The coloration toolkit of flowers
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Document Version
Publisher’s PDF, also known as Version of record

Publication date:
2015

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 4

The glossy display of buttercup flowers: thin film reflectors, filtering pigments and scattering granules

Abstract

The coloration of flowers is virtually always due to wavelength specific absorption by pigments and backscattering of light by irregularly structured petal components. Buttercup flowers are an exception to this rule because they feature a distinct gloss in addition to their matte yellow coloration. Although several anatomical features have been documented, a detailed anatomical and optical analysis on glossy buttercup flowers has not been performed. We investigated the optical properties of glossy yellow petals of several *Ranunculus* species using (micro) spectrophotometry and anatomical methods. The contribution of different petal components to the overall coloration was quantified using an optical model that treats the buttercup petal as a stack of absorbing and scattering layers, using the Kubelka-Munk theory for scattering and absorbing media. We found that the coloration of glossy buttercup flowers is due to a unique combination of structural and pigmentary coloration. A carotenoid-filled upper epidermis acts as a thin film reflector yielding the gloss and additionally serves as a filter for light backscattered by the strongly scattering underlying layers, resulting in the matte yellow color. Our optical model showed that the contribution of the gloss to the overall visual signal to insect pollinators is minor, whereas the intensity of the light reflected to the reproductive organs is large. A likely important function of the gloss is to increase light reflection to the center of the flowers, leading to a higher temperature of the reproductive organs so to enhance reproductive success.

Submitted
Plants display brightly colored flowers in order to distinguish themselves from their environment to attract pollinators (Faegri and Van der Pijl 1979, Schiestl and Johnson 2013, Shrestha et al. 2013, Papiorek et al. 2015, van der Kooi et al. 2015b). In virtually all plant species floral coloration is pigmentary, that is, the coloration is due to light scattering by irregularly structured cell complexes containing wavelength-selective absorbing pigments (Kay et al. 1981, Chittka and Menzel 1992, Lee 2007). Scattered light with wavelengths outside the absorption range of the pigment thus determines the color of the petals. For example, flowers with blue-absorbing carotenoids are yellow, and blue-green-absorbing anthocyanins yield purple colors (reviewed by Grotewold 2006).

In addition to pigmentary coloration, structural coloration can occur when structures exist that are (quasi-)regularly patterned with distances in the sub-micrometer range, that is, of the order of the light wavelength (Srinivasarao 1999, Kinoshita 2008). Structural colors arise in regularly ordered, nano-sized structures composed of materials with different refractive indices, and are common among animals, specifically birds, butterflies and beetles (Srinivasarao 1999, Vukusic and Sambles 2003, Kinoshita 2008, Seago et al. 2009, Stavenga et al. 2010, Pirih et al. 2011). The reflecting structure can be a single layer acting as a thin-film, such as in pigeon feathers and butterfly wing scales (Nakamura et al. 2008, Stavenga 2014), a multilayer, as in beetle elytra and fish scales (Seago et al. 2009, Yoshioka et al. 2011), or a complex photonic crystal, as in peacock feathers (Zi et al. 2003). Typical for structural coloration is that it is highly directional and angle-dependent, i.e. iridescent.

Recently, we studied the contribution of structural coloration to the overall coloration of flowers generated by different epidermal surface structures. We showed that in virtually all the plant species where structural coloration could be observed, this was only possible by applying very local and directional illumination. We therefore concluded that under natural viewing and illumination conditions floral coloration is almost exclusively due to absorption by pigments (van der Kooi et al. 2014, van der Kooi et al. 2015a). In other words, the coloration of the majority of flowers is pigmentary and not structural.

The flowers of many buttercups (Ranunculus spp.) are a clear exception to this rule as they feature a distinct gloss in addition to their overall yellow color (Parkin 1928; 1931; 1935, Galsterer et al. 1999, Vignolini et al. 2012, van der Kooi et al. 2014). Previous studies
demonstrated that the buttercup epidermis contains a carotenoid pigment that absorbs in the blue wavelength range and that the underlying diffusely reflecting starch layer causes an overall matte-yellow color (Parkin 1931; 1935, Brett and Sommerard 1986, Galsterer et al. 1999). Vignolini et al. (2012) suggested that the buttercup’s gloss is due to a thin epidermis that is separated from the underlying structures by an air layer, together functionally acting as a multilayer. However, their study was based on a single species and, as discussed by Vignolini et al. (2012), an optical model of the whole complex petal structure is missing. Therefore, a comprehensive overview that quantifies the contribution of different petal components to the visual signal of Ranunculus flowers has not yet been developed, leaving the complex nature of the flowers’ coloration unknown.

