the angle of illumination is identical to the angle of observation (denoted by α). Light that is reflected by the surface will not be modified by floral pigments inside the flower, so the gloss will generally be perceived as (achromatic) white. (D) Unpolarized light illuminating the flower becomes linearly horizontally polarized when it is reflected by the smooth surface of a flower; the degree of polarization depends on the angle of incidence. In glossy areas and under large angles the ratio of surface versus subsurface reflection – and thus the polarization effect – will be maximal.

in spectral purity (Lunau, 2016), by the change in hue (i.e. iridescence) or by just one hue, such as blue, green or yellow (Morehouse and Rutowski, 2009; van der Kooi *et al.*, 2015; de Premorel *et al.*, 2017). Moreover, the fact that bees can be trained to detect a particular stimulus should not be regarded as evidence for its biological relevance; bees can be trained to a suite of ecologically irrelevant stimuli, for example explosives and dynamite or different artistic painting styles (Rodacy *et al.*, 2002; Wu *et al.*, 2013; Avarguès-Weber and Giurfa, 2014; van der Kooi *et al.*, 2015). Finally, iridescent stimuli have dynamically changing visual signals, which may hamper detection and the learning process (Menzel and Shmida, 1993; Pike, 2015; Kjernsmo *et al.*, 2018). Thus, although very profound in the animal kingdom, iridescence is unlikely to play a major role in plant–pollinator signalling.

Gloss

Gloss (or specularity) is directional reflection of light at a surface. Unlike iridescence, gloss is wavelength-independent and reflection most strongly occurs under mirroring angles (Fig. 4). If the angle of observation or illumination changes to a non-mirroring angle, the observer will only perceive light scattered by the flower interior. In waxy flowers or flowers with very flat epidermises, the surface can act as a mirror, yielding a glossy appearance (Parkin, 1928; Galsterer *et al.*, 1999; van der Kooi *et al.*, 2014). Buttercups and related species (*Ranunculus* and *Ficaria* spp.) are plants with exceptionally glossy flowers (Parkin, 1928). Buttercup flowers have a very flat and smooth epidermal surface, and due to a thin air layer immediately below the epidermis (Vignolini *et al.*, 2012), the epidermis acts as an optical thin film – similar to an oil layer on water or a soap bubble – causing a very glossy appearance (van der Kooi *et al.*, 2017). Some other plant groups feature glossy flowers (e.g. *Adonis* as well as many succulent plant species), although in many species (e.g. *Tulipa*) this is due to non-ordered epidermal striations that yield an overall glossy

appearance (see above, section on structural colour and iridescence). It remains unclear how widespread glossiness is throughout the plant kingdom.

To our knowledge, there is neither evidence supporting nor evidence rejecting the hypothesis that glossiness is a signal for pollinators. On the one hand, insects that approach the flower under an angle mirroring the sun may perceive the gloss as a bright flash, increasing the flower's conspicuousness. On the other hand, the spectrum of the directionally reflected light may be colourless to pollinators, because the reflected light is not (or only weakly) modulated by pigments (Galsterer *et al.*, 1999; van der Kooi *et al.*, 2017). In buttercups, the gloss may enhance the long-distance visual signal, but for short distances, the overall yellow petal colour is probably important. Finally, flower surface properties may play a role in several non-visual functions, such as temperature regulation of floral organs, wettability of the flowers, and tactile cues or grip to pollinators (Kevan and Lane, 1985; Whitney *et al.*, 2011; van der Kooi, 2016; van der Kooi *et al.*, 2017).

In almost all plant species, for near perpendicular illumination the surface reflectance is very small (<5 %) and the visual signal thus is largely due to diffuse light reflection by the scattering structures inside the flower (Kay *et al.*, 1981; Kevan and Backhaus, 1998; Lee, 2007; van der Kooi *et al.*, 2014; Stavenga and van der Kooi, 2016). Many flower surfaces have cuticular microstructures and/or conical epidermal cells (Kay *et al.*, 1981; Lee, 2007; Costa *et al.*, 2017), prohibiting specular reflections (van der Kooi *et al.*, 2014). In *Antirrhinum* snapdragons, these conical epidermal cells were suggested to focus incident light on vacuoles containing (anthocyanin) pigments, so as to increase the saturation of the purple coloration and hence the visibility to pollinators (e.g. Gorton and Vogelmann, 1996). However, the presence of cone-shaped epidermal cells does not change the visitation rate by bees (Dyer *et al.*, 2007) or the spectral properties of the visual signal as perceived by bees (Papiorek *et al.*, 2014). Given the highly irregular shape and orientation of the cones, an alternative, possibly more important role of the cones may be to scatter incident light to many

angles, providing a matt visual signal that is visible from many directions (Wehner and Bernard, 1993; Lee, 2007; van der Kooi *et al.*, 2014). Nonetheless, further studies examining the functional significance of floral epidermal cones would be very valuable, especially as many species of flower have cone-shaped epidermal cells (Kay *et al.*, 1981; van der Kooi *et al.*, 2014).