Inspired by the probably structural origin of the glossy color of the buttercups (Vignolini et al. 2012, van der Kooi et al. 2014), we here present an in-depth study of the reflection characteristics of several buttercup species. The genus Ranunculus comprises more than 500 species with a cosmopolitan distribution (Hörandl and Emadzade 2012). We investigated the detailed spectral characteristics of three short herbaceous annual meadow buttercups, i.e. Ranunculus repens L., R. acris L. and R. lingua L., as well as the related non-glossy flowers of the kingcup Caltha palustris L. (all Ranunculaceae). We applied various spectrophotometric methods, anatomy, and optical modeling to assess the different contributions of the petal components to the buttercups’ bright-yellow coloration. We conclude that the coloration of buttercup flowers is due to a unique combination of a plate-like, pigmented upper epidermis, acting as a thin film reflector, with backscattering starch, mesophyll and lower epidermal layers, and we discuss the functional significance of the gloss.

**Materials and Methods**

*Plant material and photography*

All flower samples were obtained from meadows around Groningen, the Netherlands. Flowers were photographed with a Nikon D70 digital camera having an F Micro-Nikkor (60 mm, f2.8) macro objective (Nikon, Tokyo, Japan). Petal details were photographed with an Olympus SZX16 stereomicroscope equipped with an Olympus DP70 digital camera (Olympus, Tokyo, Japan) and a Zeiss Universal Microscope (Zeiss, Oberkochen, Germany) with a Mueller DCM510 camera (Mueller Optronic, Erfurt, Germany).
Spectrophotometry
The overall reflectance and transmittance spectra of the petals were measured with an integrating sphere. For reflectance measurements the flower was directionally illuminated from within the integrated sphere (AvaSphere-50-Refl; Avantes, Eerbeek, the Netherlands); the illuminated area had a diameter of about 5 mm. For transmittance measurements the petal was illuminated from outside the sphere with an optical fiber at an area with diameter ~1 mm. The spectrometer was an Avaspec-2048-2 CCD detector array spectrometer and the light source was a deuterium-halogen lamp (Avantes AvaLight-D(H)-S). A white diffuse reflectance tile (Avantes WS-2) was used as reference.

The spectral characteristics of very small petal areas (5-10 µm diameter) were measured with a microspectrophotometer (MSP). The MSP consisted of a xenon light source, a Leitz Ortholux microscope and the spectrometer. The microscope objective was an Olympus LUCPlanFL N 20x/0.45 (Olympus, Tokyo, Japan). The white diffuse reflectance tile was also used as a reference. For measurements of the absorbance spectrum of the buttercup petal’s pigment, small pieces of petals were first immersed in water. Transmittance spectra were measured on the isolated tissues with the MSP, from which absorbance spectra were calculated.

Cryo-electron microscopy
Cryo-electron microscopy was applied to study the anatomy of *R. acris* petals. Petals were glued in a special copper holder and quickly frozen in nitrogen slush. The samples were examined in a JEOL 5600LV scanning electron microscope (JEOL, Tokyo, Japan) equipped with an Oxford CT1500 Cryostation for cryo-electron microscopy (cryoSEM). Electron micrographs were acquired from uncoated frozen samples.

Thin film optics
We calculated the reflectance of a thin film with thickness $d$ and refractive index $n$ in air using the classical Airy formulae. With normal illumination the reflectance spectrum has extrema at wavelengths $\lambda_e = 4nd/u$, with $u$ integer; the reflectance is maximal when $u$ is odd and minimal when $u$ is even (Yeh 2005, Stavenga 2014). The wavenumbers of the extrema ($k_e = 2\pi/\lambda_e$) hence are a linear function of $u$: $k_e = su$, with slope $s = \pi/(2nd)$.

We assumed an upper epidermis with a refractive index of 1.4 (Vignolini *et al.* 2012), which contained β-carotene with peak absorbance 1.4 (at 448 nm; Results) and faced air on both
sides. In our model we considered a number of cases where the thin film had a Gaussian varying thickness with mean 2.7 µm and standard deviation σ = 0, 25, 50, 75, 100 and 125 nm.

Modeling petal reflectance and transmittance

The diffuse coloration of flowers is due to the randomized pathways of light propagating in the petal interior (Supplementary Material, Chapter 2). We modeled the petal reflectance and transmittance by considering a petal as a stack of absorbing and scattering layers. We therefore combined the Kubelka-Munk theory for absorbing and scattering media (Kubelka and Munk 1931) with a calculation procedure for a stack of absorbing and scattering layers (Yamada and Fujimara 1991, Stavenga et al. 2006, Chapter 2). The parameters of the model were based on our anatomical findings, unless specified otherwise. We assumed for the upper epidermis a Gaussian varying thickness with a mean of 2.7 µm and standard deviation of 125 nm, for the starch layer a thickness 38 µm (Parkin 1935), and for the mesophyll and lower epidermis thicknesses of 100 and 40 µm, respectively. For the pigment of the upper epidermis we estimated an absorption coefficient of $1.4/(0.4343\times2.7) = 1.2 \, \mu m^{-1}$, and the mesophyll and lower epidermal layers were assumed to contain the pigment of the lower epidermis (obtained with MSP measurements) with a peak absorption coefficient 0.02 and 0.04 µm$^{-1}$, respectively. The scattering coefficients of the starch layer, mesophyll and lower epidermis were heuristically assumed to be 10, 5 and 2 mm$^{-1}$, respectively. Finally, the surface of the lower epidermis was assumed to be 0.1 (and hence the transmittance 0.9). Implementing these parameters in the model yielded identical transmittance spectra for illumination at the adaxial and abaxial sides ($T_{ad}$ and $T_{ab}$), as expected from the reversibility of light rays (Supplementary Material, Chapter 2).

Results

Reflectance and transmittance spectra of buttercup petals indicate petal asymmetry

We investigated the general spectral characteristics of the petals of all four species with an integrated sphere. The reflectance and transmittance was low in the blue wavelength range and high above 500 nm (Fig. 1). The transmittance spectra showed a slight depression at ~680 nm, which is fairly common in yellow flowers (Chapter 3) and indicates the presence of α-chlorophyll. Whereas for the kingcup the reflectance and transmittance spectra were rather similar, for the buttercups these spectra differed from each other in the ultraviolet, suggesting that the petals are
asymmetric in pigmentation and/or anatomy (Parkin 1928; 1931; 1935, Brett and Sommerard 1986, Galsterer et al. 1999).

**Buttercup petals are both specular and matte**

The buttercups’ remarkable specularity is clearly visible in the distal part of the petal (Fig. 1a-c, 2a,c). When observed with oblique illumination, neat rows of effectively scattering granules were discernible in the main petal area (Fig. 2b). While applying epi-illumination with polarized light, the surface reflections were fully extinguished by a crossed analyzer; only a weak backscattering from the inner petal structures remained (Fig. 2d).

**Figure 1:** Reflectance and transmittance spectra of petals of four Ranunculaceae species. (a) *Ranunculus repens*; (b) *R. acris*; (c) *R. lingua*; (d) *Caltha palustris*. Green curves: reflectance, $R$; red curves: transmittance, $T$. 
The glossy display of buttercup flowers

Figure 2: Coloration of a glossy *Ranunculus acris* petal. (a) The glossiness is restricted to the main distal area of the upper (adaxial) side, which is bright yellow, while the small proximal area lacks specularity. (b) Transition area, indicated by the rectangle in (a), showing granular rows in the main petal area with oblique illumination. (c) The same area with normal epi-illumination, showing the distinct glossiness of the main petal area. (d) The same area and illumination, but with crossed polarizer and analyzer, showing extinction of the surface gloss. (e) Close-up of a petal with disrupted upper epidermis, showing bare starch cells. (f) Isolated upper epidermis observed in transmitted light, showing the continuity of the yellow pigment. Scale bars: (a) 1 cm, (b-d) 1 mm, (e) 100 µm, (f) 50 µm.
Below the upper epidermis the rows of scattering granules form together the starch layer (Parkin 1931; 1935). Removing the upper epidermis demonstrated that the starch granules are white (Fig. 2e), indicating that the yellow color of the petals is due to a short-wavelength absorbing pigment concentrated in the upper epidermis. Indeed, the isolated upper epidermis with transmitted light shows a bright, yellow color, clearly due to a homogeneously distributed, blue-absorbing pigment (Fig. 2f).