Polarization

Light is polarized when the light wave vibrations occur in a single plane. Sunlight is unpolarized and remains unpolarized when it is diffusely reflected by a rough surface, such as an irregularly shaped petal's surface. However, oblique illumination of a flat and smooth petal surface can result in strongly polarized reflected light (Fig. 4). The degree to which surface-reflected polarized light can be observed depends on its intensity relative to that of the (unpolarized) light backscattered by the petal interior. If the surface reflectance dominates, as in specular reflection of a glossy flower, polarized light can provide a visual signal to pollinators.

Although polarization vision is a common ability in many insects (reviewed by Cronin *et al.*, 2014), its significance in floral signalling may be expected to be small. A well-known case where polarization is used is that of bees that use skylight polarization as a navigational cue (Cronin *et al.*, 2014). To detect polarized light independent of the intensity of the signal, bees use specialized photoreceptors, which are located in the dorsal part of the eye (Wehner *et al.*, 1975). Polarized light reflected by flowers in front or below the foraging bee will not be perceived, as the distal and ventral parts of the bee's eyes have photoreceptors that effectively abolish polarization effects (Wehner *et al.*, 1975), so as to enhance chromatic detectability (Wehner and Bernard, 1993). In a laboratory experiment by Foster *et al.* (2014), honey bees could be trained to detect downward-facing stimuli with a strong polarization stimulus. The stimulus could only be detected when the bees approached it from below, i.e. when the dorsal part of the eyes faced the stimulus. The significance of polarization patterns in flowers under natural conditions is small, given the widespread occurrence of petal microstructures, such as striations and cones, which prevent a polarization pattern from occurring in virtually all species (Horváth *et al.*, 2002). In addition, the variable approach angle of a foraging bee to different flowers in natural conditions would probably yield polarization as an unreliable cue for identifying conspecific flowers, and thus would not serve as useful information that was evolved for plant–pollinator signalling.

Fluorescence

Fluorescence is the property of materials to absorb light at a particular wavelength and to subsequently emit light of longer wavelengths (Fig. 4). When the emitted light is in the visible wavelength range, it can potentially yield a striking visual appearance. The occurrence of fluorescence is widespread, but the quantum efficiency (the ratio of photons absorbed to the number of photons emitted) of most natural pigments is so low that fluorescence as a visual signal is rare (reviewed by Marshall and Johnsen, 2017). For flowers, fluorescence signalling was suggested to occur in nectar (Thorp *et al.*, 1975), petals (Gandía-Herrero *et al.*, 2005*a, b*) and pollen (Mori *et al.*, 2018). There is, however, no clear experimental evidence showing that plant fluorescence signalling increases the visibility to pollinators. As with most animals, fluorescence quantum efficiency of floral pigments is very low (~1 %), meaning that under natural conditions a fluorescence effect will be swamped by petal reflections (Kevan, 1976; Iriel and Lagorio, 2010*a, b*). Hence, fluorescence can currently be regarded as unimportant for visual signalling for pollinators.

CONCLUSIONS AND OUTLOOK

The plant kingdom offers a bewildering array of flower colours. Floral visual signals have different components (Table 1), each with different relevancy that may vary between environmental conditions and pollinator species. When discussing the importance of floral visual signals in the context of pollinator attraction, it is important to consider that a flower visit by a pollinator is a series of behavioural reactions that might be triggered by different signals. Some visual signals may be more important to detect flowers from long distances, whereas others are more important for reliable recognition at a close range. Navigation and foraging behaviour within the flower may subsequently be modulated by within-flower colour patterns (Kevan *et al.*, 2001; Koski and Ashman, 2014).

Different aspects of the flower's visual signals have different importance for detecting pollinators. Evidence suggests that signal intensity, perceived as brightness, plays no major role in plant–pollinator signalling, but this may differ between habitats and pollinator species. Green contrast perceived by the long-wavelength photoreceptor may play a role, especially in long-distance signalling for honey bees, and at shorter ranges chromatic contrast seems more important (Giurfa *et al.*, 1996; Hempel de Ibarra *et al.*, 2014). Chromatic contrast is determined by both the flower's hue and spectral purity. The relative importance of hue and spectral purity – which has been tested for only a few bee species – remains largely unknown and is likely to be very species- and context-specific. From an evolutionary point of view, changes in the amount of pigment produced (and thus spectral purity) may evolve more rapidly than changes in the type of pigment produced (and thus hue), as it requires only quantitative changes in an already existing pigment synthesis pathway. We note that whereas molecular techniques have been used successfully to study the genetic basis of floral pigment synthesis (Rausher, 2008; Hopkins and Rausher, 2011; Bombarely *et al.*, 2016; Sheehan *et al.*, 2016; Du *et al.*, 2018), we are much further behind in our knowledge on the genetic basis of other aspects of the visual signal (e.g. green contrast or gloss).