A somewhat homogeneous yellow coloration was seen when focusing at the lower epidermal layer (Fig. 3a). Close-up views of the petal’s abaxial side showed yellow reflections with bright contours shining through. The cell contours became distinctly visible when focusing at the mesophyll layer (Fig. 3b), suggesting that the boundaries of the mesophyll cells are strongly scattering. In addition, buttercup petals contain numerous air spaces, which markedly contribute to the light scattering (Fig. S2b-e). Reflectance spectra measured from a fully water-filled petal were about a factor 2 smaller than the spectra measured from a dry petal, highlighting the major contribution of the intercellular air spaces to the overall reflectance (Fig. S2a).

We applied imaging scatterometry to visualize the spatial reflection properties of the specular gloss and the backscattering from the petal interior (Fig. S1a). The scatterogram of the adaxial side of the *Ranunculus* flowers yielded a bright spot created by the specular upper epidermis together with a wide-field yellow light distribution due to the backscattering petal interior (Fig. S1b; see also van der Kooi et al. 2014). Interestingly, the abaxial side of the petals did not show the gloss, which can be immediately understood from the convex surface of the epidermal cells that reflect light into a wide angular space (Fig. S1c,d). Similarly, the adaxial side of the flowers of the related kingcup, *Caltha palustris*, featured a matte-yellow coloration without any gloss, due to the conically shaped epidermal cells (Fig. S1e,f; see also Papiorek et al. 2014).

The lower epidermis and mesophyll contain various pigments
To investigate the spectral characteristics of the pigmentation of the adaxial and abaxial sides, we measured reflectance spectra of very small areas (diameter 5-10 µm) with a microspectrophotometer (MSP). The shapes of the spectra measured abaxially from the bright contours and in between the contours were different (compare Fig. 3c, mesophyll and lower epidermis), which is possibly due to wavelength-dependent scattering of the mesophyll cells. Nevertheless, both spectra prominently showed a distinctly depressed reflectance at ~680 nm,
The glossy display of buttercup flowers

suggesting the presence of α-chlorophyll, which was virtually absent in the spectra measured from non-glossy areas at the adaxial side (Fig. 3c, upper epidermis), indicating an asymmetrical deposition of pigments in the petal.

Figure 3: Coloration of the abaxial side of R. acris petals and pigmentation. (a) A small area observed with bright field illumination and focus at the lower epidermis. (b) Dark field illumination with focus at the mesophyll layer; scale bar: (a, b) 50 µm. (c) Reflectance spectra measured from 5-10 µm sized areas with the microspectrophotometer (MSP) of the lower and isolated upper epidermis and a boundary area of mesophyll cells. (d) Normalized absorbance spectra measured with the MSP from an isolated upper and lower epidermis, together with the spectrum of β-carotene in hexane (obtained from http://omlc.org/spectra/PhotochemCAD/data/041-abs.txt).

Transmittance measurements on isolated upper and lower epidermal layers of R. acris obtained with the MSP showed that the absorbance spectrum of the blue absorbing pigment in the epidermal cells (Fig. 2f) was very similar to the spectrum reported for β-carotene dissolved in hexane, yet the peaks in the measured buttercup spectra (at 425, 448, and 478 nm) were more pronounced (Fig. 3d). For the upper epidermis, the estimated absorbance value at the peak
wavelength 448 nm was 1.4 ± 0.3, whereas for the lower epidermis it was more variable: 0.8 ± 0.5. The distinct peak in the absorbance spectrum of the lower epidermis at around 680 nm, indicated again that the lower epidermis contained a minor amount of α-chlorophyll (Fig. 3d; see also Fig. S2a, dry).

Anatomy of the Ranunculus petal

We examined the anatomy of buttercup flowers more closely by applying cryo-electron microscopy on *R. acris* petals. The upper epidermis thus appeared to have a very smooth surface, with a very constant thickness of ~2.8 µm (Fig. 4a). Air gaps separated the plate-like upper epidermis and the underlying starch granule layer; locally the upper epidermis touched the starch granules. The thickness of the air layer was variable, mostly due to the irregularly shaped starch cells (Fig. 4a). Figure 4b presents a diagram of the inner structure of the *Ranunculus* petals based on these new anatomical data combined with previous findings (Parkin 1928; 1931; 1935, Brett and Sommerard 1986, Galsterer et al. 1999). We consider a petal to contain four layers with different cell types and structures (Fig. 4b). The very thin upper epidermis (which contains highly concentrated carotenoid pigment) faces slanted palisade parenchyma cells, which are filled with grainy starch. The mesophyll and lower epidermis form additional layers and both contain carotenoid pigment and α-chlorophyll, mostly in boundary areas. Variably sized air spaces exist between the cells.

**Figure 4:** Anatomy of a *R. acris* petal. (a) Low-temperature scanning electron micrograph of a cut petal. The upper epidermis (arrow) is a flat layer with thickness of a few micrometer. Below the epidermis are the randomly distributed starch cells (asterisk). The thickness of the air layer beneath the epidermis is variable and large air spaces exist between the starch cells and cells of the mesophyll layer (m). Scale bar: 10 µm. (b) Diagram of a *Ranunculus* petal; ue: upper epidermis, sl: starch layer, m: mesophyll, le: lower epidermis.
The glossy display of buttercup flowers

The surface gloss is due to the upper epidermis acting as an optical thin film reflector

Figure 5 shows two flowers of the Lesser celandine, *R. ficaria*, where the lower flower has the common overall yellow color, and the upper flower is an occasional example where the petals are distally white (white glossy buttercup ‘morphs’ are found occasionally; Parkin 1928). The finding that the upper epidermis was a thin plate separated by air holes from the starch layer suggested that the upper epidermis acts as a thin film reflector. We therefore measured the adaxial reflectance spectra of very small areas with the MSP. The reflectance spectra revealed distinct oscillations, characteristic for a thin film reflector (Fig. 5b). In the yellow petals of *Ranunculus* species the oscillations were absent in the blue wavelength range, where the carotenoid pigment strongly suppressed the reflectance (Figs 5b, 6a, S3a,c). By fitting a linear function to the wavelengths of the reflectance extrema converted into wavenumbers (Materials and Methods; see also Stavenga 2014), we derived from the *R. ficaria* spectra a thin film thickness value of $\sim 2.6 \, \mu m$ (Fig. 5b, inset). Similarly, from *R. repens*, *R. acris*, and *R. lingua* thickness values of $2.3 \pm 0.3 \, \mu m$, $2.7 \pm 0.4 \, \mu m$, and $3.1 \pm 0.5 \, \mu m$, respectively, were obtained. These values are in good agreement with the thickness value obtained by anatomy (Fig. 4a).

![Figure 5](image)

**Figure 5**: Thin film optics of the upper epidermis of *R. ficaria*. (a) Two *R. ficaria* flowers, the upper flower having petals with white areas. (b) Reflectance spectra measured with an MSP from a small area of a white (a, upper arrow) and yellow petal area (a, lower arrow). Inset: the wavelengths of the reflectance extrema converted into wavenumbers (closed symbols: maxima; open symbols: minima) fitted with a linear function.
To ascertain our conclusion that the upper epidermis acts as a thin film, we performed optical modeling. We assumed an upper epidermis with a refractive index of 1.4, which contained β-carotene with peak absorbance 1.4 (at 448 nm), facing air on both sides. We applied classical thin film theory and considered a number of cases where the thin film had a Gaussian varying thickness with mean 2.7 μm and standard deviation $\sigma = 0, 25, 50, 75, \text{ and } 100 \text{ nm}$ (Fig. 6c). In the ultraviolet and long wavelength range the reflectance featured strong oscillations, depending on the variation in the thickness, but in the blue wavelength range all calculated reflectance spectra exhibited a distinct trough, due to the absorbing β-carotene (Fig. 6c). The oscillations vanished when the standard deviation of the thickness exceeded ~5% (Fig. 6c, $\sigma \geq 100 \text{ nm}$; see also Fig. S4), explaining why the oscillations are only measurable from very small areas. We also considered a model incorporating the underlying air layer, thus causing a multilayer (sensu Vignolini et al. 2012). This yielded oscillating spectra with slightly higher peak values but the same periodicity as for the thin film (Fig. S4). However, because the air gap size is highly variable (Fig. 4a) and the starch layer will act as a wide-field scatterer rather than as a plane reflector, we conclude that multilayer interference is unrealistic.