There are various other types of potential visual cues caused by the flower's surface (gloss, iridescence and polarization effects) or interior (fluorescence), but these cues are in most cases not of biological significance. This is because such effects are only very locally visible and will thus be swamped by the petal's backscattering. Moreover, visualizing these optical effects generally requires artificial (lighting) conditions, which are so specific that they are unlikely be found

in the complex visual environment where pollinators forage. This means that this is not biologically meaningful in a way that would lead to evolution of a signal. Petal gloss may be an exception in this context because it is visible under natural conditions, although there are currently no behavioural experiments that have tested whether pollinators detect and respond to gloss. The important point from an evolutionary perspective is to understand which of these potential traits are evolved signals and which are incidental as being just part of the physical properties of the floral structure. The colours of rocks, for example, can be amazing to humans, but are clearly not evolved signals for any visual system.

Flower detection by pollinators does not depend solely on chromatic and achromatic properties, but also on the flower's background (Kevan, 1972; Forrest and Thomson, 2009; Bukovac *et al.*, 2017). It is known that colour discrimination and preference is strongly dependent on the background colour in at least bumble bees (Lunau *et al.*, 1996), stingless bees (Koethe *et al.*, 2016), honey bees (Hempel de Ibarra *et al.*, 2000) and diurnal hawk moths (Kelber, 1997). Because in natural settings there is a wide range of backgrounds on which flowers may appear, it is likely that background coloration plays a role in the evolution of floral visual signals.

The anatomy of flowers and how this shapes the visual signal is relatively little studied, leaving many questions on the evolution of the optical properties of flowers unanswered. Formation of the visual signal of a flower is always an interaction of different aspects, including scattering structures, stratification, pigment localization and pigment concentration, which each have their own level of (dis)order (Figs 1 and 3). Recent studies have started to explore how these properties contribute to the visual signal (Stavenga and van der Kooi, 2016; van der Kooi *et al.*, 2016*a*), but whether there are systematic differences between pollination guilds or taxonomic groups and phylogenetic constraints is virtually unstudied. By the same token, little is known about the degree of intraspecific variation in flower colour and its effect on visitation by pollinators in natural populations. Many studies on flower colour variability focused on discrete colour morphs, whilst in many species there is continuous variability in flower colour, with small but to pollinators probably visible changes in floral visual signals (e.g. Chittka *et al.*, 1994; Frey, 2004; Rakosy *et al.*, 2012; Narbona *et al.*, 2018). Experiments with pollinators in response to real (Frey, 2004; Rakosy *et al.*, 2012) and artificial flowers (Papiorek *et al.*, 2013) suggest that pollinators respond to such (small) differences. Hence, it remains largely unknown to what extent small changes in hue, saturation or brightness correspond to changes in reproductive success, which is pivotal in understanding the effects on plant fitness.

When one studies the visual signals of flowers, one must always bear in mind the visual perception of the pollinators (Kelber and Osorio, 2010; Kemp *et al.*, 2015). At present, for model pollinator bee species there is sufficient information on colour processing, including the spectral sensitivity of photoreceptors, opponent channels and psychophysics, to build sophisticated (vision) models; however, for the vast majority of pollinators, including birds, bats and flies, a lot more work needs to be done on if and how particular species process colour. In addition, we emphasize that there is a need for studies that bring laboratory-based knowledge to field experiments. An elegant example are the studies on sexually deceptive *Ophrys*

orchids that used standardized field experiments with real pollinators in the plants' natural habitat, to study the (natural variability of) floral visual signals and (its) their importance for pollination (Streinzer *et al.*, 2009; Rakosy *et al.*, 2012). Nonetheless we emphasize that detailed optical investigations of flowers are often best carried out in laboratory environments, with controlled (illumination) conditions and high standards, to meet the needs of reproducible colour science (*sensu* White *et al.*, 2015). Indeed, whereas portable equipment such as a bifurcated probe may provide a reasonable overall impression of the reflectance spectrum, high-level precision on the absolute amount of backscattering (and thereby brightness) of flowers requires measurements with an integrating sphere (Vukusic and Stavenga, 2009; van der Kooi *et al.*, 2016*a*).

We conclude that the field of flower coloration is rapidly expanding, aided by developments in different scientific domains. In this review we have considered visual signals of flowers mostly as advertising signals to pollinators; however, these signals can also function to deter antagonists, such as florivores or nectar robbers (Strauss and Whittall, 2006; Renoult *et al.*, 2014; Papiorek *et al.*, 2016). In addition, abiotic effects may impose selective pressures on visual effects of flowers and the underlying optical properties. For example, floral pigments can protect against (harmful) ultraviolet light (Koes *et al.*, 1994) meaning that they may occur in certain floral layers for non-visual signalling purposes. We welcome further multi-disciplinary studies that combine physics, chemistry and evolutionary theory to understand the multiple functions and evolution of floral colours.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. File S1: Calculation of colour parameters. Table S1: Characteristics of the spectra shown in Fig. 2A using vision models.

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