**The buttercup petals can be treated as a stack of absorbing and scattering layers**

Quantitative insight into flower coloration can be gained by treating the petals as a stack of layers, each characterized by its specific reflectance and transmittance spectra accounted for by using the theory developed by Kubelka and Munk (1931) for tissues that absorb and/or scatter (Chapter 2). Our anatomical studies showed that whereas the buttercup’s upper epidermis can be considered as a homogenous medium with negligible scattering, the starch, mesophyll and lower epidermal layers are distinctly inhomogeneous. We applied a Kubelka-Munk-layer-stack model to the four-layer stack of a *R. acris* petal. This yielded distinctly different adaxial and abaxial reflectance spectra (Fig. 6d). The adaxial reflectance ($R_{ad}$) had a deeper trough in the blue wavelength range than the abaxial reflectance ($R_{ab}$), due to the higher concentration of carotenoid pigment in the upper epidermis compared to the lower cell layers, whereas the abaxial reflectance spectrum had a pronounced dip at ~680 nm, due to the exclusive abaxial presence of chlorophyll (Fig. 6d).
The glossy display of buttercup flowers

Discussion

The coloration toolkit: a pigmented thin film and underlying backscattering layers

When directionally illuminated, buttercup flowers are exceptional because they display a distinct gloss in addition to an overall matte-yellow color (Figs. 1, 2). We found that the coloration of buttercup flowers is due to a unique combination of a pigmented thin film reflector and a strongly
scattering underlying layer. The clearly oscillating reflectance spectra measured from very small petal areas of four *Ranunculus* species demonstrated that the epidermal layer acts as a thin film because it locally has a (quasi-)constant thickness. Therefore, the thickness of the upper epidermis can be directly derived from measurements of the petal reflectance (Figs. 5, 6).

The upper epidermis contains a high concentration of carotenoid pigment, most likely β-carotene, which creates an effective spectral filter for the light backscattered by the underlying cell layers. The backscattering starch layer in *Ranunculus* flowers was previously studied by Parkin (1928; 1931; 1935), who found that the starch cells were arranged in a slanted manner (Fig. 4b). The cells are ordered in parallel rows, and because of their irregular size and granular shape, the starch layer acts as a diffuse reflector (Figs. 2b, e). The optical model used in this study confirmed that the thickness of the upper epidermis, independent of the air gap and starch layer, determines the periodicity of the oscillations. Reflectance measurements from various areas of the same petal yielded thicknesses varying by >10%. Consequently, the reflectance spectra from larger areas are smooth (cf. Fig. 1, 6d).

*Functionally glossy?*

Although seemingly prominent, the biological function of the gloss, which is very directional, has remained enigmatic. For about normally incident light, the gloss’ reflectance amplitude is no more than ~5% (Fig. 6c), meaning that the contribution of the surface reflections to the overall visual signal is minor. It therefore remains questionable whether the gloss will be observable by insects, also because insects have compound eyes with limited spatial acuity (e.g. Giurfa *et al.* 1996, Cronin *et al.* 2014, Hempel de Ibarra *et al.* 2014). Rather, mainly the diffuse yellow color will be visible to pollinators (cf. Figs. 1, 6d). Nevertheless, Parkin (1928) noted that the occurrence of glossy flowers is widespread amongst *Ranunculus* species, and given the vast number of species within this genus (Hörandl and Emadzade 2012), the number of *Ranunculus* species exhibiting glossy flowers is likely to be very large.

Are there other explanations as to why buttercups could exhibit this complex anatomy?

It is important to realize that the reflectance amplitude of a thin film reflector considerably increases with an increasing angle of incidence (Fig. 7a). Interestingly, buttercup species are heliotropic (Stanton and Galen 1989, Kudo 1995, Totland 1996, Luzar and Gottsberger 2001, Galen and Stanton 2003, Ida and Totland 2014) and often the flowers have the shape of a paraboloid, meaning that incident sun light will be notably reflected towards the central
The glossy display of buttercup flowers

flower area where the reproductive structures are located (Fig. 7b; see also Kevan 1975). Heliotropic Ranunculaceae species (*Adonis ramose*, *Ranunculus adoneus*, *R. alpestris*, *R. montanus*, *R. glacialis*) were found to exhibit increased flower temperature, which enhances pollen germination, increases seed weight and is preferred by pollinators (Stanton and Galen 1989, Kudo 1995, Luzar and Gottsberger 2001, Galen and Stanton 2003, Ida and Totland 2014). A likely and important function of the glossy epidermis hence is to reflect incident sun light towards the center of the flower, so to achieve a higher reproductive success.

**References**


The glossy display of buttercup flowers


Supplementary material

Modelling multilayer interference
To investigate the possibility of multilayer interference, we calculated the reflectance of a multilayer consisting of a carotene-filled thin film, with fixed thickness 2.7 µm, and an air layer with constant thickness 5.0 µm facing an underlying infinite (starch) layer with refractive index 1.36. This yielded an oscillating reflectance spectrum with periodicity very similar as that for an isolated upper epidermis in air; yet, the peak values of the multilayer were distinctly higher (Fig. S4, \( m = 2, \sigma = 0 \text{ nm} \)). We also calculated the reflectance spectrum for a multilayer consisting of a fixed thin film combined with an air layer with Gaussian-varying thickness with standard deviation 100 nm (Fig. S4, \( m = 2, \sigma = 100 \text{ nm} \)). This slightly changed the overall shape of the reflectance spectrum, but the spacing of the reflectance extrema was affected negligibly (Fig. S4).

Optics of a stack of scattering and absorbing layers
The buttercups can be considered to consist of 5 layers: the upper epidermis: a pigmented thin film; the starch layer: an absorptionless, strong scatterer; the mesophyll and lower epidermis: two inhomogeneous, pigmented layers, i.e. with noticeable scattering; the lower epidermis surface facing air, i.e. a slightly reflecting layer. The reflectance and transmittance of tissues that scatter and contain pigment can be derived from Kubelka-Munk theory when the tissue’s thickness (\( d \)) as well as the scattering (\( S \)) and absorption (\( K \)) coefficients are known (for details, see Chapter 2). We calculated the reflectance and transmittance spectra of the buttercup \( R. \text{acris} \) with the above combined Kubelka-Munk-layer-stack model (Fig. 6d).
Figure S1: Imaging scatterometry of the buttercup *Ranunculus acris* and the kingcup *Caltha palustris*. (a) Diagram of a reflecting and scattering buttercup petal. Incident light is partly specularly reflected (white arrows) and diffusely scattered and transmitted (yellow arrows). (b) Scatterogram of the smooth and flat adaxial side of a petal of *R. acris*, showing a local bright spot, indicating specular reflection, and a diffuse yellow scattering pattern. (c) Epi-illumination of the lower epidermis of *R. acris*, showing the slightly rough surface. (d) Scatterogram of the abaxial side of a petal of *R. acris*, showing a very diffuse yellow pattern. (e) The upper epidermis of the kingcup *Caltha palustris* illuminated from a slightly oblique side, showing the cone-shaped epidermal cells. (f) Scatterogram of the upper epidermis of *C. palustris* demonstrating very diffuse scattering. Scale bar (c, e): 50 µm. The red circles in (a), (b), (d) and (f) indicate angular directions of 5, 30, 60 and 90° (for details, see Chapter 5).

Figure S2: Reflection changes induced by wetting cut buttercup petals. (a) Reflectance spectra measured with an integrated sphere from the adaxial and abaxial sides of a dry and fully internally wetted *R. lingua* petal. (b-e) Progressive capillary water uptake by a cut petal of *R. repens* after putting a water drop at the side of the cut (photographs taken with an interval of 15 s).
The glossy display of buttercup flowers

**Figure S3:** Reflectance spectra measured in the main distal area of buttercups with a microspectrophotometer (MSP) and wavenumber values of the oscillation extrema. (a, b) *Ranunculus repens*; (c, d) *R. lingua*. The oscillating spectra are from areas with gloss. The yellow curves were obtained from matte areas, that is, where the surface reflections were outside the aperture of the MSP’s objective.

**Figure S4:** Reflectance spectra of: i) a thin film (m = 1) with Gaussian varying thickness (σ = 125 nm) and mean thickness 2.7 µm; ii) a multilayer consisting of a thin film with fixed thickness 2.7 µm and an air gap with thickness 5.0 µm facing an infinite (starch) layer with refractive index 1.36 (m = 2, σ = 0 nm); iii) the latter multilayer but where the air gap thickness varied in a Gaussian way (m = 2, σ = 100 nm).